Planta Medica

An International Journal of Natural Products and Medicinal Plant Research

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An International Journal of Natural Products and Medicinal Plant Research

55th International Congress and Annual Meeting of the Society for Medicinal Plant Research

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Abstracts

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The Society for Medicinal Plant Research

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Editorial

The Society for Medicinal Plant Research (GA) is going to organize its 55th Annual Meeting and International Congress in Graz/Austria. It will be held at the Karl-Franzens-University of Graz and in the Convention Center Graz. As the Chairman of the congress and also currently as President of GA and Head of the Institute of Pharmaceutical Sciences of University of Graz, I am very happy that our congress has been accepted so well and that so many scientists dealing with medicinal plants research are gathering 2007 in Graz.

The main topics of the congress are
- Anti-inflammatory and immunomodulatory active natural products
- Natural products with antimicrobial activity
- Analysis and biopharmaceutics of herbal medicinal products
- Traditional herbal medicinal products
- Medicinal plants in animal healthcare

However, scientific contributions of other topics related to medicinal plant research will also be presented.
I am very happy, that Prof. Dr. H.R.H. Princess Chulabhorn Mahidol has accepted to give the opening lecture of the congress. Moreover, seventeen distinguished scientists have agreed to give plenary or keynote lectures. Besides, 34 short lectures and more than 650 poster presentations have been accepted. In addition, seven workshops will be held on specific topics. So, in total the congress will offer more that 750 scientific presentations.

We want to thank Thieme, in particular Dr. Kuhlmann, for accepting that the abstracts could be printed in Planta Medica and for the proper processing. It is a huge task to handle this high number of abstracts and I want to thank also the editorial team, in particular Prof. Franz Bucar and his assistant Mag. Marlene Monschein, for all their efforts. Also Dr. Wolfgang Schühly, Dr. Eva Wenzig and Dr. Karin Wölkart contributed a lot to edit the abstracts and helped to bring them into a form which could be accepted for Planta Medica. All colleagues who acted in the Scientific Committee as reviewers and who actively contributed to the high scientific stand of this abstract volume are also acknowledged.

Prof. Dr. Rudolf Bauer
Chairman of GrAz 2007

PL 001

Bioactive Natural Products: A journey of a Thai scientist

Mahidol C
Chulabhorn Research Institute, Vipavadee Rangsit Highway, Bangkok 10210, Thailand

Chemotherapy is one of the well-established approaches for the remedy of a large number of chronically debilitating or life-threatening diseases that urgently require improved or new medical treatments. With new emerging diseases and increasing resistance to existing drugs, there is a pressing need to discover and develop new innovative drugs with diminished side-effects to combat cancer cells, viruses and other threats. Research on natural products is essential for the discovery of lead compounds because of the incredible diversity of chemical structures that are produced by living animals, plants, micro-organisms and marine organisms. It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities. In this presentation, our current research on natural products from various bioactive Thai medicinal plants will be presented. **References:** [1] Mahidol, C.; Prawat, H.; Ruchirawat, S. Bioactive natural products from Thai medicinal plants. In Phytochemical Diversity: A source of new industrial products; Wrigley, S.; Hayes, M.; Thomas, R.; Chrystal, E., Ed.; The Royal Society of Chemistry (RSC), England (1997), 96 – 105. [2] Mahidol, C.; Prawat H.; Prachyawarakorn V.; Ruchirawat S. (2002) Phytochemistry Reviews 1: 287 – 297. [3] Prachyawarakorn, V.; Mahidol, C.; Ruchirawat, S. (2006) Phytochemistry, 67, 924 – 928. [4] Mahidol, C.; Prawat, H.; Kaweetripob, W. and Ruchirawat, S. Nat. Prod. Commun. 2007, 2, 557 – 564.

PL 002

Biodiversity and the Search for New Medicines

Cox PA
Institute for Ethnomedicine, Box 3464, Jackson Hole, Wyoming 83001 USA

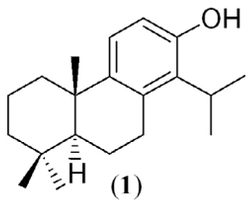
Despite remarkable progress in synthetic chemistry, human efforts have scarcely been able to document, let alone duplicate, the plethora of bioactive molecules present in biodiversity on earth. Some bioactive molecules, such as enzymes in mosquitos that serve to detoxify DDT are of very recent date, while others, such as the cyanobacterial toxin BMAA, may have occurred far before the paleozoic era. A variety of molecular structures present in plants, animals, fungi, bacteria, and viruses are difficult, if not impossible, to synthesize. Only 60 percent of the 11,500 different structural types of natural products are represented by synthetic compounds. Given this molecular diversity, it is not surprising that human cultures rely on biodiversity as a source of medicines, with 85% of the world's population relying directly on plants for their primary healthcare. Over 50% of western pharmaceuticals are derived from biodiversity. Useful bioactive molecules derived from biodiversity, however, do not appear to be equally derived from all life forms. In a convergence between strategies employed by modern pharmaceutical researchers and indigenous healers, the search for new drugs is now focused on sessile organisms, particularly plants and marine invertebrates. The discoveries of palytoxin, taxol, unique COX-2 inhibitors, artemisin, anti-spasmodic isperenoids, prostratin, and BMAA all illustrate different successful approaches to the discovery of unique bioactive molecules, including random screening, ecological screening, phylogenetic analysis, environmental genome sequencing, and ethnobotanical techniques. Compared to combinatorial chemistry, these biodiversity-based techniques appear to be far more productive avenues for the discovery of new medicines. By joining biodiversity rich nations with biotechnology rich institutions, the Convention on Biodiversity can provide incentives for discovery of exciting new medicines, while ensuring equitable sharing of benefits.

PL 003

Phytochemicals for bacterial resistance – strengths, weaknesses and opportunities

Gibbons S
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London, WC1N 1AX, U.K.

There are enormous challenges facing the pharmaceutical industry with regard to the burgeoning threat of bacterial multi-drug resistance (MDR). The proliferation of strains of methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile,* intrinsically resistant Gram-negative bacteria such as *Acinetobacter baumannii* and extremely-drug resistant *Mycobacterium tuberculosis* (XDR-TB) [1] are cause for considerable concern. Very few new antibacterials are appearing largely because the markets are both specialist and small, or are for areas of the developing world where the population is unable to pay for expensive new antibiotics. Recent new cases of XDR-TB in the US and Europe and reports of a global increase in patients with strains of MDR-TB will focus attention on the need for new agents to deal with these threats. Plants are a practically untapped source of potential new antibacterials and some of the literature examples have outstanding preliminary activity [2]. There is also an ecological rationale that plants produce antimicrobial natural products as part of their defence strategy against pathogenic microbes in their environment. Clinical strains of resistant bacteria are also unlikely to have encountered some of the new structural motifs of plant antibacterial, and this would be advantageous [3]. Additionally, some plant antibacterials such as totarol (**1**) are active against multidrug-resistant strains and are also inhibitors of the multidrug-resistance that characterises these strains. This dual mode of action would suggest that there is considerable prospect to develop new classes of antibacterial from plants and this paper will highlight some of these opportunities.



(**1**)

References: [1] Migliori, G.B. et al. (2007) Eur. Respir. 29: 423 – 427. [2] Gibbons, S. (2004) Nat. Prod. Rep. 21: 263 – 277. [3] Smith, E.C.J. et al. (2007) Phytochemistry 68: 210 – 217.

PL 004

Natural products with antimicrobial activity: from figures to facts

Maes L
Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Department of Biomedical Sciences, University of Antwerp, Belgium

Natural products provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. However, obtaining a sound 'proof-of-concept' will depend on validated *in vitro* experimental procedures in combination with realistic efficacy criteria. Of primary importance are the inclusion of parallel cytotoxicity evaluation and an IC_{50} endpoint criterion of < 100 μg/ml for crude extracts and < 25 μM for purified fractions and pure compounds [1]. Promising 'hits' should be active against the 'whole-organism' and/or in cell-based models. The next pivotal hurdle is confirmation of the *in vitro* 'hit' in matching animal models that will constitute the basis for defining "early lead" status. At least a slight

trend for activity should be demonstrated when using a suppressive treatment regimen. Resources and expertise for advanced 'lead development' are not satisfactorily available in the public and academic sector, endorsing the need for strategic networking particularly in areas with limited interest from the pharmaceutical industry. Early 'leads' should have a proper balance between pharmacological properties, metabolism-pharmacokinetic and toxicity profiles. As a preamble for *in vitro* and *in vivo* evaluation, solubility in water, simulated gastric- and intestinal fluid and in standard pharmaceutical vehicles becomes important knowledge. Since most drugs are intended for oral use, gastrointestinal absorption is best studied in rats. First-pass elimination by liver microsomes may explain unexpected lack of systemic activity. Several toxicological parameters do become available during the cell-based *in vitro* screening (cellular toxicity), *in vitro* pharmacological profiling (CYP_{450} interactions) and efficacy experiments in animals (acute toxicity in dose-titration experiments). The Ames-test carries adequate predictive power for evaluation of mutagenic potential. It is important that academic investigators become aware of the basic needs beyond the stage of 'simple' pharmacology. Early "go – no go" criteria should be introduced and fit in a "fail fast – fail cheap" rationale. Being successful in the latter will improve valorization of the current research efforts and enhance the chances that new (antimicrobial) drugs will indeed reach the patient in need. **References:** [1] Cos P, Vlietinck AJ, Vanden Berghe D, Maes L. (2006): Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J. Ethnopharmacol. 106: 290 – 302.

PL 005

Functional Plant Products in Veterinary Medicine and Animal Nutrition

Franz C

Institute for Applied Botany and Pharmacognosy of the University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria

Herbs and herbal products are also in veterinary medicine and livestock production of increasing importance. Besides of an "ethnoveterinarian revival" there are two reasons responsible for the respective development:

- the preference of pet animal and horse owners for "soft medicine"
- the restrictive use of synthetic drugs and antibiotics in farm animals

As regards some important disorders and diseases of companion animals, recent results have shown a successful treatment of male dogs suffering from BPH by a pumpkin seed extract (*Cucurbita pepo* var. *styriaca*), and chronical dermatitis could be controlled by application of tea-tree-oil (*Melaleuca alternifolia*) preparations [1]. COPD/RAO of horses could be treated by either a thymol containing preparation or a herbal medicinal product consisting of *Gentiana*-, *Primula*-, *Rumex*- and other extracts [2]. A specific challenge represent food producing animals since by 01.01.2006 the use of antibiotic growth promoters is banned throughout the European Union and in addition, since in organic farming "herbal preparations should be preferred to synthetic allopathic drugs" (EU-Directive 1804/99). Nonetheless microbial induced diarrhoea remains one of the crucial problems especially at pigs and poultry. Recent investigations resulted in significant antimicrobial and growth enhancing effects of several essential oils and herbal mixtures [3]. Special emphasis is given to the European joint research project SAFEWASTES where a number of residues of herbal extraction have been investigated on their antimicrobial and antioxidative activity. Many of the "herbal wastes" have shown anti-adhesive effects of bacteria in-vitro, and some of the materials have shown already promising results in-vivo [4]. Although there is still little information on veterinary phytotherapy, the recent results are promising presupposed a controlled quality of the herbal products in question and standardised veterinary clinical trials. **References:** [1] Reichling, J. et al. (2004): Dt. Tierärztl. Woschr. 111: 408 – 414. [2] Van den Hoven, R. et al. (2003): Veterinary Record 152: 555 – 57. [3] Kyriakis, S.C. et al. (1999): Res. Vet. Sci. 67: 223 – 28. [4] www.safewastes.info

PL 006

Immune surveillance of the central nervous system in health and disease – therapeutic intervention by natural products

Ullrich O

Institute of Anatomy, Faculty of Medicine, University of Zurich. Winterthurer Str. 190, CH-8057 Zurich, Switzerland

The way cells of the immune system defend the organism, may be dangerous for the survival and function of the neuronal network in the brain. Since neurons regenerate slowly, immune attacks inside the brain potentially imply the danger of an irreversible life-long loss of functional CNS tissue. Inflammatory reactions in the CNS, which result from a loss of control and involve a network of non-neuronal and neuronal cells, contribute significantly to the onset and progress of several major neurodegenerative diseases. On the other hand, constant immune surveillance is indispensable for neuroprotection and neurorepair, in particular during pathological conditions. To avoid inflammatory escalation, the CNS harbours an impressive arsenal of cellular and molecular mechanisms enabling strict control of immune reactions – the so-called "immune privilege". Balancing and controlling the immune response in the CNS by intervention in specific molecular mechanisms might be the key to protecting neurons from inflammatory damage. Thus, elucidating the molecular pathways of immune activation and -control in the CNS will lead to the identification of molecular targets and strategies for neuroprotective interventions, which keep or restore the well-controlled and finely-tuned balance of immune reactions, and protect neurons from inflammatory and non-inflammatory damage. Recently, the endocannabinoid system has been identified to participate crucially in CNS immune control [1] and represents a promising target of natural products-derived drugs [2] Moreover, natural products from plants turned out to be powerful modulators of inflammatory signal pathways in cell of the immune system and to be highly effective in animal models of inflammatory CNS diseases [3]. **References:** [1] Eljaschewitsch, E. et al. (2006) Neuron 49(1): 67 – 79. [2] Ullrich, O. et al. (2006) Results Probl. Cell. Differ. 43: 281 – 305. [3] Aktas, O. et al. (2004) J Immunol. 173: 5794 – 5800.

PL 007

Natural products and chronic inflammatory vascular processes

Dirsch VM

Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Inflammation is defined as a complex biological response of vascularized tissues to various exogenous or endogenous stimuli causing cell injury. Injurious stimuli range from physical to metabolic stress. Thus, various and at first glance diverse pathological conditions involve inflammatory processes. These include among 'classical' inflammatory diseases, such as arthritis also atherosclerosis, cancer, and even obesity and the metabolic syndrome. Most of these pathological conditions are connected to a "low grade" or "chronic" form of inflammation characterized by the presence and response of T cells and macrophages, proliferation of vascular cells, fibrosis and tissue destruction. Whereas a great focus was set within recent years on natural products and their effects on leucocyte response (cytokines, reactive oxygen species, nitric oxide, arachidonic acid metabolites, NF-κB signaling) this presentation will focus on natural products and their effects on vascular smooth muscle cell (VSMC) growth contributing to chronic inflammatory vascular processes, such as atherosclerosis and restenosis. To answer the question what natural compounds might interfere with VSM cell growth two cellular models were established using the pro-atherosclerotic stimulus angiotensin II leading to cellular hypertrophy in culture and the

proliferative stimulus platelet-derived growth factor (PDGF) initiating cellular signalling cascades promoting the cell cycle. The first read-out for the identification of promising compounds is the influence on VSMC growth, i.e. hypertrophy and proliferation respectively, followed by a rough identification of the signalling pathways that might be affected. Compounds utilizing novel or unexpected strategies to interfere with VSMC growth are selected for further mechanistic studies in order to understand not only their mechanism of action but also the used biological system and thus cellular mechanisms of pathology. This strategy may help to identify new pharmacological targets.

PL 008

Structures and bioactivity of alkaloids from *Stemona*

Ye Y[1], Lin L[1], Tang C[1], Lin G[2], Velten R[3]
[1]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, P. R. China; [2]Department of Pharmacology, The Chinese University of Hong Kong, Hong Kong SAR, P. R. China; [3]Bayer CropScience AG, Research Insecticides, Chemistry Insecticides, Alfred-Nobel-Str. 50, Building 6240, D-40789 Monheim am Rhein, Germany

The roots of *Stemona* species (Stemonaceae) have been used as antitussive agent and insecticide for thousands of years in Traditional Chinese Medicine. Alkaloids, present as the main components in the roots of this plant, are believed to be responsible for its bioactivities. In our systematical investigation on the five main *Stemona* species in China, more than 60 alkaloids were isolated and structure identified. Those alkaloids, according to their structural characteristics, could be classified into seven subtypes, namely stemofoline-type, protostemonine-type, maistemonine-type, tuberostemonine-type, croomine-type, stemoninine-type and parvistemonine-type. On the basis of the clue of traditional applications, most of the isolated compounds were screened for two bioactivity indications. One is the *in vivo* screening against agricultural relevant insect pests; the other is the guinea pig cough model. The results indicated that some stemofoline-type alkaloids showed good insecticidal activities, while some stemoninine-type alkaloids exhibited significant antitussive activities. Through our investigation, antitussive and insecticidal activities of this traditional medicine have been well demonstrated by relevant distinct bioactive alkaloids in certain species. The phytochemical and pharmaceutical investigations of these *Stemona* species offered the substantial basis to disclose the relationships between certain *Stemona* species, the alkaloids of different types and the corresponding bioactivities. **References:** [1] Li-Gen Lin et al. (2006), J. Nat. Prod., 69: 1051 – 4. [2] Chang-qiang Ke et al. (2003), Chinese Chem. Lett., 14: 173 – 5. [3] Yang Ye et al. (2003), Tetrahedron Lett., 44: 7171 – 3. [4] Yang Ye et al. (1994), Phytochemistry, 37: 1205 – 8.

PL 009

Chips and Qi: microchip-based authentication of traditional Chinese medicinal plants

Sucher NJ[1]
[1]Herbal Analysis and Pharmacology Laboratories, Centre for Complementary Medicine Research, University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW 1797, Australia

Traditional Chinese herbal medicine (TCM) is the most widely practiced form of herbalism worldwide. Quality control and assurance are of great importance for the modern scientific development and clinical use of TCM. Problems are the variation in the content of pharmacologically active ingredients in the herbal medicines and their contamination or adulteration with morphologically similar toxic plants. Authentication of the medicinal plants used for the preparation of herbal medicines is essential. The identification of medicinal plants has long relied on morphological and chemical assays (chemical fingerprinting). More recently, DNA-based assays have been used for the authentication of herbal medicines [1,2]. In this approach, plant DNA is amplified by the polymerase chain reaction and the reaction products are analyzed by gel electrophoresis, sequencing, or hybridization with species-specific probes. It has been demonstrated in the last ten years or so that these molecular biological techniques can be performed in miniature analytical devices or "microchips" [3]. We have developed silicon chip-based analytical micro devices for the DNA-based identification of TCM materials [4 – 6]. Technological progress has made DNA sequence analysis cost effective and easy to perform. However, care has to be exercised in the choice of the particular DNA region to be analyzed and the interpretation of the results for taxonomic purposes. Molecular taxonomy has proved to be considerably more challenging in plants than animals [7]. Generation of molecular "barcodes" [8] for medicinal plants, however, would appear to be worth the concerted effort of the multidisciplinary medicinal plant research community. DNA-based authentication can complement morphological and chemical methods and will be useful for research, production, clinical use and forensic examination of herbal medicines. *Acknowledgements:* Hong Kong University of Science & Technology, Hong Kong Jockey Club, Innovation and Technology Commission of the Hong Kong SAR, China. **References:** [1] Shaw, P.C., But, P.P.H. (1995) Planta Med. 61: 466 – 469, [2] Cai ZH et al. (1999) Planta Med. 65: 360 – 364, [3] Vilkner, T. et al. (2004) Anal. Chem. 76: 3373 – 3386, [4] Lenigk, R. et al. (2001) Langmuir 17: 2497 – 2501, [5] Trau et al. (2002) Anal. Chem. 74: 3168 – 3173, [6] Carles, M. et al. (2005) Planta Med. 71: 580 – 584, [7] Chase, M.W. et al. (2005) Phil. Trans. R. Soc. B 360: 1889 – 1895, [8] Savolainen, V. et al. (2005) Phil. Trans. R. Soc. B 360: 1805 – 1811.

Keynote lectures

KL 001

Emerging Strategies to Enhance the Competitiveness of Natural Products in Drug Discovery

Wang Y
Novartis Institute for Biomedical Research, Lichtstrasse 35, CH-4002, Basle, Switzerland

With continuous advancements in molecular biology, cellular biology and genomics, the number of molecular targets has significantly increased in high throughput screening. Diversity oriented synthesis in chemogenomics and high throughput synthesis in combinatorial chemistry are taking the dominant position for "screen friendly" libraries in drug discovery. Facing increasing competitions, the problem how to enhance the competitiveness of natural products in drug discovery is the major challenge to ensure their further integration into contemporary drug R&D processes. The emerging strategies in our natural product research include miniaturized fraction screening with high throughput bioassays to make full use of the biodiversity advantage; knowledge based screening in rational ways to interact directly with various disease areas; high performance chemical screening to increase the compound library diversity; and state-of-the-art virtual screening including evolving cheminformatic and bioinformatic technologies in scaffold hopping, *in silico* docking and target identification. The confluence of these strategies provides exciting new possibilities to exploit the unrivaled chemical diversity of natural products evolved through millions of years of natural selection by interaction with biomolecules.

KL 002

Natural product discovery via chemical genetics in zebrafish

Crawford AD[1], Breyne A[1], Oosterlynck J[1], Maes J[1], Dewaele M[2], Ruzzene M[8,9], Kamuhabwa AR[6], Nshimo CM[6], Mbwambo ZH[7], Verbruggen AM[1], Pinna LA[8,9], Agostinis P[2], Busson R[1,4], Rozenski J[1,4], Esguerra CV[3,5], de Witte PAM[1]

[1]Department of Pharmaceutical Sciences, [2]Department of Molecular Cell Biology; [3]Department of Molecular and Cellular Medicine, [4]Rega Institute for Medical Research, [5]Stem Cell Institute, Katholieke Universiteit Leuven, Flanders, Belgium; [6]Department of Pharmacognosy, [7]Institute of Traditional Medicine, Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania; [8]Department of Biological Chemistry, Università di Padova, [9]Venetian Institute for Molecular Medicine, Padova, Italy

Natural products represent a significant – and currently underexploited – source of chemical diversity for the discovery and development of novel pharmaceuticals. One of the remaining bottlenecks in natural product discovery is the limited availability of biomedically relevant assays for high-throughput screening and rapid, bioactivity-guided fractionation of extracts. Recently, zebrafish have emerged as an effective system for the systematic *in vivo* identification and validation of both drug targets and small molecules. We have used the advantages of zebrafish (including small size, optical transparency, rapid development, high fecundity, and high genetic, physiologic, and pharmacologic similarity with humans) to establish a powerful platform for the discovery of bioactive natural products. Using a zebrafish-based, chemical genetic assay for anti-angiogenic activity relying on a sub-effective dose of the VEGF receptor inhibitor SU5416, we screened extracts from a collection of East African medicinal plants. Two extracts were identified that potentiated SU5416 in a dose-dependent manner. Using zebrafish bioassay-guided HPTLC fractionation, we rapidly identified the respective bioactive compounds as emodin (a known inhibitor of the protein kinase CK2) and a rare abietane diterpenoid with no previously described bioactivity. Intruigingly, CK2 has recently been described as an important modulator of the PI3K/AKT/FOXO pathway, one of the two main routes for VEGF signaling. We also determined the abietane diterpenoid to diminish AKT phosphorylation and downstream signaling events. These results substantiate the ability of chemical genetic screening in zebrafish to efficiently identify drug-like, pathway-specific inhibitors, and introduce the zebrafish as a novel platform for microgram-scale, *in vivo* natural product discovery.

KL 003

Chemopreventive effects of a Brazilian traditional medicinal plant, *Tabebuia avellanedae*, on in vitro and in vivo carcinogenesis systems

Tokuda H[1], Iida A[2]

[1]Kyoto Prefectural Uiversity of Medicine, Kawaramachi-dori, Kamigyo-ku, Kyoto, 602 – 084, Japan, [2]Takasaki University of Health and Welfare, Takasaki, 370 – 0033, Japan

As a part of an ongoing project, an investigation of the anti-tumor and tumor promoting properties of *Tabebuia avellanedae* spray dry powder essence and its active compound, 5-hydroxy-2-{(1-hydroxymethyl1)-naphtho 2,3-b} furan-4,9-dione (NFD) was carried out. *Tabebuia avellanedae* (TA) (Bignoniaceae), which is native in South America from Brazil to northern Argentina, is well known in traditional folk medicine and has been used for the treatment of various diseases since five hundred years. The inner bark of this plant produced in Brazil is widely used in Asia as a herbal tea and for health promotion. The application of a new screening procedure which utilizes the synergistic effects of short-chain fatty acid and phorbol esters enabled rapid and easy detection of naturally occurring substances (anti-tumor promoters, chemopreventive agents) with inhibition of Epstein-Barr virus (EBV) activation, using human lymphoblastoid cells. In addition, we have now extended these investigations to a new carcinogenesis model in which we initiated the tumors with 7,12-dimethylbenz(a)anthracene (DMBA) and promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA) a in two-stage mouse skin test and other models. These results provide a basis for further development of these botanical supplements for human cancer chemoprevention and demonstrate the utility of this system as model for testing of therapies designed to prevent or delay the malignancy of certain human tumors.

KL 004

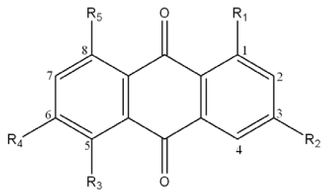
Antimicrobial activity of some lichen substances

Manojlovic N[1], Gritsanapan W[2], Milosev M[1], Manojlovic I[3]

[1]Faculty of Medicine, University of Kragujevac, S.Markovica 69, Kragujevac, 34000, Serbia; [2]Faculty of Pharmacy, Mahidol University, Bangkok, Thailand, [3]Faculty of Science, University of Kragujevac, R.Domanovica 12, Kragujevac, 34000, Serbia

Lichen substances comprise quite different classes of compounds like aliphatic acids, amino acid derivatives, polyols, lactones, quinones, depsides, depsidones, depsones, dibenzofuranes, chromones, terpenoids, steroids and carotenoids [1]. In general, lichens produce some characteristic metabolites, which have yet to be found in higher plants [1]. The secondary metabolites possess a broad spectrum of biological activities including antibiotic, antiviral, anti-inflammatory, antiproliferative, analgesic and cytotoxic effects [1]. For example, usnic acid has antitumour, antimutagenic, analgesic, antipyretic and enzyme inhibitory activity. In the present study, some *Caloplaca*, *Evernia*, *Thamnolia*, *Usnea* and *Cetraria* lichens growing in Serbia and *Laurera benguelensis* collected in Thailand were examined. Until now, there are no reports of phytochemical and antimicrobial studies for *L. benguelensis*. Metabolites from methanolic extracts were separated on a silica gel column using benzene-acetone mixtures as eluents. Usnic acid, evernic acid, protolichesterinic acid, thamnolic acid, squamatic acid, some naphthaquinones and anthraquinones 1 – 7 were isolated and identified. Extracts and compounds were tested against 12 bacterial and 12 fungal strains.

AQ	R_1	R_2	R_3	R_4	R_5
1	OH	OCH_3	H	CH_3	OH
2	OH	OH	H	CH_3	OH
3	OH	CHO	H	OCH_3	OH
4	OH	CH_2OH	H	OCH_3	OH
5	OH	OCH_3	OH	CH_3	OH
6	OCH_3	OCH_3	H	CH_3	OH
7	OH	OCH_3	H	CH_3	OCH_3



Chemical structures were characterized by UV, EI-MS and ^{1}H NMR. Parietin (1) was the primary anthraquinone of all *Caloplaca* extracts [2]. In the case of *L. benguelensis*, parietin (the main anthraquinone), fallacinal and emodin were isolated and identified. The antimicrobial screening [3] of crude *Caloplaca* and *Laurera* extracts showed the MIC values to be 80 – 320 μg/ml for bacteria and 40 – 320 μg/ml for fungi tested. The anthraquinones (1 – 7) exhibited considerable antibacterial effects on the species tested with MICs ranging from 10 to 320 μg/ml. Testing of these anthraquinones on fungi showed the MIC to be 10 – 160 μg/ml. Our investigations confirmed a broad spectrum antimicrobial activity of above mentioned lichenic extracts and acids. For example, the MIC values of usnic acid were found between 1 and 80 μg/ml. The anthraquinones, lichenic acids and other lichen compounds showed significant antimicrobial activity, which confirms the hypothesis that lichens represent an important source for the production of new antimicrobial agents. *Acknowledgements:* Ministry of Science and Environment of Serbia, pro-

ject Nº 142025 **References:** [1] Huneck, S. et al. (1997) Identification of lichen substances. Springer. Berlin. [2] Muzychkina, R.A. (1998) Natural anthraquinones, Biological and Physicochemical Properties. House Phasis, Moscow. [3] Manojlovic, T.N. et. al. (2002) Lichenologist 34: 83 – 85.

KL 005

Hyphenated NMR techniques and NMR-based metabolomics in studies of medicinal plants

Jaroszewski JW
Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

Plant extracts, including pharmaceutical preparations from medicinal plants, represent extremely complex mixtures containing numerous chemical entities in strongly varying concentrations. It is important to obtain a detailed knowledge of the chemical composition of such mixtures, i.e., the plant metabolome, for a number of reasons. One is that pharmacological activity of herbal medicine often cannot be related to a single constituent, as many different compounds – directly or indirectly – may affect the biological effect. Commercial herbal preparations are often standardized using a single or a few constituents, but may be extremely variable with respect to other constituents. It is therefore important to achieve means of comprehensive description of these complex mixtures and to establish methods for rapid identification of constituents that account for observed variability. Another reason for seeking rapid identification of plant metabolome components, often present in very small amounts, is derived from the drug discovery perspective. Traditionally, natural-product based drug discovery often involves very laborious preparative-scale fractionations, unfortunately often ending with purification of already known or trivial constituents. Exact knowledge of extract composition achieved at an early stage of an investigation, including rigorous structure determination of constituents without actually isolating them, can solve many frustrating problems inherent to natural product research. This lecture will demonstrate how multivariate analysis of NMR data and hyphenated NMR techniques, in particular HPLC-SPE-NMR, can provide fairly comprehensive global description as well as in-depth knowledge of individual chemical structures present in complex mixtures of natural origin. Examples from author's laboratory will include plant extracts as well as commercial herbal preparations (*Hypericum perforatum, Ginkgo biloba, Harpagophytum procumbens, Echinacea purpurea, Warburgia salutaris, and Kanahia laniflora*).

KL 006

The impact of natural products on drug discovery in the pharmaceutical industry

David B
Institut de Recherche Pierre FABRE, ISTMT, 3 rue des Satellites, 31432 Toulouse, France

Natural compounds have been a major source of bio-active molecules and leads for the development of synthetic compounds [1]. Over the last twenty years, about 60% of the anticancer chemical entities commercially available have been natural products or natural product derived molecules [2]. Recently, screening capacities have greatly increased in the pharmaceutical industry with the introduction of Ultra High Throughput Screening (u-HTS) robotic systems which can routinely scan thousands of molecules a day for biological activities. Subsequently, the enthusiasm for using natural products in the discovery of novel pharmaceutical leads has declined in the last decade, with many big pharma companies stopping or significantly scaling down their natural substances programs. On average, more than 500,000 different compounds are screened on one HTS pharmacological target and the research cycles have become much shorter. The majority of these small molecules are now provided by combinatorial or parallel chemistry. Despite the acceleration and the more wide-spread use of robotics as well as the massive introduction of combinatorial chemistry, the output of annually launched drugs has fallen while the amount of money currently invested in R&D has skyrocketed. In this context, natural compounds are needed to be revisited as a rich source of promising model lead molecules. They represent a unique reservoir of bio-active chemodiversity. Nevertheless, natural products present some drawbacks. Drug discovery from nature is a time and resource consuming activity. Collection, identification of the biological material, extraction, primary pharmacological screening, bioguided fractionation, dereplication of active components, structure elucidation of active compounds, their supply and so on take months of intensive efforts. The specific challenges faced by a pharmaceutical company, the strategic choices and general processes will be presented. **References:** [1] Butler, M.S. (2004) J. Nat. Prod. 67: 2141 – 2153. [2] Newman, D.J. et al. (2007) J. Nat. Prod. 70: 461 – 477.

KL 007

Exploring mechanisms of grapefruit juice drug interactions and its potential toxicity

Butterweck V, Zdrojewski I, De Castro WV, Derendorf H
College of Pharmacy, Department of Pharmaceutics, University of Florida, Gainesville, FL 32610, POBox 100494

Grapefruit juice (GFJ) inhibits not only the drug-metabolizing enzyme cytochrome P-450 3A4 (CYP3A4) in the small intestine [1], but also interacts with intestinal P-glycoprotein (P-gp), a membrane efflux-transporter [2]. Using a Caco-2 cell culture model we determined the permeability of talinolol across monolayer membranes in absence and presence of GFJ and several of its components. 6',7'-epoxybergamottin was the most potent inhibitor ($IC50 = 0.7\,\mu M$), followed by 6',7'-dihydroxybergamottin ($IC50 = 34\,\mu M$). Naringenin was around 10-fold more potent than its glycoside naringin with IC50 values of 207 and 2814 μM, respectively. Whereas the effect of a single dose of GFJ on the pharmacokinetics of several drugs has been studied extensively [3], data concerning the effect of grapefruit juice on the pharmacokinetics of CYP3A4 substrates after repeated use are limited. We investigated the interaction between GFJ and simvastatin in a time and dose dependent manner in rats. When simvastatin was administered alone in a concentration of 200 mg/kg (p.o.), the 50% survival rate after 10 days limited the further duration of the experiment. When simvastatin (20 mg/kg, p.o.) and GFJ (5 ml/kg) were given concomitantly for 4 consecutive weeks, no signs of toxicity were observed. Although it has been shown that consumption of a single glass of GFJ (200 ml) can increase the serum levels of the parent drug simvastatin up to 16fold [4], it is possible that this pharmacokinetic interaction does not necessarily have to result in a pharmacodynamic interaction and may occur only under single dose conditions. **References:** [1] Bailey, D.G. et al. (1998) J. Clin. Pharmacol. 46: 101 – 10. [2] Linand, H. et al. (2003) Clin. Pharmacokinet. 42: 59 – 98. [3] Saito, M. et al. (2005) Drug. Saf. 28: 677 – 94. [4] Lilja, J.J. et al. (1998) Clin. Pharmacol. Ther. 64: 477 – 83

KL 008

Evaluation of anti-inflammatory activity and identification of bioactive compounds from *Vitex negundo* L., *Cardiospermum halicacabum* L. and *Tridax procumbens* L

Jachak SM, Selvam C, Srivastava A, Ahuja V
Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER), Sector-67, SAS Nagar (Mohali)-160 062, Punjab, India

Several plant species find mention in traditional medicine focusing on relief from pain and inflammation. India, a country represented by rich culture, traditions, and natural biodiversity, offers a unique

potential for the drug discovery researchers. In India we have two (Eastern Himalaya and Western Ghats) of the 18 worlds' hotspots of plant biodiversity and interestingly, India is 7th among the 16 countries where 70% of the world's species occur collectively. India is rich in the flora with respect to endemic plant species, the distribution of which is determined as 5725 angiosperms (of 17,500), 10 gymnosperms (of 64), 193 pteridophytes (of 1022), 678 bryophytes (of 2584), 466 lichens (of 2450), 3500 fungi (of 23,000), and 1924 algae (of 2500) [1]. Out of the 17,500 flowering plant species, around 8000 are medicinal and a large number of these are mentioned in Ayurveda (Indian traditional medicine system) for treating inflammatory disorders [1]. For our studies medicinal plants which are reported in Ayurveda for treating inflammatory and related disorders have been selected. We are engaged in the characterization of natural COX-2/COX-1 inhibitors through bioassay-directed fractionation [2, 3] and in design and synthesis of natural product-derived analogues as potential COX-2 inhibitors e.g. curcumin and chalcone [4, 5]. As examples of our efforts to find COX inhibitors from Indian medicinal plants, the studies on anti-inflammatory activity of *Vitex negundo* L., *Cardiospermum halicacabum* L., and *Tridax procumbens* L. using COX-1 and COX-2 catalyzed prostaglandin biosynthesis *in vitro* assay and carrageenan-induced rat paw edema *in vivo* assay along with isolation of bioactive compounds, are described herein. **References:** [1] Sanjappa, M. Plant diversity in India – status, conservation and challenges (P. Maheshwari Medal Award Lecture). XXVIII Conference of Indian Botanical Society, Oct. 24 – 26, 2005, B.S.I., Dehradun, India, p. 5 – 6. [2] Selvam C., Jachak S. M. et al. (2004) Tetrahedron Lett. 45: 4311 – 4314. [3] Selvam C., Jachak S. M. (2004) Phytother Res. 18: 582 – 584. [4] Selvam C., Jachak S. M. et al. (2005) Bioorg. Med. Chem. Lett. 15: 1793 – 1797. [5] Jachak S. M. (2006) Curr. Med. Chem. 13: 659 – 678.

KL 009

Quality and safety should go hand in hand to monitor herbal products: examples from Chinese medicinal materials (CMM)

Chan K[1], *Leung KSY*[2], *Lu GH*[3]
[1]*Pharmacy Department, School of Applied Sciences, University of Wolverhampton, UK;* [2]*Department of Chemistry,* [3]*Department of Chinese Medicine, Baptist University, Hong Kong SAR, China*

The Pros and Cons in using Chinese Medicines: Increasing use of Chinese medicines has created debates on skepticism and support of the practice of Chinese medicine (CM) in the west (1). These debates mainly focused on herbal formulae containing Chinese medicinal materials (CMM) or ready made proprietary Chinese medicinal products (PCM) since the successful randomised clinical trial of a 10-CM herb prescription in treatment of atopic eczema has been published in 1992 (2). PCM adulterated with pharmaceutical drugs and crude CMM wrongly supplied or substituted by CMM possessing liver and kidney toxicity have been found in the market. Many reviews indicate that there is a lack of information on how to use CMM safely and that many CMM are related to adverse reactions when used as complementary or alternative treatments (3). These do not reflect the recent increase in the number of CM clinics in major cities in Austria, Finland, Germany and the United Kingdom. Global efforts in good practices: Qualified CM practitioners prescribe CMM as a mixture of herbs to individual patients. They are often taken as freshly prepared decoction. PCM may be used, though not frequently, as convenient substitutes to CMM mixtures. The efficacy of CM treatment depends very much on the quality of CMM used. These products contain numerous chemical compounds whose identities, structures and bioactivities are often unknown. Good practices should be put into action, including good agricultural practice, good supply practice (as traceability), good laboratory practice, good manufacturing practice and implemented regulations. Regulatory agencies produce monographs to set standards. These aspects are important to the healthcare and wellbeing of the public in the EU, China and worldwide. Therefore, a worldwide coordinating effort is needed. This paper addresses the recent progress in setting standards for CMM from the source of raw materials to the market and providing quality assurance and safety, through regulatory control. **References:** [1] Chan, K. (2005) J Ethnopharmacol. 96: 1 – 18. [2] Atherton, D. (2002) In: Chan K. Lee H (eds). The Way Forward for Chinese Medicine, Francis & Taylor; London pp 397 – 411. [3] Ernst, E. (2005) In: Leung P.C. Xue C.C. (eds) Chinese Medicine, Modern Practice, World Scientific. Singapore, pp1 – 22.

Short lectures

SL 001

Dual protective compounds from "vasoactive" Chinese herbs

Li H[1], *Steinkamp-Fenske K*[1], *Bollinger L*[1], *Yao Y*[1], *Xu H*[1], *Bauer R*[2], *Förstermann U*[1]
[1]*Department of Pharmacology, Johannes Gutenberg University, Mainz, Germany;* [2]*Department of Pharmacognosy, Karl Franzens University, Graz, Austria. Correspondence to Dr. Huige Li, Department of Pharmacology, Johannes Gutenberg University, Obere Zahlbacher Strasse 67, D-55131 Mainz, Germany*

Cardiovascular diseases are associated with reduced NO bioactivity and enhanced oxidative stress. Therefore, a combined upregulation of endothelial NO synthase (eNOS) and downregulation of NADPH oxidase may have therapeutic potential. The purported effects of "circulation-improving" herbs according to the Traditional Chinese Medicine show striking similarities with the vascular actions of eNOS-derived NO. In the present study, we tested extracts of 17 Chinese herbs known to have potential effects on the vasculature for their effect on eNOS gene expression. The results demonstrated that aqueous extracts of *Salvia miltiorrhiza L.*, *Prunella vulgaris L.*, and *Zizyphus jujuba spinosa* significantly increased eNOS promoter activity, eNOS mRNA and protein expression, as well as NO production in EA.hy 926 cells, a cell line derived from human umbilical vein endothelial cells (HUVEC). We then analyzed the effects of known constituents of these three herbs on eNOS expression. Among such compounds, ursolic acid[1], betulinic acid, luteolin and cynaroside were capable to increase eNOS expression in HUVEC and EA.hy 926 cells. Interestingly, ursolic acid and betulinic acid also attenuated the expression of NADPH oxidase subunit Nox4, thereby reducing oxidative stress and improving eNOS functionality. Consequently, ursolic acid- or betulinic acid-treated endothelial cells showed an increased production of bioactive NO (as indicated by a higher efficacy in stimulating cGMP generation in RFL-6 reporter cells). Thus, some natural compounds from Chinese herbs possess dual protective effects: upregulation of eNOS and a parallel downregulation of NADPH oxidase. The resulting increase in bioactive NO could mediate some of the beneficial effects of such medicinal plants. **References:** [1] Steinkamp-Fenske K. et al. (2007) Atherosclerosis; in press.

SL 002

HPLC-based activity profiling of plant and fungal extracts for $GABA_A$ receptor ligands using a functional assay with *Xenopus* oocytes

Kim HJ[1], *Khom S*[2], *Baburin I*[2], *Hering S*[2], *Hamburger M*[1]
[1]*Institute of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland;* [2]*Institute of Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*

Drugs acting on $GABA_A$ receptors are effective for the treatment of anxiety, sleep disorders, insomnia and convulsive disorders. For pharmacological studies on these ionotropic receptors we have recently developed a method for 'concentration jump' applications of neurotransmitters (or drugs) during two-microelectrode voltage-clamp experiments on *Xenopus* oocytes [1]. Automation, fast per-

fusion rates, and economical use of compounds make the system suitable for screening studies on ligand-gated ion channels. Here we made use of this approach for rapid HPLC-based profiling of new $GABA_A$ ligands of natural origin. Active extracts are separated by a single injection of 3 – 6 mg of active extract onto a semi-preparative (150 x 10 mm i.d.) HPLC column, gradient elution and time-based fractionation. This provides sufficient material for repeated testing of fractions in the oocyte assay. Structural information on active peaks is obtained via PDA and MS detectors. The protocol has been validated by spiking experiments with inactive extract and increasing amounts of the $GABA_A$ receptor ligand valerenic acid (2). Presence of GABA in extracts is a rather frequent problem when screening for GABAergic activity. To eliminate false positives, we have established a rapid and simple dereplication procedure by which GABA in extracts is analyzed as OPA derivative using RP-HPLC. The dereplication protocol has been validated with active and inactive plant and fungal extracts. The profiling approach has been successfully used to identify $GABA_A$ ligands in selected active extracts which had been prioritized after screening a library of approx. 1000 plant and fungal extracts. **References:** [1] Baburin, I. et al. (2006) Pflugers Arch. Eur. J. Physiol. 453: 117 – 123. [2] Khom, et al. in preparation

SL 003

In vitro and in vivo antifibrotic effects of triptolide on rats

Chong LW[1,2], Huang YT[3]
[1]Institute of Clinical Medicine, National Yang-Ming University, 155, Li-Nong Street, Section 2, Taipei, Taiwan; [2]Division of Gastroenterology, Department of Internal Medicine, Shin Kong Wu Ho- Su Memorial Hospital, Taipei, Taiwan; [3]Institute of Traditional Medicine, National Yang-Ming University, 155, Li-Nong Street, Section 2, Taipei, Taiwan

Aims: Hepatic fibrosis is characterized as a chronic inflammatory response to repeated insults. Triptolide ($C_{38}H_{42}O_6N_2$, a diterpene triepoxide derived from a Chinese herb *Tripterygium wilfordii*), is a potent immunosuppresive and anti-inflammatory agent with inhibitory activity on NFκB pathways. In this study, we investigated the *in vitro* and *in vivo* effects of triptolide on hepatic fibrosis. **Methods:** A cell line of rat hepatic stellate cells (HSC-T6) was stimulated with transforming growth factor β1 (TGF-β1) or TNF-α. The inhibitory effects of triptolide on the NFκB signaling cascade and fibrosis markers including α-smooth muscle actin (α-SMA) and collagen, were assessed. *In vivo* therapeutic study was conducted in dimethylnitrosamine (DMN)-treated rats. The rats were randomly assigned to 1 of 3 groups: control rats, DMN rats receiving vehicle (0.7% carboxyl methyl cellulose, CMC) or triptolide (20 μg/kg), each given by gavage twice daily for 3 weeks starting after 1 week of DMN administration. **Results:** Triptolide (5 – 100 nM) concentration-dependently inhibited the NFκB transcriptional activity induced by TNF-α, lipopolysaccharide and phorbol 12-myristate 13-acetate in HSC-T6 cells. In addition, triptolide also suppressed TNF-α- and TGF-β1-induced α-SMA secretion and collagen deposition in HSC-T6 cells. Fibrosis scores of livers from DMN-treated rats receiving triptolide (1.15 ± 0.15) were significantly reduced in comparison with DMN-treated rats receiving vehicle (1.92 ± 0.24). In addition, hepatic collagen contents and α-SMA protein expression in DMN rats were significantly reduced by triptolide treatment. **Conclusion:** Our results showed that triptolide inhibited TNF-α-induced activation of HSC-T6 cells and ameliorated liver fibrosis in DMN-intoxicated rats.

SL 004

From molecular diagnostics to molecular targeted therapy with natural product small molecule inhibitors in oral squamous cell carcinoma

Konkimalla VB[1], Suhas VL[2], Chandra NR[2], Gebhart E[3], Efferth T[1]
[1]German Cancer Research Center, C015, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany; [2]Bioinformatics Centre, Indian Institute of Science, Bangalore, India; [3]Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany

Oral squamous cell carcinoma (SCC) ranks among the top ten cancers worldwide. Despite the success in diagnosis and therapy during the past 30 years, oral SCC still belongs to the tumor types with very unfavorable prognosis. In an effort to identify genomic alterations with prognostic relevance, we applied the comparative genomic hybridization (CGH) technique on oral SCC. The tumors exhibited five and up to 47 DNA copy number alterations (CNAs), indicating a considerable degree of genomic imbalance. Nineteen of 35 tumors showed a gain of chromosome band 7p12. Genomic imbalances were investigated by hierarchical cluster analysis and clustered image mapping to investigate whether genomic profiles correlate with clinical data. Results of the present investigation show that profiling of genomic imbalances in general, and especially of EGFR on 7p12, may be suitable as prognostic factors. In order to identify small molecule inhibitors for EGFR, we established a database of 531 natural compounds derived from medicinal plants used in traditional Chinese medicine (TCM). Candidate compounds were identified by correlation analysis using Kendall τ test of IC_{50} values of tumor cell lines and microarray-based EGFR mRNA expression. Further validation was done by molecular docking studies using AutoDock program with crystal structure of EGFR tyrosine kinase domain as docking template. We estimate these results to be a further step toward the ultimate goal of individualized, patient-adapted tumor treatment based on tumor molecular profiling.

SL 005

Ethnopharmacological survey of Chazuta valley (Peruvian Amazon): a potential source for anti-inflammatory herbal drugs

Sanz-Biset J[1], Campos de la Cruz J[2], Epiquién Rivera MA[3], Cañigueral S[1]
[1]Unitad de Farmacologia i Farmacognosia, Universitat de Barcelona, E-08028 Barcelona, Spain; [2]C/ Sánchez Silva 156 Urb. St. Luzmilla-Comas, Lima, Peru; [3]Jr Ricardo Odonova 157 Urb Villa Sol-Los Olivos, Lima, Peru

Acculturation and the consequent loss of ethnomedical knowledge are particularly critical in the Peruvian Amazon, where 11 ethnic groups became extinct from 1950 [1]. In Chazuta valley dwells the major concentration (47.4% of its population) of San Martin Quechuas or Lamas Quechuas, one of the biggest indigenous groups of the Peruvian Amazon [2]. With the aim of recording the medicinal plants used in the Chazuta region, an ethnopharmacological field survey was performed in the area from October 2004 to August 2005. Through interviews with 140 adults, 326 plants were collected and identified, with 1076 medicinal uses reports. In the present work, results concerning the use of plants for inflammatory disorders are presented. These were the type of ailments most times treated: 335 use reports, which involved 126 plants. Most likely, the region's climate and rural lifestyle (Fig.1) make these disorders especially prevalent, the majority of which were recorded as unspecified rheumatism, broken bones and inguinal hernia. The plants (plant parts) most times cited were: *Maytenus* sp. (bark), *Mansoa alliacea* (root bark), *Brunfelsia grandiflora* (root bark), *Tovomita* aff. *stylosa* (bark), *T. foldatsii* (bark), *Zygia longifolia* (bark), *Calliandra angustifolia* (bark), *Phthirusa stelis* (stem), *Chloroleucon mangense* (root bark) and *Forsteronia graciloides* (latex). Often, remedies were taken with norms on food consumption and behavioural control, so strict in a few cases that implied fasting, severe rest, and social

seclusion. Informants stated "diets" as fundamental for therapeutic success.

Fig. 1: Chazutian carrying heavy weights through long distances, a daily routine.

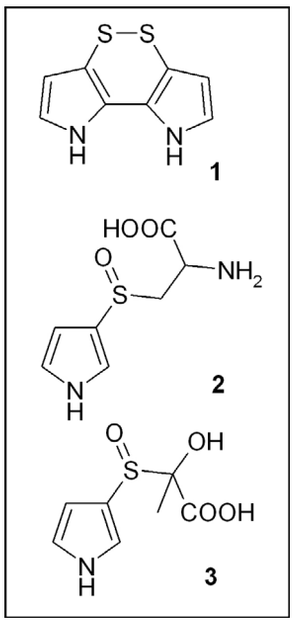
Acknowledgements: To the informants for sharing their knowledge, and J. Vallès and the other botanists who helped in the plant identification. **References:** [1] GEF/PNUD/UNOPS (1997). Amazonía peruana: comunidades indígenas, conocimientos y tierras tituladas. Atlas y base de datos. GEF and PNUD, Lima. [2] INEI (1993). Censos nacionales 1993. Lima. http://www.inei.gob.pe/biblioineipub/bancopub/Est/Lib0001/capit306.htm.

SL 006

Nyctanthes arbor-tristis Linn. – Spectrum of its bioactivity potential

Banerjee A, Poddar A, Ghanta S, Chakraborty A, Chattopadhyay S
Drug Development, Diagnostics and Biotechnology Division, Indian Institute of Chemical Biology, Kolkata 700 032, India

Nyctanthes arbor-tristis L. is a traditionally used Indian medicinal plant. Traditional reported uses include antihelmintic, antibilious, diaphoretic etc. and in the treatment of various diseases such as fever, rheumatism, intestinal worm disinfections etc. [1,2]. Tribal people of central India used various parts of this plant for relieving cough, dysentery, snakebite, sores etc. [3]. In our laboratory we have screened the methanolic extract of leaves of *N. arbor-tristis* L. against various human ailments [4]. Bioactivity guided fractionation lead us to isolate two pure compounds with novel activity against visceral leishmaniasis (*kala-azar*) and oxidative stress. To assess the free radical scavenging activity and oxidative DNA damage protecting activity of *N. arbor-tristis* L. we screened PE (bioactive fraction), NA1 and NA2 (pure compounds) as a source of natural antioxidant. Free radical scavenging property of PE, NA1 and NA2 also showed significant activity for ABTS and hydroxyl radical scavenging assay. We have also examined the antigenotoxic and protective effects of PE, NA1 and NA2 against pBlueScriptII SK (-) supercoiled DNA strand scission by hydroxyl radicals. Strikingly protective activity may explain its therapeutic potential towards cancer drug development. It could also provide a valuable biomarker of overall oxidative stress. Dose dependent inhibitory effect of PE and NA1 on growth of axenic promastogote culture of *Leishmania donovani* Ag83 showed potentially significant activity [4]. In vivo efficacy of PE and NA1, against *L. donovani* infected golden hamster model was performed. Results showed clearly that PE at 100 mg/kg body weight effectively reduced hepatic parasite burden up to 83% whereas spleenic burden up to 77%, respectively, in comparison to untreated infected control.



Acknowledgment: This work was supported by grants from the Indian Council of Medical Research, New Delhi, India. **References:** [1] Ambasta SP (1986) The useful plants of India. Council of Scientific and Industrial Research, Govt. of India, New Delhi. [2] Chopra, R.N., et al. (1956) Glossary of Indian Medicinal Plants. C. S. I. R., Govt. of India, New Delhi. [3] Jain, S.K. (1991) Dictionary of Indian Folk Medicine and Ethnobotany, Deep Publications, New Delhi. [4] Patent Filled: Chattopadhyay S, Poddar A, Kumar A, Achari B (2005) Leishmanicidal activity of Calceolarioside A containing night jasmine leaf extract, (2725 DEL 2005).

SL 007

Chemical composition and free radical scavenging activity of saffron

Baser KHC[1], Kosar M[1], Demirci B[1], Kara I[2]
[1]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskişehir, Turkey; [2]Anadolu Agricultural Research Institute, 26010 Eskişehir, Turkey

The dried red stigmata (saffron) of *Crocus sativus* L. flowers are used as a precious spice due to their aroma and coloring properties in different cuisines of the world. Saffron is also used for its pharmacological activities such as sedative and analgesic (1 – 2). Since spices are generally considered to have antioxidant activity, it was decided to test saffron also for this activity due to its high content of carotenoids (3 – 4). Cut stigmas from fresh flowers of *Crocus sativus* L. cultivated in Eskisehir and Safranbolu regions in Turkey were oven dried. The steam volatiles of saffron obtained by microdistillation were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), simultaneously. The colour compounds of saffron were extracted with 80% methanol and analyzed by reversed phase high pressure liquid chromatography (HPLC) using an acidic mobile phase. The activities of the extracts of saffron against 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radical (ABTS) free radical were investigated using on-line (HPLC-ABTS) and off-line methods. Safranal (62.1 and 49.3%), α-isophorone (10.0 and 16.3%) and β-isophorone (6.2 and 8.4%) were found as the characteristic aromatic volatiles in Eskisehir and Safranbolu samples, respectively. The carotenoids especially crocin

derivatives were determined as colouring components in methanol extracts of saffron. Free radical scavenging activities of methanol extracts were determined by on-line and off-line ABTS assays and crocins were found to be the active constituents. **References:** [1] Zareena, A.V et al. (2001) J. Agric. Food Chem 49: 687 – 691. [2] D'Auria, M. et al. (2004) Flavour Fragr. J. 19: 17 – 23. [3] Lozano, P. et al. (1999) J. Chromatog. A 830: 477 – 483. [4] Li, N. et al. (1999) J. Chromatog. A 849: 349 – 355.

SL 008

Sulphur pyrroles from *Allium* subgenus *Melanocrommyum* – a new class of pigments

Keusgen M[1], Vogt A[2], Jedelská J[1], Reinscheid U[2]
[1]University of Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany; [2]Max-Planck-Institut für biophysikalische Chemie, Am Fassberg 1, D-37077 Göttingen, Germany

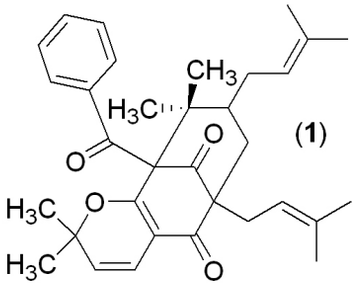
Since ancient times, onions, garlic and some other species of the genus *Allium* L. (onions) have been used as phyto-pharmaceutics, seasonings, and vegetables. The health benefits of *Allium* vegetables are mainly related to sulphur containing compounds as well as saponins [1]. The species-rich genus *Allium* has a main centre of distribution reaching from Southwest Asia to the high mountains of Middle Asia. In this area, several wild species are used by the local population, as can be concluded from casual remarks in some floras. The so-called cysteine sulphoxides of these plants are believed to be mainly responsible for these health benefits. Besides these known substances, also a new sulphur compound containing two pyrrole ring systems and a disulpho-bridge were isolated (**1**). This dithiodipyrrole is typical for a number of members of the subgenus *Melanocrommyum* like *A. macleanii*, *A. giganteum*, *A. jesdianum*, *A. rosenorum*, *A. winklerianum* and *A. rosenbachianum* and has a deep red colour, which occurs after heating or wounding of plant material. It must be assumed that an alliinase-like enzyme cleaves the cysteine sulphoxide **2**. The pyrrole **3** seems to be an intermediate of this reaction. Many of these species containing the dithiodipyrrole are used as traditional medicinal plants. *Acknowledgements:* Thanks are due to Dr. RM Fritsch, IPK Gatersleben, Germany, for collecting and careful determination of plant material. This research was kindly supported by the VolkswagenStiftung, Hannover, Germany, as part of the program "Zwischen Europa und Orient-Mittelasien/Kaukasus im Fokus der Wissenschaft". **References:** [1] Keusgen, M. (2002) Health and Alliums. In: Allium Crop Science – Recent Advantages (Edited by H.D. Rabinowitch and L. Currah), 357 – 378, CABI Wallinford, Oxon.

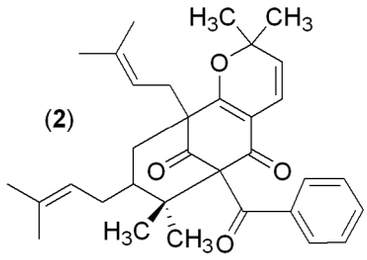
SL 009

Elucidating the chemistry of floral resins from *Clusia valerioi* Standl. (Clusiaceae)

Crockett SL[1], Kunert O[2], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, 8010 Graz, Austria; [2]Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-University Graz, 8010 Graz, Austria

Clusia (Clusiaceae) is a neotropical genus that consists of approximately 250 species of frequently hemi-epiphytic trees and shrubs. Species of this genus are often dioceous, meaning that separate plants bear male and female flowers. In order to facilitate pollination, several species offer floral resins as a reward for pollinating bees. The resins are, in turn, used by certain species of social bees in the construction of their nests. Other resins from species of *Clusia* contain phloroglucinol derivatives with interesting biological activities [1]. The female and male floral resins of *Clusia valerioi* Standl., collected from Costa Rica, were investigated and revealed the presence of several phloroglucinol derivatives, including new epimers of Plukenetione F (**1**) and G (**2**), compounds previously isolated from *Clusia plukenetii* [2].





Acknowledgements: Thanks go to the Tropical Research Station in La Gamba, Costa Rica, for allowing the collection of resin from flowers of *Clusia valerioi*. Partial support for this research from the Österreichische Forschungsgemeinschaft (ÖFG) (Projekt 06/9688) is gratefully acknowledged. **References:** [1] de Oliveira, C., et al. (1996) Tetrahedron Lett., 37: 6427 – 6430. [2] Henry, G., Jacobs, H., Carrington, C., McLean, S., Reynolds, W. (1999) Tetrahedron, 55: 1581 – 1596.

SL 010

Induction of apoptosis through reactive oxygen species mediated inhibition of topoisomerase II by plumbagin from Drosera

Kawiak A[1], Piosik J[2], Gwizdek-Wisniewska A[1], Stasilojc G[3], Bigda J[3], Lojkowska E[1]
[1]Department of Biotechnology, [2]Department of Molecular and Cellular Biology, [3]Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Kladki 24, Gdansk 80 – 822, Poland

Reactive oxygen species (ROS) have been recognized as key molecules, which can selectively modify proteins and therefore regulate cellular signalling including apoptosis. Plumbagin, a naphthoquinone present in the *Drosera* genus, is known to generate ROS and has been found to inhibit the activity of topoisomerase II (Topo II) through the stabilization of the Topo II-DNA cleavable complex. The objective of this research was to clarify the role of ROS and Topo II inhibition in the induction of apoptosis mediated by plumbagin. As determined by the comet assay, plumbagin induced DNA cleavage in HL-60 cells, whereas in a cell line with reduced Topo II activity (HL-60/MX2), the level of DNA damage was significantly decreased. The onset of DNA strand break formation in HL-60 cells was delayed in comparison with the generation of intracellular ROS. In HL-60/MX2 cells, ROS were generated at a similar rate, whereas a significant reduction in the level of DNA damage was detected. The pretreatment of cells with N-acetylcysteine (NAC) attenuated plumbagin-induced DNA damage, pointing out to the involvement of ROS generation in cleavable complex formation. The induction of apoptosis was significantly delayed in HL-60/MX2 cells indicating the involvement of Topo II inhibition in plumbagin-mediated apoptosis. Direct interactions between plumbagin and DNA were excluded based on spectroscopic analysis. Thus, these results suggest that ROS generated by plumbagin at low concentrations (3 μM) act as signal-

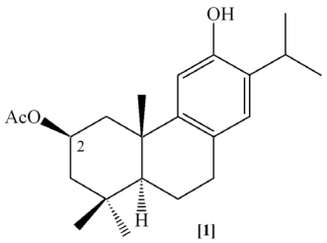
ling molecules mediating apoptosis through Topo II inactivation rather than through direct DNA damage.

SL 011

2β-acetoxyferruginol – a new antibacterial abietane diterpene from *Prumnopitys andina*

Smith E[1], Gibbons S[1]
[1]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London WC 1N AX, UK

The leaves and bark of the conifer *Prumnopitys andina* were investigated for antibacterial activity, as part of an ongoing project to investigate rare conifer species for new antibacterials. Hexane, $CHCl_3$, acetone and MeOH extracts were assayed against *Staphylococcus aureus* and *Propionibacterium acnes,* which resulted in an active methanol extract from the bark. Prep-HPLC on a C_{18} column led to the isolation of a new abietane diterpene, 2β-acetoxyferruginol **[1]**.



[1]

This new compound was active against *P. acnes* standard strain ATCC 6919 with a minimum inhibitory concentration (MIC) of 4 μg/ml. Against *S. aureus*, the compound inhibited the growth of two multidrug-resistant effluxing strains at 8 μg/ml, but was inactive against EMRSA-15 and a standard ATCC strain. This was a surprising result since the related diterpene ferruginol, which has a methylene at carbon 2 rather than an acetoxy group, is active against all four *S. aureus* strains at 4 – 16 μg/ml (1). The results show that modification of the basic ferruginol structure at carbon 2 on the A ring, can have a dramatic effect on activity against some *S. aureus* strains. Structural studies have focused on the B and C ring of abietanes and totaranes (2,3,4), but this result suggests that carbon 2 could be a suitable position for modification. Synthetic analogues could be developed, for example, with differing hydrocarbon chain lengths or the introduction of different functional groups to try to improve activity and bioavailability. *Acknowledgements:* We thank Stiefel International R & D Ltd for funding this study. **References:** [1] Smith, E. et al. (2007) Phytochemistry 68: 210 – 217; [2] Evans, G. et al. (1999) Bioorg. Med. Chem. 7: 153 – 164; [3] Evans, G. et al. (2000) Bioorg. Med. Chem. 8: 1663 – 1675; [4] Yang, Z. et al. (2001) Bioorg. Med. Chem. 9: 347 – 356.

SL 012

Antimicrobial activity of Angocin® *Anti-Infekt N*, a combination of nasturtium (*Tropaeoli majoris herba*) and horseradish (*Armoraciae rusticanae radix*)

Conrad A[1], Kolberg T[1], Richter H[1], Engels I[1], Frank U[1]
[1]Institute of Environmental Medicine and Hospital Epidemiology, University Medical Center Freiburg, Breisacher Str. 115, 79106 Freiburg, Germany

Aims: Angocin® *Anti-Infekt N* (Repha, Langenhagen, Germany) is a native preparation comprised of the haulm of nasturtium (N) and the root of horseradish (H). The active ingredients are isothiocyanates that are cleaved from precursors (glucosinolates) after oral intake. Clinical trials have shown that this drug is effective for treatment of urinary and upper respiratory tract infections [1]. **Objective:** Antimicrobial susceptibility testing of the preparation. **Methods:** A gas-test was used to investigate the antimicrobial activity of the volatile mustard oils that are released by the preparation. Gently dried and ground N and H were applied to the lids of Mueller Hinton-agar plates (ratio of 2.5:1) and mixed with sterile H_2O. Amounts of 3.31 mg N/1.25 mg H to 400 mg/160 mg were tested in 65 mm, amounts of 200 mg/80 mg up to 1200 mg/480 mg in 95 mm plates. Various clinically relevant bacterial species were tested (20 isolates each). The organisms were plated onto the agar plates and placed above the test substances. The plates were sealed with tape and incubated at 37 ° C for 92 h. Colony forming units were counted after 24 h and 92 h, and the MIC_{90} was determined for each bacterial species. Susceptibilities were checked by use of synthetic mustard oils and with agar- and broth dilution methods. **Results:** *H. influenzae*: MIC_{90} 50 mg N/20 mg H, *M. catarrhalis:* 100 mg/40 mg, *E. coli:* 400 mg/160 mg, *P. aeruginosa:* 400 mg/160 mg, *P. vulgaris* 400 mg/160 mg, MSSA and MRSA 400 mg/160 mg, *S. pyogenes* 400 mg/160 mg, *S. pneumoniae* 400 mg/160 mg, *K. pneumoniae* 1000 mg/400 mg. **Conclusion:** A broad in vitro antibacterial activity was found against both, gram-negative and gram-positive bacteria, including MRSA and *P. aeruginosa*. Given that one capsule of the preparation contains 200 mg N/80 mg H and that the advised daily intake averages out at 15 – 25 capsules, clinical efficacy can be explained. *Acknowledgements: This study was financed by a grant from Repha GmbH, Langenhagen, Germany.* **References:** Goos, KH. et al. (2006) Arzneimittelforschung 56: 249 – 57.

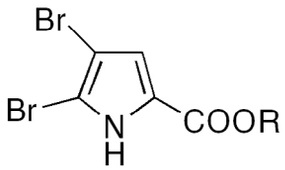
SL 013

The first marine natural products from *Agelas oroides* inhibiting the FabI enzymes from *Plasmodium falciparum*, *Mycobacterium tuberculosis* and *Escherichia coli*

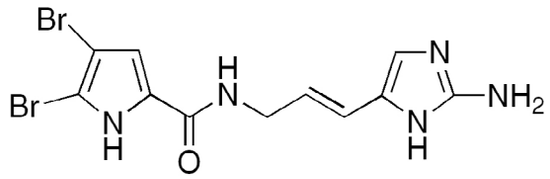
Tasdemir D[1], Topaloglu B[2], Perozzo R[3], Brun R[4], O'Neill R[5], Carballeira NM[5], Zhang X[6], Tonge P[6], Rüedi P[7]
[1]Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK; [2]Faculty of Fisheries, Istanbul University, Istanbul TR-34480, Turkey; [3]School of Pharmaceutical Sciences, University of Geneva CH-1211, Switzerland; [4]Swiss Tropical Institute, Basel CH-4002, Switzerland; [5]Department of Chemistry, University of Puerto Rico, San Juan 00931 – 3346, Puerto Rico; [6]Department of Chemistry, State University of New York at Stony Brook, New York 11794 – 3400, USA; [7]University of Zurich, Institute of Organic Chemistry, Zurich, Switzerland

The type II fatty acid pathway (FAS-II) is a validated target for antimicrobial drug discovery. In continuation of our efforts to discover natural inhibitors of the enoyl-ACP reductase enzyme from *P. falciparum (Pf*FabI*), M. tuberculosis* (InhA) and *E. coli* (*Ec*FabI), we studied the *n*-hexane, $CHCl_3$ and aq. MeOH extracts of *Agelas oroides* sponge. An activity-directed fractionation based on *Pf*FabI inhibition on all three extracts afforded six pure metabolites, 24-ethyl-cholest-5α-7-en-3β-ol (**1**), 4,5-dibromopyrrole-2-carboxylic acid (**2**), its methyl ester (**3**), (*E*)-oroidin (**4**), 3-amino-1-(2-aminoimidazoyl)-prop-1-ene (**5**), taurine (**6**), as well as some minor, inseparable fatty acid (methylated) mixtures (**FAMA-FAMG**). **FAMA** consisting of a 1:2 mixture of 5*Z*,9*Z*-tricosadienoic (**7**) and 5*Z*,9*Z*-tetracosadienoic acid (**8**), and **FAMB** composed of **8,** 5*Z*,9*Z*-pentacosadienoic (**9**) and 5*Z*,9*Z*-hexacosadienoic acid (**10**) (3:3:2) appeared as *Pf*FabI inhibitory principles of the hexane extract (IC_{50}s 0.35 μg/ml)33 Oroidin isolated in free base form (**4a**) was the active principle of the $CHCl_3$ extract, and had a greater enzyme inhibitory effect than oroidin isolated as TFA salt (**4b**), the major and the active component of the aq. MeOH extract (IC_{50}s 0.3 and 5.0 μg/ml, resp.). The enzyme kinetic studies identified **4a** as an uncompetitive *Pf*FabI inhibitor. All compounds showed antimalarial activity, and were generally non-toxic to mammalian cells. **FAMA** and **FAMD** also inhibited the InhA

and *Ec*FabI enzymes with IC_{50} values ranging between 0.07 – 9.4 μg/ml.



2 R: H, **3** R: Me



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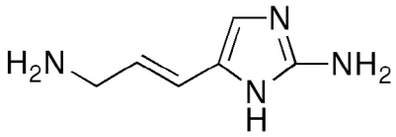

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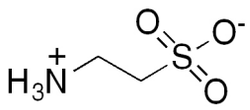


6

SL 014

The possible interaction between an edible insect and five antibacterial kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. *angustifolia* (Sapindaceae) leaf extracts

Eloff JN[1], Teffo LS[1], Toms RB[2], Aderogba AM[1]
[1]Phytomedicine Programme, University of Pretoria; Private Bag X04, Onderstepoort 0110 South Africa; [2]Transvaal Museum, Northern Flagship Institution, P.O. Box 413, Pretoria, 0001 South Africa

Dodonaea viscosa Jacq. var. *angustifolia* (Sapindaceae) a medicinal plant used in folk medicine to treat diseases and inflammatory conditions was investigated for its antioxidant and antibacterial properties because it is the sole host plant for the edible stinkbug, *Encosternum delegorguei* Spinola, a traditional delicacy for the Vhavenda tribe of Limpopo Province of South Africa. As insects are known to sequester compounds from their host plants [1], we were interested to investigate the medicinal properties of *D. viscosa* and determine if the same compounds occur in the insect. *D. viscosa* methanol leaf extracts has antibacterial activity against several bacteria and viruses [2]. Bioassay guided fractionation of dichloromethane and acetone fractions from serial extraction of *Dodonaea viscosa* Jacq. var. *angustifolia* leaf powder yielded 3, 5, 7-trihydroxy-4′-methoxyflavone (kaempferide) (**1**); 5, 7, 4'-trihydroxy-3, 6-dimethoxyflavone (**2**); 5, 7-dihydroxy-3, 6, 4'-trimethoxyflavone (santin) (**3**); and 5-hydroxy -3, 7, 4'-trimethoxyflavone (**4**) and kaempferol (**5**). MIC of isolated compounds against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* varied from 16 μg/ml to more than 250 μg/ml. Good structure activity relationships could be established. There were no ninhydrin positive compounds present in insect extracts (i.e. peptides frequently responsible for antibacterial activities in many insects probably absent). From bioautography of insect extracts zones of inhibition coinciding with the R_f of some isolated compounds were found indicating that some of the compounds present in *D. viscosa* could be present in the insect. *Acknowledgements: The NRF provided funding.* **References:** [1] Harborne, J.B., 1982. The Flavonoids: Advances in Research. In: T.J. Mabry (Ed.), Chapman and Hall Ltd. Cambridge, UK. [2] Getie, M., et al. (2003) Fitoterapia 74: 139 – 143

SL 015

An improved HPLC/MS method for the analysis of escins in Common Horse-chestnut (*Aesculus hippocastanum*)

Perrone P[1], Schaneberg B[1]
[1]ChromaDex, Inc., 2830 Wilderness Place, Boulder, CO, 80301, USA

The deciduous tree, *Aesculus hippocastanum* L. [Sapindaceae] known as the Common Horse-chestnut is native to a small area in the mountains of the Balkans, but is now widely cultivated in temperate regions around the world. The extract from the seed finds widespread use in the treatment of chronic venous insufficiency, edema, and sprains. The components of the extract primarily responsible for the pharmacological effect are a group of saponin compounds collectively known as escins, and is comprised of over thirty glycosidic triterpene compounds derived from the aglycone protoaescigenin or barringtogenol C. HPTLC is the current analysis method for total escins in raw material or products. Through the use of HPLC/MS, an analysis method has been developed which also allows for the determination of total escins. By selecting specific fragments due to ionization the percent total escins can be quantified reproducibly. A number of products on the market in addition to raw material were tested and the values were compared to specifications when available. In most cases, products achieved label claim, but not all. Extract procedures can also cause variation in products. This method is able to determine if the product is representative of the raw material extract, or if it has been changed due to extreme extract procedures.

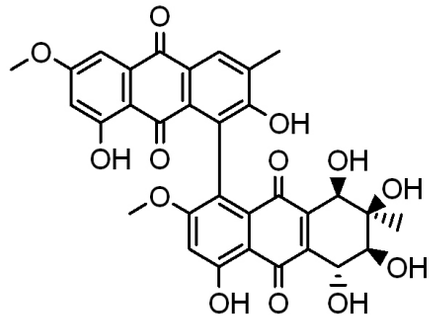
SL 016

New bioactive metabolites isolated from the endophytic fungus *Stemphylium globuliferum* isolated from *Mentha pulegium* growing in Morocco

Debbab A[1,4], Edrada RA[1], Wray V[2], Müller WEG[3], Hakiki A[4], Mosaddak M[4], Proksch P[1], Ebel R[1]
[1]Heinrich-Heine-Universität, Institut für Pharmazeutische Biologie und Biotechnologie, Universitätsstrasse 1, Geb. 26.23, D-40225 Düsseldorf, Germany; [2]Helmholtz-Zentrum für Infektionsforschung GmbH, Inhoffenstraße 7, D-38124 Braunschweig, Germany; [3]Johannes-Gutenberg-Universität, Institut für Physiologische Chemie und Pathobiochemie, D-55099 Mainz, Germany; [4]Université Mohamed V-Agdal, Faculté des Sciences, Laboratoire des Substances Naturelles et Thermolyse Éclair, 4 Avenue Ibn Battouta B.P. 1014 RP, Rabat, Morocco

Endophytic fungi constitute one of the most interesting sources of bioactive natural products. They are synergistic to their respective host and at least some of them are thought to play an important role in the plant's defence by producing secondary metabolites that protect the host from being attacked by pathogenic fungi and pests. Besides the known compounds altersolanol A, altersolanol J, alterporriol D and macrosporin, four new metabolites 1, 2, 3 and 4 were identified from the EtOAc-extract of a solid rice culture of the endophytic fungus *Stemphylium globuliferum* isolated from the stem of mint plant *Mentha pulegium*. The structures of the new compounds were elucidated on the basis of comprehensive NMR spectral analysis (^1H and ^{13}C NMR, COSY, HMBC, ROESY) as well as mass spectrometry. The crude organic extract and some of the pure com-

pounds showed moderate to strong toxicity toward L5178Y mouse lymphoma cells.



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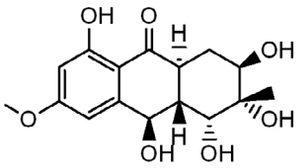

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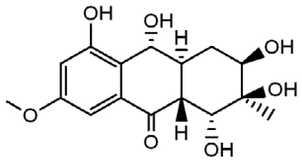

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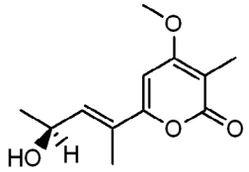


4

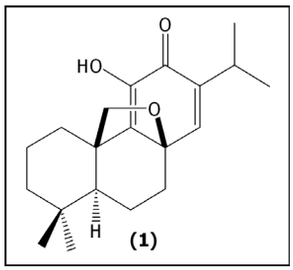
SL 017

Isolation, chemical transformations, and antiproliferative activity against human cancer cell lines of abietane diterpenes from two Mexican *Salvia* species

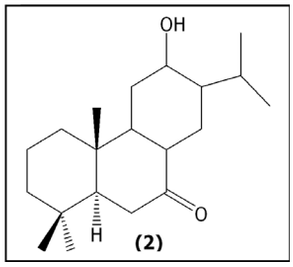
Córdova I[1], *Padrón JM*[2,3], *Andrés LS*[2], *Delgadillo J*[4]

[1]*Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California. Calzada Universidad No. 14418, Mesa de Otay, 22390, Tijuana Baja California México;* [2]*Instituto Universitario de Bio-Orgánica "AG", Universidad de La laguna, Avenida Astrofísico Francisco Sánchez, 2, 38206, Tenerife Canary Islands, Spain;* [3]*Instituto Canario de Investigación del Cáncer (ICIC), Avenida Astrofísico Francisco Sánchez, 2, 38206; Facultad de Ciencias, Universidad Autónoma de Baja California, Unidad Universitaria de Ensenada, Ctra Tijuana-Ensenada KM. 103, Ensenada México*

Salvia, one of the largest plant genera of about 900 species, has been used in traditional medicinal systems for generations [1]. In the course of a study of the chemical composition of the flora used in Latin America popular medicine, we have performed the phytochemical studies of extracts from two *Salvia* species, *S. pachyphylla* Munz and *S. clevelandii* (A. Gray) E. Greene. Both are endemic from the North of Baja California (Mexico) and South of California (USA). *Salvia pachyphylla* is used by indigenous communities for its medicinal properties [2]. In this research the major secondary metabolites isolated from these species and their biological studies against five human cancer cell lines are described. A cold acetone extract of the aerial parts of *S. pachyphylla* was chromatographed on silica gel to give nine known natural products and the new diterpene (1) identified on the basis of spectroscopic data analysis. From the aerial parts of *S. clevelandii,* seven known diterpenes were obtained and identified by comparison with the spectroscopic data found in the bibliography. The antiproliferative activity of some of the isolated compounds were evaluated *in vitro* against A2780 ovarian cancer, SW1573 non-small-cell lung cancer, WiDr colon cancer, T-47D breast cancer, and HBL-100 breast cancer cells. The growth inhibition (GI_{50}) was determined using the National Cancer Institute (NCI) protocol with slight modifications [3]. Finally a series of β-amino alcohol analogs of sugiol (2) were synthesized in a straightforward manner. The *in vitro* cytotoxic activities were examined, and the most potent analogs induced considerably growth inhibition in the range of 1.5 – 6.7 μM [4].



(1)



(2)

Acknowledgements: This research was supported by the EU INTERREG III-MAC initiative (05/MAC/2.3/c14 biopolis), Ministerio de Educación y Ciencia of Spain, co-financed by the European Regional Development Fund (CTQ2005 – 09074-C02 – 01/BQU), and the Gobierno Autónomo de Canarias. I.C.G, is thankful to the Banco de México, Secretaría de Educación Publica and Universidad Autónoma de Baja California en México for predoctoral fellowships. **References:** [1] Scott, G. et al. (1985) Chem. Br. 648 – 653., [2] I. Córdova. et al. (2006) J. Nat. Prod., published on Web., [3] Miranda, P.O. et al. (2006) Chem Med Chem, I: 323 – 329., [4] I. Córdova. et al. (2006) European Journal of Medicinal Chemistry. 41: 1327 – 1332.

SL 018

Willow bark extract (BNO 1455) and its fractions promote apoptosis in human lung cancer cells irrespective their basal level of COX-2, p53 and Bcl-2 genes

Hostanska K[1], *Jürgenliemk G*[2], *Nahrstedt A*[2], *Abel G*[3], *Saller R*[1]

[1]*University Hospital Zurich, Institute for Complementary medicine, Rämistrasse 100, 8091 Zurich, Switzerland;* [2]*WWU Münster, Institute for Pharmaceutical Biology and Phytochemie, Hittorfstrasse 56, 48149 Münster, Germany;* [3]*Bionorica AG, Kerschensteiner Strasse 11 – 15, 92318 Neumarkt, Germany*

Aim of this study was to examine effects of compounds (salicylalcohol derivates F1, flavonoids F2, proanthocyanidins F3), isolated from willow bark extract BNO 1455 on proliferation and apoptosis in human lung cancer cells. We used COX-2 proficient human non-small cell lung cancer (NSCLC) A549 cells with wild type p53 and low level Bcl-2 genes (6.5%) in opposite to small cell lung cancer (SCLC) SW2 cells with negligible COX-2 basal level (4%), mutation on p53 and high expression of Bcl-2 genes (93%). Cytotoxicity and growth inhibitory activity of BNO 1455 and its fractions were investigated after 72 h exposure with propidium iodide uptake by flow cytometry and with WST-1 assay, respectively. Apoptosis induction was detected by Annexin V adherence and morphological changes in cell scatter characteristics using flow cytometry in cell lines at

their established GI_{50} concentrations. As controls acetylsalicylic acid (ASA), salicin (SAL) and quercetin were used. Dose dependent antiproliferative effects were observed on both cancer cell lines. Comparative studies indicate quantitative differences concerning the GI_{50}. The GI_{50} (µg/ml) of BNO 1455 was 206.7 and 52.8 for A549 and SW2 cells, respectively. GI_{50} values of ASA were comparable being between 2.2 – 3.4 mM. Fractions F1, F2 and F3 contributed to the inhibitory activity of BNO 1455 in an additive manner according to their amount as expressed in inhibitory units of 100 mg substances. Apoptosis induction after 72 h treatment of cells with all substances was confirmed by Annexin V adherence in both cell lines at GI_{50}. However, the F2 and F3 fractions were more potent inducers of apoptosis. Results of this study demonstrate antiproliferative and apoptosis-inducing effects of willow bark extract.

SL 019

In vitro antiprotozoal activity of organic waste materials against *Cryptosporidium parvum*

Teichmann K[1], Klimitsch A[1], Schatzmayr G[1], Hadacek F[2], Joachim A[3]
[1]BIOMIN Research Center, Technopark 1, A-3430 Tulln, Austria; [2]Department for Chemical Ecology and Ecosystem Research, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; [3]Institute for Parasitology and Zoology, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria

Plant residues and by-products from pharmaceutical or food production may be cost-efficient sources of functional feed ingredients in animal nutrition. In the course of the EU-funded project SAFEWASTES numerous materials that usually are treated as wastes are evaluated for their potential for further usage. The present study investigated *in vitro* antiprotozoal effects against *Cryptosporidium parvum,* an intestinal parasite (Apicomplexa) that infects a wide range of hosts including humans. Especially young or immunocompromised animals are susceptible to infection and mass propagation of the parasite which is often accompanied by loss of weight and water, retarded development and increased susceptibility to other diseases. Among several methods a cell culture assay with HCT-8 host cells was chosen for determination of anti-cryptosporidial effects of test substances. Intracellular parasite development was evaluated microscopically after labelling by indirect fluorescent antibody technique (IFAT). Cell vitality assays were conducted simultaneously to assess eventual deleterious effects on the host cells, since it is important to discriminate unspecific cytotoxic effects from direct effects against the parasites. 53 extracts prepared from 18 different organic waste materials were screened in this assay. An ethanol extract from olive press-cake effectively inhibited *C. parvum* development while being tolerated by the host cells in the applied concentrations. Monensin as a positive control inhibited parasite development in a highly reproducible way. Bioassay-guided fractionation was done to identify the active principles of the materials. *Acknowledgements:* Prof. Dr. Arwid Daugschies (University of Leipzig) and his team for provision of parasite material and methodological advice. The SAFEWASTES project is funded by the 6th Framework Programme of the European Union.

SL 020

The need of *in vivo* experiments to validate the effects noticed *in vitro* of certain plant extracts against *Histomonas meleagridis, Tetratrichomonas gallinarum* and *Blastocystis* pp.

Grabensteiner E[1], Liebhart D[1], Arshad N[1], Hess M[1]
[1]Clinic for Avian, Reptile and Fish Medicine, Department of Farm Animals and Herd Management, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria

In the Safewastes project, a total of 45 plant substances were tested for their *in vitro* activity against clonal cultures of *Histomonas meleagridis, Tetratrichomonas gallinarum* and *Blastocystis* spp. (1), three protozoan parasites, at two different concentrations (10 mg/ml and 5 mg/ml). Ethanolic extracts were found to be most efficacious. However, only three of them proved to be effective against all parasites at both tested concentrations. In addition, two ethanolic extracts were effective against histomonads and blastocysts at both tested concentrations. However, these two substances did not inhibit the growth of tetratrichomonads. The minimal lethal concentrations of these five substances were determined for all parasites as they were selected for further *in vivo* studies. The first *in vivo* experiments were conducted using ethanolic extracts of thyme and saw palmetto administered through the drinking water from the first day onwards to one-day-old BUT-T9 turkeys. At the age of 14 days the birds were infected with a cloned *Histomonas meleagridis* isolate through the cloacal route. The clinical outcome of both substances was somewhat similar: within five weeks p.i. all birds of both groups had died or had to be euthanized. Sectioning revealed in all cases a severe typhlohepatitis, indicative for Histomonosis caused by *Histomonas meleagrids*. These studies demonstrate the need of *in vivo* experiments in order to assess *in vitro* results. Currently investigations are performed using ethanolic extracts of two additional substances and the outcome and the relevance for practical purposes will be placed in context with the obtained *in vitro* results. **References:** [1] Hess. M., et al. (2006) Parasitology 133: 547 – 554

SL 021

Effect of extracts of carrot waste on gut microflora

Stella S, Tedesco D
Dep. Veterinary Sciences and Technologies for Food Safety, University of Milan, Italy

The bacterial community within the gastrointestinal tract (GIT) crucially influences the host health status. To prevent disease and reduce morbidity and mortality, inhibition of pathogenic bacteria proliferation is needed. Bioactive compounds from plants showed an activity on standard gut strains.The aim of the present study was to evaluate the effects of water and ethanol extracts from carrot waste on the growth and viability of gut microbiota in weaning piglets. Carrot extract samples were solubilized into deionized water or ethanol and sterilized by filtration. Different shares of culture media were prepared and additioned with the extract solutions. The concentrations tested were 10, 100 and 1000 µg/mL; also a blank share was prepared. Fecal samples were withdrawn from the rectal ampulla of healthy piglets (60 – 70 days of age), diluted and plated onto Petri dishes containing specific culture media. The following microbial parameters were considered: Total Bacterial aerobic Count, Total and Faecal Coliforms, *Escherichia coli*, Enterococci, Total Anaerobic bacterial Count, *Clostridia*, *Lactobacilli*. The evaluation of the effect of extracts was performed considering the number and the size of colonies grown on the media. The effect of the two tested carrot waste extracts on gut microflora was different, especially on coliforms. Water extract showed a slight inhibitory effect on Total coliforms growth, while ethanol extract had a growth-promoting activity. The effects observed were dose-dependent. Our results emphasize the different activity of the two extracts tested and are a prerequisite for future studies on the activity of their components. *Acknowledgements:* The research was supported by EU: SAFEWASTES, project n. 513949.

SL 022

Ethnoveterinary plants with antiviral activity against feline herpesvirus type 1

McGaw LJ, Bagla VP, Eloff JN
Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04 Onderstepoort 0110, South Africa

Ethnoveterinary studies conducted in South Africa commonly record indications of medicinal plant use for non-specific, general ailments. Few studies suggest that rural livestock-owners are able

to successfully diagnose viral diseases in their animals. Plants widely used to treat undefined ailments in ethnoveterinary medicine (EVM) may have antiviral activity. Sixteen plants featuring prominently in EVM were collected and acetone extracts of the leaves prepared. These extracts were tested against feline herpesvirus type 1, an enveloped virus sensitive to environmental effects. Cytotoxicity tests on the host cells, Crandell feline kidney (CRFK), were performed concomitantly with the antiviral assays. Each extract, at a concentration of 1 mg/ml redissolved in 0.5% DMSO, was incubated with the virus for a defined contact time of 20 minutes before a serial dilution in cell culture medium was performed. The dilutions were then applied to confluent monolayers of CRFK cells and observed for cytopathic effect (CPE) after incubation. One extract, *Croton gratissimus*, was highly toxic to the CRFK cells and antiviral effects could not be detected. Six plants (37.5%), including the commonly used medicinal plants *Ziziphus mucronata*, *Rhus lancea* and *Leonotis leonurus*, displayed no effect on the virus, and three extracts (19%, *Pouzolzia mixta*, *Pterocarpus angolensis* and *Hippobromus pauciflorus*) showed a 1 log reduction in viral growth. A 2 log reduction in viral growth was shown by *Pittosporum viridiflorum* and *Cussonia spicata* extracts (12.5%). A quarter of the extracts, namely *Combretum caffrum, Ricinus communis*, *Schotia brachypetala* and *Sclerocarya birrea*, resulted in a 3 log reduction in viral effect. The positive results obtained in this screening exercise potentially justify the use of these plants in EVM, and provide impetus for testing the extracts against more resistant viruses encountered in livestock animals such as lumpy skin disease, and this is being pursued. *Acknowledgements:* Claude Leon Foundation, National Research Foundation (South Africa).

SL 023

Assessment of hepatotoxicity for regulatory affairs

Tegtmeier M[1,2], Siegers CP[2]
[1]Schaper & Brümmer GmbH & Co. KG, Bahnhofstr. 35, 38259 Salzgitter, Germany; [2]Institute of Experimental and Clinical Pharmacology and Toxicology, Ratzeburger Allee 160, 23538 Lübeck, Germany

On three recent occasions in Germany drug regulatory decisions for herbal drugs (*Meliloti herba*, *Piperis methystici rhizoma*, *Chelidonii herba*) have been based on possible hepatotoxicity as suspected adverse effects. These decisions have been contested by the reason that adverse effect attributions have not been based on scientific evidence but mainly on animal experimental data and time coincidence. Pathologically elevated or decreased hepatic parameters are common, especially among patients, with aminotransferase-increases observed in up to 1/3 of all outpatients. For hepatotoxicity as a drug effect two principally different modes of actions can be separated with relevance to incidence and population risk. For liver damage exerted by toxic mechanisms (i.e. direct reaction of a drug or its metabolites) only an increase in liver damage (e.g. GPT-increase, GOT-increase, coagulation decrease) should be taken as proof for a causal correlation. The detection of elevated enzymes at the time of hospitalization alone cannot be sufficient. Additionally, in the cohort of affected patients a dose response correlation should be observed. Idiosyncratic liver damage on the other hand is dependent upon antibodies or leukocytes specifically reacting with drugs or drugs bound to liver membranes. In both cases the presence of specific antibodies or immune cells can be proven by rather simple laboratory methods (antibody binding, lymphocyte proliferation). The presence of specific reaction in either of these tests strongly suggests immunologically mediated mechanisms, whereas the absence does not necessary rule out immunologically mediated hepatotoxicity. Whereas toxic liver damage may be limited by intake limitation, restricting daily intake is no preventive strategy for idiosyncratic reactions. Whether or not regulatory actions should be taken ought to be based on proven (or likely) causal association indicated by the parameters given above; also the availability of safer therapeutic alternatives should be considered in a risk benefit analysis.

SL 024

Coumarin derivatives from plant extracts – margin of exposure

Schulze J
Medical Faculty, Johann Wolfgang Goethe-University Frankfurt/Main, Theodor Stern-Kai 7, D-60590 Frankfurt/Main, Germany

Coumarin derivatives have been discussed as potential carcinogens; they are present in plants and plant extracts and are taken up by a variety of routes. Recently a potential carcinogenic risk from coumarin derivatives in plant extracts has been discussed. In order to relate the coumarin uptake from herbal extracts, data for coumarin concentrations in food, in plant extracts and in oral PUVA therapy have been compiled. Based on these data, lifetime exposures from these sources have been calculated, based on average intake from these sources, in accordance with current carcinogenic risk assessment. For the risk evaluation of food ingredients the margin of exposure (MOE) has been taken for source comparisons. Based on the 8-MOP dose used during a treatment cycle in oral PUVA therapy (MOE = 1, no epidemiological evidence for increased carcinogenicity) lifetime exposure from food sources with an average Western European diet results in a MOE of 0.43. For the use of frequently used therapeutic doses of Angelica extracts and an assumed content of 1% psoralens in the total furocoumarin content, the MOE is calculated at 0.033. This calculation includes a number of assumptions which all have been rated conservatively, i.e. resulting in an overestimation. Therefore, no carcinogenesis warning can be based on the furocoumarin content of herbal extracts, as well as it can not be based on the furocoumarin content of foodstuff.

SL 025

Different methods to investigate plant extracts in regard to their physiological activity on human skin cells: an overview

Deters AM
Institute for Pharmaceutical Biology and Phytochemistry, Westphalian Wilhelms University of Muenster, Hittorfstr. 56, 48149 Muenster, Germany

The skin is the greatest organ build up from dermis and epidermis. Their barrier function is given by the cornified envelope, as result of keratinocyte terminal differentiation. Dermal fibroblasts produce collagens, elastins and various other proteins essential for elasticity skin and for the fixation of the epithelia. *In vivo* both cell types are very close in contact and interact with each other via a variety of different signal molecules. Since the mesenchymal fibroblasts may respond to in another way than the epithelial keratinocytes, it is obvious necessary to investigate specific effects of medicinal plant extracts or single isolated compounds on both cell types to obtain specific information on cell behaviour and physiology influenced by test compounds. *In vitro* cultures of both cell types are well established methods in dermatology but only a few people use them for investigation of plants traditionally used for treatment of skin disorders, diseases and wound healing. Since the main limitating parameter for submerged cultures is their water solubility, extracts of Avocado pits, and polysaccharides of St. James Worth were tested in regard to their effects on human skin fibroblasts and keratinocytes. The investigations focussed on a possible cytotoxicity determined as necrosis, to induce apoptosis as well as their ability to influence the cellular proliferation and mitochondrial activity of dermal and epidermal cells. The obtained results showed that neither compounds of avocado pits nor polysaccharides from *Hypericum perforatum* exert necrotic effects. The strong acidic polysaccharides of St John's worth enhanced the proliferation of fibroblasts but not of keratinocytes while the weak acidic polysaccharides and the neutral one's stimulated only the keratinocyte proliferation. Similar results were obtained after treatment with Avocado pit extracts. Avocado pit extracts improved only the keratinocyte proliferation. The metabolic activity of fibroblasts was stimulated by al Avocado fractions but only the fractions obtained after HSCCC with ethyl acetate respective a methanol/water. Obtained data were supported by qRT-PCR.

SL 026

Measurement of synergistic effects of a phytopharmaceutical by microarray-analysis

Ulrich-Merzenich G[1], Jobst D[1], Zeitler H[1], Müller J[2], Vetter H[1]
[1]Medizinische Poliklinik der Rheinischen Friedrich-Wilhelms-Universität Bonn, Wilhelmstr. 35 – 37, D-53111 Bonn, Germany, [2]Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, D-64295 Darmstadt, Germany

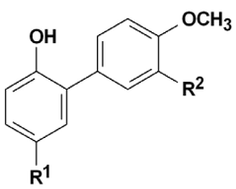
Phytopharmaceuticals are complex compounds. Microarray-based gene/protein expression analyses have been proposed to be capable of relating complex mixtures to complex effects, thereby revealing synergistic effects [1,2]. Phytodolor (PD) is a standardized alcoholic plant extract of *Populus tremulus* (S1), *Solidago virgaurea* (S2) and *Fraxinus excelsior* (S3), indicated in rheumatic pain. With the aim of revealing molecular mechanisms of PD action, we examined the effect of PD, its single extracts and acetylsalicylic acid (ASS) on gene-expression profiles in human fibroblasts. Fibroblasts were synchronized for 72 hrs and stimulated with PD (0.05 and 0.1%), S1, S2, S3 (0.1%), ASS (30 µg/ml) and LPS (10 µg/ml) for 6 hrs. RNA-expressions were analysed by Piquor® Skin Patho Microarray (1126 genes) and data analyzed by Piquor Analyser and microarray ImaGenesoftware. Values above 1.7- or below 0.58-fold compared to controls were judged as up- or downregulation. The application of 0.05% PD revealed a downregulation of only two genes, whereas 0.1% PD modulated additionally 36 further genes. Identified genes (eg. PGH2, TNC, LPS stimulated: MMP-1, IL-5,-6,-8, Gro-1) are involved in immunoregulation, inflammation and apoptosis. S1, S2, S3 and ASS modulated 51, 24, 31 and 44 genes respectively. Regulated genes of PD and its single extract show taken together an overlap of 57.9%. S1 has the maximum individual overlap with PD (36.5%) and greater overlap with ASS (52.9%). Initial protein expression analyses (PGH2, IL-5, -6, -8, Gro-1) support gene expression data. Microarray analyses will develop our understanding of the functional mechanisms of complex mixtures like PD and synergism. **References:** [1] Ulrich-Merzenich G. et al. (2007) Phytomedicine 14: 70 – 82. [2] Ulrich-Merzenich G, Jobst D. (2007) Genchipanalysen an humanen Fibroblasten unter Einwirkung definierter Rindenextrakte. Vortrag: Forum univ. Arbeitsgruppen f. Naturheilverfahren u. Komplementärmedizin, München.

SL 027

Investigations on the main neolignan constituent methylhonokiol of *Magnolia grandiflora* as a new anti-inflammatory lead compound

Schühly W[1], Hüfner A[2], Wenzig EM[1], Kunert O[2], Haslinger E[2], Bauer R[1]
Institute of Pharmaceutical Sciences, [1]Department of Pharmacognosy and [2]Department of Pharmaceutical Chemistry, Karl-Franzens-University Graz, 8010 Graz, Austria

The genus *Magnolia* plays a role in Asian medicinal systems as TCM and Japanese Kampo medicine [1]. One main lignan constituent in the seeds of the North American *Magnolia grandiflora* is 4'-*O*-methyl-honokiol (**1**) which is known to possess anti-inflammatory activities with COX-2 inhibitory activity (IC_{50}) of 1.5 µg/mL. Honokiol – another lignan component of *Magnolia* species – is active in the same range.



	R^1, R^2
1	CH_2-CH=CH_2
2	CH_2-CH_2-OH
3	CH=CH-CH_3
4	CH_2-CHOH-CH_3

In search of even better anti-inflammatory substances and SAR in a COX-1 /COX-2 and a 5-LOX system [2, 3], we synthesized and tested an array of derivatives of the lead compound **1** with a focus on side chain modification including length, polarity, number and/or position of the double bonds. The predominantly new and hitherto undescribed compounds were purified by means of HPLC and their structure elucidation was obtained with NMR and MS. **References:** [1] Schühly, W. et al. (2001) Pharm. Biol. 39: 63 – 9. [2] Rollinger, J. et al. (2005) Planta Med. 71: 399 – 405. [3] Adams, M. et al. (2004) Planta Med. 70: 904 – 8

SL 028

Evaluation of the activity profile of the sulfated polysaccharides extracted from the red alga *Delesseria sanguinea* (Hudson) Lamouroux

Groth I, Alban S
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

Delesseria sanguinea (D.s.), a red alga occurring in the Baltic Sea, contains sulfated polysaccharides (SP). As known from heparin, SP exhibit not only anticoagulant, but a wide range of biological activities. The aim of the study was to examine the activity profile of these alga-derived SP (D.s.-SP). The D.s.-SP were obtained by a standardised water-extraction procedure. By performing the extraction with 13 algae batches harvested over two years, the D.s.-SP were shown to be of reproducible quality. The biological effects of D.s.-SP were compared with unfractionated heparin (UFH) in various *in vitro* test systems recording relevant steps within the (patho-)physiological network of inflammation, metastasis, and haemostasis: (1) elastase- and (2) hyaluronidase activity assays, (3) haemolytic complement modulation assay (CMA), further the coagulation assays (4) APTT and (5) thrombin time (TT), and finally, the cytotoxicity assays (6) MTT and (7) LDH. All tests were performed at least three times on different days. Compared to UFH, the D.s.-SP revealed to be superior in all the assays except for APTT and TT. The corresponding IC50 and concentration for doubling the coagulation time in APTT and TT (mean ± SD [µg/ml]) were:

	Elastase	Hyaluronidase	CMA	APTT	TT
UFH	0.272 ± 0.015	12.1 ± 2.87	8.15 ± 5.05	1.03 ± 0.05	0.58 ± 0.03
D.s.-SP (n = 13)	0.223 ± 0.033	3.40 ± 0.65	0.061 ± 0.051	5.62 ± 1.26	4.59 ± 0.91

Cytotoxicity studies with tumour and human blood cells showed no negative effects on cell viability. Regarding the selected effects discussed to contribute to anti-inflammatory and antimetastatic activity (1 – 4) the D.s.-SP were shown to be superior to UFH. In contrast, they were less anticoagulant which may indicate a reduced risk to induce bleeding. *Acknowledgements:* This project is financed by the EU (FIAF/EFF) and the LFALF Mecklenburg-Vorpommern.

SL 029

Investigations on the mechanisms of action of 4-methylepigallocatechin in the guinea-pig ileum

Marçal RM[1], Rocha MB[1], Souza FVM[1], Estevam CS[1], Pizza C[2], Santána AEG[3]
[1]Physiology Department, Federal University of Sergipe, Av Marechal Rondon, s/n, São Cristóvão, Sergipe, 49.100 – 000, Brazil; [2]Università degli Studi di Salermo, Fisciano (Salerno), 84084, Italy [3]Chemistry Department, Federal University of Alagoas, Maceió, Alagoas, 57072 – 97, Brazil

We have recently reported that 4'-methylepigallocatechin, isolated from the stem bark of *Maytenus rigida* Mart. (Celastraceae), showed an opiate-like analgesic effect in rats [1]. In the present work, we have investigated the mechanism of action of 4'-methylepigallocatechin in the terminal segments of the guinea-pig ileum, a well-known model to study opiate agonists [2]. The terminal portion of the guinea-pig ileum (n= 5 – 8) was mounted in an organ bath and isotonic contractions were recorded. In the guinea-pig ileum, electrically-induced contractions (0.1 Hz; 0.5 ms; 40 V) were significantly ($p < 0.001$) reduced by morphine (1 µM). Unlike morphine, 4'-methylepigallocatechin (1 nM-100 µM) did not modify the electrically-elicited contractions. The contractions induced by histamine (2 µM) or $BaCl_2$ (0.03 M) were significantly reduced in the presence of

4'-methylepigallocatechin (8 μM). On the other hand, the contractions elicited by 20 and 60 mM KCl were not modified by 4'-methylepigallocatechin (1 nM-100 μM). Catechin (8 μM) reduced, in a non-reversible fashion, the E(max) of $CaCl_2$ contractile response without changing the CE_{50}. Verapamil (1 nM), an L-type channel blocker, as well as 4'-methylepigallocatechin (8 μM) significantly ($p < 0.001$) reduced the contraction induced by carbachol (100 μM). The inhibitory effect of verapamil (1 nM) on the carbachol-induced contractions was potentiated ($p < 0.05$ vs. verapamil) by co-treatment with 4'-methylepigallocatechin (8 μM). In conclusion, an opiate-like mechanism of action could not be detected in the guinea-pig ileum. Indeed, these observations support the hypothesis that 4-methylepigallocatechin acts by molecular mechanisms that modulate calcium entry or reduce sensitivity of contractile machinery to [Ca2+](i). *Acknowledgements: CNPq, FINEP* **References:** [1] Dias, K.S.. et al. (2007) Fitoterapia, (accepted); [2] Paton, W.D. (1956) Br. J. Pharmacol. Chemother. 12: 119.

SL 030

In vivo and in vitro evidence for antidiabetic activity of *Desmodium gangeticum*

Govindarajan R[1], Houghton PJ[2], Asare-Anane H[3], Persaud S[3], Jones P[3]
[1]Dept Pharmacognosy and Ethnopharmacology, NBRI Lucknow 226001, India [2]Pharmacognosy Laboratories, Pharmaceutical Sciences Division, Kings College London, London SE1 9NH, UK [3]Reproductive Health, Endocrinology and Development Division, Kings College London, London SE1 1UL UK

Extracts of the aerial parts of *Desmodium gangeticum* (L.) DC. (Papilionaceae) are used traditionally in India to treat diabetes but little scientific investigation has been performed. A 50% aqueous ethanol extract (DG) was prepared and its effects measured on rats (n = 6 for each group) rendered diabetic with streptozocin 50 mg/kg, using a group as non-treated rats as a non-diabetic control. Diabetic rats were administered vehicle alone, DG 200 mg/kg or glibenclamide 600 μg/kg daily by the oral route daily for 2 weeks. Blood glucose levels were measured by the *o*-toluidine test [1] on days 0, 7 and 15. Blood glucose levels (mg/dL) in diabetic rats given DG decreased from 202.15 ± 2.86 (Day 0) to 127 ± 3.09 (Day 15) compared to 210.27 ± 3.44 to 246.46 ± 3.16 for diabetic with no treatment and 202.03 ± 7.22 to 99.56 ± 2.76 for those treated with glibenclamide. Cultured MIN6 β-islet pancreatic cells, grown in 2mM glucose, were treated with a range of concentrations of DG (0.25 to 2.0 mg/ml) and the amount of insulin secreted after 30 min was measured using RIA [2]. The insulin secreted increased in a dose-dependent fashion, 2 mg/ml giving an increase of 155 ± 19% compared with untreated cells. HPLC analysis of the extract reveled a high concentration of chlorogenic acid, which has been reported to be hypoglycaemic and this may explain some of the activity noted. These results support the traditional use of *D. gangeticum* in treating diabetes. *Acknowledgements:* Indian Government for BOYSCAST Fellowship for RG **References:** [1] Sasaki, T. et al. (1972) Rinsho Kagaku 1: 346 – 353. [2] Hauge-Evans, A.C. et al. (2002) Mol. Cell. Endocrin. 191: 167 – 171.

SL 031

Clinical Efficacy of Phytodolor under special consideration of double-blind trials and their meta-analysis

Gundermann KJ[1]
[1]Department of Pharmacology and Toxicology, Chair of Pharmacology, Pomeranian Medical Academy, al. Powstańcow 72, 70111 Szczecin, Poland

The effects of Phytodolor, a fixed combination of extracts from aspen leaves and bark (*Populus tremula*), common ash bark (*Fraxinus excelsior*), and golden rod herb (*Solidago virgaurea*), have been widely verified in experimental and clinical investigations in painful inflammatory and degenerative rheumatic diseases. Its mode of action includes antiinflammatory, antioedematous, antioxidative and analgesic properties. Due to the synergistic action of its components it is considered to be broader than that of synthetic antirheumatics. Twenty one open clinical studies and 18 randomised, placebo- or verum-controlled double- and single-blind trials have been performed in different subtypes of rheumatic diseases and confirm the pharmacological evidence of efficacy, such as by reducing the intake of non-steroidal antiinflammatory drugs (NSAIDs) [1]. A meta-analysis of randomised, placebo-controlled double-blind studies, covering four trials of comparable design with the main variables of efficacy pain at movement, permanent pain and pain at rest, and of two trials on the savings of NSAIDs demonstrated a clear and highly significant overall therapeutic effect of Phytodolor [2]. The herbal combination product was shown to be effective for the treatment of inflammatory and degenerative diseases, soft tissue rheumatism as well as for fibromyalgias. Its efficacy is clinically relevant primarily for the treatment of musculoskeletal inflammation and pain at very good tolerability. **References:** [1] Gundermann, K.-J., Müller J. (2007) subm. for publ. in Wien. Med. Wochenschr. [2] Gundermann, K.-J., Godehardt E., Ulbrich M. (2007) Efficacy of a herbal preparation in patients with painful inflammatory and degenerative rheumatic diseases: meta-analyses of double-blind, randomised, clinical trials, paper under preparation

SL 032

Rhodiola SHR-5 extract and the treatment of depression

Darbinyan V[1], Aslanyan G[2], Amroyan E[2], Gabrielyan E[2], Malmstrom C[3], Panossian A[4]
[1]Department of Neurology, Armenian State Medical University, Koryun 2 St., Yerevan 375025, Armenia; [2]Scientific Centre of Drug &Medical Technology Expertise, 15, Moscovyan Street, Yerevan, 375001, Armenia; [3]The PBM Clinic, Institute of Health Competence, Arenavägen 41, 12177 Stockholm-Globen, Sweden; [4]Swedish Herbal Institute, Prinsgatan 12 5tr, SE-413 05 Göteborg, Sweden

Rhodiola (*Rhodiola rosea*) is a medicinal plant exhibiting significant adaptogenic properties that have been extensively studied and exploited in Scandinavia and Russia with particular reference to increasing mental performance against a background of fatigue and asthenia. In a randomised, double-blind, placebo-controlled phase III clinical trial, the effects of standardised extract SHR-5 of Rhodiola rhizome on mild to moderate depression were examined. Medication with SHR-5 was administered in the form of 400 mg tablets each of which contained 170 mg of Rhodiola extract that had been quantified by HPLC with respect to triandrin, rhodioloside, tyrosol and rosavin content. Male and female participants, aged between 18 – 70 years, were selected according to DSM-IV diagnostic criteria for depression, the severity of which was determined by scores gained in Beck Depression Inventory and Hamilton Rating Scale for Depression (HAMD) questionnaires. Patients with initial HAMD scores between 21 and 31 were randomised into three groups, one of which (group A: $n = 30$) received two tablets daily of SHR-5 (340 mg/day), a second (group B: $n = 29$) received two tablets twice-per-day of SHR-5 (680 mg/day), whilst a third (group C: $n = 29$) received two placebo tablets daily. The efficacy of the extract with respect to the reduction of depressive condition was assessed from the total and specific subgroup HAMD scores obtained on days 0 and 42 of the study period. For individuals in groups A and B, overall depression together with insomnia, emotional instability and somatisation, but not self-esteem, improved significantly following medication, whilst the placebo group showed no such improvements. No serious side effects were recorded in any of the study groups, and we were able to show that in animal systems SHR-5 does not interact with other drugs sensitive to CYP1A2, CYP2C9 and CYP3A4 enzymes. Results from a series of experiments involving rabbits have demonstrated that SHR-5 inhibits the over-activation of p-SAPK/p-JNK induced by emotional stress. The SAPK/JNK pathway is known to be involved in the pathogenesis of glucocorticoid resistance (GR), found in subgroups of patients with major depression, and the activation of SAPK/JNK has been reported to inhibit GR function. Hence it can be hypothesised that the antidepressant effects of SHR-5 are associated with the inhibition of stress activated protein kinases. This

study constitutes the first report that Rhodiola extracts exhibit antidepressive potency in patients with mild to moderate depression.

SL 033

CNS dopamine agonistic action of the *Vitex agnus castus* extract Ze 440 in freely moving, chronically instrumented animals

Brattström A[1], Dimpfel W[2], Koetter U[1], Käufeler R[1]
[1]Max Zeller Söhne AG, CH-8590 Romanshorn [2]NeuroCode AG; D-35578 Wetzlar

Dopamine agonistic action is intensively under investigation because of their usefulness in movement disorder. Extracts prepared from Vitex agnus castus (VAC) showed remarkable dopamine agonistic actions believed to inhibit exaggerated prolactin release in premenstrual syndrome. In order to investigate whether orally administered VAC besides inhibition of stimulated prolactin release induces general dopamine action within the central nervous system (CNS), the pattern of field potentials were recorded from electrodes positioned in frontal cortex, hippocampus, striatum and reticular formation. All four electrodes were placed 3 mm lateral within the left hemisphere (anterior coordinates are 12.2, 5.7, 9.7 and 3.7, according to the atlas of Paxinos and Watson). Animals were given 2 weeks for recovery from the surgical procedure. The electrical activity was amplified and transmitted wireless from the freely moving rats. Signals were collected in sweeps of 4 s duration and submitted to Fast Fourier Transformation. The resulting power spectra were divided into 6 frequency ranges (delta, theta, alpha 1, alpha 2, beta 1 and beta 2). Spectra were averaged in steps of 3 minutes each and displayed on-line. In an off-line procedure spectra were averaged to provide 60 minutes periods for data presentation and further statistical analysis. Changes of electrical power ($\mu V2/\omega$) are expressed as % of the 45 min absolute pre-dose electrical power values within each frequency band. Mobility was measured by a video tracking system (GJB Datentechnik, D). Oral administration of saline did not show any changes in the EEG power spectrum. Administration of 10, 25 and 50 mg of the special VAC extract Ze 440/kg body weight (BW) dose related changed the alpha 2 frequency band as well as delta and theta frequencies predominantly in frontal cortex and striatum. The largest effect was caused by 25 mg Ze 440/kg BW. Administration of a potent dopamine D2 receptor blocker (L 741,626; 2 mg/kg BW) prior to oral administration of 25 mg Ze 440/kg BW abolished the EEG responses in the frontal cortex but not those in the striatum. Oral administration of 10, 25 or 50 mg Ze 440/kg BW prevented the normal decrease of mobility with time, whilst the combination of 25 mg Ze 440/kg BW with the dopamine D2 antagonist led to higher mobility.

SL 034

Flavonoids from chokeberry fruits (*Aronia melanocarpa*) reduce oxidative stress and inflammation in patients with coronary artery disease treated with statins

Naruszewicz M[1], Dłużniewski M[2]
[1]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha St., 02 – 097 Warsaw, Poland; [2]II Cardiology Clinic, Medical University of Warsaw, 8 Kondratowicza st., 03 – 242 Warsaw, Poland

Recent studies have shown that chronic flavonoids treatment improves vascular function and cardiovascular remodeling by decreasing superoxide anion production as well as by increasing level NO derived from endothelial cells. Progressive decrease in systolic blood pressure and reduction of low-density lipoprotein oxidation (Ox-LDL) has also been reported. However, none of these studies has been done in patients with coronary artery disease treated with statins. Ours was a double-blind, placebo-controlled, parallel trial. Forty- four patients (11 women and 33 men, mean age 66 years) who had survived myocardial infarction and had received statin therapy for at least 6 months (80% dose of 40 mg/day simvastatin) were included in the study. The subjects were randomized to receive either 3 x 85 mg/day of chokeberry flavonoids extract (Aronia melanocarpa E) or placebo for a period of 6 weeks. The study extract was a commercially-available (OTC) product of the following declared composition: anthocyanins (about 25%), polymeric procyanidins (about 50%) and phenolic acids (about 9%). Compared to placebo (ANOVA and Tukey's test), flavonoids significantly reduced serum F2-isoprostans ($p<.000$) and ox-LDL levels ($p<.000$) (by 38% and 29%, respectively), as well as hsCRP ($p<.007$) and MCP-1 ($p<.001$) levels (by 23% and 29%, respectively). In addition, a significant increase in adiponectin ($p<.03$) levels and a reduction in systolic and diastolic blood pressure by a mean average of 11 and 7.2 mm Hg, respectively, was observed. In view of the fact that chokeberry flavonoids reduce the severity of inflammation, independently of statins treatment, they can be used clinically for secondary prevention of ischaemic heart disease.

Workshops

Workshop for Young Researchers

YRW 001

Extraction and analysis of non-derivatized glucosinoates in plant extracts – a validated PLE/LC-MS Protocol

Mohn T[1], Rüster U[2], Hamburger M[1]
[1]Institute of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland; [2]Institute of Pharmacy, University of Jena, Semmelweisstrasse 10, D-07743 Jena, Germany

Glucosinolates are a class of secondary metabolites which is characteristic of the order Capparales. More than 120 glucosinolates have been described so far, mostly from the family Brassicaceae. Glucosinolates have attracted significant interest due to the chemopreventive properties of some of their transformation products [1]. Numerous protocols for the extraction and analysis of glucosinolates have been published. Typically, the plant material is heat-pretreated and/or extracted at high temperature to inactivate myrosinase, followed by enzymatic desulfatation on a solid support, elution of desulfoglucosinolates and separation by reversed phase HPLC. More recently, analysis of intact glucosinolates has been attempted using ion-pair chromatography [2]. However, peak shape and separation usually were not satisfactory, and method validation insufficient. In all published methods for glucosinolate analysis, little effort has been devoted to optimize and validate crucial extraction parameters and sample preparation steps. We carried out a systematic optimization and validation of an assay for the direct analysis of non-derivatized glucosinolates in *Isatis tinctoria* leaves (woad, family Brassicaceae). Various parameters such as solvent composition, particle size, temperature, and number of required extraction steps were optimized using pressurized liquid extraction (PLE). We observed thermal degradation of glucosinolates at temperatures above 50 °C, and loss of > 60% within 15 min at 100 °C, but no enzymatic degradation in the leaf samples at ambient temperature. Excellent peak shape and resolution was obtained by reversed-phase chromatography on a Phenomenex Aqua column using 10mM ammonium formate as ion pair reagent. Detection was carried out by ESIMS (negative ion mode). The purity of reference glucosinolates was determined with qNMR. Analysis of cruciferous vegetables and spices such as broccoli (*Brassica oleracea* var. *silvestris* L.), garden cress (*Lepidium sativum* L.) and black mustard (*Sinapis nigra* L.) demonstrated the general applicability of the method. **References:** [1] Halkier, B., Gershenzon, J. (2006) Annu. Rev. Plant Biol. 57: 303 – 333. [2] Song, L. et al. (2005) Anal. Biochem. 347: 234 – 243.

YRW 002

Determination of aflatoxins in herbal drugs by post-column fluorescence enhancement in a reverse phase liquid chromatographic method

Rashmi K[1], Bhatnagar SP[2], Katiyar CK[3]
[1]Ranbaxy Laboratories Limited, Gurgaon 122 001, Haryana, India; [2]Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, 835 215, India; [3]Ranbaxy Laboratories Limited, Gurgaon 122 001, Haryana, India

In the proposed study, Thomas Romers' [1] method for extraction of aflatoxins in mixed feed and other agricultural commodities has been modified for application in medicinal herbs. The medicinal herbs studied include *Bacopa monniera*, *Centella asiatica*, *Gymnema sylvestre* and *Tinospora cordifolia*, the method utilizes extraction of aflatoxins by a mixture of acetone and water in the ratio 85:15 and removal of interferences by adding cupric carbonate and ferric chloride gel [2]. The aflatoxins were extracted from the aqueous phase with chloroform. The chloroform extract was washed with a basic aqueous solution. A florisil column or immunoaffinity column was used for clean-up. The aflatoxins were confirmed by using trifluoroacetic acid [3]. Francis *et al* [4] method using beta-cyclodextrin for post column fluorescence enhancement of aflatoxins by a reverse phase liquid chromatographic method in corn was extended for the determination of aflatoxins in the medicinal herbs. In order to avoid choking and minimising frequent washing of the column, hydroxypropyl beta-cyclodextrin was used in place of beta-cyclodextrin which has increased solubility over beta-cyclodextrin. The post column derivatisation instrument, PCX5200, Pickering laboratories Inc. was utilized. The aflatoxins B_1, B_2, G_1 and G_2 were determined without preparing derivatives of B_1 and G_1. The aflatoxins were dissolved in hydroxypropyl beta-cyclodextrin solution and using a mobile phase of methanol/hydroxypropyl beta-cyclodextrin (1:1), the aflatoxins were resolved on a C 18 column. Fluorescence of the aflatoxins was enhanced by post column introduction of an aqueous concentrated hydroxypropyl beta-cyclodextrin solution. The method has been validated to study accuracy, precision (system and method precision), specificity, linearity, limit of detection and limit of quantitation as per the ICH guidelines on analytical validations. The limit of detection for aflatoxin B_1 was found to be 0.1 ppb. *Acknowledgement:* Spectro Laboratories, New Delhi, India, Kuldeep Dhingra, Shushant Gupta, Ravinder Bhardwaj and Umesh. **References:** [1] T.R. Romer (1975) J. Assoc. Off. Anal. Chem., 58: 500 – 505. [2] J. Velasco (1972) J. Assoc. Off. Anal. Chem., 55: 1359 – 1360. [3] W. Przybylski (1975) J. Assoc. Off. Anal. Chem., 58: 163 – 164. [4] Francis et al. (1988) J. Assoc. Off. Anal. Chem., 71: 725 – 728.

YRW 003

Comparative isolation and structural investigations of polysaccharides from *Boswellia serrata* ROXB. and *Boswellia carteri* BIRDW

Herrmann A[1], Lechtenberg M[1], Hensel A[1]
[1]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

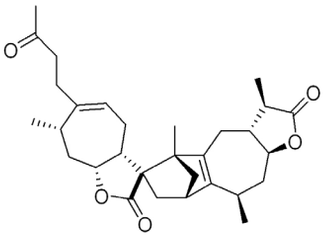
Resins from different species of *Boswellia* sp. are used for therapy of chronic inflammations. While the lipophilic part of the resin is characterized limited data are available on the water-soluble part. For detailed investigations comparative studies were performed with resins from *Boswellia serrata* ROXB. and *Boswellia carteri* BIRDW., Burseraceae. Quantification of the carbohydrate content indicated the presence of 20 – 30% of polysaccharides in the resins of both plants. Isolation of polysaccharides was achieved by cold aqueous extraction after defatting. Raw polysaccharides were fractionated by IEC in 8 subfractions followed by GPC on Superose®6. Sugar composition of all fractions was determined by ion exchange HPLC-PAD and GLC of the acetylated monomers. HPLC analysis was shown to have numerous advantages to GLC but leading to comparable results. Dominant compounds found in all fractions were Gal and Ara besides small amounts of Fuc, Man, Xyl, Rib and Glc. Also glucosamine was detected. Acid fractions showed higher amounts of 4-O-methyl-GlcA and GlcA, while GalA was absent. Detailed structural analysis by methylation analysis, ^{13}C-NMR and D,L-configuration analysis by CE indicated the presence of arabinogalactan proteins, which was manifested by positive tests with Yariv reagent. Also neutral 1,5-arabans, different type-II-arabinogalactans and methylglucuronoarabinogalactans were identified. Differences between polysaccharides from *B. carteri* and *B. serrata* were found concerning the protein amounts of AGP and the Ara-Gal ratios.

YRW 004

Isolation and characterisation of guaianolides from *Helichrysum montanum* and *H. splendidum*

Lourens ACU[1], Van Heerden FR[1], Viljoen AM[2], Munro OQ[1]
[1]School of Chemistry, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, South Africa, [2]School of Pharmacy, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa

The genus *Helichrysum* (Asteraceae) consists of approximately 500 species worldwide, with 245 species indigenous to South Africa [1].These plants are often used medicinally and are chemically diverse [2,3]. The aim of this study was to investigate the phytochemistry of *H. montanum*, a species morphologically closely related to *H. splendidum*, which is used traditionally to treat rheumatism [4]. An extract of *H. montanum* yielded four guaianolides, a flavonoid and a coumarin. The complex stereochemistry of the guaianolides presented a challenge during structure elucidation since several stereoisomers have been reported from other *Helichrysum* species and from other genera [3,5]. The guaianolides from *H. splendidum* were also reisolated and a crystal structure obtained for helisplendidilactone (a dimeric guaianolide, $C_{30}H_{40}O_5$, monoclinic $P2_1$, a= 9.0738(5), b= 11.4764(7), c= 12.2960(9) Å, β= 96.933(5)°, Z= 2, R_1= 0.0516, T= 100(2) K), which assisted in the structure elucidation of dimer **1** from *H. montanum*. The presence of the guaianolides is significant, since this type of compound is rare in this genus and also in the subtribe (Gnaphaliineae) [3].



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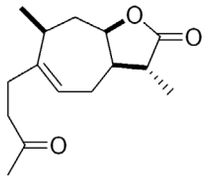

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Acknowledgements: National Research Foundation, University of KwaZulu-Natal, SANBI **References:** [1] Hilliard, O.M. (1983) Asteraceae. Inuleae. Gnaphaliineae. Flora of Southern Africa 33 (7,2). South African Botanical Institute. Pretoria. [2] Van Wyk B-E. et al. (2000) Medicinal Plants of South Africa. Briza. Pretoria. [3] Jakupovic J. et al. (1989) Phytochemistry 28: 1119 – 1131. [4] Pooley E. (2003) Mountain Flowers: A field guide to the flora of the Drakensberg and Lesotho. The Flora Publications Trust. Durban. [5] Zdero C. et al. (1989) Phytochemistry 28: 3105 – 3120.

YRW 005

Optimisation of a fluorimetric assay for the screening of potential α-glucosidase inhibitors in coloured plant extracts and foods

Payn DN[1,2], Deseo MA[1,2], Thompson DR[1], Morris CA[1], Leach DN[1,2]
[1]Centre for Phytochemistry and Pharmacology, Southern Cross University, PO Box 157, Lismore, NSW, 2480, Australia; [2]CRC for Sugar Industry Innovation through Biotechnology, University of Queensland, St Lucia, QLD 4072

The frequently used method for the *in vitro* screening of inhibitors of yeast α-glucosidase is based on the hydrolysis of p-nitrophenol-α-D-glucopyranoside to yield p-nitrophenol and glucose. After adjusting to pH 8 to form the yellow nitrophenolate anion, the absorbance at 405 nm is measured [1,2]. During our investigations into potential α-glucosidase inhibitors from sugarcane products, we found a decrease in sensitivity when highly coloured extracts were assayed. Therefore, an optimised method of the α-glucosidase assay was developed, using the fluorogenic substrate 4-methylumbelliferyl-α-D-glucopyranoside (4-MUG), to ensure a more reproducible and reliable screening tool for highly coloured plant extracts and foods. The optimised conditions for the assay were determined: α-glucosidase (2mU/mL), 4-MUG (84 μM), incubation time (20 minutes), incubation temperature (37 °C) and assay buffer pH (5.5). A molasses extract was screened for α-glucosidase inhibition using the optimised conditions with 80% inhibition seen at 150 μg/mL and no negative quenching effects at concentrations within the linear range of the instrument. The optimised assay was also used to determine IC_{50} values for acarbose and fucoidan. The results suggest that acarbose does inhibit yeast α-glucosidase, which contradicts earlier work [3]. Overall, this optimised assay will be a valuable tool for the screening of highly coloured plant extracts and foods for α-glucosidase activity. **References:** [1] Kim, Y. M. et al. (2005). Nutrition 21: 756 – 761. [2] Shim, Y. J. et al. (2003). J. Ethnopharmacol. 85: 283 – 287. [3] Oki, T. et al. (1999). J. Agr. Food Chem. 47: 550 – 553.

YRW 006

Chemopreventive activity of *Angelica sinensis* root extracts and its alkylphthalides

Dietz B, Schinkovitz A, Liu D, Edirisinghe PD, Deng S, Hagos G, Pauli GF, van Breemen RB, Farnsworth NR, Bolton JL
UIC/NIH Center for Botanical Dietary Supplements Research in Women's Health, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60605, USA

The roots of *Angelica sinensis* (Oliv.) Diels (Dang Gui; Apiaceae) have a long history in traditional Chinese medicine as an ailment for women's disorders and are often called "lady's ginseng". Today, Dang Gui is popular worldwide with numerous dietary supplements used for women's health and as anti-aging products. In the current study, we examined the chemopreventive activity of a methanol extract of Dang Gui by measuring the relative ability to induce the detoxification enzyme, quinone reductase-1 (QR1) assay. The lipophilic fractions of this extract showed strong QR induction with concentrations to double activity (CD) of 5.5 ± 0.7 μg/mL (ether) and 3.9 ± 0.5 μg/mL (chloroform). Further fractionation led to the isolation of phenolic esters and alkylphthalides, especially a ligustilide rich fraction (CD = 6.8 ± 1.2 μM), with strong QR inducing properties. One mechanism of QR induction could be through alkylation of Keap1 by electrophiles resulting in higher concentrations of Nrf2 in the nucleus. Enhanced levels of free Nrf2 will facilitate the transcription of chemopreventive enzymes through the antioxidative response element (ARE). Using MALDI mass spectrometry and LC-MS-MS, we demonstrated that the lipophilic extracts of *A. sinensis* and some alkylphthalides alkylated human Keap1 protein. The alkylphthalides were then incubated with glutathione and characterized using LC-UV-MS-MS to elucidate the structure of the ultimate electrophile reacting with Keap1 and thus inducing detoxification enzymes. These data suggest that lipophilic Dang Gui extracts might function as chemopreventive agents through induction of detoxification enzymes such as QR. *Acknowledgements:* This work was supported by NIH Grant P50 AT00155.

YRW 007

Bioactivity and cysteine sulphoxides of some wild *Allium* species from Central Asia

Jedelská J[1], Fritsch RM[2], Keusgen M[1]
[1]University of Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany; [2]Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany

The very diverse genus *Allium* L. shows a nearly exclusive distribution across the northern hemisphere with a main centre of diversity in Southwest and Central Asia. Rhizomes or bulbs, extracts of those or green parts of several species are intensively used by the native population of Central Asia [1]. Investigations concerning radical scavenger activity and antibiotic activity were focused on the subgenera *Allium*, *Rhizirideum,* and *Melanocrommyum*. Remarkable high scavenger activity (larger than 100%, related to BHT standard) was reported for *A. giganteum* Regel, *A. alaicum* Vved. and *A. komarowii* Lipsky, all belonging to the subgenus *Melanocrommyum*. But also an extract obtained from *A. pskemense* B. Fedt., a close relative of common onion (*A. cepa* L.), exhibited a high radical scavenger activity. Cysteine sulphoxides of investigated species were also analysed and correlated with results for bioactivity testing. These substances might be responsible for radical scavenger activity but also other compounds must be considered. Antibiotic activity was visualized by an inhibition zone surrounding a filter paper soaked with ethyl acetate extracts. Of all tested samples, *A. rosenorum* was most active despite this species showed rather low (about 0.1%) cysteine sulphoxide content. Extracts of *A.* aff. *cristophii* were only active against *Streptococcus pyogenes*. *A. hymenorrhizum* Ledeb. was rich in cysteine sulphoxides and showed an antibacterial activity against most of the tested bacteria strains. Also antifungal and antialgal activities were found. *Acknowledgements:* This research was kindly supported by the VolkswagenStiftung, Hannover, Germany, as part of the program "Zwischen Europa und Orient-Mittelasien/Kaukasus im Fokus der Wissenschaft". **References:** [1] Keusgen, M. et al. (2006) J. Ethnobiol. Ethnomedicine 2: 18.

YRW 008

Pro-senescent effect of resveratrol in human endothelial cells is associated with S-phase arrest and increased ROS production

Schilder YDC[1], Heiss EH[1], Sorescu D[2], Dirsch VM[1]
[1]Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria; [2]Emory University, School of Medicine, Division of Cardiology, 1639 Pierce Drive, Atlanta GA 30322, USA

Resveratrol, a polyphenol found among others in red wine, has cardiovascular protective properties, such as being anti-inflammatory or stimulating NO-production. Recently, resveratrol has been shown to increase life-span in simple organisms. We hypothesized that resveratrol exerts an additional vaso-protective effect by delaying endothelial cell senescence. Human umbilical vein endothelial cells (HUVECs) were chronically exposed to 10 μM RV. RV-treated cells hereby showed a shorter replicative life span (75 vs. 90 days) with lower maximum cumulative population doublings (30 vs. 60) compared to control cells, RV-treated cells showed more senescence-associated-β-galactosidase positive cells than controls at passage 19 (70 ± 8 vs. 27 ± 7%). Since oxidative stress has been implicated in senescence, we measured reactive oxygen species (ROS). RV increased intracellular levels of ROS after 24 h incubation to about 150%. Mitochondria and NADPH-oxidases (Nox) are the main sources of ROS in HUVECs. We therefore employed various mitochondrial (CCCP, mitoQ and rotenone) and Nox-inhibitors (apocynin and DPI), respectively, and found that only apocynin and DPI reduced ROS to

control levels. Moreover, RV-triggered elevation of ROS and induction of senescence were associated with cell cycle arrest in S-phase (130% vs. control). Conclusion: RV accelerates senescence in HUVECs by causing cell cycle arrest in S-phase and oxidative stress via activation of Nox.

YRW 009

Selective inhibition of platelet-derived growth factor-induced signalling cascades by indirubin-3'-monoxime

Schwaiberger AV, Heiss EH, Dirsch VM
Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

In Traditional Chinese Medicine the recipe Danggui Longhui Wan is used as an antileukaemic drug. As active compound the bisindol indirubin was identified, which shows in enzyme assays inhibitory effects on CDKs (cyclin-dependent kinases), key regulators of the cell cycle [1]. Targeting CDKs may be a promising treatment of vasculoproliferative disorders [2]. Therefore, we tested indirubin-3'-monoxime (I3MO), a cell permeable derivative of indirubin, for antiproliferative activity in platelet-derived growth factor (PDGF-BB)-treated rat vascular smooth muscle cells (RVSMCs) and investigated underlying mechanisms. Examination of BrdU-incorporation in RVSMCs evinced a concentration-dependent inhibition of PDGF-BB (20 ng/ml)-induced DNA-synthesis by I3MO, which is caused by an arrest of cells in G0/G1-phase of cell cycle [3]. To investigate whether I3MO interferes with phosphorylation and activation of the PDGF-receptor, cells were preincubated with I3MO (3 μM) and treated with PDGF-BB for up to 30 min. Western blot analysis with a specific antibody against phospho-tyrosine residues revealed a sustained decrease in receptor phosphorylation. Surprisingly we found, that even though phosphorylation of critical downstream signalling kinases Akt and Erk1/2 is delayed by I3MO for two and five minutes, respectively, the extent of activation of these kinases is not affected. Focusing on other proteins involved in signal transduction of PDGF-receptor, however, showed a significant abolishment of STAT3 phosphorylation at Y705 within the first 30 min as well as in long-time stimulations for up to 18 hours. Further examination of nuclear extracts by western blot revealed a clear decrease in phosphorylated STAT3 in the nucleus. Recapitulating these results, blockade of STAT3 phosphorylation might be at least one mechanism for the antiproliferative effect of I3MO in PDGF- stimulated RVSMCs. *Acknowledgements:* CNRS, Station Biologique, Amyloïds and Cell Division Cycle, Meijer L. **References:** [1] Hoessel, R. et al. (1999) Nat. Cell Biol. 1: 60 – 67. [2] Dzau, V.J. (2002) Nat. Med. 8: 1249 – 1256. [3] Schwaiberger, A.V. et al. (2006) Planta Med. 72: 991

YRW 010

Effects of *Byrsocarpus coccineus* (Connaraceae) extract on the central nervous system (CNS)

Akindele AJ, Adeyemi OO
Department of Pharmacology, College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria

In establishing the pharmacological profile of *Byrsocarpus coccineus*, we investigated the CNS effects of the aqueous leaf extract using the Y-maze, hexobarbitone sleeping time and hole board models in mice. In the Y-maze test, *Byrsocarpus coccineus*, BC (50 – 100 mg/kg, p.o.) produced significant ($P < 0.05$) increases in the time spent in the open arm at post-treatment times of 30 – 90 min. Peak effect was elicited at 100 mg/kg 30 min post-treatment, with mice spending 2.63 ± 0.21 min in the open arm vs. 0.62 ± 0.21 min for control. This effect was significantly higher than that of diazepam, 1 mg/kg p.o. (2.00 ± 0.20 min). At doses of 200 and 400 mg/kg, the effect of BC in increasing the time spent in the open arm diminished with the effect at the higher dose (1.24 ± 0.29 min) being not significantly different from the control. In the hexobarbitone sleeping time test, BC (50 – 100 mg/kg) increased the onset and decreased the duration of sleep, with effects only being significant at 100 mg/kg (onset = 7.00 ± 1.18 min and duration = 10.00 ± 1.84 min vs. onset = 3.43 ± 0.81 min and duration = 20.71 ± 2.34 min for control). At doses of 200 and 400 mg/kg, BC decreased onset and significantly increased sleeping time with the effect at the higher dose (onset = 3.29 ± 0.61 min and duration = 39.86 ± 3.78 min) being significantly lower than that of diazepam, 3 mg/kg p.o., in respect of duration of sleep only (onset = 3.14 ± 0.26 min and duration = 87.29 ± 9.87 min). In the modified hole board experiment, BC (50 – 100 mg/kg) caused an increase in both exploratory activity and locomotion, while at 200 and 400 mg/kg the reverse was the case. These effects were not significant. Diazepam, 1 mg/kg p.o., caused a non-significant increase in exploratory activity and a significant increase in locomotion (25.71 ± 4.44 sectional crossings in 5 min vs. 10.86 ± 2.30 sectional crossings in 5 min for control). Results obtained suggest that the aqueous leaf extract of *Byrsocarpus coccineus* possess a dose determined anxiolytic – sedative activity with no effect on exploratory activity and locomotion.

YRW 011

The stability of *Z*-ligustilide and its relevance for the biological evaluation of *Angelica* botanicals

Schinkovitz A, Dietz B, Deng S, Chen SN, Pro S, Lankin D, Nikolic D, van Breemen RB, Bolton JL, Farnsworth NR, Pauli GF
UIC/NIH Center for Botanical Dietary Supplements Research, MCP/PCRPS, College of Pharmacy, University of Illinois at Chicago, Chicago IL 60612, USA

Originating from traditional Chinese medicine, *Angelica* species are commonly used herbals [1]. Phthalide monomers such as *cis*-3-butylidene-4,5-dihydro-phthalide (*Z*-ligustilide, **1**) are often associated with the active principle in botanical preparations [2, 3]. Because pure **1** is known to undergo rapid chemical degradation [4, 5], the purpose of this study was to elucidate whether the observed bioactivity may be attributed to **1** by itself, or associated with its degradation products. Therefore, a three-prong approach was taken, which involved: 1) the isolation of pure **1**, 2) careful stability monitoring, and 3) the evaluation of biological activity using a quinone reductase (QR) assay. The use of an optimized liquid-liquid chromatography protocol employing 2-step FCPC/HSCCC permitted the isolation of high-purity **1** (99.4 by GC, 99.6% by qHNMR), from the roots of *Angelica sinensis* (Oliv.) Diels. (Apiaceae). When the stability of **1** was monitored by GC-MS and [q]NMR to evaluate the impact of different storage conditions, **1** was found stable when stored in organic solutions at temperatures -30° C or below. However, once dried, **1** undergoes significant degradation within 24 hours, even when kept in the dark at -30 ° C. Interestingly, **1** of high-purity, when subjected to biological testing, showed no measurable activity in the QR assay, while chemically degraded samples exhibited significant activity (CD value: 6.5 – 11.7 μM ± 0.4 – 1.2) in the same assay. Our results demonstrate that the storage conditions can have a profound impact on the quality of phthalide-containing herbals as well as on the biological activities attributed to the phthalide class of compound. **References:** [1] Deng, S. et al. (2006) Phytochem. Analysis 17: 398 – 405 [2] Cao, Y-X. et al. (2006) Vasc. Pharmacol. 45: 171 – 6. [3] Lui, L. et al. (2005) Planta Med. 71: 808 – 13. [4] Cui, F. et al. (2006) Drug. Dev. Ind. 32: 747 – 55. [5] Zhou, C. et al. (2001) Act. Pharm. Sinic. 36: 793 – 95.

YRW 012

Changes in the lignan pattern of flax (*Linum usitatissimum* L.) in the course of plant development

Klaes M[1], Schmidt TJ[1]
[1]Westfälische Wilhelms-Universität Münster, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstraße 56, D-48149 Münster, Germany

Flax seed (*L. usitatissimum* L.) is one of the richest sources of precursors for enterolignans which possess chemopreventive potential

against malignant tumours as well as cardiovascular and other diseases [1]. Secoisolariciresinol diglucoside (SDG) is the major lignan in the seeds where it occurs mainly in the form of oligomeric esters with hydroxymethylglutaric acid [1]. The major part of flax seed SDG possesses 8S, 8'S-configuration (SS-SDG) corresponding to (+)-secoisolariciresinol [1]. In the course of our ongoing studies on lignan diversity in the genus *Linum*, we have shown that adult *L. usitatissimum* plants contain a variety of lipophilic dibenzylbutyrolactone lignans, e.g. yatein, and that these compounds possess 8R, 8'R-configuration [2]. As a result of our present study, the latter compounds are not present in the seeds. In accordance with literature [1,3], SS-SDG along with p-coumaric- and ferulic acid glucosides (CAG, FAG) were detected as major constituents besides a minor amount of RR-SDG. Changes in lignan biosynthesis and accumulation must hence occur during development, i.e. from 8S, 8'S-configured lignans to such with R,R-configuration and from polar dibenzylbutane glucosides to lipophilic dibenzylbutyrolactones. We have hence begun to investigate changes in lignan pattern of *L. usitatissimum* during plant development. Seeds were germinated and samples harvested in one-day intervals over two weeks and analysed by HPLC using two different systems for polar [3] and non-polar constituents [2]. It was found that the formation of lipophilic lignans is neither initiated during germination nor related to the onset of photosynthesis or lignin formation. Neither the amounts of SS-SDG nor those of its stereoisomer are significantly increased in this phase. Developing plantlets contain only minor amounts of SDG, CAG and FAG, which appear to be restricted to the seed remnant. Instead, several polar phenolic components, some with lignan-like UV spectra, displayed a marked increase. Their identification is in progress while the study is continued over the whole lifetime of the plants. *Acknowledgement*: We acknowledge financial support from Deutsche Forschungsgemeinschaft (DFG) grant # Schm 1166/2 – 2 and the valuable help by A. Frehe and C. Knickenberg, Münster, during an undergraduate research project. **References:** [1] Westcott, N., Muir, A.D. (2003) Flax-The genus Linum. Taylor & Francis. London. [2] Schmidt, T.J. et al. (2006) Phytochem. Anal. 17: 299 – 311. [3] Eliasson, C. et al. (2003) J. Chromatogr. A. 1001: 151 – 159.

WS 1: Importance of HMPC community monographs and community list entries for the marketing authorisation and registration of herbal medicinal products in Europe

(Permanent Committee on Regulatory Affairs on Herbal Medicinal Products)

WS 1-01

Importance of HMPC community monographs and community list entries for the marketing authorisation and registration of herbal medicinal products in Europe

Chair: Vlietinck AJ[1], Alban S[2]
[1]Laboratory of Pharmacognosy, University of Antwerp, B-2610 Wilrijk, Belgium; [2]Pharmaceutical Institute, Christian Albrechts-University, 24118 Kiel, Germany

Recently the HMPC Working Party on Community Monographs and Community List (MLWP) finalised the procedures and templates for the preparation of an entry to the Community List of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products (EMEA/HMPC/57137/2007), and released the document for the three-month public consultation. By now, about ten community herbal monographs and/or list entries have been adopted for publication and twenty more are in several phases according to the timetable viz. between drafting to release for public consultation and from public consultation to finalisation by the MLWP. It is therefore appropriate in this workshop to discuss the implementation of these documents at the level of the national authorities and the manufacturers of herbal medicinal products, especially in terms of legal basis, rational and feasibility. More specifically, will these documents not only represent current scientific knowledge, but might they also serve as guidance on harmonized criteria for the evaluation of herbal medicinal products in Europe? Moreover, will these documents not only allow to discriminate between well-established and traditional use for herbal medicinal products but also between traditional herbal medicinal products and herbals containing food supplements throughout Europe? It is expected to gain critical feedback from all participants in terms of harmonisation across the EU, so that more successful applications on herbal medicinal products to the regulatory authorities would be submitted in the future.

WS 2: Pharmacokinetics of herbal medicinal products – useful or not?

(Permanent Committee on Manufacturing and Quality Control of Herbal Remedies)

WS 2-01

Topics:
View of the national competent authority of Austria (AGES PharmMed)
Pharmacokinetics of different *Echinacea purpurea* Moench and *Ginkgo biloba* L. preparations
Biopharmaceutical characterisation of HMPs- some aspects from the (researching) industry

Chair: Meier B[1],
Panelists: Länger R[2], Lang F[3], Woelkart K[4]
[1]University of Applied Sciences, Grüental, CH-8820 Wädenswil, Switzerland, [2]AGES PharmMed, LCM Herb, Schnirchgasse 9, A-1030 Wien, Austria; [3]Dr. Willmar Schwabe Pharmaceuticals, Willmar-Schwabe-Str. 4, D-76227 Karlsruhe, Germany; [4]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University, A-8010 Graz, Austria

View of the authority: According to the Directive 2001/83/EC and the Austrian medicines law the dossier for application for a marketing authorization for medicinal products has to include data on distribution, biotransformation and elimination of pharmacologically active substances. The essential facts should be presented in the SPC of the product. Exceptions are explicitly tolerated for the registration of traditional herbal medicinal products where data on pharmacological properties (including pharmacokinetic particulars) are not mandatory. In most herbal medicinal products the entire extract is considered as the active ingredient. Therefore differing requirements should apply compared to synthetic drugs. The extent of the data which should be submitted depends on several criteria, for example:

1. Type of application (full applications with new active substances need more comprehensive data compared to a bibliographic application)
2. Type of active ingredient (a standardised herbal preparation is better accessible for studies on pharmacokinetics than an extract with analytical markers only)
3. Duration of use (short term treatment bears a lower risk for pharmacokinetic interactions than treatment of chronic diseases)
4. Congruence with the usual (traditional) dosage scheme (a differing single daily dose should be substantiated by pharmacokinetic data)
5. Safety concerns (e.g. evidence from literature on possible pharmacokinetic interactions)

Scientific approach Establishing the pharmacological basis for efficacy of herbal medicinal products (HMPs) is a constant challenge. In this context, also the question of bioavailability, the elucidation of metabolic pathways and their pharmacokinetics is of major interest. These data are relevant to link results from pharmacological *in vitro* assays and clinical studies. A better understanding of the pharmacokinetics and bioavailability of phytopharmaceuticals can also help

in designing rational dosage regimens. [1] The preparations used in our pharmacokinetic single dose studies are different *Echinacea purpurea* Moench (Echinaforce™) and *Ginkgo biloba* L. (Geriaforce™, EGb 761™) liquid formulations and tablets with diverse excipients. The concentrations of the active compounds in the administered products are in the low mg range per dose. The resulting plasma concentrations are in the ng per mL range. Therefore a validated sensitive and selective LC-ESI-MS-based method that is capable of monitoring plasma levels of the traces of active constituents in humans was developed and used. In all studies performed taking *Echinacea* or *Ginkgo* the initial concentrations of the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides, the main alkamides, or bilobalide, ginkgolide A and B, respectively, could be already detected in the blood 10 or 15 minutes after oral administration. [2,3,4] The assessment of the bioavailability, especially the determination of pharmacokinetic characteristics and further pharmacokinetic/pharmacodynamic modelling contribute in more rational use of these products. **Aspects of the (research)-industry** In the case of traditional and well established HMPs pharmakokinetical investigations may be waived in many cases and restricted to modified release pharmaceutical forms. For standardised or quantified extracts biopharmaceutical and pharmacokinetical aspects are desirable during product development especially with products showing low solubility. In the case of new plant extracts and for toxicological and clinical trials pharmacokinetical studies are obligatory. From a scientific point of view such pharmacokinetical results related to markers are however questionable and should not be overrated by the competent authorities. **References:** [1] Bhattaram, V.A. et al. (2002) Phytomedicine 9: 1 – 33. [2] Woelkart, K. et al. (2005) J. Clin. Pharmacol. 45: 683 – 689. [3] Woelkart, K. et al. (2006) Int. J. Clin. Pharm. Th. 44: 401 – 408. [4] Biber, A. (2003) Pharmacopsychiatry 36:S32-S37. **Acknowledgement:** The Workshop is sponsored by **Zeller AG**, Herbal Medicinal Products, CH-8590 Romanshorn and by **Schwabe AG, Karlsruhe.** The research work of K. Woelkart was kindly supported by Bioforce AG, Roggwil, Switzerland

WS 3: Models for testing addictive behaviour
(Permanent Committee on Biological and Pharmacological Activity of Natural Products)

WS 3-01
Alcohol and nicotine preference, alcohol and nicotine deprivation effect; test systems and results with desoxypeganine

Doetkoette R, Winterhoff H
Institute for Pharmacology and Toxicology, Domagkstr. 12, D 48149 Münster, Germany

Deoxypeganine (DOP), an alkaloid from *Peganum harmala* L. is a weak inhibitor of acetyl- and butyrylcholinesterase as well as of monoaminooxidase A. Two test models will be presented in which this substances was tested and the possible therapeutic targets discussed. I. Alcohol/nicotine preference: This test checks whether a substance is able to reduce particularly alcohol preference, i.e. without affecting total fluid or food intake. AA* rats were used in the free choice limited access paradigm. These genetically determined alcohol-preferring AA rats show a pronounced preference for alcohol, which could be reduced specifically and dose dependently by DOP. No hints on a development of tolerance were observed. AA rats were switched to nicotine drinking and developed a nicotine preference too. The nicotine preference tested in the same experimental design could be reduced by DOP also. II. The deprivation effect: The alcohol deprivation effect is a short term increase in the relative intake of alcohol after an alcohol deprivation period and is regarded as an animal model for alcohol craving. Selected Wistar- and Fawn-hooded rats of known alcohol intake had no access to alcohol for a period of two weeks. After this period a clear increase in alcohol intake in controls was observed in contrast to the DOP treated animals. In nicotine drinking AA rats a pronounced increase in nicotine intake was observed following a three weeks deprivation period. These results are of special interest owing to the pronounced comorbidity of alcohol- and nicotine dependency. * The AA rats were a kind gift from the National Public Health Institute, Department for Mental Health and Alcohol Research, Helsinki, Finland

WS 3-02
Operant animal models of drug-seeking behavior

Bäckström P
National Public Health Institute, Department of Mental Health and Alcohol Research, POB 33, FI-00251 Helsinki, Finland

Relapse prevention is one of the major challenges for the treatment of drug addiction. In abstinent drug users the likelihood of relapse is increased by stress, re-exposure to the drug, and drug-associated stimuli. These factors increase drug-seeking behavior also in laboratory animals. Operant animal models of drug seeking include the extinction/reinstatement models as well as the fixed-interval second-order and progressive ratio schedules of drug self-administration. In the extinction/reinstatement model animals are trained to self-administer the drug e.g. by pressing a lever. Drug availability or delivery can be paired with a stimulus, e.g. a light or a tone. After repeated pairings, the light and tone acquire conditioned reinforcing properties and are able to guide behavior on their own. Lever pressing is then extinguished by withdrawing the drug and the drug-associated stimuli. Drug-seeking behavior, measured by resumption of lever pressing, can be triggered by stress, a priming injection of the drug, or presentations of the conditioned light or tone stimuli in the absence of the drug. Fixed-interval second-order schedules of self-administration are based on the ability of stimuli to become conditioned to drug effects. Under these schedules, long sequences of behavior can be maintained in the absence of the primary reinforcer. A fixed number of responses leads to a stimulus presentation and, additionally, the first response after a predetermined time interval results in drug delivery. The number of responses during the interval is considered a measure of the strength of drug-seeking behavior. Progressive ratio schedules measure the motivation to self-administer the drug. Under these schedules the response requirement for drug delivery is progressively increased according to a predetermined schedule so that very few responses are required for the first drug reinforcer but eventually even hundreds of responses per reinforcer may be required. A session is considered completed when the animal does not respond for the drug within a specified time period. The final response ratio reached represents the maximal effort the animal is willing to make to receive the drug. Supported by the Finnish Foundation for Alcohol Studies, the Ella and Georg Ehrnrooth Foundation, and the Yrjö Jahnsson Foundation.

WS 4: Identification and authentication of plant starting materials
(Permanent Committee on Breeding and Cultivation of Medicinal Plants)

WS 4-01
Identification and authentication of herbal substances

Franz C[1], Klier B[2], Reich E[3], Novak J[1]
[1]Institute for Applied Botany and Pharmacognosy, Veterinärplatz 1, A-1210 Vienna; [2]Phytolab GmbH & Co. KG, D-91487 Vestenbergsgreuth; [3]CAMAG-Laboratories, CH-4132 Muttenz

Due to the increasingly high number of plant species used as starting materials for herbal medicinal products and the risk of admixtures and adulterations with related or similar species identification along the production and processing chain is a crucial problem. Identity and purity is usually tested by macro- and microscopic as well as chromatographic methods, preferably TLC. The most recent

developments in HPTLC and the characterisation of herbal materials by specific chromatographic fingerprints will be discussed. In addition, DNA analysis has become a novel technique for identification and authentication of raw materials and monitoring throughout the production chain. Depending on their practicability the different DNA based methods may supplement or substitute other identity tests.

WS 5: Nasal innate and adaptive immune responses and their influence by herbal preparations

WS 5-01

Overview on the immune response

Szelenyi I
Institute of Experimental and Clinical Pharmacology, University of Erlangen, Fahrstr. 17, 91054 Erlangen, Germany

The major task of the immune system is the protection of organisms from infection by identifying and killing pathogens. To meet this challenge, organisms develop different mechanisms with staggered defences of increasing specificity. The nose as first target of respiratory tracts is covered by epithelial cells representing the most physical barrier for pathogens. If pathogens penetrate cell layers the innate immune system provides a rapid but non-specific response. Epithelial cells are not only simple barriers but also involved in biological cleaning processes via producing various mediators. The innate immune system produces non-specific, immediate responses to pathogens. This innate, or natural, immunity includes two parts. One part, called humoral innate immunity, includes a variety of substances in body fluids. They are also important mediators in the activation of the adaptive immune system. The other, called cellular innate immunity is mediated by phagocytes. Phagocytes (e.g. neutrophils, macrophages, dendritic cells, etc.) belong to this immune system without long-lasting immunity. Only vertebrates have additional and more sophisticated defense mechanisms. The adaptive immunity can recognize and destroy organisms or substances and is antigen-specific with delay between exposure and response. Different types of lymphocytes are tools of the adaptive immunity. The B cells are involved in humoral and T-cells in cell-mediated immune responses. Specificity is one of the two properties that distinguish adaptive immunity from innate immunity. The other one called immunologic memory is the ability of the adaptive immune system to induce stronger and more effective immune responses against antigens after their first contact. In this way the organism is primed against future attacks.

WS 5-02

Modulation of tlr signalling pathways in pursuit of therapy

Gessner A
Mikrobiologisches Institut, Medizinische Mikrobiologie, Immunologie und Hygiene, Friedrich-Alexander-University Erlangen- Nürnberg, Fahrstr. 17, 91054 Erlangen, Germany

The innate immune system provides the first line of defense against infection. Cells of the innate immune system recognize and are activated by highly conserved structures expressed by large group of microorganisms called pathogen-associated molecular patterns (PAMPs). A limited number of germline-encoded pattern recognition receptors (PRRs) are involved either in recognition (scavenger receptors, C-type lectins) or in cell activation (Toll-like receptors or TLR, helicases and NOD molecules). TLRs play a pivotal role in cell activation in response to PAMPs. TLR are type I transmembrane proteins that are expressed not only by innate immune cells but also lymphocytes comprising the adaptive immune system and non immune cells. In all the cell types analyzed, TLR agonists, alone or in combination with costimulatory molecules, induce cell activation. The ability of different cell types to respond to TLR agonists is related to the pattern of expression of the TLRs and its regulation as well as their intracellular localization. Ligation of TLR controls innate and adaptive immune responses by inducing synthesis of pro- as well as anti-inflammatory cytokines and activation of effector as well as regulatory lymphocytes. TLRs are therefore considered as major targets for the development of vaccine adjuvants, but also of new immunotherapies. The potential of TLR ligands as a novel class of pharmaceuticals for the prevention or treatment of allergic disorders, the enhancement of anti-tumour-immune responses and the therapeutical modulation of infectious diseases will be discussed.

WS 5-03

Antimicrobial activity and medical plant extracts: relevance for sinusitis

Maune S
Department of Otorhinolaryngology, Head and Neck Surgery, Municipal Hospital of Cologne, Neufelder Str. 32, 51058 Köln, Germany

Sinusitis is a common disease in public. About 85 – 90% of antimicrobial drugs are used in the community; up to 80% are used to treat respiratory tract infections. Antibiotics are getting limited in case of bacterial resistance. Plants are known to be active against many infectious microbials and are widely used in public, with high evidence for safety and efficacy due to clinical day-to-day experience. Therefore it seems to be very interesting which effects phytomedical extracts induce in respiratory cells and against relevant bacterials. Therefore it is interesting whether or not plant extracts induce activity in the first and second line defense of nasal mucosa. We addressed our interest to the capabilities of these substances to get influence in either the human antimicrobial activity in nasal mucosa or the antimicrobial activity by self. The antimicrobial activities of plant extracts were determined by ***in vitro*** bioassays using agar diffusion-method. The minimal bactericidal concentration (MBC) and lethal dose, LD 90/50, were calculated for gram-positive and -negative bacterial standard strains. Bacteria strains were selected according to their relevance on upper airway infection. We could find out either the induction of human antimicrobial peptides or direct antimicrobial activity against some of the bacteria. This has been shown concerning MBC, LD 90, and LD 50. In conclusion, phytomedical extracts revealed distinct antibacterial activities. The astonishing, hitherto unknown, antibacterial activities of the commercially phytochemical and its extracts gave some promising clues for the anti-infective efficacy of the drug observed in clinical experience and encourage us to elucidate the antimicrobial efficacy of phytochemical drugs.

WS 5-04

Immune functions of nasal epithelial cells and their modulation by herbal extracts

Pahl A
Institute of Experimental and Clinical Pharmacology, University of Erlangen, Fahrstr. 17, 91054 Erlangen, Germany

Airway epithelial cells have been considered to play a role as a mucosal barrier. However, studies suggest that these cells can also act as immune effector cells in response to several stimuli and play a key role in the immunologic interaction between the airway and the environment. Epithelial cells in allergic individuals are in an activated state, as shown by the increased expression of adhesion molecule-like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and the increased production of IL-6, IL-8, GM-CSF and TNF-α thus contributing to the allergic reaction. However, epithelial cells may also play important roles via direct cell-to-cell interactions. Chronic rhinosinusitis (CRS) is defined as an inflammatory condition involving the paranasal sinuses for longer than 12 weeks. CRS patients suffer from rhinitis and olfactory dysfunction, suggesting that nasal inflammation and abnormalities of the olfactory epithelium contribute to sinusitis.

Stimuli which induce exacerbations of sinusitis may include infections, but the role in CRS is uncertain. Furthermore, immunologic inflammatory responses play major roles in the pathophysiology of CRS. Based on the findings of the immune function of nasal epithelial cells, we established various *in vitro* models mimicking pathological mechanisms of CRS. We demonstrated the validity of these *in vitro* models by showing their sensitivity to known anti-inflammatory compounds. These preclinical models are now used to screen herbal extracts for anti-inflammatory activities on the immune function on nasal epithelial cells. These investigations may allow the identification of herbal compounds with the ability to treat patients suffering from CRS.

WS 5-05

Immunomodulating properties of Tonsilgon® N

Melnikov OF, Zabolotny DI
Institute of Otolaryngology of Medical Sciences of Ukraine, Zoologicheskaya str. 3, 03680, Kiev, Ukraine

Experimental studies on 35 Wistar rats with immunodeficiency caused by cyclophosphamide were conducted to determine the immunomodulating properties of Tonsilgon® N, a fixed herbal combination of *Radix Althaeae, Flores Chamomillae, Herba Equiseti, Folia Juglandis, Herba Millefolii, Cortex Quercus and Herba Taraxaci*, defining its therapeutic influence. The preparation was administered orally for 10 days, twice daily, in the dosage for adults. The control group included both normal animals that also were given preparation, and the group of animals that underwent any of interferences. Antibody-forming activity (Jerne method), level of natural cellular cytotoxicity (CF- method) and activity of blood cell phagocytosis (latex method) were examined. In statistics a nonparametric test "U" was used. Administration of cyclophosphamide led to a 2.5-fold antibody decrease and a decrease of natural cytotoxicity by 80% in comparison with control animals. Activity of phagocytosis was inhibited to a lesser degree. Administration of Tonsilgon® N resulted in partial recovery of antibody response in animals with immunodeficiency and in quantity stimulation of antibody producers in normal animals. As regards natural cellular cytotoxicity, Tonsilgon® N renewed the destructive activity of cytotoxic blood cells regarding red cells of chickens (150) in animals with immunodeficiency and increased this activity in normal animals. In group of normal animals the stimulation of phagocytosis was more expressed and defined. It may be assumed that Tonsilgon® N has a broad spectrum of immunomodulating influence either in normal condition or by chemically induced immunodeficiency. Tonsilgon® N seems to modulate both adaptive and innate immunity.

WS 6: State of the art in clinical and preclinical studies with EPs® 7630 (Umckaloabo®)

WS 6-01

A detailed view on the constituents of EPs® 7630

Germer S, Hauer H, Erdelmeier C, Schoetz K
Preclinical Research, Dr. Willmar Schwabe GmbH & Co. KG, PO Box 410925, 76209 Karlsruhe, Germany

EPs® 7630 is a liquid herbal drug preparation from the roots of *Pelargonium sidoides* DC. (DER = 1:8 – 10), which is produced by extraction of milled roots with 11% (m/m) ethanol in water. This solvent leads to a polar spectrum of constituents which differs significantly from extracts obtained by extraction with nonpolar solvents. EPs® 7630 is composed of five main groups of constituents, namely inorganic salts, monomeric and oligomeric carbohydrates, highly substituted benzopyranones, purine derivatives as well as unsubstituted and substituted oligomeric prodelphinidins. The main benzopyranones of EPs® 7630 are highly oxygenated at the phenyl moiety (three to four oxygens) and in addition sulphated at distinct positions. A disulphate of 5,6,7-trihydoxy-benzopyranone has been identified for the first time in plants. The oligoprodelphinidins, frequently supposed to be compounds with unspecific tanning interactions, show, in contrast to other polyphenols, an amazing variety of substructures and connectivities which results in an uncommon diversity even at a low degree of polymerization. In addition, some oligomers contain to a minor content a further substituent as demonstrated by a series of oligomers displaying additional constant masses in electrospray mass spectroscopy. Three distinct purine derivatives, probably intermediates of DNA-syntheses, were identified and characterized by phytochemical means. Taken together, these constituents amount to about 60 to 70% of the total weight of EPs® 7630, the active ingredient of the phytopharmaceutical Umckaloabo®.

WS 6-02

Anti-infective mode of action of EPs® 7630 at the molecular level

Thäle C[1], Kiderlen AF[2], Kolodziej H[1]
[1]Institute of Pharmacy – Pharmaceutical Biology, Freie Universität Berlin, Königin -Luise-Straße 2+4, D-14195 Berlin, Germany; [2]Cellular Immunology Unit P22, Robert Koch-Institut, Nordufer 20, D-13353 Berlin, Germany,

Clinical data have shown that EPs® 7630, an aqueous ethanolic extract from roots of *Pelargonium sidoides* DC., is an efficacious treatment of respiratory tract infections such as acute bronchitis. However, the mode of action at the cellular and molecular level is still insufficiently defined. Previous *in vitro* studies with murine bone marrow-derived macrophages (BMMΦ) and *Leishmania* infected murine macrophage-like RAW 264.7 cells applying functional assays and gene expression experiments by RT-PCR (1,2), respectively, have provided evidence for an immunomodulatory activity of EPs® 7630. More recently, *in vitro* studies using BMMΦ infected with *Listeria monocytogenes* also established the immunomodulatory potential of EPs® 7630 at the protein level. Single cell analysis by flow-cytometry (FACS) of non-infected and *L. monocytogenes*-infected BMMΦ demonstrated enhanced production of IL-1α, TNF-α and IL-12 within 6 h of treatment with EPs® 7630 compared to non-treated controls. These observations were confirmed by ELISA-analysis of cell culture supernatants which revealed increased levels of IL-12 and TNF-α in the presence of EPs® 7630. In order to obtain further information on the responsible signal-transduction pathways, changes in the expression pattern of membrane receptors, which are among the first events taking place after cell activation, were evaluated. Thus, it was observed that independent of the infection status BMMΦ treated with EPs® 7630 expressed significantly higher levels of CD40 compared to controls. CD40 is a member of the TNF-α receptor family and transfers activating signals into the cell. Its expression is strictly correlated to the activation status of the cell. In conclusion, these *in vitro* observations on cytokine production and cell surface receptor expression highlight EPs® 7630 as a potent modulator of macrophage activity and provide a rational basis for the beneficial effects of EPs® 7630 in infectious conditions at the molecular level. *Acknowledgement:* Part of this work was kindly supported by Dr. Willmar Schwabe GmbH & Co KG, Karlsruhe, Germany **References:** [1] Kolodziej, H., et al., (2003) Phytomedicine. 10 Suppl 4, 18 – 24. [2] Trun, W. et al., (2006) Phytomedicine 13, 570 – 575.

WS 6-03

Extract of *Pelargonium sidoides* (EPs® 7630) displays anti-infective properties by enhanced phagocytosis and differential modulation of host – bacteria interactions

Conrad A[1], Engels I[1], Frank U[1]
[1]Institute of Environmental Medicine and Hospital Epidemiology, University Medical Center Freiburg, Breisacher Str. 115, 79106 Freiburg, Germany

Background: EPs® 7630 is an extract derived from the roots of *Pelargonium sidoides* DC. Clinical trials have shown that the preparation is effective in the treatment of respiratory tract infections such as acute bronchitis. **Objectives:** *In-vitro* study to assess the impact of EPs® 7630 on human peripheral blood phagocytes (PBP) and on the host-bacteria interaction using A-Streptococci (GAS) as a model. **Methods:** A whole-blood based flow cytometric assay was used to analyze phagocytosis of *C. albicans* and oxidative burst. Intracellular killing of yeast cells was assessed by a microbiological assay. Adhesion of GAS on human HEp-2 and buccal epithelial cells (BEC) was determined by flow cytometry and GAS invasion of HEp-2 cells was analyzed by a penicillin/gentamicin-protection assay. EPs® 7630 was applied at concentrations between 0 and 30 µg/ml. **Results:** EPs® 7630 increased the number of phagocytosing PBP with a maximum effect of 56% at 2 min and led to an increase of burst-active PBP (up to 120% at 4 min). Intracellular killing was enhanced, as demonstrated by a 31% reduction in the number of surviving target organisms after 120 min. Interestingly, EPs® 7630 displayed a differential effect with respect to adhesion of GAS to HEp-2 cells and BEC. While adhesion to HEp-2 cells was inhibited with a maximum effect of 46% at 120 min, adhesion to BEC was increased up to 7-fold. EPs® 7630-pre-treatment of HEp-2 cells or GAS revealed that the preparation targets GAS rather than epithelial cells. In addition, EPs® 7630 significantly reduced GAS invasion of HEp-2 cells at 60, 120, and 180 min. **Conclusions:** The essential anti-infective properties of EPs® 7630 can explain its clinical effect. Thus, the enhanced function of phagocytes represents an important effector mechanism in order to repel pathogens. Furthermore, modulated host-bacteria interaction may prevent from bacterial super- and recurrent infections. Reduced bacterial adhesion to intact epithelia (HEp-2) protects from bacterial colonization and infection/super-infection whereas enhanced attachment of bacteria to decaying BEC may inactivate pathogens by swallowing. Thus the inhibition of GAS invasion of epithelial cells prevents from microorganisms that evade host defences and antibiotic treatment.

WS 6-04

Pelargonium sidoides extract EPs® 7630 in the treatment of respiratory tract infections in children and adults – an overview of recent results

Kamin W
Children's Hospital, University of Mainz, 55101 Mainz, Germany

EPs® 7630 is a liquid herbal drug preparation from the roots of *Pelargonium sidoides* DC. In Germany, EPs®-solution is marketed under the trade name Umckaloabo®. EPs® 7630 shows antiviral, antibacterial and immunomodulatory properties, which account for its therapeutic effect in respiratory tract infections (RTI) demonstrated in patients (pts) suffering from acute bronchitis [1, 2, 3]. It also proved efficacious in a study with 103 pts suffering from an acute maxillary rhinosinusitis, showing a significant decrease in the mean sinusitis severity score of 5.5 vs. 2.5 points in the placebo (PL) group ($p < 0.00001$) [4]. Results of further recently finished trials investigating EPs® 7630 in acute bronchitis are now available. In 2 studies with 200 resp. 220 pts aged 1 – 18 yrs, a significantly reduced total score of the 3 typical symptoms cough, dyspnoea, and pulmonary rales at auscultation was shown for EPs® 7630-solution on day 7 (main outcome measure; PL vs. EPs® 7630: -1.2 vs. -3.4 points resp.- 2.9 vs. -4.4 points; $p < 0.001$). In a further study with 399 pts aged 6 – 18 yrs, EPs® 7630 tablets (3 x 10 mg, 3 x 20 mg or 3 x 30 mg/d vs. PL) significantly improved the Bronchitis Severity Score (BSS) between day 0 and day 7 in the two higher dosages (PL vs. EPs® 7630 (10/20/30): -3.3 vs. -3.6/-4.4/-5.0 points; $p < 0.001$ for the comparisons PL vs. EPs® 7630 (20/30)). A similar designed trial confirmed these results in 405 adults, showing a significant BSS improvement for all 3 dosages (PL vs. EPs® 7630 (10/20/30): -2.7 vs. -4.3/-6.1/-6.3 points; $p < 0.001$ for all comparisons of PL vs. EPs® 7630). The results also underline the good tolerability of EPs® 7630; especially, serious adverse events did not occur. EPs® 7630, therefore, is an efficacious and well-tolerated herbal medicine for the treatment of RTI in children and adults outside the strict indication for an antibiotic therapy. **Ref.:** [1] Wagner, H. et al. (Eds.) (2007) Phytomedicine 14 SVI. [2] Chuchalin, A.G. et al. (2005) Explore 1:437. [3] Matthys, H., Heger, M. (2007) Curr. Med. Res. Opinion 23: 323. [4] Bachert, C. et al. (2006) Focus Alternat. Complement. Ther. 11: 4

WS 7: Echinacea: Update on current research

WS 7-01

Echinacea for preventing and treating the common cold

Linde K[1], Barrett B[2], Woelkart K[3], Bauer R[3], Melchart D[1]
[1]Centre for Complementary Medicine Research, Technische Universität München, Germany; [2]Department of Family Medicine, University of Wisconsin-Madison, USA; [3]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl Franzens-University Graz, Austria

Preparations of the plant *Echinacea* (familiy *Compositae*) are widely used in some European countries and in North America for common colds. Most consumers and physicians are not aware that products available under the term *Echinacea* differ appreciably in their composition, mainly due to the use of variable plant material, extraction methods and addition of other components. Now we assessed whether there is evidence that *Echinacea* preparations are 1) more effective than no treatment; 2) more effective than placebo; 3) similarly effective to other treatments in A) the prevention and B) the treatment of the common cold. Outcomes of interest in prevention trials were: number of individuals with one or more colds, and severity and duration of colds; and in treatment trials: total symptom scores, nasal symptoms and duration of colds. Sixteen trials including a total of 22 comparisons of an Echinacea preparation and a control group met the inclusion criteria. The majority had reasonable to good methodological quality. A variety of different Echinacea preparations were used. None of the three comparisons in the prevention trials showed an effect over placebo. Comparing an Echinacea preparation with placebo as treatment, a significant effect was reported in nine comparisons, a trend in one, and no difference in six. More than one trial was available only for preparations based on the aerial parts from *Echinacea purpurea*. Therefore we concluded that preparations based on aerial parts of *E. purpurea* might be effective for the early treatment of colds in adults but results are not fully consistent. Beneficial effects of other *Echinacea* preparations and for preventative purposes might exist but have not been shown in independently replicated, rigorous randomized trials. [1,2] Again a recent meta-analysis reported that standardized extracts of Echinacea were effective in the prevention of symptoms of the common cold after clinical inoculation, compared with placebo. [3] **References:** [1] Linde K., Barrett B. et al. (2006) The Cochrane Database of Systematic Reviews Issue 1. [2] Gillespie E.L., Coleman C.I. (2006) Conn. Med. 70: 93 – 97. [3] Schoop R., Klein P. et al. (2006) Clin. Ther. 28: 174 – 183.

WS 7-02

Anti-bacterial properties of Echinacea extracts

Sharma M[1], Hudson JB[1], Arnason JT[2]
[1]Department of Pathology & Laboratory Medicine, University of British Columbia, Vancouver; V5Z 1M9, Canada; [2]Department of Biology, University of Ottawa, Ottawa, Canada

Anti-bacterial properties have traditionally been attributed to *Echinacea* extracts, although there seem to be few experimental reports to support these claims. We decided to carry out a comprehensive analysis of several chemically defined *Echinacea* extracts, acquired from commercial sources, for their ability to inactivate (colony forming unit assays) 15 different human pathogenic bacteria. Six extracts were used, representing 3 types of preparation: two *E. angustifolia* root preparations, containing high levels of alkylamides and very low in polysaccharides, two preparations of *E. purpurea* aerial parts, containing high levels of polysaccharides but no alkylamides, and two preparations of mixed *E. purpurea* roots plus aerial parts, with moderate alkylamides and low in polysaccharides. A variety of caffeic acid related compounds were present in all the extracts, but in different concentrations. Three bacteria associated with upper respiratory infections, *Hemophilus influenzae, Streptococcus pyogenes, Legionella pneumophila,* and *Propionibacterium acnes*, associated with skin inflammation, were susceptible to most of these extracts, but particularly to the *E. purpurea* root + aerial preparations (moderate alkylamide and low polysaccharides), which were capable of inactivating more than 4 $\log_{10}$ colony forming units. Optimal activity required the presence of light (applied as a combination of UVA and visible light), suggesting that the active compounds were photosensitizers. The other 11 bacteria, together with the fungi *Candida albicans* and *Trichoderma viridans*, were relatively resistant to all the extracts. These data support the concept that certain *Echinacea* preparations are capable of inactivating selected species of bacteria, particularly those commonly associated with respiratory symptoms, but the active components are not alkylamides or polysaccharides.

WS 7-03

The contribution of alkylamides to the overall effect of Echinacea

Gertsch J[1]
[1]Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland

This presentation examines recent developments in Echinacea research, with a particular focus on N-alkyl amides (alkylamides). These natural products are known to quickly accumulate in blood plasma upon ingestion and can reach nM concentrations. Data indicate that alkylamides may exert potent anti-inflammatory effects in vivo. It is shown, that at least part of these effects are mediated via the cannabinoid type-2 receptor, which is expressed on leukocytes. The amphiphilic alkylamides tend to segregate in aqueous environment and their pharmakodynamics is influenced by the composition of complex mixtures, i.e. other constituents in plant extracts. An example of a superadditive effect on signal transduction is provided with data obtained with the alkylamide-containing tincture EchinaforceTM. Overall, the effects of alkylamides on the innate immune response are summarized and put into context with the use of Echinacea for common cold and upper respiratory infections.

WS 7-04

CYP inhibitory action of Echinacea preparations differs widely, but is it clinically relevant?

Heinrich M[1], Modarai M[1], Kortenkamp A[2]
[1]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy University of London, 29/39 Brunswick Square London WC1N 1AX, United Kingdom; [2]Centre for Toxicology, The School of Pharmacy University of London, 29/39 Brunswick Square London WC1N 1AX, United Kingdom

Widespread use of any (herbal) medical product calls for the investigation of possible interactions with other medicines. While pharmacovigilance allows the continuous monitoring of potential signals, the increasing simultaneous use of diverse medications makes this monitoring difficult to interpret and, of course, does not explain possible mechanism of actions of such interactions. Many of these interactions occur via induction or inhibition of the CYP P450 enzyme system, a superfamily of membrane-bound, heme-thiolate proteins, which are principally responsible for phase 1 oxidative metabolism and detoxification of xenobiotics and endobiotics. The German regulatory authority, for example, requires in vitro assessment of herbal medicinal products on the main cytochrome P450 enzymes involved in drug metabolism [1]. In a fluorometric supersome assay, products containing Echinacea ethanol water extracts were evaluated. These extracts vary widely in their inhibitory activity on CYP3A4 (IC50 values: 12.71 μg/ml -1812 μg/ml) [2]. Therefore, conclusions drawn using one specific extract cannot necessarily be generalized to others, similarly extracted ones derived from the same botanical drug. Individual alkylamides showed moderate inhibitory activity against CYP enzymes, but these effects are – at the doses of Echinaforce® normally used – unlikely to be of clinical concern. Other constituents contribute to the extract's observed in vitro effects and are currently under investigation [3]. These data provide a sound basis for assessing the interaction potential of Echinacea preparations studied. While additional studies on other potential targets may be desirable, overall, the data contribute important information to the safety profile of this drug and indicate that these preparations are likely to be safe if used under a normal therapeutic regimen. Funding: Our studies on herbal medicines and the cytochrome system are partially sponsored by Bioforce, CH and by the European Union (FP6). **References:** [1] BfArM (2004) http://www.bfarm.de/cln_042/nn_424630/DE/Arzneimittel/besTherap/amPflanz/ampflanz-node.html (Last accessed 20.08.2006). [2] Modarai, M. et al. (2007) J. Pharm. Pharmacol. 59: 567 – 573. [3] Modarai, M., et al. (2007) GA congress, poster abstract

Poster Presentations

1. Anti-inflammatory and immunomodulatory active natural products

P 001

Effects of Ginseng polysaccharides and polyporus polysaccharides on TNF-α and IFN-γ-production by enteric mucosal lymphocytes of rats with collagen induced arthritis

Zhang W[1], Zhao H[1], Lu C[1], Liu Z[1], Yang D[2], Chen S[2], Jiang M[1], Lu A[1,2]
[1]China Academy of Chinese Medical Sciences, Beijing 100700, China; [2]State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Shenzhen, Guangdong 518057, China

The effects of Ginseng and Polyporus polysaccharides (from *Ginseng* and *Polyporus umbellatus* (Pers) Fries) [1,2] on the TNF-α and IFN-γ-production by enteric mucosal lymphocytes in collagen induced arthritis (CIA) rats were investigated. The Peyer's patches lymphocytes (PPL), intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) of SD rats were isolated, and checked with flow cytometry after co-culturing with Ginseng and Polyporus polysaccharides at different dosages. ELISA was used to measure the levels of TNF-α and IFN-γ in the supernatants. The arthritic incidence of CIA in SD rats was 17/20 at the week 4 after the immunization. More CD4 cells and more CD8 cells were detected in PPL, IEL and LPL in CIA models. Gingseng polysaccharides at 0.15 g/L, 0.30 g/L and 0.60 g/L can significantly lower the secretion of TNF-α in PPL of CIA rats. At 0.15 g/L and 0.30 g/L, it can lower the secretion of TNF-α in IEL of CIA rats, and it can lower the secretion of TNF-α in LPL of CIA rats. Polyporus polysaccharides at 1.0 g/L and 2.0 g/L can significantly lower the secretion of TNF-α in PPL of CIA rat and the secretion of TNF-α in IEL of CIA rats. At 1.0 g/L and 2.0 g/L, it can lower the secretion of TNF-α in LPL of CIA rats. Ginseng polysaccharides at 0.15 g/L, 0.30 g/L and 0.60 g/L can significantly lower the secretion of IFN-γin IEL and LPL of CIA rats, and increase the secretion of IFN-γin PPL of CIA rats. Polyporus polysaccharides at 0.5 g/L, 1.0 g/L and 2.0 g/L can significantly lower the secretion of IFN-γin PPL, IEL and LPL of CIA rat, and increase the secretion of IFN-γin PPL of CIA rats. Obvious dose dependent responses in PPL were found in ginseng and polyporus polysaccharides. The results indicate that Ginseng and Polyporus polysaccharides could affect the enteric mucosal immune response in CIA rats. *Acknowledgements:* Supported in part by the projects of National Natural Science Foundation of China (No 30171133) **References:** [1] Ma, L., et al. (1995) Zhongguo Zhong Xi Yi Jie He Za Zhi. 15: 411 – 3. [2] Huang, D.N., et al. (2004) Zhong Xi Yi Jie He Xue Bao. 2: 350 – 2.

P 002

The effectiveness of AKL1, a herbal treatment for asthma: a randomised placebo controlled double-blinded cross-over trial

Thomas M[1], Sheran J[1], Fonseca S[1], Lee A[1], Larkins N[2]
[1]University of Aberdeen, Aberdeen, Aberdeenshire – UK; [2]AKL Technologies, 60 – Westminster Gardens, Marsham Street, London – UK

Aims: The purpose of this study was to provide scientific evidence regarding the efficacy and safety of AKL 1, an herbal mixture with anecdotal reports of effectiveness in asthma, as an 'add-on' therapy for adult patients whose asthma remains uncontrolled on standard medication. **Methods:** 32 asthmatics (7 male, median (range) age 40.5 (22 – 73) yrs., median (range) FEV1 (forced expiratory volume over 1 s; %) predicted 87.5 (33 – 93)%, median (range) daily ICS (inhaled corticosteroid) dose 800 (0 – 4000) mcg beclomethasone) completed a 36 week randomized double blinded placebo controlled cross-over trial consisting of; four week baseline, twelve-week treatment with AKL or identical placebo, eight week washout and further twelve-week cross-over treatment period. The change occurring over treatment periods was observed for lung function, Asthma Control Questionnaire (ACQ), Asthma Quality of Life Questionnaire (AQLQ), Leicester Cough Questionnaire (LCQ) scores. The mean (95% Confidence Interval) individual patient changes between active minus placebo periods was calculated. **Results:** Trends to clinical improvements favoring active treatment were consistently seen in the patient-centered outcomes: ACQ mean difference (active – placebo)=-0.35 (-0.78 to 0.07, p = 0.10, AQLQ difference 0.42 (-0.08 to 0.93, p = 0.09), LCQ difference 0.49, (-0.12 to 1.16, p = 0.15). A change in ACQ and AQLQ score of 0.5 signified clinically relevant changes in asthma control or health status. On the ACQ, 28% were unchanged, 22% better on placebo and 50% better on AKL 1. On the AQLQ 29% had no change, 29% were better on placebo and 42% better on AKL 1. No significant differences in lung function were found (FEV1: (active – placebo) mean (95% CI) difference = 0.01 (-0.12 to 0.14)L, p = 0.9. PEF: -3 (-22 to 28)L/min, p = 0.9). Nine exacerbations occurred during placebo treatment and five whilst on AKL. No significant treatment associated adverse events were noted. **Conclusions:** AKL1 treatment was well tolerated. It is now well established that asthma symptoms correlate poorly with the level of airway obstruction as determined by the FEV1 and PEF (peak expiry volume). Following treatment, subjective improvement in asthma symptoms may occur without improvement in the level of airway obstruction. AKL1 provided consistent trends to symptom and quality of life improvements. When taken these were taken together a statistical significance with a 99.9% of certainty was shown. While these results support recommendations to measure airway obstruction objectively they additionally point to a need for further investigational studies with AKL 1.

P 003

Inhibitory effect of an Indian medicinal plant on pro-inflammatory cytokines and NO production in cell lines

Kanwar AS, Bhutani KK
Department of Natural Products, National Institute of Pharmaceutical Education & Research, S.A.S. Nagar (Mohali), Punjab – 160 062, India

Phyllanthus amarus Schumach. & Thonn. (family *Euphorbiaceae*), is a small herb used in Indian traditional systems of medicine for painful disorders related to inflammatory diseases**.** Scientists have reported effective activity of *Phyllanthus* species in inhibiting both neurogenic and inflammatory components of pain response caused by formalin [1]. Inflammatory diseases represent a significant proportion of illnesses. The pro-inflammatory cytokines and the reactive free radical nitric oxide (NO) have been reported to be involved in the development of inflammatory diseases [2]. This led us to investigate the hexane, methanolic and aqueous various extracts of the whole plant extracts on the production (pg/ml) of interleukin 1β (IL-1β), interleukin 2 (IL- 2), tumor necrosis factor α (TNF- α) and on the production (μM) of nitric oxide (NO). Extracts (100 μg/ml) of *Phyllanthus a.* have shown significant ($P < 0.001$) inhibitory effects on different pro-inflammatory cytokines, interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α) and nitric oxide (NO) in mouse macrophage cells, RAW 264.7 stimulated by lipopolysaccharides (LPS). An inhibition ($P < 0.001$) was also found in the production of interleukin 2 (IL- 2) in EL 4 lymphoma cells stimulated with 4 μg/ml of Concavalin A (Con-A) with all the extracts. Our finding suggests that both aqueous and organic solvent extracts of the herb may have therapeutic potential in the control of inflammatory disorders. **References:** [1] Filho, V. C. et al. (1996) J. Pharm. Pharmacol. 48: 1231 – 1236. [2] Freeman, B. D., Natanson, C. (2000) Expert. Opin. Investig. Drugs 9: 1651 – 1663.

P 004

Acetyl-boswellic acids inhibit NFκB activation and TNF-α release by monocytes. Rationale for the treatment of chronic inflammatory diseases

Syrovets T, Estrada AC, Laumonnier Y, Büchele B, Simmet T
Institute of Pharmacology of Natural Products & Clinical Pharmacology, University of Ulm, D-89081 Ulm, Germany

In traditional Ayurvedic medicine extracts from the oleogum resin from *Boswellia serrata* Roxb. have been used for the treatment of various chronic inflammatory diseases. Such extracts have been successfully employed in clinical pilot studies for the treatment of ulcerative colitis and rheumatoid arthritis. We have isolated and characterized various acetyl-boswellic acids (ABA), which constitute the active principle of *Boswellia* extracts. Proinflammatory cytokines such as tumor necrosis factor (TNF) α are prevalent at the sites of chronic inflammation, where they are critically involved in the chronification process. Specifically TNF-α has been identified as a highly relevant pharmacotherapeutic target. The expression of cytokines including TNF-α is tightly regulated by transcription factors including NF-κB. Here we show that in endotoxin (LPS)-activated human monocytes ABA inhibit the TNF-α expression through inhibition of the NF-κB signaling. The activity of other transcription factors remained unaffected implying specificity. ABA had no effect on the binding of NF-κB proteins to DNA binding sites as analyzed by surface plasmon resonance. Instead, ABA inhibited the LPS-induced degradation of the NF-κB inhibitor and the translocation of NF-κB to the nucleus. Furthermore, ABA inhibited the IκB kinases (IKK), which are crucial for the activation of NF-κB. Thus, via their direct inhibitory effects on IKK, ABA exert inhibition of NF-κB and subsequent the down-regulation of TNF-α expression in LPS-activated human monocytes. These findings provide a novel molecular basis for the anti-inflammatory properties ascribed to drugs containing boswellic acids and suggest that ABA might be used for treatment of chronic inflammation.

P 005

Anti-inflammatory activity of an aqueous extract of *Grewia tiliaefolia* leaves

Juvekar AR, Sakat SS, Shah AS, Wakade AS
Department of Pharmaceutical Sciences & Technology, University Institute of Chemical Technology, Matunga, Mumbai 400019, India

The leaves of *Grewia tiliaefolia* vahl. (Tiliaceae) have been used in vitiated conditions of pitta, kapha and burning sensation. It is one of such plants used in skin diseases, inflammatory bowel diseases, diarrhoea and pruritis. The aim of the present study was to evaluate the anti-inflammatory activity of an aqueous leaf extract of *Grewia tiliaefolia* (GT) in rodents using carrageenan, histamine and serotonin induced paw edema, prostaglandin inhibitory activity by castor oil induced diarrhoea, acetic acid induced capillary permeability, cotton pellet granuloma and sodium CMC induced leukocytes emigration tests. Results showed a dose dependent anti-inflammatory activity in carrageenan, histamine and serotonin induced paw edema at the dose of 250 and 500 mg/kg. It also showed dose dependent prostaglandin inhibitory activity by preventing castor oil induced diarrhoea. The extract also exhibited dose dependent inhibition of vascular permeability induced by acetic acid; however it did not show any significant effect on growth of granuloma tissue. It also significantly reduced leukocyte counts at the dose of 500 mg/kg. Phytochemical studies indicated the presence of tannins, phenolics and flavonoids. These findings suggest that the leaves of *Grewia tiliaefolia* possess anti-inflammatory activity in acute and subacute phase of inflammation, which may result from inhibition of prostaglandin synthesis, inhibition of inflammatory mediators, inhibition of increased vascular permeability and inhibition of leukocyte emigration into inflamed tissues.

P 006

Immunomodulatory activity of methanolic extract of *Nyctanthus arbortristis* leaves

Juvekar AR, Nachankar RS, Wakade AS, Shah AS
Department of Pharmaceutical Sciences & Technology, University Institute of Chemical Technology, Matunga, Mumbai 400019, India

The leaves of *Nyctanthus arbortristis* L. (NA) are reported to be useful in rheumatism, menorrhagia, dysentry, asthma and allergic reactions. They were proven experimentally to have antibacterial, antiinflammatory and anthelmintic activity. The aim of the present study was to investigate the effect of methanolic leaves extraxt (MNA) on immune cells by using *in-vitro* and *in-vivo* models. The MNA was examined for the ability to induce secretory and cellular responses in murine peritoneal macrophages. Macrophages treated with the extract exhibited increased acid phosphatase and myeloperoxidase activity as well as significant increase in the production of nitric oxide (NO), hydrogen peroxide (H_2O_2), and superoxide (O_2^-). Hence, *in vivo* studies were carried out to confirm the immunomodulatory potential of MNA in mice. Oral administration of MNA at doses of 250 and 500 mg/kg, significantly increased in total leukocyte count and in weight of spleen indicating an uplift of innate immunity. It has significantly increased carbon clearance index and ovalbumin induced delayed type hypersensitivity (DTH) reactions. It also produced a significant increase in serum globulin content and specific antibody titer against ovalbumin. Treatment with MNA increased the number of bone marrow cells positive for nonspecific esterase and peroxidase activity. Leaves of NA showed the presence of carbohydrates and glycosides (iridoids glucosides and flavanol glycosides), which suggest the major role of glycosides for the immunostimulant activity. In conclusion MNA has shown to stimulate both innate and adaptive immune response either through stimulation of macrophages or through stimulating the release of factors that are involved in proliferation of bone marrow cells.

P 007

Immune modulation by Wormwood (*Artemisia absinthium*) – results of a double blind, placebo controlled trial on Crohn's disease patients

Omer B[1], Krebs S[2], Omer LM[3], Noor TO[4]
[1]Department of Internal Medicine, Yale University School of Medicine, 06519 New Haven, CT, USA; [2]Department of Internal Medicine I, Faculty of Medicine, University of Saarland, Kirrbergstr. 66424 Homburg/Saar, Germany, [3]Clinic Zaehringerstr. 12, 79618 Rheinfelden, Germany; [4]Medical Faculty, University of Freiburg, 79111 Freiburg Germany,

Wormwood extract (*Artemisia absinthium* L.) was found to inhibit tumor necrosis factor α (TNF-α) production [1]. In a multi centre double-blind study, forty patients suffering from Crohn's disease (CD) were given an herbal blend of wormwood (Seda Crohn 3 x 500 mg/day) or placebo for 6 weeks under double blind experimental conditions. Minimum score of 200 on Crohn's Disease Activity Index (CDAI) was required at baseline. The patients were evaluated with the help of CDAI, an Inflammatory Bowel Disease Questionnaire (IBDQ), the 21-item Hamilton Depression Scale (HAMD) and an 8-item Visual Analogue Scale (VA-Scale) at two-week intervals. All concomitant anti-Crohn medications were maintained at the same dose level. After 6 weeks there was almost complete remission of symptoms in 13 (65%) patients in the wormwood group as compared to only 3 (15%) in the placebo group. Average CDAI-score fell from 275 ± 15 to below 175 ± 12 (in placebo group from 262 ± 11 to 230 ± 14). In IBDQ, HAMD, and VAS scores there was a similar significant difference between the two treatment groups at 6th evaluation week ($p \geq 0.05$). The results strongly suggest that wormwood has a remarkable immune modulating effect on Crohn's disease. The improvements in HAMD scores indicate that wormwood also has an effect on mood and quality of life of CD patients, which is not achieved by any other standard medication. The use of wormwood in other TNF-α targeting diseases such as rheumatoide

arthritis is yet to be investigated. **References:** [1] Lee H.G., et al. (2004) Ann. N. Y. Acad. Sci.; 1030: 555 – 568.

P 008

The differential effect of *Eriobotrya japonica* hydrophilic extract on cytokines production and modulation

Matalka KZ, Ali D, Qadan F
Faculty of Pharmacy and Medical Technology, University of Petra, Amman, Jordan

Stimulating or modulating the release of cytokines by immunomodulators or immunostimulating agents is an attractive mode for treating several diseases such as viral infections. For instance, patients with viral infections are in need of increasing or inducing T helper 1 (Th1) or proinflammatory cytokines, which ultimately activate T cytotoxic and Natural killer lymphocytes to kill virally infected cells. Of these agents, we found that the water extract (hydrophilic extract) from the leaves of *Eriobotrya japonica* (EJHE) can induce and modulate cytokines in a dose-dependent manner. Twenty-four hour exposure of increasing concentrations of EJHE enhanced significantly ($p < 0.001$) the production of IL-12 p70, IFN-γ and TNF-α from PHA+LPS-stimulated whole blood. However, the production of IL-12 p70, IFN-γ and TNF-α was reduced at high EJHE concentrations (10 – 100 μg/ml). No significant changes in the production of IL-10 were seen. In addition, EJHE reversed significantly the inhibitory effect of hydrocortisone on the cytokines production from PHAS+LPS stimulated whole blood. Without PHA and LPS, EJHE was found to induce IFN-γ, IL-12 p70, TNF-α, and IL-10 from whole blood culture in a concentration dependent manner. The maximum induction of IFN-γ, IL-12 p70, and TNF-α by EJHE was between 1 – 10 μg/ml. On the other hand, IL-10 induction kept increasing even at the highest concentration used 100 μg/ml of EJHE. Furthermore, intra-peritoneal injection of EJHE into mice increased significantly serum cytokines level mainly at 1 and 100 μg/ml. Two-hour post i.p. injection, EJHE increased serum IFN-γ, TNF-α, and IL-10 to ~750, 1000, and 250 pg/ml, respectively. However, 24 h post i.p. injection, the levels of TNF-α, and IL-10 were similar to basal levels but IFN-γ levels were 200 pg/ml. These results indicate that EJHE induces proinflammatory and Th1 cytokines in a concentration dependent manner and the effect of this induction should be studied further in viral models to check the efficacy of such cytokine induction.

P 009

Chemical constituents and anti-inflammatory activity of the aerial parts of *Calliandra haematocephala* Hassk

Abou Zeid AHS[1], Hifnawy MS[2], Sleem AA[3], Mohamed RS[1]
[1]Pharmacognosy Dept., [3]Pharmacology Dept., National Research Centre, El-Tahrir St., Dokki (12622), Cairo, Egypt; [2]Pharmacognosy Dept., Faculty of Pharmacy, Cairo Univ., Cairo, Egypt

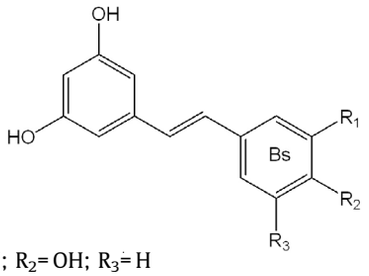
The carbohydrate content *of Calliandra haematocephala* was determined as glucose by the phenol-sulphuric acid method. It showed that the percentage of total carbohydrates and free sugars was 5.4% and 2.3% respectively. HPLC analysis of low molecular weight carbohydrates extracted by 80% ethanol [1] revealed the presence of ten sugars representing 99.48% of total sugars. The major sugars were ribose (19.12%), galacturonic acid (10.78%), glucose (8.15%), arabinose (7.72%), and fructose (7.64%). The polysaccharide hydrolysate of *C. haematocephala* extracted by cold and hot water revealed the identification of six and seven sugars representing 83.83% and 82.56% of total sugars, respectively. The major sugars of the cold extract were rhamnose (53.89%), ribose (11.49%), and mannose (10.24%), while those of the hot extract were ribose (27.17%), rhamnose (14.56%), glucuronic acid (13.71%), mannose (11.47%), and glucose (6.44%). The protein content of *C. haematocephala* was found to be 14.25%, as determined by Micro-Kjeldal method [2]. Total amino acids were determined by an amino acid analyzer, which allowed the identification of 17 amino acids. The major ones were glutamic acid (38.80%), aspartic acid (9.72%), isoleucine (6.1%) and tyrosine (5.37%). Sulphosalicylic acid hydrolysis for determination of free amino acids revealed the presence of 17 amino acids, of which glutamic acid (24.10%), aspartic acid (10.71%), isoleucine (7.91%) and lysine (6.12%) were most dominant. The anti-inflammatory activity of the total ethanol and successive extracts of the plant at two dose levels was determined by the rat paw oedema assay [3]. Results were statistically analyzed. The highest activity was found at 100 mg/kg b.wt. of the total ethanol extract (87.80% potency) followed by 100 mg/kg b.wt. of chloroform extract (81.81% potency) in comparison with indomethacin (100% potency). The rest of the extracts showed moderate dose dependant activities. **References:** [1] Gertz, C.H. (1990). HPLC tips and tricks, Great Britain at the add press, Oxford p. 608. [2] Pearson, D. (1970); "The Chemical Analysis of Food" 6th ed. Churchill Ltd., London. [3] Winter, G.A. et al. (1962) Proc. Soc. Exp. Biol. Med. III, 1544.

P 010

Phenolic constituents from *Yucca schidigera* inhibit COX-1, COX-2 and 5-LOX *in vitro*

Wenzig EM[1], Bauer R[1], Stochmal A[2], Oleszek W[2]
[1]Institute of Pharmaceutical Sciences, Dept. Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria; [2]Institute of Soil Science and Plant Cultivation, State Research Institute, ul. Czartoryskich 8, 24 – 100 Pulawy, Poland

Yucca schidigera Roezl (Agavaceae) is a plant native to the South-Western United States and Mexico. It has been used for centuries in traditional medicine against a variety of inflammatory disorders like headaches, gonorrhoea, arthritis and rheumatism. Apart from a high level of saponins, the plant contains considerable amounts of phenolics which occur in the stem bark [1]. In order to investigate the anti-inflammatory potential of this plant, we have tested a yucca bark extract concentrated of phenolics, as well as the major phenolic constituents of this extract, resveratrol (**1**), trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene (**2**) and yuccaols A (**3**), B (**5**) and C (**4**), for their inhibitory activity against cyclooxygenase (COX)-1, COX-2 and 5-lipoxygenase (LOX) [2,3]. The extract significantly inhibited all three enzymes (IC_{50} COX-1 18.48 μg/ml, COX-2 65.95 μg/ml and 5-LOX 42.22 μg/ml). The pure compounds also inhibited COX-1, COX-2 and 5-LOX to a different extent. In general, they were more active against COX-1 than against COX-2 and 5-LOX, and the inhibitory potential seems to be strongly influenced by the substitution of ring B in the stilbenic moiety.



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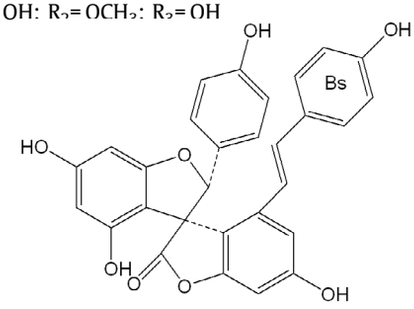
2 R_1 = OH; R_2 = OCH_3; R_3 = OH



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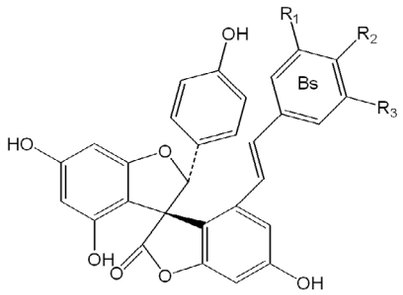


4 R_1= OH; R_2= OCH_3; R_3= OH
5 R_1= H; R_2= OH; R_3= H

References: [1] Oleszek, W. et al. (2001) J. Agric. Food Chem. 49: 747 – 752. [2] Fiebich, B.L. et al. (2005) Planta Med. 71: 12 – 19. [3] Adams, M. et al. (2004) Planta Med. 70: 904 – 908.

P 011

Immunomodulatory effects of β-sitosterol on human Jurkat T cells

Aherne SA[1], Daly T[1], O'Connor T[1], O'Brien NM[1]
[1]Department of Food and Nutritional Sciences, University College Cork, Republic of Ireland

Plant sterols are specific phytochemicals that resemble cholesterol in structure but are found exclusively in plants [1]. The most common phytosterols in the human diet are β-sitosterol, campesterol and stigmasterol. Although plant sterols have been described as immunomodulatory compounds [2] there are a limited number of studies reporting the effects of these compounds on immune modulation. In the present study we investigated the effects of β-sitosterol on cytokine release in mitogen-treated Jurkat cells. Human Jurkat T cells (2×10^5/ml) were supplemented with increasing concentrations of β-sitosterol (10 – 100 μM) in the presence or absence of the mitogen phorbol-12-myristate-13-acetate (10 ng/ml) plus ionomycin (350 ng/ml) (PMA/IoM) for 24 h. Cell viability and growth were determined by the MTT assay. IL-2 release from the cells was measured by enzyme-linked immunosorbent assay (ELISA). Supplementation with the phytosterol alone did not induce IL-2 release from the cells. The presence of the mitogen PMA/IoM increased IL-2 release 100-fold (648.5 pg/ml) compared with untreated cells (6.9pg/ml). β-sitosterol significantly decreased IL-2 release from mitogen-stimulated Jurkat cells in a dose-dependent manner. In the presence of 10 μM β-sitosterol IL-2 release was only slightly reduced whereas at a higher concentration (50 μM) IL-2 release was reduced by 76% ($P< 0.01$). At a concentration of 100 μM, β-sitosterol significantly inhibited the release of IL-2 from PMA-IoM treated Jurkat cells by 50% ($P< 0.01$). These findings suggest that β-sitosterol exhibits immunomodulatory effects in human Jurkat T cells and that further research is warranted on the role of phytosterols in modulation of the immune system. *Acknowledgement:* Department of Agriculture and Food under the Food Institutional Research Measure (FIRM) as administered by the National Development Plan 2000 – 2006. **References:** [1] Bradford, P.G., Awad, A.B. (2007) Mol. Nutr. Food Res. 51: 161 – 170. [2] Bouic, P.J. (2001) The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years. Curr Opin Clin Nutr Metab Care 4: 471 – 475.

P 012

Cytotoxic and antiviral activities of *Magnolia grandiflora* L. leaves growing in Egypt

Mohamed SM[1], Ibrahim NA[2], Ali MA[3]
[1]Medicinal and Aromatic Plants, [2]Pharmacognosy, [3]Water Pollution, Department, National Research Center, Tahrir St. Dokki, 12311, Cairo, Egypt

Magnolia grandiflora L. (Magnoliaceae), is used in traditional medicine for treatment of fever, diarrhea, rheuma and arthritis. A number of biologically active alkaloids, sesquiterpenes, phenolics and neolignans have been isolated from this species. Bioassay guided fractionation of a methanol extract of *Magnolia grandiflora* L. leaves led to isolation and characterization of four aporphine alkaloids, magnoflorine, lanuginosine, liriodenine and anonaine. The structure of these compounds was determined on the basis of spectroscopic studies. The Cytotoxicity of the pure compounds magnoflorine and lanuginosine were determined in tumor cell lines, Hela (cervix tumor cell line), HEPG2 (hepatocellular carcinoma cell line) and U251 (brain tumor cell line). The cell viability tests were performed as described previously (1). Magnoflorine and lanuginosine displayed a significant activity in the *in vitro* cytotoxic assay against (HEPG2) cancer cell line. Magnoflorine was more cytotoxic (IC_{50} 0.4 μg/ml) than lanuginosine (IC_{50} 2.5 μg/ml) against HEPG2 in comparison with the standard doxorubacin (IC_{50} 0.27 μg/ml). In addition, magnoflorine and lanuginosine exhibited cytotoxicity against brain tumor cell line U251 with IC_{50} 7 μg/ml and 4 μg/ml respectively. The two compounds were found to have no cytotoxic activity against Hela cancer cells. On the other hand, the antiviral activity of the methanol extract was investigated against two viruses: Herpes simplex virus type -1 (HSV-1) and Poliovirus type-1 (PV-1) using Plaque reduction assay (2). The activity was calculated by percentage of viral plaque inhibition at non-cytotoxic concentrations of the extract. Methanol extract showed high antiviral activity against herpes simplex virus (HSV-1) (83.7%) inhibition at 2.2 μg/ml whereas the extract exhibited a moderate antiviral activity against Poliovirus type-1 (47%) inhibition at 1.1 μg/ml. In conclusion, magnoflorine and lanuginosine might be valuable antitumor promoting agents and the methanol extract exhibited potent anti-viral activity against HSV-1 virus that can be exploited for development of an alternative remedy for HSV-1 infection. **References:** [1] Skehan, P.et al., (1990) J. Nat. Cancer Inst., 82 (13), 1107 – 1112**.** [2] Bermejo, P. et al., (2002) Planta Med. 106 – 110.

P 013

Anti-inflammatory activity of *Aegopodium podagraria* L

Prior RM[1,2], Lundgaard NH[1,2], Light ME[2], Stafford GI[2], van Staden J[2], Jäger AK[1]
[1]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, 2 Universitetsparken, 2100 Copenhagen O, Denmark; [2]Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

In the middle ages *A. podagraria* was used for the treatment of podagra by Friars [1]. Present-day cases of anecdotal evidence of use of *A. podagraria* (Ground elder) for treatment of gout and inflammatory diseases prompted us to investigate the anti-inflammatory activity of the species. Water, methanol, acetone, dichloromethane, ethyl acetate and hexane extracts were prepared from *A. podagraria* roots, leaves, stems and flowers. The extracts were screened *in vitro* for cyclooxygenase-1 (COX-1) inhibitory activity [2]. Flowers of *A. podagraria* extracted with hexane had the highest percentage COX-1 inhibitory activity. All extracts of all plant parts, except aqueous extracts which were inactive, exhibited activity in the assay. For bioassay-guided isolation, dried, powdered root material (20 g) was defatted with 200 ml petroleum ether. The material was then extracted four times with 200 ml ethyl acetate. The extract was filtered and taken to dryness and the residue fractionated on a VLC column (25 g silica gel 60), eluted with mixtures of hexane:

ethyl acetate. The fractions were tested in the COX-1 assay. The active fraction eluting from the VLC at hexane 80 – 70%, was applied to a preparative TLC plate, and developed in hexane: ethyl acetate (1:1). Fractions were scraped off the plate, eluted off the silica gel and tested in the COX-1 assay. The active fraction (R_f 0.7 – 0.86) was investigated by ^{1}H-NMR and ^{13}C-NMR. By comparison with literature data [3,4] the compound was identified as falcarindiol. The IC_{50}-value of falcarindiol was 0.3 μM in the COX-1 assay. A quantitative determination of the seasonal variation in the content of falcarindiol in different plant parts was carried out by HPLC-analysis. The flowers from *A. podagraria* collected in June 2006 had the highest concentration of falcarindiol (88 mg/g plant material). **References:** [1] Olesen, A., 2000. Lægeplanter fra danske urtehaver. Frydenlund, København, p. 134. [2] Jäger, A.K. et al. (1996) J. Ethnopharm. 52, 95 – 100. [3] Lechner, D. et al. (2004) Phytochemistry 65, 331 – 335. [4] Crepa, A., Hofmann, T. (2003) J. Agric. Food Chem. 51, 3865 – 3873.

P 014

Pectin of common pondweed *Potamogeton natans* L. prevents endotoxin shock in mice

Popov S, Popova G, Paderin N, Ovodova R
Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya str., 167982 Syktyvkar, Russia

The pectic polysaccharide named potamogetonan PN has been earlier obtained from the *Potamogeton natans* L. (Potamogetanaceae) and has been found to possess strong preventative anti-inflammatory activity at oral administration. It can be suggested that altered cytokine balance may be involved in the preventative effect of PN. To test this idea, an endotoxin-induced shock model was used. Mice orally received drug vehicle (saline) or PN (50 mg/kg). They were challenged with 25 mg/kg of LPS (from *Yersinia enterocolitica*) administered i. p. 24 h later. For lethality studies, survival was recorded at 6, 12, 18, 24, 48, and 72 h after LPS injection. Concentration of cytokines (ELISA) and a number of leukocytes were measured in the lavage from peritoneal cavity 1.5 h after LPS injection. PN was found to improve survival of mice subjected to a lethal dose of LPS (64% *vs* 7% in control at 72 h). In the absence of PN, lethal LPS challenge resulted in an early production of proinflammatory TNF-alfa and IL-1beta cytokines and anti-inflammatory IL-10, all occurring at 1.5 h. Oral pre-treatment with a dose of PN that prevented death attenuated the TNF-alfa and IL-1beta bursts and increased the IL-10 concentration. PN was found to diminish the amounts of neutrophils migrating to the peritoneal cavity after LPS injection. Decreased accumulation of neutrophils is in agreement with the shift toward an anti-inflammatory cytokine profile by potamogetonan. Thus, potamogetonan PN was found to prevent endotoxin-induced shock in mice and the effect of PN on cytokine responses is suggested to mediate improved survival in this model.

P 015

Preventative anti-inflammatory effect of pectic galacturonans after oral administration

Popov S, Popova G, Golovchenko V, Ovodova R
Institute of Physiology, Komi Science Centre, Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya str., 167982 Syktyvkar, Russia

Pectic substances are well known to be the major structural components of plant cell walls and to possess anti-inflammatory activity. The segments of alfa-1,4-D-galacturonan are well known to make up the obligatory backbone of all pectins and to inhibit leukocyte adhesion *in vitro*. The aim of the study is to elucidate an anti-inflammatory effect of pectic galacturonans after oral administration. Galacturonans deprived of side sugar chains were obtained by partial acidic hydrolysis (2 M TFA, 100 °C, 5 h) of pectins from marsh cinquefoil *Comarum palustre* (comaruman), siberian tea *Bergenia crassifolia* (bergenan), duckweed *Lemna minor* (lemnan), seagrass *Zostera marina* (zosteran) and commercial citrus pectin. The glycuronic acid content was determined using interaction with 3,5-dimethyl phenol in the presence of conc. sulphuric acid (calibration curve was obtained for D-galacturonic acid). The mol. weights of polysaccharides were analyzed by HPLC. The fractions with Mw > 300, 100 – 300 and 50 – 100 kDa were obtained from galacturonans using membrane ultrafiltration. Anti-inflammatory capacity of galacturonans obtained was assessed in the carrageenan paw edema test in mice. Positive control and reference groups received indomethacin and apple pectin, respectively. The resulting galacturonans consist of residues of galacturonic acid (approximately 98%) and rhamnose (traces, ~0.5%); the protein impurities are absent. Galacturonans were determined to present about one third of the macromolecule, $[alfa]_D^{20}$ of galacturonans (+226 – +278) were higher compared with those of the parent pectins (+113 – +198). The parent pectins, except comaruman, failed to possess an anti-inflammatory effect. All galacturonans with Mw > 300 were found to reduce inflammatory reaction. The maximal effect of galacturonans was observed at 1 h after carrageenan injection (*ca.* 60% reduction of footpad swelling) and was comparable with that of indomethacin. The delayed edema (5 h) was less affected by the pre-administration of galacturonans (*ca.* 33% reduction). Galacturonans with Mw less than 300 kDa failed to exhibit anti-inflammatory activity. Thus, the galacturonans with Mw > 300 kDa were shown to possess an anti-inflammatory activity after oral administration. An elimination of side sugar chains of the pectic macromolecule appeared to be useful in development of an anti-inflammatory remedy.

P 016

Interaction of dicaffeoylquinic esters with reactive nitrogen species and cytokine release

Giner RM, Olmos A, Máñez S
Departament de Farmacologia, Facultat de Farmacia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain

Protein tyrosine nitration is clearly established under disease conditions [1]. It is mediated by reactive nitrogen species (RNS) such as peroxynitrite and nitrite, formed as secondary products of NO metabolism in presence of oxidants including superoxide and hydrogen peroxide (H_2O_2). Provided that the content of some naturally occurring phenolics, such as hydroxycinnamates, in medicinal plants and dietary supplements is actually high, and taking into account the interaction with RNS [2,3], these principles have become increasingly important. The present communication reports further investigations of two phenolics, isolated from *Phagnalon rupestre* (Asteraceae) [4]: 3,5-dicaffeoylquinic acid (DCA) and its methyl ester (DCE), on a myoglobin-catalyzed nitration process by nitrite/hydrogen peroxide using a cell-free system [5]. Both compounds exerted a weak inhibition on the nitration of free tyrosine while the reference epigallocatechin gallate (EGCG) exhibited an IC_{50} value of 40 μM. Additionally, in order to achieve the influence into the genesis of NO production, we examined their effect on inducible nitric oxide synthase (NOS-2) expression and nitrite levels as well as on interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) production in cultured macrophages stimulated with lipopolysaccharide (LPS) [5]. DCA and EGCG decreased the nitrite levels as well as the expression of NOS-2, while DCE showed no effect. DCE and EGCG inhibited IL-1β production by 54% and 57%, respectively, and moderately reduced the levels of TNF-α while DCA only produced a slight decrease in the level of IL-1β. The moderate inhibition of IL-1β production by DCE was scarcely detectable at the RNA message level. These results indicate that these principles are able to modulate NOS-2 activation in part via reduction of cytokine release. In contrast, they seem to be poorly efficient on peroxide-mediated tyrosine nitration. *Acknowledgements:* This work was supported by the Spanish Ministry of Science and Technology (SAF 2002 – 00723) and by the Generalitat Valenciana (Project GV 353/06). **References:** [1] Radi, R. et al. (2004) PNAS 101: 4003. [2] Olmos, A. et al. (2005) Nitric Oxide 12: 54. [3] Olmos, A. et al. (2007) Planta Med. 73: 20. [4] Góngora, L. et

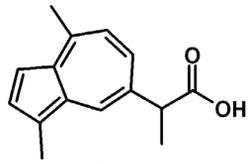
al. (2002) Planta Med. 68: 561. [5] Olmos, A. et al. (2007) Eur. J. Pharm. Sci. 30: 220.

P 017

Synthesis of derivates of chamazulene carboxylic acid as potential inhibitors of inflammation via interaction with CB receptors

Oehler C, Imming P
Institut für Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Wolfgang-Langenbeck-Straße 4, 06120 Halle (Saale), Germany

The natural profen chamazulene carboxylic acid (CCA) was shown to be an antiinflammatory substance [1]. CCA (**1**) is isolated from chamomile or yarrow. It is a degradation product of different sesquiterpene lactones such as matricin. In artificial gastric fluid half of the amount of matricin is converted to CCA [1]. Therefore matricin can be applied as a prodrug of **1**.



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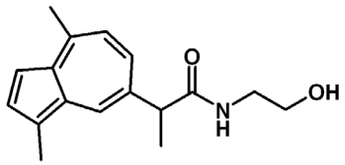

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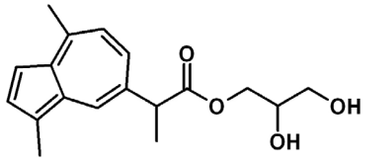

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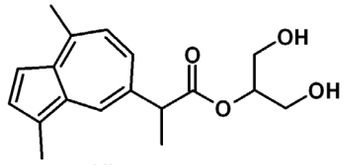

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In the process of inflammation not only the cyclooxygenase enzymes COX 1 and COX 2 are involved but also other systems such as the endocannabinoid system. Surprisingly, it was found that both, an (unspecific) CB *agonist* and a CB2 receptor *antagonist* after topical application suppressed inflammatory reactions in mice [2]. CCA was shown to inhibit COX 2, but not COX 1. In order to elucidate the possible involvement of **1** in the endocannabinoid system, we prepared possible metabolites of **1**: **2**, **3**, **4**, and the dopamide of **1**. Compounds **2** and **4** might exert their effect through binding at CB receptors similar to anandamide and 2-arachidonoylglycerol. We will present the syntheses of **2**, **3** and **4** using CCA as the educt. To check for antiinflammatory effects, compounds **2**, **3** and **4** will be tested *in vitro* and in the TPA-induced mouse ear oedema, a model for acute inflammation. **References:** [1] Ramadan, M. et al. (2006) J. Nat. Prod. 69: 1041 – 5. [2] Oka, S. et al. (2006) Eur. J. Pharmacol. 538: 154 – 62

P 018

Anti-inflammatory and analgesic activity of *Memecylon edule* Roxb

Nualkaew S[1], Thongpraditchote S[2], Wongkrajang Y[2], Rattanamanee K[3]
[1]*Faculty of Pharmacy, Mahasarakham University, Kantarawichai, 44150, Mahasarakham, Thailand;* [2]*Faculty of Pharmacy, Mahidol University, Phayathai, 10400, Bangkok, Thailand;* [3]*Faculty of Pharmaceutical Sciences, Naresuan University, 65000, Phitsanulok, Thailand*

The anti-inflammatory and analgesic activities of the leaves of the plant *Memecylon edule* Roxb. (Melastomataceae), which are used against burns by traditional practioners in Northeast of Thailand, were studied using *in vivo* models of inflammation and pain. Anti-inflammatory activity was determined by ethyl-phenylpropiolate-induced mouse ear edema; analgesic activity was studied by acetic acid-induced writhing test in mice [1]. Four sequential extracts of *M. edule* obtained with hexane, ethyl acetate, ethanol and 50% ethanol were studied. The results showed that the ethyl acetate extract of *M. edule* possessed strong anti-inflammatory and analgesic activities. The topical application of 1.0 mg/ear of ethyl acetate extract inhibited the edema induced with ethyl-phenylpropiolate by 47.8% after 4 hours, an effect which is less intense than that produced by indomethacine at the same dose (62.4%). The ethyl acetate extract at a dose of 200 mg/kg orally produced significant inhibition of acetic acid induced writhing response by 56.6% which is of the same intensity as indomethacin at the dose of 10 mg/kg. These results provide support for the use of *M. edule* leaves in relieving inflammation and pain. The ethyl acetate extract was also screened for secondary constituents. The extract showed the presence of flavonoids and terpenes. The research will continue with the isolation of the pure active chemical constituents. *Acknowledgements:* The Thailand Research Fund (TRF). **References:** [1] Recio, MC. et al. (2000) Life Sci.; 16: 2509 – 18.

P 019

Topical anti-inflammatory effect of acetone rhizome/root extract of *Potentilla malýana* Borbas

Pilipović S[1], Bosnić T[1], Redžić S[2], Mijanović M[3]
[1]*Institute for Quality Control of Medicines, M.Tita 9, 71000 Sarajevo, Bosnia and Herzegovina,* [2]*Faculty of Sciences, Zmaja od Bosne 44, 71000 Sarajevo, Bosnia and Herzegovina,* [3]*Institute for Pharmacology, Faculty of Medicine, University of Sarajevo, Èekaluža 90, 71000 Sarajevo, Bosnia and Herzegovina*

Potentilla malýana is an endemic plant from Balkan Peninsula**,** and there are no data about usage of this *Potentilla*. Plant material was collected in autumn 2006. The Acetone extract of the rhizome and root of Potentilla *malýana* has been evaluated for anti-inflammatory potential using the mouse ear edema model [1]. Animals (groups N = 6) had food and water ad libitum during the test. Albino mice (6 – 7 weeks of age, average body weight 25 g) were supplied from Institute for Pharmacology, Faculty of Medicine in Sarajevo. The animals were housed in climate-controlled quarters (24 ± °C at 50% humidity) with a 12-h light/12-h dark cycle. Ear inflammation was induced by 3% *Oleum crotonis* acetone solution, in quantity of 10 µL on both mouse ears. Application of extract was only once on left ear, two hours after starting the inflammation. The right ear was the control (without any additional treatment). The ear was observed for three days, and appearance changes were expressed in scores from 0 to 14 [2]. Before application to a mouse ear, the acetone extract was prepared, which had total 5% of phenolic compounds [3]. Hydrocortisone 1% ointment was used as control on second group of the animals (N = 6). The pharmacological reaction was found with the acetone extract of the rhizome *Potentilla malýana,* whose pharmacological reaction was weakest to hydrocortisone ointment. The score for treated ear was 8 ± 1, for untreated ear 12 ± 2, and for hydrocortisone ointment 5 ± 1. The acetone extract of the plant *Potentilla malýana* exhibited significant reduced inflammation in time for 30 – 50% in relation to control ear. **References:** [1] Grujić Vasić J., Pilipović S., Zulić I., Mijanović M., Redžić S.

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P 020

Activity of *Stachys germanica* L. subsp. *salviifolia* (Ten.) Gams on mutagenesis

Menghini L[1], Pagiotti R[2], Moretti M[3], Tirillini B[4], Menghini A[2], Dominici L[3]
[1]Dipartimento di Scienze del Farmaco, Via dei Vestini, 31, 66013, Chieti Scalo (CH), Italy; [2]dipartimento di biologia vegetale e biotecnologie agroambientali e zootecniche, Borgo XX giugno, 06100 Perugia, Italy; [3]Dipartimento di Specialità Medico-Chirurgiche e Sanità Pubblica, Via del giochetto, 06100 Perugia, Italy; [4]Istituto di Botanica ed Orto Botanico, via Bramante, 61023 Urbino, Italy

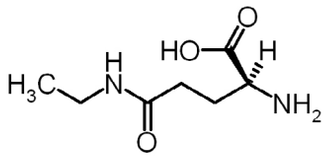
Stachys germanica L. subsp. *salviifolia* (Ten.) Gams (Lamiaceae) is an herbaceous plant widespread in Central and Southern Italy [1]. The fresh plant was used against wart and pustule but few references deal with this plant, perhaps because the subsp. *salvifolia* wasn't distinguished from the other subspecies of the *S. germanica.* There isn't any reference about the biological activity of the plant. Aerial parts were extracted with dichloromethane or subjected to sequential extraction with *n*-hexane, chloroform, chloroform/methanol (1:1) and methanol. To assess their genotoxic/antigenotoxic properties, the extracts were tested *in vitro* by applying the alkaline single-cell microgel-electrophoresis (comet) assay on HepG2 cells. These hepatic cells, originating by a primary hepatoblastoma, retain many of the morphological characteristics of liver parenchymal cells, and contain phase I and phase II drug metabolizing enzymes which play an essential role in the activation/detoxification of promutagens/procarcinogens [3]. The following approaches were performed: (i) co-treatment, (ii) pre-treatment and (ii) post-treatment protocol by challenging the extracts with the model mutagen 4-nitroquinoline-*N*-oxyde (4NQO). The tested extracts neither affected cell viability nor induced DNA damage, except chloroform extract which was highly cytotoxic and genotoxic (2.5 μg/mL). As regard the antigenotoxic properties, the dichloromethane extract was active in the co-exposure experiments (2.5 μg/mL) and in the post- exposure experiments (20 μg/mL); the *n*-hexane extract was active only in the co-exposure experiments (2.5 μg/mL); the chloroform/methanol extract was active in the co- and post- exposure experiments (2.5 μg/mL and 10 μg/mL respectively); the methanol extract was active only in the co- exposure experiments (10 μg/mL). **References:** [1] Conti, F., et al. (2005) An annotated checklist of the italian vascular flora, Palombi ed., Roma [2] Knasmüller et al. (1998) Mutat. Res. 18: 402, 185 – 202

P 021

Synthesis of potential theanine metabolites, related structures and their affinity for CB receptors

Schneider R[1], Sinning C[1], Cascio MG[2], Di Marzo V[2], Imming P[1]
[1]Institut für Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Wolfgang-Langenbeck-Straße 4, 06120 Halle (Saale), Germany; [2]Istituto di Chimica Biomolecolare CNR, Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy

Tea (*Camellia sinensis* (L.) O. Kuntze) contains up to 1.6% of the rare amino acid L-theanine (**1**) [1], which has the attribute to change mood and, contrary to caffeine, a relaxing effect [2]. As with other amino acids, metabolic decarboxylation is possible, leading to a derivative of γ-aminobutyric acid (GABA), viz. 4-amino-N-ethyl-butyramide. This in turn could be converted to 4-arachidonyl-N-ethyl-butyramide (**2**). For **2** and similar structures, we found an affinity for CB1 and CB2 receptors. Cannabinoid receptor ligands are of crucial importance in inflammation and due to this also in carcinogenesis, and show psychopharmacological activity [3,4]. **2** was synthesized starting with aminolyis of boc-4-aminobutyryl-p-nitrophenyl ester (**3**) [5] by ethylamine leading to boc-4-aminobutyrethylamide. This was transformed by gaseous hydrogen chloride to 3-ethylcarbamoyl-propyl-amide hydrochloride. From this and arachidonic acid, compound **2** was synthesized. We also synthesized 4-oleoyl-N-ethyl-butyramide, oleoyl and arachidonoyl amides of 5-amino-pentanoic acid ethyl amide and 4-amino-butyric acid ethyl ester. All compounds were tested for displacement of [^{3}H]CP55,940 by scintillation counting, using HEK-293 cells transfected with human CB1 and CB2 receptors. Rates of displacement for **2** were: 44% (CB1, 1 μM), 100% (CB1, 10 μM), 59% (CB2, 1 μM) and 94% (CB2, 10 μM).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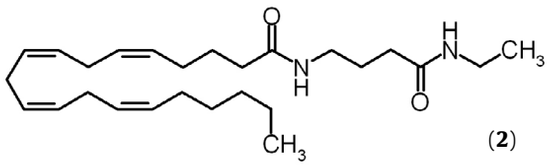
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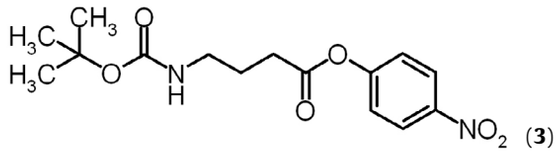
(**3**)

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P 022

In vitro anti-inflammatory activity of extracts from seeds of some *Nigella* species

Landa P[1], Marsik P[1], Vanek T[1], Kokoska L[2]
[1]Laboratory of Plant Biotechnologies, Joint Laboratory of Institute of Experimental Botany AS CR, v.v.i. and Research Institute of Crop Production, v.v.i., Rozvojova 263, 165 02 – Prague 6, Czech Republic, [2]Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6 – Suchdol, Czech Republic

The seeds of *Nigella sativa* L., commonly known as black seed or black cumin, are used in the Indian subcontinent, Arab countries and Europe in folk medicine and for culinary purposes [1]. Among wide spectrum of biological activities, also the anti-inflammatory activity of seeds and their constituents has been determined *in vitro* as well as *in vivo* [2, 3, 4]. In the present study *n*-hexane (H), chloroform (C) and methanol (M) Soxhlet extracts from seeds of *N. sativa* and other five less known species of the genus (*N. arvensis* L., *N. damascena* L., *N. hispanica* L., *N. nigellastrum* Willk., and *N. orientalis* L.) were examined (in concentration 100 μg/mL) for *in vitro* inhibition of cyclooxygenase-1 (COX-1) and -2 (COX-2) enzymes. The strongest inhibitory activity on COX-2 possessed H extracts from *N. orientalis* (87.13%) followed by *N. arvensis* (82.31%), *N. hispanica* (80.13%), and *N. sativa* (78.13%). The best activity in COX-1 assay was found out for H extracts from *N. sativa* (100% inhibition) followed by *N. orientalis* (90.41%) and C extract from *N. hispanica* (89.29%). H and C extract from *N. arvensis* showed better efficiency on COX-2 then COX-1. In both assays, H extracts were the most efficient (with exception of *N. hispanica* and *N. nigellastrum* in COX-1 test) which indicates that mainly non-polar constituents par-

ticipate on inhibitory activity of plant extracts tested on both COX forms. *N. orientalis*, *N. arvensis* and *N. hispanica* showed better inhibitory activity on COX-2 than in folk medicine widely used *N. sativa*. It indicates that these plants should be further studied as potential source of anti-inflammatory compounds. *Acknowledgements:* This work was supported by project 1P04OC926.001. **References:** [1] Ali, B. H., Blunden, G. (2003) Phytother. Res. 17: 299 – 305. [2] Al-Okbi, S. Y. et al. (1997) Egypt J. Pharm. Sci. 38: 451 – 469. [3] El-Dakhakhny, M. (2002) J. Ethnopharmacol. 81: 161 – 164. [4] Marsik et al. (2005) Planta Med. 71: 739 – 742.

P 023

Analysis of natural and synthetic cannabinoid receptor ligands via [^{32}P]GTPase assay

Nickl K[1], Seifert R[2], Heilmann J[1]
[1]Institute of Pharmaceutical Biology, Universitätsstraße 31, D-93053 Regensburg, Germany; [2]Institute of Pharmacology and Toxicology, Universitätsstraße 31, D-93053 Regensburg, University of Regensburg, Germany

Two cannabinoid receptors are known, the cannabinoid receptor 1 (CB_1R) and the cannabinoid receptor 2 (CB_2R). Both regulate important effects, such as appetite and motor coordination (CB_1R), as well as inflammation and host defence (CB_2R). Finding new ligands at CBRs is of high importance treating diseases such as multiple sclerosis, obesity or immune deficiencies. Regarding natural products it was recently shown that alkamides of *Echinacea* bind to CB_2Rs [1, 2]. We established a highly-sensitive test system using the baculovirus/*Sf*9 cell system to evaluate the potency of natural and synthetic compounds. To study ligands we used the [^{32}P] GTPase assay. Co-expressing CBRs with $G\alpha_{i2}$-, $G\beta_1\gamma_2$- and RGS4-protein resulted in the highest GTPase activation (CB_1R: 70%, CB_2R: 83% vs. blank value: membrane and solvent without ligand). CP 55,940 (5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol), a synthetic compound derived from the natural ligand Δ^9-THC, is a strong partial agonist at CBRs (nM range). Anandamide (arachidonoylglycerol), the endogenous agonist, is a full (CB_1R)/partial (CB_2R) agonist and WIN 55212,2 (*R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]-pyrrolo-[1,2,3,-de]-1,4-benzoxazin-yl]-(1-naphtalenyl)-methanone-mesylate) is a high potency agonist at CB_2Rs. AM 251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) is a selective CB_1R inverse agonist/antagonist and AM 630 (6-iodo-2-methyl-1-[2 – 4(morpholinyl)-ethyl]-[1*H*-indol-3-yl](4-methoxyphenyl)methanone) is a selective CB_2R antagonist. At present we are studying Δ^9-THC and alkamides by [^{32}P] GTPase assay and are characterising the binding properties of all ligands by [^{3}H] CP 55,940 binding. **References:** [1] Raduner, S. et al. (2006) J. Biol. Chem. 281: 14192 – 206. [2] Wölkart, K. et al. (2005) Planta Med. 71: 701 – 5.

P 024

Exopolysaccharide from cyanobacterium *Synechocystis aquatilis* – production rate, chemical characterization and biological testing

Alban S, Venzke K, Blaschek W, Volk RB
[1]Pharmaceutical Institute, Department of Pharmaceutical Biology, University of Kiel, Gutenbergstraße 76, D-24118 Kiel, Germany

Cyanobacteria are a very abundant source of structurally diverse polysaccharides which are characterized by a great variety in both number and type of constitutive monosaccharides (acidic and neutral sugars) [1]. The strain *Synechocystis aquatilis* Sauvageau B90.79 is a unicellular cyanobacterium (order Chroococales, family Chroococaceae) which was found to produce and release high amounts of polysaccharides (so called released polysaccharides, RPSs) into its watery environment during cultivation. After 60 days of 8-L-batch cultivation, the average yield of the RPS was 0.5 g/L and hence the mean RPS productivity was 8 mg L^{-1} d^{-1}. According to chemical analysis, the *S. aquatilis* RPS belongs to the group of sulfated fucose-rich polysaccharides: it was mainly composed of arabinose (45%) and fucose (47%) and had a degree of sulfation of 0.43. Biological testing of the RPS revealed a moderate anticoagulant activity of less than 10% compared to the reference compound unfractionated heparin. However, distinctive effects on the complement activation were observed: its inhibitory effect on the classical pathway of complement activation was 600fold stronger than that of unfractionated heparin, whereas that on the alternative pathway of complement activation was 2 – 3fold weaker. The results indicate that this biotechnologically producible RPS represents a specifically acting complement modulator. **References:** [1] DePhilippis, R., Vincenzini, M. (1998) FEMS Microbiol. Rev. 22: 151 – 75.

P 025

Anti-inflammatory triterpenoids from *Alchornea cordifolia* leaves

Osadebe PO, Ebi GC, Okoye FBC
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, 41001, Enugu State, Nigeria

The n-hexane extract of *Alchornea cordifolia* Müll.Arg. leaves has been shown to exhibit a significant and dose dependent inhibition of oedema [1]. In the present investigation, the anti-inflammatory effect of three triterpenoids isolated from the n-hexane extract was evaluated. The pulverized leaves of *Alchornea cordifolia* were extracted with n-hexane and the crude n-hexane extract (A) precipitated with absolute ethanol to obtain n-hexane residue (A1) and ethanol soluble (A2). Both A1 and A2 were subjected to phytochemical investigation. Column chromatographic separation of A2 on silica gel using mixtures of n-hexane, ethyl acetate and methanol afforded three major triterpenoids A2I, A2II and A2III. These triterpenoids were confirmed with Liebermann – Buchard reaction. The anti-inflammatory effects of the triterpenoids were evaluated using egg-albumen-induced rat hind paw edema as a model of inflammation. A2I (50 mg/kg) and A2III (60 mg/kg), given intraperitoneally, showed high and significant ($p < 0.01$) activity with percent inhibition of edema values of 73.85 and 49.23 respectively. The activity of A2I (50 mg/kg) is comparable to that of Aspirin (100 mg/kg). The two compounds also showed dose – dependent inhibition of edema with ID_{50} of 36.6 mg/kg and 52.2 mg/kg respectively. In conclusion, the anti-inflammatory effects of the n-hexane extract of *Alchornea cordifolia* leaves may be attributed to the presence of the triterpenoids A2I and A2III. *Acknowledgments:* The authors are grateful to Mr. Ozioko of Bio-resource Development and conservation centre, Nsukka, Enugu State, Nigeria, for authenticating the plant material. **References:** [1] Osadebe, P.O., Okoye, F.B.C. (2003). J. Ethnopharmacol. 89: 19 – 24

P 026

Sesquiterpene lactones from Arnica flowers down-regulate IL-1β stimulated matrix metalloproteinase genes in human and bovine chondrocytes

Jäger C[1], Zwingmann J[2], Humar M[3], Suter A[4], Merfort I[1]
[1]Department of Pharmaceutical Biology and Biotechnology, University of Freiburg, 79104 Freiburg, Germany; [2]Department of Orthopedics and Traumatology, University Hospital Freiburg, 79106 Freiburg, Germany; [3]Department of Anesthesiology and Critical Care Medicine, University Hospital Freiburg, 79106 Freiburg, Germany; [4]A. Vogel Bioforce AG, 3925 Roggwil, Switzerland

The major pathologic manifestations of rheumatoid arthritis and osteoarthritis are joint inflammation and articular cartilage degradation by proinflammatory cytokine-stimulated matrixmetalloproteinases (MMPs) and aggrecanases [1]. In a pilot clinical study phytomedicines from *Arnica montana* flowers have been proven to exert beneficial effects in patients suffering from mild to moderate osteo-

arthritis of the knees [2]. Here, we show that helenalin and dihydrohelenalin derivatives, sesquiterpene lactones (SLs) from Arnica flowers, were highly active in inhibiting IL-1β induced mRNA expression of matrixmetalloproteinase (MMP)-13 and MMP-1 at micromolar concentrations in primary bovine articular chondrocytes and human articular chondrocytes using real time qPCR analyses. A similiar dose-dependent inhibition on DNA binding capacity of IL-1β-stimulating transcription factors AP-1 and NF-κB using the EMSA was also noted. Moreover, further experiments revealed that AP-1 was directly targeted. EMSA experiments were also carried out with different preparations from Arnica flowers. The strenght of DNA binding inhibition of both transcription factors was dependent on the type and content of SLs. In summary, besides its antiinflammatory activity SLs from Arnica may also be effective in blocking cartilage matrix degradation by MMPs by impairing AP-1 and NF-κB binding activities. *Acknowledgements:* Financial support by Bioforce, Switzerland is gratefully acknlowledged **References:** [1] Burrage, P.S. et al. (2006) Front. Bioscience 11: 529 [2] Knuesel, O. et al. (2002) Adv. Ther. 19: 209 – 218.

P 027

Structural and immunological comparison of arabinogalactan-proteins from *Viscum album* L. berries and herb

Herbst B[1], Classen B[1], Ulmer A[2], Blaschek W[1]
[1]Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany; [2]Research Center Borstel, Parkallee 22, 23845 Borstel, Germany

Mistletoe (*Viscum album* L.) is used as a medicine since ancient times. Today mistletoe extracts play an important role in adjuvant cancer treatment [1] because of their immunostimulating properties. Besides lectins, different polysaccharides from mistletoe berries are discussed to contribute to the *in vivo* activity [2]. For arabinogalactan-proteins (AGPs), especially from *Echinacea*, different immunomodulating activities have been proven *in vitro*, e.g. enhanced production of cytokines [3]. Therefore AGPs from mistletoe herb and berries were purified, characterized and immunologically tested. AGPs were isolated from the high molecular weight fraction of an aqueous extract after protein removement, tangential flow filtration (MWCO 30.000Da), dialysis and precipitation with ß-glucosyl-Yariv reagent. Methylation analysis, partial acid hydrolysis and uronic acid reduction were performed to characterize the chemical structure of the carbohydrate moiety of the AGPs. Methylation analysis of AGP from herb detected as main components 1,3,6-Gal*p* and 1,3-Gal*p*, in the side chains 1,5-Ara*f* and terminal 1-Ara*f* and a total arabinose: galactose ratio of 1: 1.8. Determination of uronic acids showed 1,4-GalA*p* and 1-GlcA*p* to be the acidic compounds in AGP from herb. The AGP from berries has a completely different structure rather untypical for AGPs with arabinose being the dominating monosaccharide. Both AGPs were tested in a competitive ELISA for cross-reactivities with monoclonal antibodies raised against *Echinacea purpurea* AGP [4], to gain further information on structural similarities or differences. First investigations on biological activities of mistletoe AGP from herbal drug preparations revealed interaction with human Toll-like receptor 2. **References:** [1] Büssing, A. (2000) Mistletoe the genus Viscum. Harwood Academic Publishers. Amsterdam. [2] Stein, G.M. et al. (1999) Anticancer Res. 19: 3907 – 3914. [3] Classen, B. et al. (2006) Phytomed. 13: 688 – 694. [4] Classen, B. et al. (2004) Planta Med. 70: 861 – 865.

P 028

Effects of semisynthetic pullulan sulfates on the release of inflammatory cytokines *in vitro*

Partschefeld J, Alban S
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

An important step of an inflammatory response is the production and release of cytokines like tumor necrosis factor-alpha (TNF-α) or interleukin-6 (IL-6) by human polymorphonuclear neutrophils (PMN) and monocytes (MC). Therefore, the inhibition of that release represents a promising approach to treat inflammatory diseases. Heparin was shown to exhibit anti-inflammatory activities and to affect the release of cytokines [1]. However, due to its high anticoagulant activity, its clinical application in inflammatory diseases is limited [2]. To develop glucan sulfates with an improved activity profile compared to heparin, series of pullulan sulfates (PulS) were produced by sulfation of the linear α-1,4/1,6-glucan from the yeast *Aureobasidium pullulans*. In the present study, the structure-dependent effects of PulS on the cytokine release were investigated. PMN and MC, isolated from blood of healthy volunteers, were activated with 0.1 μg/ml LPS in presence or absence of the test compounds (conc. ranging from 0.005 to 50 μg/ml). The conc. of released TNF-α and IL-6 were measured by ELISA. PulS showed to reduce the release of TNF-α and IL-6 from PMN and MC, resp., stronger than unfractionated heparin (UFH). Already at 0.005 μg/ml, inhibitory effects between 30 and 60% were observed. With increasing conc. the inhibition only slightly increased. Comparing PulS with a similar MW of 10 kDa, but degrees of sulfation (DS) ranging from 0.2 to 2.0, the inhibitory activity improved up to a DS = 1.5. In the absence of LPS, PulS do not induce any significant cytokine release. Moreover, the cytokine detection by ELISA is not impaired by the test compounds. In summary, semisynthetic PulS with reduced anticoagulant activity are superior to heparin in inhibiting the cytokine release of activated leukocytes, which is in line with their increased anti-inflammatory activity *in vivo*. **References:** [1] Hochart, H. (2006) Br. J. Haematol. 133: 62 – 67 [2] Becker, M. et al. Thromb. Haemost. (2003) 89: 915 – 25

P 029

Sulfated polysaccharides from *Delesseria sanguinea* (Hudson) Lamouroux inhibit the release of inflammatory cytokines *in vitro*

Partschefeld J, Alban S
Pharmaceutical Institute, Christian-Albrecht-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

Sulfated polysaccharides (SP) like the prime example heparin are known to exhibit a wide range of biological activities. Unfractionated heparin (UFH) was shown to significantly attenuate the production of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) by human polymorphonuclear neutrophils (PMN) and monocytes (MC) *in vivo* and *in vitro* [1,2], and thus to inhibit an important step of inflammatory processes. The aim of this study was to investigate whether also SP extracted from *Delesseria sanguinea* inhibit the cytokine release *in vitro*. Furthermore, the influence of the extraction method (aqueous extract or alkaline extract) on these effects was examined. Reference substances were UFH and PS3 (a semisynthetic β-1, 3 glucan sulfate (US7008931-B2) with proven anti-inflammatory activity *in vivo*. PMN and MC isolated from blood of healthy volunteers were activated with LPS in presence or absence of increasing concentrations of the test compounds. The concentrations of TNF-α, IL-6 and IL-8 in the cell supernatant were quantified by ELISA. The SP from D.s. showed a conc.-dependent (0.005 – 1 μg/ml) reduction by up to 70% of the LPS-induced release of TNF-α and IL-6 by MC and PMN as well as of IL-8 by MC. They were as active as PS3 and superior to UFH. Further, the inhibition by SP extracted with water was stronger than that by SP extracted with NaOH. By control experiments, both any influence on the cytokine

measurement by ELISA and any effect on non-stimulated cells could be excluded. In conclusion, the SP isolated from D.s. reduce the cytokine release by stimulated PMN and MC. This activity and further *in vitro* effects like inhibition of complement activation, elastase and hyaluronidase suggest an anti-inflammatory potential. *Acknowledgements:* this project is financed by the EU (FIAF/EFF) and the LFALF Mecklenburg-Vorpommern **References:** [1] Anastase, S. et al. (2003) J. Biomed. Mater. Res. 66: 376 – 84 [2] Masihi, et al. (1997) Int. J. Immunopharmac. 19: 463 – 468

P 030

Anti-inflammatory activity of four *Staphylea* L. species

Laciková L'[1], Wenzig EM[2], Bauer R[2], Mažterová I[1], Granèai D[1]
[1]Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovakia; [2]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria

Staphylea preparations are used in Traditional Chinese Medicine as a cough remedy, and they have been used by native Americans for the treatment of rheumatic conditions. Based on the significant cytotoxic, antibacterial and antioxidant activity of some *Staphylea* species [1, 2, 3] the anti-inflammatory activity of petrol ether extracts from leaves of *Staphylea colchica* Stev., *S. elegans* Zab., *S. holocarpa* Hemsl. and *S. pinnata* L. has been determined. The anti-inflammatory activity was tested using *in vitro* assays for inhibition of cyclooxygenase (COX-1 and COX-2) [4] and 5-lipoxygenase (5-LOX) [5]. From the extracts possessing > 50% inhibitory activity at a screening concentration of 50 µg/ml, IC_{50} values were determined. All extracts inhibited the activities of COX-1 and COX-2 in a similar extent, with *S. holocarpa* performing best (IC_{50} 10.1 and 5.2 µg/ml against COX-1 and COX-2, respectively). 5-LOX inhibition of the extracts was less pronounced. The highest inhibitory activity was shown by the *S. colchica* extract (IC_{50} 26,7 µg/ml). *Acknowledgements:* University of Graz, Grant Agency VEGA SR No. 1/4289/07 and Cátedra Dubcek grant 2006 sponsored by Complutense University of Madrid. **References:** [1] Jantová, S. et al. (2001) Phytother. Res. 15: 22 – 25. [2] Jantová, S. et al. (2000) Phytother. Res. 14: 601 – 603. [3] Laciková, Ľ. et al. (2007) Molecules 12: 98 – 102. [4] Fiebich, et al. (2005) Planta Med. 71: 12 – 19. [5] Adams, M. et al. (2004) Planta Med. 2004 70: 904 – 908.

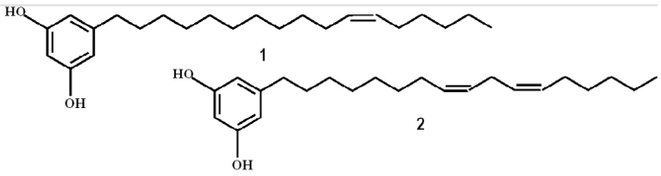
P 031

Cyclooxygenase inhibitory 5-alkenylresorcinols isolated from mango (*Mangifera indica* L.) peels

Knödler M[1], Wenzig EM[2], Bauer R[2], Conrad J[3], Beifuß U[3], Carle R[1], Schieber A[1]
[1]Institute of Food Science and Biotechnology, Section Plant Foodstuff Technology, Hohenheim University, August-von-Hartmann-Straße 3, 70599 Stuttgart, Germany; [2]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria; [3]Institute of Chemistry, Section Bioorganic Chemistry, Hohenheim University, Garbenstraße 30, 70599 Stuttgart, Germany

Fruits of *Mangifera indica* L. (Anacardiaceae) as well as other parts of the plant have widely been used in traditional medicine. As a part of our continuing research project aimed at the discovery of bioactive compounds from food- and pharmaceutical processing by-products [SAFEWASTES (www.safewastes.info)], the anti-inflammatory activities of aqueous, hydroethanolic and lipophilic mango peel extracts have been examined by means of *in vitro* assays [1,2]. Bioassay-directed fractionation of the dichloromethane extract revealed the presence of the novel 5-(11*Z*-heptadecenyl)-resorcinol (**1**) and the known 5-(8*Z*,11*Z*-heptadecadienyl)-resorcinol (**2**) beside a series of recently described C_{15}-, C_{17}, and C_{19}-substituted alk(en)ylresorcinols [3] in low abundance. The structures of the two isolates were elucidated by 1D and 2D NMR studies, MS and chemical methods. Both compounds exhibited potent COX-1 and COX-2 inhibitory activity with IC_{50} values given in table 1. Structure activity relation by referring to synthetic saturated homologues indicated that degree of unsaturation in the alkyl chain plays an important role for the inhibitory activity. **Table 1**: IC_{50} values of compounds 1 and 2

Compound	IC_{50} COX-1 (µM)	IC_{50} COX-2 (µM)
1	3,54	4,42
2	1,93	3,52



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P 032

Flower oil components from four *Staphylea* L. species

Laciková L'[1], Švajdlenka E[2], Mažterová I[1], Granèai D[1]
[1]Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovakia; [2]Department of Natural Drugs, Pharmaceutical Faculty, Veterinary and Pharmaceutical University, Palackého 1 – 3, 612 42 Brno, Czech Republic

Traditional Chinese medicine uses preparations from *Staphylea* species as a cough remedy and in America they have been used for the treatment of rheumatic, gynecological and dermatological problems. Significant cytotoxic, antibacterial and antioxidant activity of some *Staphylea* species is known as well [1, 2, 3]. For the first time the chemical composition of the hydrodistilled volatile fraction from the flowers of *Staphylea colchica* Stev., *Staphylea elegans* Zab., *Staphylea holocarpa* Hemsl. and *Staphylea pinnata* L. was investigated by GC/MS method. 27 compounds were identified in oil from the fresh and dried material, stored for 1 year. The qualitative and quantitative content of investigated compounds was similar in the fresh and dried flowers of four *Staphylea* species. Mostly the structures of different C_{19} – C_{32} oxygenated aliphatic hydrocarbons were identified with the dominating content of tricosane (44.0% and 45.7% in *S.elegans* and *S. pinnata*, respectively), *n*-hexadecanoic acid (36.6% in *S. colchica*) and heneicosane (24.5% in *S. elegans* and *S. pinnata*) in dried flowers. Table 1: The qualitative content overview of identified compounds in dried and fresh flowers

Species	dried flowers, stored for 1 year	fresh flowers
S. colchica	C_{20}-C_{30} aliphatic hydrocarbons, *n*-hexadecanoic acid	C_{20}-C_{30} aliphatic hydrocarbons, *n*-hexadecanoic acid, aldehydes, ketones
S. elegans	C_{21}-C_{29} aliphatic hydrocarbons	C_{21}-C_{29} aliphatic hydrocarbons
S. holocarpa	C_{20}-C_{30} aliphatic hydrocarbons, *n*-hexadecanoic acid	C_{20}-C_{30} aliphatic hydrocarbons, *n*-hexadecanoic acid, aldehydes, ketones, esters of higher fatty acids
S. pinnata	C_{21}-C_{29} aliphatic hydrocarbons	C_{19}-C_{32} aliphatic and nonaliphatic hydrocarbons

Acknowledgements: Grant Agency VEGA SR No. 1/4289/07 and Cátedra Dubcek grant 2006 sponsored by Complutense University of Madrid. **References:** [1] Jantová, S. et al. (2001) Phytother. Res. 15: 22 – 25. [2] Jantová, S. et al. (2000) Phytother. Res. 14: 601 – 603. [3] Laciková, Ľ. et al. (2007) Molecules 12: 98 – 102.

P 033

Elastase inhibition assay – the choice of the synthetic substrate determines the result

Groth I, Alban S
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

It is well known that many factors like buffer concentration, pH, or enzyme concentration influence the inhibitory activity of test compounds in *in vitro* enzyme inhibition assays. The aim of the present study was to investigate whether different synthetic substrates have an influence on the inhibitory data as well. As an example, we have chosen a well established validated *in vitro* PMNE (polymorphonuclear neutrophil elastase)-inhibition assay. The basic assay was modified by using the following synthetic peptide-substrates: 1. S-2484 (Chromogenix), 2. MOSAAPVPNA (Bachem), 3. I-1270 (Bachem). The concentration-dependent elastase inhibition by unfractionated heparin (UFH), PS3 (a semisynthetic glucan sulfate, US7008931-B2), and a sulfated polysaccharide from the red alga *Delesseria sanguinea* (D.s.-SP) was determined.

	IC50[µg/ml]/*relative IC50 values*		
	S-2484	**MOS**	**I-1270**
PS3	0.19	0.21	0.16
	1.0	*1.0*	*1.0*
UFH	0.59	1.21	0.28
	3.1	*5.9*	*1.7*
D.s.-SP	0.41	0.40	0.18
	2.2	*1.9*	*1.1*

The inhibitory potency of each inhibitor showed to be clearly dependent on the used substrate: this concerns both its maximal inhibitory activity, the gradient of its concentration-dependent inhibition, and its IC50-value. In addition, even the ratios of the IC50-values of any inhibitors alter by change of the substrate (see table). Only the order of the inhibitors concerning their activity is independent of the substrate and always keeps constant. Thus also activities of inhibitors given in relation to a reference compound may vary in dependence on the respective assay procedure. As a consequence, even the use of reference substances allows only a rough estimation of an inhibitor's potency. In conclusion, *in vitro* enzyme assays with synthetic substrates are useful tools in pharmacological research, but one should always be aware of their manifold limitations. IC50 values are strongly dependent on the respective test system and even relative declarations are not essentially reproducible in a modified test system.

P 034

Clinical evaluation of the efficacy and safety of Bioaron C® in children with recurrent bacterial and viral infections of the upper respiratory tract

Pampura A[1], Beuscher N[2], Smirnova M[1], Horoszkiewicz-Hassan M[2], Schönknecht K[2]
[1]Paediatric Medicine and Surgery Research Institute, Department of Allergology and Clinical Immunology, Rosszdrav, Russia; [2]Europlant PhytoPharm Sp. z o.o. Klêka 1, 63 – 040 Nowe Miasto nad Wartą, Poland

Bioaron C® syrup is a popular herbal product having an immunostimulant effect. The product has been used in Poland for the treatment of upper respiratory tract infections and in the prevention of recurrent bacterial and viral infections of the upper respiratory tract. Bioaron C® syrup contains water extract of aloe leaf (*Aloe arborescens* Mill.), chokeberry fruit juice (*Aronia melanocarpa* Elliot.) and vitamin C. Pharmacological studies in Balb/c mice have shown a stimulant effect on the B-cell and T-cell responses, a post marketing clinical study good tolerability (1,2). Clinical evaluation of the efficacy of Bioaron C®, in terms of the frequency and duration of infection in children with recurrent upper respiratory tract infections, was performed in a clinical hospital in Moscow. The study was completed by 60 children. The group comprised 21 children aged from 3 to 6 years and 39 children aged from 6 to 12 years. The total disease duration within 6 months after medicinal product administration was reduced from 35.64 ± 1.18 to 31.33 ± 1.84 days ($p = 0.045$) compared to the same individual calendar period in the previous year. In the T-cell immunity test, an increase in the relative and total T-cell counts ($CD3^+$, $CD4^+$, CD8) was seen; there was a statistically significant increase in the relative number of $CD3^+$ and $CD4^+$ and total T-cell count ($CD4^+$, CD8) as well. A statistically significant reduction in the relative number of CD16+ cells was seen. In the drug safety tests, no biochemical abnormalities in blood parameters have been seen. **References:** [1] Skopińska-Różewska, E., Demkow, U., Sommer, E., Nowak, K., Sievers, H. (2003), Symposium Muzyna Aloe arborescens i Aronia melanocarpa. Aloe arborescens and Aronia melanocarpa. The Role of Natural Immunomodulators in the Prevention and Treatment of Diseases. [2] Horoszkiewicz-Hassan, M. et al. (2005), Herba Polonica 2005; 5 (1/2): 45 – 54. The tolerance and efficacy of Bioaron C® syrup in the treatment of upper respiratory tract infections in children. The results of a postmarketing surveillance study carried out in Poland.

P 035

Rapid electrochemical method to screen antioxidative capacity of biological matrices (RESAC)

Héritier J, Zonnevijlle F, Andlauer W
HES-SO Valais/Wallis, Institue of Life Technologies, Route de Rawyl 64, CH-1950 Sion

One aspect involved in initiation and development of cardiovascular diseases and cancers are oxidative processes, leading to the generation of hydroxyl radicals and peroxyl compounds [1]. There is growing body of evidence that the chemopreventive effects of antioxidants contribute to inactivate "reactive oxygen species" [2 – 4]. In all cases, assessment of antioxidant capacity of our food or other biological matrices is of utmost importance. The majority of free radicals are good oxidants. Therefore, a good antioxidant maybe at first a reductant. The wide mixture of antioxidants with varying redox potentials in plant foods may interact in a synergistic manner, enabling effective protection against oxidation at low concentration levels [5]. Measurement of electrochemical oxidizability should be an appropriate method to assess antioxidant capacity of a biological sample. The aim of the present study was to apply a recently developed electrochemical method for a rapid screening of antioxidant capacity (RESAC) of biological matrices. The electrochemical detector used was equipped with a glassy carbon electrode, operating at a potential of +600 mV. Calibration of the electrochemical method with common photometric standard methods DPPH [6] and FRAP [7] has been done. Results obtained indicate a good correlation between RESAC and these conventional photometric methods (DPPH $r = 0{,}935$, $n = 12$; FRAP $r = 0{,}992$, $n = 12$). After calibration, applicability of the method has been shown with selected tea and green coffee samples. The RESAC-method is a rapid and convenient assay based on electrochemical oxidation for the assessment of antioxidant capacity. The method is well suited for quality and specification control and may facilitate future studies regarding alteration of antioxidant capacity during growth, storage and processing of food or medicinal plants. **References:** [1] Andlauer, W. et al. (1998) Curr. Opin. Clin. Nutr. Metabol. Care 1: 539 – 547 [2] Droy-Lefaix, M.T. (1997) Age 20: 141 – 149 [3] Cheng, T.O. (1998) J. Am. Coll. Cardiol. 31: 1214 [4] Tanaka, K. et al. (1998) Mutat. Res. 412: 91 – 98 [5] Soleas, G.J. et al. (1997) J. Agric. Food Chem. 45: 3995 – 4003 [6] Brand-Williams, W. et al. (1995) Food Sci. Technol. 28: 25 – 30 [7] Benzie, I.F.F., Strain, J.J. (1996) Anal. Biochem. 239: 70 – 76

P 036

Characterisation of anti-inflammatory effects of STW5 and STW6 on rat small intestine *in vitro*: Involvement of adenosine A_{2A} receptors

Nieber K[1], Michael S[1], Müller CE[2], Kelber O[3]
[1]Institute on Pharmacy, University of Leipzig, Talstr. 33, D-04103 Leipzig, Germany; [2]Institute of Pharmacy, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany; [3]Steigerwald Arzneimittel GmbH, Havelstr. 5, D-64295 Darmstadt, Germany

The phytotherapeutic agent STW 5 is successfully used in the therapy of motility disorders but its effect on inflammatory processes in the gut remains unclear. The aim of the present study was to compare possible anti-inflammatory properties of STW 5 and its lead component, STW 6 (Iberis amara fresh plant extract) on the rat small intestine *in vitro* and to study the involvement of adenosine A_{2A} receptors (A_{2A}R). The experiments were performed on isolated ileum/jejunum preparations in conformity with the German Guidelines for Animal Care. The inflammation was induced with 2,4,6 trinitrobenzolsulfonic acid (TNBS, 0.01 M). The ACh-evoked (1 mM) contractions were measured isometrically. TNFα-mRNA was assayed using quantitative RT-PCR with SYBR® Green as detecting agent. Morphological changes were visualized after van Gieson staining. Radioligand binding assays were carried using [^{3}H] CGS 21680 as radioligand. Preincubation of the tissue preparations with TNBS (30 min) resulted in an inhibition of the ACh-evoked contraction. STW 5 (64 – 512 µg/ml) and STW 6 (3 – 24 µg/ml) prevented this inhibition concentration dependently, when they were coapplied with TNBS. TNBS induced morphological changes of the smooth muscular layers and the mucosa. The coincubation of STW 5 and STW 6, respectively, resulted in more intact mucosal and muscular layers. The protective effects of the two herbal preparations on contractile and morphological disturbances were blocked by the A_{2A}R antagonist 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC 0.2 µM). To evaluate an anti-inflammatory mechanism TNF-α mRNA and its LPS-stimulated releases from monocytes were investigated. STW 5 but not STW 6 diminished the TNBS-induced increase of TNFα-mRNA level and inhibited the LPS-stimulated release. These effects were also blocked by CSC (0.2 µM). Radioligand binding assays confirmed the affinity of STW 5 to the A_{2A}R, whereas STW 6 did not bind. The results indicate that STW 5 and STW 6 act anti-inflammatory by direct or indirect activation of A_{2A}R. The involvement of other adenosine receptors subtypes cannot be excluded, especially in the action of STW 6.

P 037

Investigations on the anti-leukemia and immunomodulating activities of *Agaricus blazei* extract

Jiang JJ[1], Kim CF[1], Fung KP[2], Leung KN[2], Lau CBS[1]
[1]School of Pharmacy; [2]Department of Biochemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

The medicinal mushroom *Agaricus blazei* (AB) is traditionally used as a remedy against various cancer, infection, and immune related diseases [1 – 3]. In present study, we investigated both the anti-leukemia and immunomodulatory effects of an ethanol-water extract of *Agaricus blazei*. In MTT and tritiated thymidine incorporation assays, the AB extract showed statistical significant suppression on the growth of human acute promyelocytic leukemia NB-4 (IC_{50} values at 82.2 ± 23.5 µg/ml and 109.6 ± 51.7 µg/ml respectively) and the chronic myelogenous K-562 cells (IC_{50} values at 219.2 ± 87.6 µg/ml and 138.3 ± 16.5 µg/ml respectively) but has no growth-inhibitory effect on human normal liver cells WRL 68. In an attempt to clarify the underlying mechanisms, NB-4 cells were treated with AB extract and resulted in apoptosis characteristic DNA laddering using gel electrophoresis (20, 100 and 400 µg/ml, 72 h) and enrichment of nucleosome to maximum of 2.5-folds (250 µg/ml, 48 h) using cell death detection ELISA. To investigate the immunomodulatory effect of AB extract, BALB/c mice were orally fed with water (as control) or extract (100 or 500 mg/kg/day, n = 7) for 28 days. Splenic lymphocytes isolated from these mice on day-14 and day-28 were incubated with mitogens LPS or Con A for 72 h. Our results indicated that both AB extract treated groups significantly lowered the *ex-vivo* mitogen-stimulated lymphocyte proliferation in tritiated thymidine incorporation assay, with $p < 0.0001$ in all cases using two-way ANOVA. In conclusion, AB extract inhibited the growth of leukemia cells, at least in part, by induction of apoptosis. This is also the first report of AB extract in suppressing *ex-vivo* mitogen-stimulated lymphocyte proliferation. *Acknowledgements:* This study is supported by HKSAR RGC Competitive Earmarked Research Grant CUHK4531/05 M. **References:** [1] Bernardshaw, S. et al. (2005) Scand. J. Immunol. 62(4):393 – 8. [2] Higaki, M. et al. (1997) Nippon Yakurigaku Zasshi 110 Suppl 1: 98 – 103. [3] Mizuno, T. (2002) Int. J. Med. Mushroom 4: 299 – 312.

P 038

Immunopharmacological properties of the polysaccharide fraction isolated from *Ginkgo bilobae* folium

Hancianu M, Pavelescu M, Grigorescu E, Miron A, Stanescu U
University of Medicine and Pharmacy, Faculty of Pharmacy, Iasi, Univesrsity Street, No. 16, 700115, Romania

Many macromolecular compounds of the vegetal polysaccharidic type possess important immunostimulatory properties [1]. The crude macromolecular fraction was isolated from the leaves of *Ginkgo biloba* L. (codified as PGfol) harvested at the Botanical Gardens of Iasi, Romania. The hot water extract was partially purified and submitted to chemical and immunopharmacological studies. Purification started with anion-exchange-column chromatography, followed by HP-GPC. We found out that the polysaccharide fraction contains one macromolecular component with the molecular weight of 260.000D. The immunostimulatory activity of PGfol was further investigated by a series of tests on rats (after 7 days p.o. treatment with 100,0 mg/kg): phagocytosis capacity of PMN cells by NBT test, serum complement activity and determination of the activity on spleenic T-lymphocyte [2]. In all experimental procedures Levamisole® (10,0 mg/kg p.o.) was the reference compound; statistical significance was established by the Student's "t" test. The results showed that PGfol has a good immunostimulatory activity, equal or higher to the Levamisole. The protection against *Klebsiella pneumoniae* 507 experimental infection was measured on mice after 10 days p.o. treatment with 10,0 mg/kg PGfol; the survival rate after the treatment with PGfol was 72,7% compared to 68,7% of the control group. **References:** [1] Flandroy, L. (1996) Biofutur, 159, 35 – 41. [2] Caesar, W. (1989) Dtsch. Apoth. Ztg. 129: 2430.

P 039

NO-, TNF-, and IL-12-inducing activity of fractions of EPs® 7630

Janecki AJ[1], Kiderlen AF[2], Kolodziej H[1]
[1]Freie Universiät Berlin, Institute of Pharmacy, Pharmaceutical Biology, Königin-Luise-Straße 2+4, 14195 Berlin, Germany; [2]Robert Koch-Institut, Nordufer 21, 13353 Berlin, Germany

The efficacy of EPs® 7630, a herbal medicinal product for the treatment of upper respiratory tract infections, has been demonstrated in a number of clinical studies [1,2]. Despite considerable efforts, the remedial effects cannot be related to a chemically defined principle. Using several functional bioassays, EPs® 7630 has shown appreciable immunomodulatory activities *in vitro* [3]. In continuation of our previous work regarding the search for immunologically active constituents, EPs® 7630 was subjected to a variety of extraction procedures (EtOAc, CH_2Cl_2, H_2O) giving a range of subfractions. In a modified protocol, EPs® 7630 was initially separated into a MeOH-soluble (MSP) and -insoluble (MIP) fraction which were subsequently similarly treated as above. Each sample was tested for its NO-, TNF- and IL-12-inducing capacity using non-infected and GFP-trans-

fected-*Leishmania major*-infected bone marrow-derived macrophages (BMMΦ). Following a 48 h incubation period, significant TNF levels (ca 79 pg/ml vs. 200 pg/ml with IFN+LPS as positive control) were induced by EPs® 7630 and the H_2O phase of MIP, respectively, while only negligible NO and IL-12 productions were determined in all the samples. In flow cytometric (FACS) analysis the GFP-signal indicating viable parasites decreased to 29 and 21%, when cells were treated with EPs® 7630 and the H_2O phase of MIP, respectively. Untreated cells were used as a control. The conspicuous intracellular killing of parasites, commonly related to NO, suggested that extracellular NO radicals were non-detectable in the Griess assay, while these cytotoxic effector molecules were effective intracellularly. This may be attributed to radical scavengers present in this fraction or to relatively low but efficient NO levels. Attention is currently paid to the chemical constituents of this active fraction. *Acknowledgements:* EPs® 7630 was kindly provided by Dr Willmar Schwabe GmbH & Co, Karlsruhe, Germany. **References:** [1] Matthys, A. et al. (2007) Curr. Med. Res. Opin. 23: 323 – 31. [2] Chuchalin, A.G. et al. (2005) Explore (NY) 1: 437 – 45 [3] Kolodziej, H., Kiderlen, A.F. (2007) Phytomedicine; 14 Suppl 1: 18 – 26

P 040

Phytocompounds from *Cryptomeria japonica* exhibit potent bioactivities on modulating inflammatory mediators and hepatoprotection

Shyur LF[1], Huang CC[1], Lo CP[1], Chiu CY[1], Wang SY[2], Chang ST[3], Yang NS[1]
[1]Agricultural Biotechnology Research Center, Academia Sinica, Taipei 115, Taiwan, R.O.C.; [2]Department of Forestry, National Chung-Hsing University, Taichung, Taiwan, R.O.C.; [3]School of Forestry and Resource Conservation, National Taiwan University, Taiwan, R.O.C

Cryptomeria japonica D.Don is an industrially important plantation tree species in Asia countries. This study aims to characterize the anti-inflammatory and hepatoprotective activities of the phytocompounds from *C. japonica* wood on LPS- or TPA-induced expression of proinflammatory mediators and CCl_4-induced acute liver injury in mice. A CJH7 – 2 fraction was purified from the wood hexane extract of *C. japonica* using silica gel column chromatography, which exhibits significant activities, at 5 μg/ml, on inhibition of NO production and iNOS expression and up-regulating HO-1 expression in LPS-stimulated macrophages. CJH7 – 2 also potently inhibits TPA-induced COX-2 protein expression in mouse skin (1 mg/200 μl/site) and COX-2 enzymatic activity (IC_{50}= 5 μg/ml). CJH7 – 2 (10 mg/kg BW) can prevent CCl_4-induced liver injury and aminotranferases activities in mice. Chemical fingerprinting analysis showed that terpenes are the major bioactive compounds in the CJH7 – 2 fraction. This is the first study to demonstrate that chemical constituents from wood extract of *C. japonica* possess anti-inflammatory activity *in vitro* and *in vivo* that may play a role in hepatoprotection. The results also suggest that the great potential of *C. japonica* compounds need further development as therapeutic agent for inflammatory disorders.

P 041

Free radical scavenging activity and the content of phenolic compounds in mints and balms cultivated in South-West Slovakia

Fialová S, Tekeľová D, Mrlianová M, Tóth J, Nagy M, Granèai D
Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovakia

Oxidative stress is an instigator of cell death and causes numerous human diseases. The effort of current medicine tries to eliminate oxidative stress by applying antioxidants in the therapy as well as in the prevention. Lamiaceae plants are important sources of natural antioxidants. The free radical scavenging activity of 50%-hydroalcoholic extracts of leaves of three *Mentha* L. species (*M.*x *piperita* L., *M. spicata* L., *M. longifolia* (L.) Huds. subsp. *lavanduliodora*) and two *Melissa officinalis* L. subspecies (*M. officinalis* L. subsp. *officinalis* cv. 'Citra', *M. officinalis* L. subsp. *altissima* Sibth. & Sm.), harvested in July and September, in the DPPH (2,2-diphenyl-1-picrylhydrazyl) test was determined. The content of total hydroxycinnamoic derivates [THD] [1], rosmarinic acid [RA] (by HPLC) and flavonoids [1] was determined as well. Hydroalcoholic extracts exhibited significant antioxidative activity (AA). AA was correlated with the content of the respective phenolic compounds.

	THD [%]		RA [%]		flavonoids [%]		AA SC_{50} [μg/ml]	
	VII	IX	VII	IX	VII	IX	VII	IX
Mentha L.								
M. x piperita	9.3	8.6	1.77	1.91	0.88	0.84	3.1	4.2
M. spicata	7.3	6.2	1.95	2.88	0.42	0.40	4.9	5.3
M. longifolia	4.0	1.8	1.19	0.80	0.53	0.51	5.8	9.8
Melissa officinalis L.								
M. off. subsp. officinalis cv. 'Citra'	13.1	10.2	3.79	4.56	0.46	0.47	2.6	3.8
M. off. subsp. altissima	13.1	11.5	4.75	4.68	0.29	0.31	2.4	2.8

Acknowledgements: VEGA grant 1/4289/07 and VEGA grant 2/5052/25 **Reference:** 1. European Pharmacopoeia. 5th edition (PhEur 5), Strasbourg: Council of Europe (2004) S 1496 – 97, S 1989 – 90

P 042

Anti-inflammatory and antinociceptive activities of *Salvia halophila* and *Salvia virgata* from Turkey

Küpeli E[1], Göger F[2], Kosar M[2], Baser KHC[2]
[1]Gazi University, Faculty of Pharmacy, Department of Pharmacognosy 06330 Etiler-Ankara, Turkey; [2]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy 26470 Eskisehir, Turkey

Chronic inflammatory diseases remain one of the world's major health problems (1). Currently, both steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammatory disorders. Steroids have an obvious role in the treatment of inflammatory diseases, but due to rate limiting toxicities, can only be prescribed over short periods except in very severe cases where the risks are acceptable. Prolonged use of NSAIDs also associated with severe side effects, notably gastrointestinal haemorrhage (2). The recently developed cyclooxygenase-2 (COX-2) selective drugs introduced into therapy, however, do not seem to be free of risk (3). Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. Turkey is an important country for *Salvia* species. The flora of Turkey includes 88 species of the genus *Salvia*. The aerial parts of *S. halophila* and *S. virgata* were used in Soxhlet extraction with different solvents such as *n*-hexane, ethylacetate, methanol and aqueous methanol (50%). Plants were also extracted with water under reflux. In the present study, the potential effects of the extracts from the aerial parts of *S. halophila* and *S. virgata* were studied in *p*-benzoquinone-induced writhing reflex for the assessment of antinociceptive activity and carrageenan-induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate (TPA)-induced ear edema models in mice for the anti-inflammatory activity. Results have shown that methanol extract of *S. virgata* significantly inhibited carrageenan-induced paw edema and *p*-benzoquinone-induced writhing reflex at 100 mg/kg dose, while this extract showed no effect in the TPA-induced ear edema. On the other hand, the other exracts did not show any inhibitory antinociceptive and anti-inflammatory activities in these *in vivo* models. **References:** [1] Yesilada, E., Ustun, O, Sezik, E., Takaishi, Y., Ono, Y., Honda, G. (1997). J. Ethnopharmacol. 58: 59 – 73. [2] Miller, T. A. (1983) Am. J. Physiol. 245: 601 – 623. [3] Wallace, J.L. et al. (1998) Gastroenterology 115: 101 – 109.

P 043

Preliminary investigation of cytotoxic and antioxidant activity of some medicinal plants growing in Serbia and Montenegro

Menković N[1], Zdunić G[1], Šavikin K[1], Stanojković T[2], Juranić Z[2], Janković T[1]
[1]Institute for Medicinal Plants Research, T. Kožćužka 1, 11000 Belgrade, Serbia; [2]Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia

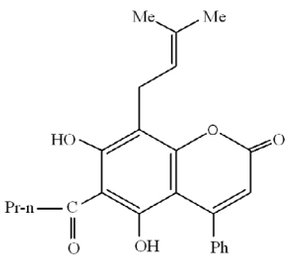
Six medicinal plants growing in Serbia (*Sanguisorba officinalis* L., *S. minor* Scop., *Anthyllis aurea* Weld., *Erica carnea* L., *Hypericum richeri* Vill. and *Lathyrus binatus* Panè.) were investigated for antioxidant and antiproliferative activity. Due to the literature data there are a little information about antiproliferative activity of those plants. Roots of *S. minor*, flowers of *S. officinalis* and *E. carnea* (mountain Suvobor); flowers of *A. aurea* and aerial parts of *H. richeri* (Èakor mountain); aerial parts of *L. binatus* (mountain Tara) were collected in 2005. Air-dried powdered material was extracted with methanol in Soxhlet apparatus. Dry extracts were used for experiments. Antioxidant activity was tested in reaction with DPPH radical [1] and in lipid peroxidation test [2]. Trolox® was used as positive controls. Total phenolic content (gallic acid equivalent) of extracts was estimated using the Folin-Ciocalteu's reagent. Neoplastic HeLa cells were used for the investigation of cytotoxic effects. Cell survival was determined indirectly by measuring total cellular protein by the Kenacid Blue R dye binding method [3]. IC_{50} concentration was defined as concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. All experiments were done in triplicate. The amount of total phenolics varied from 108.9 ± 3.1 to 201.5 ± 4.4 mg GAE/g dry weight of extracts. All extracts showed strong antioxidant activity in reaction with DPPH (IC_{50}= 16.2 ± 0.8 – 37.7 ± 2.1 µg/ml) and in inhibition of lipid peroxidation. The most active was extract of *S. officinalis*. There was a correlation between the phenolic content and antioxidant activity. Investigated extracts, also, exhibited significant cytotoxic effects with an IC_{50} ranging from 66.9 ± 2.3 to 147.7 ± 19.1 µg/ml. Preliminary results showed that all extracts possess the potential for antiproliferative action against human cervix carcinoma cells *in vitro*. Extracts of *S. officinalis* and *S. minor* were the most potent. **References:** [1] Silva, B.A. et al. (2005) Food Chem. 90: 157 – 167. [2] Liu, F. et al. (1997) Life Sci. 60: 763 – 771. [3] Clothier, R.H. (1995) The FRAME cytotoxicity test. Methods in Molecular Biology, 43: 109 – 118.

P 044

The quality standard of *Mammea siamensis* flowers for medicinal use

Noysang C[1], Woelkart K[1], Luanratana O[2], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University, Universitaetsplatz 4, 8010 Graz, Austria; [2]Faculty of Pharmacy, Department of Pharmacognosy, Mahidol University, 10400 Bangkok, Thailand

Mammea siamensis (Miq.) T. Anderson (local name Sarapi), Clusiaceae, is used in Thai indigenous medicine as a heart tonic. The *n*-hexane faction of *M. siamensis* flowers and subfractions thereof were reported to exhibit very strong cytotoxic effect against human CCRF-CEM leukemia cells [1]. The main compounds of *M. siamensis* flowers are coumarins like mammea A/AC **1**, mammea A/AC cyclo D **2**, mammea B/AC cyclo D **3**, mammea E/AC cyclo D **4**, mammea E/BA cyclo D **5**, mammea E/BC cyclo D **6**, mammea E/BD cyclo D **7**, mammea A/AA cyclo F **8** and mammea A/AC cyclo F **9** [2 – 4]. Pharmacognostic and chemical characterisation of the floral parts were performed for the quality control of the plant. Analytical investigations were performed to determine the constituents of the flowers of *M. siamensis* by using thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) on the basis of previously published spectral data [5]. By these investigations, it will be possible to control and improve the quality of *M. siamensis* flower raw materials for medicinal use.



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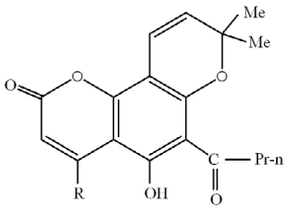

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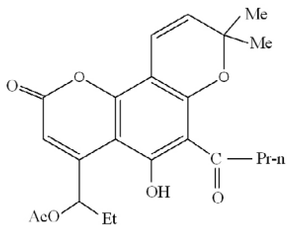
3 R = Pr-n



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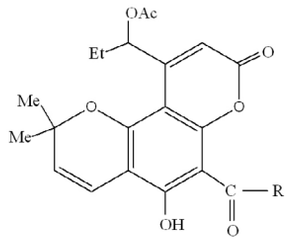
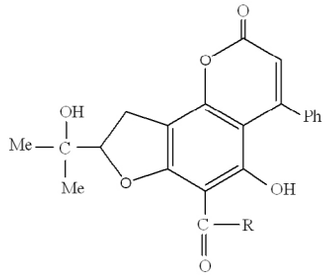


5 R = Bu-i
6 R = Pr-n
7 R = Pr-i



8 R = Bu-i
9 R = Pr-n

Acknowledgements: C.N. is thankful to Rajamangala University of Technology Thanyaburi for a Ph.D. scholarship and Faculty of Pharmacy, Mahidol University. **References:** [1] Noysang, C. et al. (2006) The 9th International Congress on Ethnopharmacology, Nanning, Guangxi, P.R. China [2] Mahidol, C. et al. (2002) J. Nat. Prod. 65: 757 – 60. [3] Phuwapraisirisan, P. et al. (2001) ACGC Chem. Res. Commun. 13: 28 – 32. [4] Thebtaranonth, C. et al. (1981) Phytochem. 20: 2305 – 6. [5] Yang, H. et al. (2006) J. Agric. Food Chem. 54: 4114 – 20.

P 045

Steroids from *Vernonia nigritiana* Oliv. & Hiern. with topical anti-inflammatory activity

Sosa S[1], De Tommasi N[2], Cioffi G[2], Merfort I[3], Della Loggia R[1], Tubaro A[1]
[1]Dipartimento dei Materiali e delle Risorse Naturali, Università di Trieste, Via A. Valerio 6, 34127, Trieste, Italia; [2]Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, 84084, Fisciano, Salerno, Italia; [3]Lehrstuhl für Pharmazeutische Biologie und Biotechnologie, Universität Freiburg, Stefan-Meier-Straße 19, 79104 Freiburg, Germany

Vernonia nigritiana Oliv. & Hiern. (Asteraceae) is a widely distributed plant of West Africa where is traditionally used against dermatoses, digestive insufficiency, fever, rheumatism and headache [1]. Previous studies revealed a topical anti-inflammatory activity of a chloroform extract from the aerial parts of *V. nigritiana*, containing stigmastane-type steroids [2]. Continuing the investigation of *V. nigritiana* constituents, nine polyhydroxylated stigmasterol glycosides (**1**-**9**) and six (**10**-**15**) polyhydroxylated stigmastanes having a $\Delta^{7(8),\,9(11),\,24(28)}$ -steroid cyclic system, were isolated. Their structure was elucidated by NMR, 1D-TOCSY, 2D-HOHAHA, COSY-DQF, HSQC, HMBC, 1D-ROESY and 1D-NOESY. The molecular formula of the compounds was confirmed by MS analysis. All the compounds were screened for their topical anti-inflammatory activity as inhibition of the Croton oil-induced ear oedema in mice [3]. Each compound provoked a significant oedema reduction, the most active being compounds **1** and **12** (ID_{50}= 0.10 and 0.21 μmol/cm^2, respectively). Their effect was only two and five fold lower than that of the steroidal drug hydrocortisone (ID_{50}= 0.04 μmol/cm^2). Compounds **2**-**11** and **13**-**15** provoked inhibitions in the range of 31 – 83% at the highest dose (0.5 μmol/cm^2). As reference, the parent compounds stigmasterol and stigmastanol (0.5 μmol/cm^2) were inactive. To clarify a possible mechanism of action, these compounds were evaluated for their ability to inhibit NF-κB, a transcription factor regulating the expression of inflammatory macromolecules. Studies, carried out in an electrophoretic mobility shift assay [4], showed a complete NF-κB inhibition induced by compounds **1** and **5** (50 μM), and a slight inhibition for compounds **6** (50 μM) and **8** (20 – 100 μM). **References:** [1] Igile, G. et al. (1995) J. Nat. Prod. 58: 1438 – 1443. [2] Cioffi G. et al. (2001) International Symposium of the Phytochemical Society of Europe. Lausanne, 12th-14th September 2001. [3] Tubaro A. et al. (1985) Agents Actions 17: 317 – 319. [4] Lyss G. et al. (1997) Biol. Chem. 378: 951 – 961.

P 046

Activity of plant wastes on acute phase and immune response in heifers

Sgorlon S, Colitti M, Farinacci M, Gaspardo B, Stefanon B
[1]Dipartimento Scienze Animali, Università di Udine, Italy

The effect on acute phase and immune response of a patented protected plant waste (PW), containing mainly terpenes (linalool, p-cimol, myrcene) has been evaluated in dairy heifers under ACTH challenge (AC). The experimental protocol involved 3 groups of 5 heifers that were fed the same basal diet. The CTR group received no PW integration, while experimental groups received 0.3 kg/head/day (LOW) or 0.9 kg/head/day (HIGH) of PW. After 21 days, all the heifers were treated with ACTH (0.5 mg/head of Synachten – Novartis – twice a day) from day 22 to day 26. Blood was sampled before (days 19 and 22) and during (days 24 and 26) AC, and analysed for cortisol, employing an immunoenzymatic kit, and for glucose, ceruloplasmin (Cp), haptoglobin (Hp) and Zn by an automatic analyser (ILab 600, Instrumentation Laboratory) according to Bertoni et al. [1]. Real Time PCR (Sybr® Green chemistry) was performed on total RNA isolated from whole blood, to analyse the transcriptional pattern of TNF-α, IFN-γ, IL-2 and IL-6. Biochemical data were analysed with a factorial model (GLM) with fixed effects for dose of PW (3 levels), ACTH treatment (2 levels) and their interaction (SPSS, [2]). Biomolecular data were calculated as relative expression (*n*-fold) compared to animals without PW integration. The *n*-fold variations before and during AC were analysed with T-test. In the CTR group, cortisol, glucose, Hp, Cp increased whereas Zn decreased ($P < 0.01$) after AC [3,4] as well as the proinflammatory cytokines. The increase of Hp after AC was lower ($P < 0.05$) in PW groups compared to the CTR group. The administration of high dose of PW (HIGH group) reduced Cp and increased Zn concentrations both before and during AC ($P < 0.01$). No relevant modifications of cytokines expression patterns were observed in relation to PW administration. These results show a positive effect of the administration of PW on the acute phase response, especially at the highest dose. *Aknowledgments*: The research was supported by SAFEWASTES, EU Project number 513949, Bruno Stefanon **References:** [1] Bertoni, G., Trevisi, E. (1998) Zoot. Nutr. Anim. 24: 17 – 29. [2] SPSS (1997) Advanced Statistics 7.5. SPSS Inc., Chicago, IL. [3] Arthington, J. D., Eicher, S. D. (2003) J. Anim. Sci. 81: 1120 – 1125 [4] Cebra, C. K., Heidel, J. R. (2003) J. Vet. Intern. Med. 17:902 – 7.

P 047

Biological study and flavonoids of *Pongamia pinnata* (L.) Pierre (Leguminosae)

Marzouk MA[1], Ibrahim MT[2], El-Gindi OR[2], Mahmoud M[3], Abou Bakr MS[2]
[1]Natural Products Group (Nobel Project), National Research Centre, El-Behoos St. 31, Dokki, Cairo, Egypt; [2]Department of Pharmacognosy, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt; [3]Department of Pharmacology, Theodor Bilharz Research Institute, Giza, Egypt

In two recent phytochemical studies [1,2], the flavonoides of *Pongamia pinnata* fruits were studied. Because of the broad structural and biological variation of *Pongamia* extracts [3 – 6], our study aimed at the isolation of flavonoids in the 70% aqueous methanol extract (AME) of *Pongamia pinnata* leaves and evaluation of its hypoglycaemic, anti-inflammatory, hepatoprotective and antioxidant activities. Successive column chromatographic separation has led to isolation of two new isoflavonoids, genistein 4'-*O*-methyl ether 7-*O*-β-D-rutinoside and 2',5'-dimethoxy-genistein 7-*O*-β-D-apiofuranosyl-(1'''→6'')-*O*-β-D-glucopyranoside and a new rotenoid, 12a-hydroxy-α-toxicarol, together with seven metabolites, vicenin-2, kaempferol 3-*O*-β-D-rutinoside, rutin, vitexin, isoquercitrin, kaempferol 3-*O*-β-D-glucopyranoside, 11,12a-dihydroxy-munduserone, kaempferol, and quercetin. The structures were established by UV, ESI-MS, 1D and 2D NMR [1 – 3,5,7]. As a conclusion for the biological studies on male Swiss Albino mice (18 – 20 g) infected with Egyptian *Schistosoma mansoni* 100 ± 10 *cercariae*/mouse [8] and rats (100 – 150 g), it was found that the AME is non-toxic to mice (LD_{50} up to 4 g.Kg^{-1} b.wt, the maximum soluble dose). At the two dose levels (100 and 200 mg Kg^{-1} b. wt.), it improved significantly the liver functions i.e reduced the elevated ALT and GGT serum levels in comparison with praziquantel and reduced the MDA and GSH levels in liver homogenate, in comparison with silymarin. Also, it showed significant hypoglycemic and anti-inflammatory activities at the dose levels of 50 and 200 mg Kg^{-1} b.wt, respectively. Moreover, a significant reduction of granuloma diameter and esenophilic counts was recorded through a histopathological investigation on treatment with AME (200 mg Kg^{-1} b. wt.), in comparison with mofebutazone. **References:** [1] Ahmed, G. et al. (2004). Phytochemistry 65: 921. [2] Yadav, P. P. et al. (2004). Phytochemistry 65: 439. [3] Sekine, T. et al. (1999). Phytochemistry 52: 87. [4] Ito, C. et al. (2004). Planta Med. 70: 585. [5] Laupattararakasem, P. et al. (2004). Planta Med. 70: 496.. [6] Ito, C. et al. (2004). J Ethnopharmacol. 105: 39. [7] Agrawal, P. K. (1989) Studies in organic chemistry 39,^{13}C NMR of flavonoids. Elsevier science, New York, pp. 283 – 364. [8] Liang, Y.S. et al. (1987) Proceeding of the first Sino-American Symposium, 1: 34.

P 048

Biological examination and novel biflavone di-*C*-glycosides from *Jatropha multifida* L. leaves

Moharram FA[1], Marzouk MS[2], Haggag EG[1], El-Batran S[3], Ibrahim RR[1]
[1]Pharmacognosy Department, faculty of Pharmacy, Helwan University, Helwan, Egypt; [2]Chemistry of Natural Products Group (Nobel Project) and [3]Pharmacology Department, National Research Centre, Dokki, Cairo, Egypt

Many *Jatropha species* have been used as local or popular remedies [1]. Some extracts and compounds of different *J.* species were found to have anti-inflammatory, bronchodilator of tracheal muscles, antiarraythmic [2], and antihypertensive activities [3]. Also, several research programs are directed towards certain *J.* species to identify their antitumor active diterpenes and lignans [4 – 7]. This study aimed at the identification of polyphenols of *J. multifida* leaves and examination of some biological activities. Three novel biflavone di-*C*-glycosides have been identified as 6,6"-di-*C*-β-D-$^4C_1$-glucopyranosyl-methylene-(8,8")-biapigenin (jatrophenol I, **7**), 3,6"-di-*C*-β-D-$^4C_1$-glucopyranosyl-methylene-(6,8")-biapigenin (jatrophenol II, **8**), and 6,6"-di-*C*-β-D-$^4C_1$-glucopyranosyl-methylene-(3,8")-biapigenin (jatrophenol III, **9**) on the basis of UV, HRESI-MS and NMR analyses. The total aq. 80% MeOH extract of *J. multifida* leaves was defatted under reflux with pet. ether (60 – 80 °C) and then fractionated on polyamide column using H_2O-MeOH mixtures starting with H_2O up to pure MeOH. The mean frs. were then subjected to repeated column chromatography on cellulose, sephadex LH-20 and or polyamide with different convenient solvent systems e.g. aq. MeOH or MeOH, *n*-BuOH saturated with H_2O, BIW (*n*-BuOH-IsoprOH-H_2O, 4:1:5, organic layer) or MeOH/Me_2CO-H_2O (1:1). Together with the novel biflavones seven phenolic compounds have been identified as apigenin 7-*O*-β-D-neohespredoside (**1**), ferulic acid (**2**), quercetin (**3**), vicenin-II (**4**), isoorientin (**5**) vitexin (**6**), and luteolin (**10**) on the basis of comparison with the published data [8]. The investigated extract and that of *J. integrima* were found to be non-toxic up to the maximum soluble dose (LD_{50} 4 g/Kg b wt). In a dose dependant manner (at 20, 40 mg/100 g b. wt) from the two extracts, significant analgesic and anti-inflammatory effects in comparison to indomethacine were recorded and showed significant hypotensive effect at the same doses, as well. **References:** [1] Baily, L.H (1953). The standard cyclopedia of harticulture. The Mcmillan Co., New york. [2] Ojewole, J.A.O. (1983). Fitoterapia 54: 153. [3] Abreu et al. (2003). Fitoterapia 74: 650. [4] Taylor, et al. (1983). J. Am. Chem. Soc. 105: 3177. [5] Endo, Y. et al. (1991). Tetrahedron Lett 32: 3083. [6] Ravendranath et al. (2003). Chem Pharm Bull 51: 870. [7] Das, B., Anjani, G. (1999). Phytochemistry 51: 115. [8] Harborne, J.B. (1984). Chapman & Hall Ltd, New York, pp. 49 – 50, 255.

P 049

The effects of flavonoids and phenolic acids on superoxide dismutase activity

Jasprica I[1], Medić-Šarić M[1], Šitum K[2], Marković S[2], Mornar A[1], Dumić J[3]
[1]Department of Medicinal Chemistry Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovaèića 1, 10000 Zagreb, Croatia; [2]GlaxoSmithKline Research Centre Ltd., Prilaz baruna Filipovića 29, 10000 Zagreb, Croatia; [3]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovaèića 1, 10000 Zagreb, Croatia

Flavonoids and phenolic acids are compounds ubiquitous in photosynthesizing organisms and many *in vitro* studies have demonstrated significant interferences with mammalian enzyme systems and signaling pathways. In this study, we investigated the effects of 35 flavonoids (flavones, flavonols, flavanones and isoflavonoids) and 9 phenolic acids on the activity of superoxide dismutase (SOD), which is one of the key enzymes in antioxidant defense system. All experiments were performed on differentiated THP-1 cell line (human macrophage-like cells). Cytotoxicity of chosen compounds was determined using bioluminiscent cytotoxicity assay by which the release of adenylate kinase from damaged cells was measured, while [6-H^3] thymidine incorporation assay was used to determine the effect of tested compounds on cell proliferation. The effects of chosen polyphenols in 30 μM concentration on SOD activity were measured 24 hours after the addition of compounds using RANSOD® antioxidant kit. Lipopolysacharide from *E. coli* was used as a positive and un-stimulated cells as a negative control. None of the tested polyphenols exhibited cytotoxic effects after 24 hours of incubation. Generally, flavones and flavonols suppressed the proliferation of differentiated THP-1 cells, flavanones and isoflavonoids exhibited mild inhibitory effects, while phenolic acids did not affect the proliferation. Among all tested compounds only morin suppressed SOD activity, since 25% inhibition comparing to the un-stimulated cells was found when the cells were exposed to this flavonol. The majority of the tested substances induced SOD activity (1.2 to 2 times comparing to the negative control) while acacetin, baicalein and diosmetin were shown to be powerful inducers of SOD since they enhanced the enzyme activity more than twofold.

P 050

Pelargonium sidoides for the treatment of acute bronchitis: a systematic review and meta-analysis

Jimoh TB, Guo R, Ernst E
Complementary Medicine. Peninsula Medical School, Universities of Exeter and Plymouth. 25 Victoria Park Road, Exeter. EX2 4NT, UK

Due to the limited success of antibiotics, cost, adverse effects and antibiotic resistance more attention is now focused on alternative medications for acute bronchitis, hence the search for effective herbal options. The aim of the study was to systematically assess the efficacy of *Pelargonium sidoides* preparations for the treatment of acute bronchitis. Systematic literature searches were conducted in Medline, Amed, Embase, Cinahl and Cochrane controlled trials register from inception up to April 2007 without language restrictions. Reference lists of retrieved articles were searched, and manufacturers contacted for published and unpublished materials. Randomized clinical trials (RCTs) testing *P. sidoides* extract against placebo or standard treatment in patients with acute bronchitis and assessing clinically relevant outcomes were included. Two reviewers extracted and validated relevant data independently. Methodological quality was evaluated with the Jadad score [1]. Six RCTs met the inclusion criteria, of which 4 (n = 1012) reported enough detail for inclusion in the meta-analysis. One study compared EPs®7630 against conventional non-antibiotic treatment (acetylcysteine); the other five studies tested EPs®7630 against placebo. All studies reported findings suggesting the effectiveness of *P. sidoides* in treating acute bronchitis. Meta-analysis of the four placebo- controlled RCTs suggested that EPs®7630 significantly reduced bronchitis symptom scores in patients with acute bronchitis by day 7 (weighted mean difference 2.80, 95% CI 2.44 to 3.15). No serious adverse events were reported. The overall quality of trials was good and reporting of outcomes consistent. There is encouraging evidence that *P. sidoides* may reduce the symptoms of acute bronchitis. Acknowledgements TBJ's research fellowship is funded by Dr Willmar Schwabe Pharmaceuticals. Karlsruhe, Germany. **References:** [1] Jadad, A.R et al. (1996), Assessing the Quality of Reports of Randomized Clinical Trials: Is Blinding Necessary? Control. Clin. Trials 17: 1 – 12.

P 051

Anti-inflammatory and analgesic activities of *Feijoa sellowiana* Berg. leaves and investigation of their phenolic constituents

El Dib RA[1], Moharram FA[1], Marzouk MS[2], El-Shenawy S[3], El-Sayed H[1]
[1]Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Helwan, 11795 Cairo, Egypt; [2]Chemistry of Natural Products Group (Nobel Project); [3]Pharmacology Department, National Research Centre, Dokki, 12311 Cairo, Egypt

Pineapple guava (*Acca* or *Feijoa sellowiana*) is a bushy shrub relative of the tropical guava [1,2]. There are many *Feijoa* industrialized pro-

ducts on the market in the form of jam, syrup, or crystallized fruits [2]. Previous few studies on the leaf, fruit and stem extracts of *F. sellowiana* showed antimicrobial, antitumoral, immunomodulatory and antioxidant effects [3 – 6]. However few phytochemical studies were reported on different parts of this plant [7,8], e.g. flavonoids [9], volatile components [10, 11], lipids [2] and tannins [12]. We report herein an extensive phytochemical study on the 70% methanol extract of the leaves of *F. sellowiana* cultivated in Egypt, in which twelve phenolic metabolites were isolated. The structures were elucidated as nilocitin (**1**), 4,6-hexahydroxydiphenoyl-β-D-$^4C_1$-glucopyranose (**2**), castalagin (**3**), ellagic acid (**4**), ellagic acid pentoside (**5**), ellagic acid β-D-$^4C_1$-glucopyranose (**6**), 3-methoxyellagic acid 3'-sulphate (**7**), trimethoxyellagic acid sulphate (**8**), methylflavogallonyl sulphate (**9**), avicularin (**10**), quercitrin (**11**) and hyperin (**12**), on the basis of chromatographic, chemical and spectroscopic evidences (UV, HRESI-MS and NMR). The investigated extract was orally non-toxic to mice up to 5 g/kg body weight (max. soluble dose). In a dose dependant manner (250, 500 and 1000 mg/kg b. wt.), it exhibited significant analgesic effect using both chemical and thermal stimulus. The extract showed significant anti-inflammatory effect by measuring the decrease in paw volume as compared to control. No pharmacological studies were performed on the isolated compounds. **References:** [1] Umberto Quattrocchi, F. (2000) CRC Word dictionary of plant names. CRC Press. New York. [2] Ruberto, G., Tringali, C. (2004) Phytochemistry 65: 2947. [3] Vuotto, M. et al. (2000) Int. J. Antimicrob. Ag. 13: 197. [4] Ielpo, M. et al. (2000) Fitoterapia 71: 101. [5] Castaldo-Cobianchi, R. (1997) Int. J. Antimicrob. Ag. 8: 199. [6] Koshimizu, K. et al. (1988) Cancer Lett. 39: 247. [7] Romero-Rodriguez, M. et al. (1994) Food Chem. 49: 23. [8] Romero-Rodriguez, M. et al. (1994) Food Chem. 49: 251. [9] Ferrara, L. et al. (1999) Cosmet. News 22: 392. [10] Binder, R., Flath, R. (1989) J. Agric. Food Chem. 37: 734. [11] Shaw, G. et al. (1989) Phytochemistry 28: 1529. [12] Okuda, T. et al. (1982) Phytochemistry 21: 2871.

P 052

Polysaccharides from Plantaginaceae and Brassicaceae: effects on innate immunity

Miron A, Lupusoru CE, Pavelescu M, Hancianu M, Stanescu U, Grigorescu E
"Gr. T. Popa" University of Medicine and Pharmacy, Universitatii Str. 16, Iasi, 700115, Romania

Many pathological states are characterized by a decrease in innate immunity [1]. In this respect, five raw water-soluble polysaccharides were investigated for the effects on innate immunity: PM, Pm and Pl – polysaccharides isolated from leaves of *Plantago major* L., *Plantago media* L. and *Plantago lanceolata* L., respectively, PB and PR – polysaccharides isolated from fresh pressed juice obtained from leaves of *Brassica oleracea* L. convar. capitata (L.) Alef. var. alba DC and roots of *Raphanus sativus* L. var. niger. The raw polysaccharides were administrated orally to normal mice in a dose of 50 mg/kg (PM, PM, Pl, PR) and 100 mg/kg (PB) for a seven day period. Levamisole (10 mg/kg) was used as positive control. The effects on innate immunity were assessed by evaluating serum opsonic capacity, phagocytic and bactericide activities of peritoneal macrophages, phagocytic activity of neutrophil granulocytes and hemolytic activity of seric complement [2]. PR was found to be the most active polysaccharide; its immunostimulating effects were comparable with those of levamisole. PR increased serum opsonic capacity by 20%, phagocytic and bactericide activities of peritoneal macrophages by 33.3% and 80.9%, respectively, phagocytic activity of neutrophils by 44.3% and decreased hemolytic activity of seric complement by 30.4%. The immunostimulating effects of tested polysaccharides partially justify therapeutical uses of plantain leaves, white cabbage leaves and black radish roots in traditional medicine. Besides, these polysaccharides could be used as complementary therapy in many diseases in which innate immunity is significantly decreased. **References:** [1] Romagne, F. (2007) Drug Discov. Today 12: 80 – 87. [2] Ghiciuc, C.M. et al. (2004) Ann. Pharm. Fr. 62: 43 – 48.

P 053

Bioassay-guided evaluation of anti-inflammatory and antinociceptive activities of *Rhododendron ponticum* L. leaves

Erdemoglu N[1], Küpeli E[1], Yesilada E[2], Çalış I[3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler 06330, Ankara, Turkey; [2]Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, Kayışdağı 34755, Istanbul, Turkey; [3]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Sıhhıye 06100, Ankara, Turkey

Aim: *Rhododendron ponticum* L. is used for the treatment of inflammatory diseases and to alleviate rheumatic pain and against toothache in Turkish traditional medicine. The present study was to evaluate the anti-inflammatory and antinociceptive effects of *R. ponticum* leaves using *in vivo* models, and isolation and chemical characterization of the biologically active constituents. **Methods:** Carrageenan-induced hind paw edema model was used for anti-inflammatory activity and *p*-benzoquinone induced abdominal contractions model for the antinociceptive activity. Bioassay-guided fractionation procedures were used for isolation and chemical characterization of the biologically active constituents. **Results:** The ethyl acetate fraction displayed marked anti-inflammatory (28.4 – 40.7% inhibition) and antinociceptive (50.7% inhibition) acitivity as compared to references. Flavonols [a mixture of hyperoside and isoquercitrin (**1**) and, quercitrin (**2**)] as well as one flavanon glycoside [6-C-glycosylnaringenin (**3**)] were isolated from ethylacetate fraction and their structures were elucidated by spectral techniques. In addition, the anti-inflammatory activity TPA-induced mouse ear edema model was employed for the isolated compounds. Compounds **1** and **2** showed a potent activity profile against all the *in vivo* models employed, while **3** was only possessed significant activity against carrageenan-induced edema. All flavones were investigated according to anti-inflammatory activity, compounds **1 – 3** were found to show significant inhibitory activity on carrageenan-induced edema, while except from compound **3** they did not display potent inhibitory activity on TPA-induced ear edema model. **Conclusion:** Results of the present study have supported the utilization of the plant in Turkish folk medicine and revealed that flavones are the major anti-inflammatory and antinociceptive principles of the plant.

P 054

Polyphenolic profile and *in vitro* immunomodulatory effect of Croatian native propolis

Vladimir-Knežević S[1], Marković S[2], Sreènik G[3], Kadija A[4], Kropek M[4], Kőszegi T[5], Kosalec I[6]
[1]Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry, University of Zagreb, Marulićev trg 20, HR-10000 Zagreb, Croatia; [2]Glaxo Smith-Kline Research Centre Ltd., Prilaz baruna Filipovića 29, HR-10000 Zagreb, Croatia;[3]Krka Farma d.o.o., DPC Jastrebarsko, Cvetkovići bb, HR-10450 Jastrebarsko, Croatia; [4]Belupo d.d., Danica 5, HR-48000 Koprivnica, Croatia; [5]University Medical School, Pécs, Ifjúság u. 13, H-7624 Pécs, Hungary; [6]Derpartment of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39, HR-10000 Zagreb, Croatia

Propolis, the resinous product collected by honey bees from various plant sources, has been found to have numerous biological activities which are mainly attributed to the presence of polyphenolic compounds. Our study aimed to evaluate the polyphenolic composition and *in vitro* modulation effect of propolis on cytokines production. Four samples of raw propolis originated from the continental region of Croatia were analysed by reversed-phase HPLC-DAD method. The content of flavonoids (chrysin, galangin, apigenin, kaempferol, luteolin and naringenin), phenolic acids (caffeic, ferulic and coumaric acids) and caffeic acid phenyl ester (CAPE) significantly varied among examined samples and were in a wide concentration range between 0.13 mg/g and 66.15 mg/g. Modulation of cytokine production in concavalin A-stimulated splenocytes from Balb/C mice by propolis ethanolic extracts (0.3 – 90 μg/mL), chrysin (0.1 – 30 μg/

mL) and CAPE (0.01 – 3 μg/mL) was evaluated using the enzyme-linked immunosorbent assay (ELISA). Treatment of cells with the propolis extracts (≥ 10 μg/mL) that contained high quantities of chrysin, galangin and CAPE significantly (85 – 100%) suppressed IL-4, lL-5, IL-10 and IFN-γ expression. Our results demonstrated that propolis affects the key messengers of immune response which play a very important role in the pathogenesis of immune system-associated diseases.

P 055

Inhibition of antigen-induced degranulation and IL-4 production from RBL-2H3 cells by *Lavandula hybrida* and *L. angustifolia* extracts

Blažeković B[1], Marković S[2], Vladimir-Knežević S[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry, University of Zagreb, Marulićev trg 20/2, HR-10000 Zagreb, Croatia; [2]Glaxo Smith-Kline Research Centre Ltd., Prilaz baruna Filipovića 29, HR-10000 Zagreb, Croatia

Allergic diseases are considered to be multifactorial inflammatory diseases that present a serious health problem worldwide while their prevalence have continuously increased during the past few decades. They are results of type I hypersensitivity mediated through the effects of antigen-specific IgE which binds to mast cells and, on subsequent cross-linking by allergen reexposure, causes cell degranulation with the release of preformed mediators in the early-phase as well as the production of cytokines in the late-phase. The aim of our study was to investigate the antiallergic activities of cultivated *Lavandula hybrida* Rev. and *L. angustifolia* P. Mill. using the rat basophilic leukaemia cell line (RBL-2H3), a well-characterised *in vitro* model of mast cells**.** The ethanolic extracts of flowers of *L. hybrida* (LHE) and *L. angustifolia* (LAE) were found to significantly inhibit release of β-hexosaminidase, the marker of antigen-IgE-mediated degranulation in RBL-2H3 cells, showing similar activities (IC_{50}= 37 μg/mL and IC_{50}= 42 μg/mL, respectively). Furthermore, using the enzyme-linked immunosorbent assay (ELISA), we established that LHE and LAE inhibit antigen-induced production of anti-inflammatory cytokine IL-4 (IC_{50}= 60 μg/mL and IC_{50}= 45 μg/mL, respectively). Among ten investigated principal bioactive compounds, luteolin, apigenin and ursolic acid were found to be the most potent inhibitors of degranulation as well as cytokine production. In conclusion, the tested extracts showed antiallergic effects acting on the both phases of type I reaction. Therefore, our results suggest a possible therapeutic application of *Lavandula* species in allergy and allergy-related diseases.

P 056

Brine shrimp cytotoxicity of tropical medicinal plants and the role of gum arabic

Taha A

Department of Chemistry, College of Science, University of Bahrain, P.O. Box 32038 Sakheer, Kingdom of Bahrain

Cytotoxicities of five medicinal plants, known in local tradition for their anti-inflammatory and anti-tumor properties, were evaluated against brine shrimp, *Artemia salina*. The medicinal plants tested were *Aristolochia brachteolata* (AB), *Acanthospermum hispidum* (AH), *Azadirachta indica* (AI), *Nerium oleander* (NO) and *Calatropis procera* (CP). Ethanol extracts of the plants showed a marked significant activity as shown by the values of $p \leq 0.05$ obtained using one-way analysis of variance (ANOVA). Their lethal concentrations (LC_{50}) were in the order of 36.9 μg/ml (for AI seeds), 50 μg/ml (for AI whole plant), 131.6 μg/ml (for CP latex), 135 μg/ml (for CP leaves), 159 μg/ml (for AH seeds), 166 μg/ml (for AH whole plant), 175.6 μg/ml (for CP whole plant), and 670 μg/ml (for NO whole plant). The role of gum Arabic (GA) in alleviating the cytotoxicities of these plants was investigated since studies have linked some of these plants, such as *Aristolochia* [1,2] with nephrotoxicity and GA diet supplementation with kidney malfunctioning symptoms reduction [3]. Our results show that the toxicity of these plants extracts and that of known toxic chemicals such as $CuSO_4$ (Cu-LC_{50} 2.88 ppm) and the fish poison rotenone (LC_{50} 1.23×10^{-2} ppm) have been significantly reduced by GA. Toxicity reduction ranged between 30% to 280%. **References:** [1] Steward, M.J. Steenkamp, V. (2001) Therap. Drug Monitor. 23: 698 – 706. [2] Vanherweghem JL,. et al.(1993) Lancet 341: 387 – 391. [3] Bliss, Z. et al. (1996) Am. J. Clin. Nut. 63: 392 – 398.

P 057

Free radicals scavenging activity of Mongolian endemic and Vietnamese medicinal plants

Batmunkh T[1], Juan QH[2], Nga DT[2], Kyung KE[1], Ah KY[3], Wan SY[3], Ah KK[2], Burm-Jong L[1,2]

[1]Department of Chemistry, [2]Biohealth Products Research Center, Inje University, Gimhae 621 – 749, [3]Division of Marine Environment and Bioscience, Korea Maritime University, Busan 606 – 791, Republic of Korea

In a purpose to study Vietnamese medicinal and Mongolian endemic plants exhibiting antioxidant activity against oxidative stress, we have prepared 50% ethanol extracts from air-dried 25 Mongolian plants and, water and dichloromethane fractions from 50% ethanol extracts of air-dried 14 Vietnamese plants. The samples have been screened for free radical scavenging activity *in vitro* assay systems: DPPH radical and NO radical scavenging assays. Interestingly, 50% ethanol extract of *Garcinia oblongifolia* (IC_{50}= 6 μg/ml) and its dichloromethane fraction (IC_{50}= 6.9 μg/ml), 50% ethanol extract of *Ficus racemosa* (IC_{50}= 6.9 μg/ml) among Vietnamese plants showed significant radical scavenging activities with the IC_{50} value lower than that of α-tocopherol (IC_{50}= 8.4 μg/ml) using as positive control. And, 50% ethanol extracts of *Limonium aureum* (IC_{50}= 39.6 μg/ml), *Thymus gobicus Tscern* (IC_{50}= 43.5 μg/ml), and *Rheum uninerve Maxim* (IC_{50}= 43.7 μg/ml) showed good radical scavenging activities. The result of NO scavenging activity showed that all 18 plants showed strong free radical scavenging activity in NO scavenging assay. The most significant activities were the ethanol extracts of *Asparagus gobicus* Ivanova ex Grubov (IC_{50}= 2.68 mg/ml), *Rheum uninerve* Maxim. (IC_{50}= 2.75 mg/ml), *Salsola collina* Pall. (IC_{50}= 31.3 mg/ml) in Mongolian samples and *Hiptage benghelensis* (L.) (IC_{50}= 1.67 mg/ml), *Gouania leptostachya* DC. (IC_{50}= 2.34 mg/ml), and *Asarum petelotii* O.C.Schmidt (IC_{50}= 2.63 mg/ml) in Vietnamese samples. Several Vietnamese plants already in therapeutic use might exert some of their effects as natural antioxidants. The majority of the tested Mongolian endemic and sub-endemic plants were never studied for bioactivity before. *Acknowledgement:* This study was supported partly Brain Korea 21 (BK21) fund of the Ministry of Education and by a grant from the Ministry of Commerce, Industry and Energy (MOCIE) and the Korea Institute of Industrial Technology Evaluation & Planning (ITEP) through the Biohealth Products Research Center (BPRC) of Inje University. **References:** [1] Nakagawa, T., Yokozawa, T. (2002) J. Food & Chem. Tox. 40: 1745 – 1750.

P 058

Cannabis tinctures and extracts – *in vitro* profiling for cytotoxic and anti-inflammatory effects

Peschel W[1], Politi M[1], Wilson N[1], Constanti A[2], Prieto Garcia JM[1], Heinrich M[1]

[1]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK; [2]Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK

Cannabis is frequently used as a co-medication by patients with cancer or chronic inflammatory diseases such as rheumatoid arthritis [1]. Accordingly, it has been shown that cannabinoids modulate apoptotic signalling, and the activity of pro-inflammatory factors [2]. The advantages of using whole plant preparations compared to pure cannabinoids, particularly in terms of less toxic side effects,

has also been reported [1]. Traditional Cannabis preparations (TCPs) such as tinctures (*e.g.* British pharmacopoeia 1932) may thus combine useful biological effects of cannabinoid and non-cannabinoid constituents. We therefore tested the ability of TCPs to produce cytotoxicity in an *in vitro* (MTT) assay and also to modulate the activation of Nuclear Factor kappa B (NF-κB, IL-6 reporter gene luciferase assay). Our results showed that TCP toxicity in cancer cells (HeLa, PC12) increased in proportion with the cannabinoid content of the starting material and decreasing solvent polarity. The LC15 of Cannabis tinctures were between 100 µl/ml for high polar extracts and low cannabinoid content and < 0.02 µl/ml for lipophilic extracts of high cannabinoid material. Crude extracts/tinctures had a moderate NF-κB-inhibiting effect at non-toxic concentrations in HeL*aluc* cells, whereas fractions thereof (specifically medium polar preparations) either activated or inhibited NF-κB more effectively at concentrations between 0.2 and 100 µg/ml. ^{1}H-NMR fingerprints with suppression of solvent signals reflected those effects distinguishing between five starting materials and the type extract. The ratio between major cannabinoid and phenolic constituents in TCPs as relevant marker for cytotoxic and anti-inflammatory effects is suggested. *Acknowledgements:* William Ransom & Son Plc, European commission (grant code COOP-CT-2004 – 512696). **References:** [1] Russo, E., Guy, G.W. (2006) Medical Hypotheses 66: 234 – 246. [2] Juttler, E. et al. (2004) Neuropharmacol. 47: 580 – 592.

P 059

Action of the herbal combination STW 5 in a model of 5-HT$_3$ receptor mediated ileal contraction *in vitro*

Abdel-Aziz H[1], Kelber O[2], Okpanyi SN[2], Weiser D[2], Khayyal MT[3]
[1]Al-Ahliyya Amman University, Faculty of Pharmacy and Medical Sciences, Al Salt Road, P.O.Box 183, 19328 Amman, Jordan; [2]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295 Darmstadt, Germany; [3]Depts. of Pharmacology, Faculty of Pharmacy, Cairo University, Kasr-El-Aini Street, 11562 Cairo, Egypt

The herbal combination STW 5 (Iberogast®) has shown to be effective against functional gastrointestinal diseases in several randomised controlled double blind studies. It is widely used in therapy for more than four decades, having a very favourable safety profile [1]. Recent experiments showed that STW 5 was able to decrease 5-HT mediated sensory nerve stimulation in the GIT [2] and that it has binding affinities to 5-HT$_4$ and to a lesser extent to 5-HT$_3$ receptors [3]. Our present study aimed to investigate the possible effects of STW 5 on 5-HT$_3$ receptors of the guinea pig ileum in order to further elucidate of its mechanisms of action. The response of the isolated guinea pig ileum to the specific 5-HT$_3$ agonist, SR 52772 (3 – 100 µM) was tested *in vitro* by measuring the contractions evoked by increasing doses of the agonist. The measurement of dose response relationship was repeated in the presence of different concentrations of STW 5, applied as an ethanol free lyophilisate, in the bath. At a bath concentration of 1 and 3 µg/ml, STW 5 induced a parallel shift of the dose response curve of SR 52772 to the right. The contraction induced by SR57227 (100 µM) was decreased by STW 5 to 77% (1 µg/ml) and to 51% (3 µg/ml), respectively. Effects were statistically significant and indicate an antagonistic effect of STW 5 on 5-HT$_3$ receptors. The results indicate a possible involvement of 5-HT$_3$ receptor antagonism in the gastrointestinal effects of STW 5 (Iberogast®). **References:** [1] Rösch, W., et al. (2006) Phytomedicine 113 S V 114 – 121. [2] Liu, C. et al. (2004) Neurogastroenterol.Motil. 16: 759 – 764. [3] Simmen, U. et al. (2006) Phytomedicine 13 S V:51 – 55

P 060

STW 5 is effective in an experimental model of esophagitis

Khayyal MT[1], Abdel-Aziz H[2], Wadie W[1], Okpanyi SN[3], Kelber O[3]
[1]Depts. of Pharmacology, Faculty of Pharmacy, Cairo University, Kasr-El-Aini Street, 11562 Cairo, Egypt; [2]Al-Ahliyya Amman University, Faculty of Pharmacy and Medical Sciences, Al Salt Road, P.O.Box 183, 19328 Amman, Jordan; [3]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295 Darmstadt, Germany

STW 5 (Iberogast®), a phytomedicine used in functional gastrointestinal diseases, has been shown to significantly alleviate symptoms of functional dyspepsia in several randomized controlled double blind studies [1], having a significant impact also on heartburn as a symptom [2]. Therefore the effect in esophagitis, which can be involved in the etiology of this symptom, was studied in a pharmacological model *in vivo*. STW 5, as a lyophilisate dissolved in water, was administered orally to male Wistar rats fasted for 18 h. 1 h later, animals were anesthesised and a ligation was formed between forestomach and corpus as well as between stomach and pylorus for induction of an inflammation of the esophageal mucosa. 4 h later, rats were sacrified and the inflamed area of the esophagus was measured. The gastric ligations led to a marked esophagitis, measurable as inflamed area of the esophageal mucosa. STW 5 (200 mg/kg b.w.) led to a significant reduction of the inflamed area to 30% of inflamed controls. The results indicate that the beneficial effect of STW 5 (Iberogast®) in heartburn as a symptom of functional dyspepsia could in part result from an anti-inflammatory effect on the mucosa of the esophagus. **References:** [1] Gundermann, K.J. et al. (2004) MMW-Fortschr. Med. 146: 33/34. [2] Kelber, O. et al. (2006), Short Lecture, DPhG Annual Joint Meeting, Marburg, Germany

P 061

Anti-inflammatory effects of *Populus tremula, Fraxinus excelsior, Solidago virgaurea* extracts and their combination Phytodolor® in human monocytes

Bonaterra G[1], Kinscherf R[1], Kelber O[2], Weiser D[2], Metz J[1]
[1]Anatomy and Cell Biology, University of Heidelberg, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany; [2]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295 Darmstadt, Germany

Populus tremula, Fraxinus excelsior and *Solidago virgaurea* extracts are components of Phytodolor® (STW 1), a phytomedicine used in the therapy of painful inflammatory diseases especially of rheumatic origin [1,2]. Aim of this study was to analyse the effects of these components and their combination in the regulation of inflammatory processes in activated human monocytes. Monocytes from buffy coats of healthy human donors were isolated by Histopaque-density gradient centrifugation and adhesion. The monocytes were preincubated for 90 min with 0.05, 0.1 or 0.2% of the respective extract, Phytodolor® or 30 µg/ml diclofenac. Thereafter they were incubated in serum-free RPMI 1640 medium with interferon-gamma (INF-γ; 50 U/ml; 45 min) and lipopolysaccharide (LPS; 1 µg/ml) for 5 to 48 hours. Apoptosis of monocytes (YO-PRO-1 staining), gene (real time PCR) and protein (Cell Elisa) expression of cyclooxygenase-2 (COX-2) and tumor necrois factor-alpha (TNF-α) were analyzed. *Populus tremula* and Phytodolor® inhibited the increase of survival time of monocytes by IFN-gamma/LPS significantly and concentration dependently. The increase of pro-inflammatory gene and protein expression of COX-2 and TNF-α in activated monocytes was significantly reduced by Phytodolor® and its component extracts. Effects were comparable to diclofenac, which was used as reference. The observed inhibition of pro-inflammatory effects by Phytodolor® and its components may be also involved in the analgetic effect of this phytomedicine. The results allow the conclusion on a supraadditive, synergistic effect of the three components. **References:** [1] Klein-Galczinsky, C. (1999) Wien. Med. Wochenschr. 149: 248 – 253. [2] Gundermann, K.J., Müller, J. (2007) subm. for publ. in Wien. Med. Wochenschr.

P 062

Effect of *Achillea millefolium* L. extract, fractions, and isolated compounds on inflammatory cytokine production in LPS-stimulated human mononuclear cells

Baldia AM[1], Benedek B[2], Kopp B[2], Butterweck V[1]
[1]Department of Pharmaceutics, College of Pharmacy, University of Florida, POBox 100494, Gainesville, FL 32610, USA; [2]Department of Pharmacognosy, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Yarrow (*Achillea millefolium* L.) is traditionally used not only for the treatment of gastro-intestinal and hepato-biliary disorders, but also as an antiphlogistic drug [1]. The production of cytokines (e.g. IL-1, IL-6) is associated with the inflammatory process and plays a role in the genesis of rheumatoid arthritis and other inflammatory diseases [2]. The present study was undertaken to determine whether yarrow has an effect on the interleukin (IL)-6 secretion using a human pro-monocytic cell line (U937). PMA-differentiated cells were incubated with or without different concentrations of the extract and fractions (10, 1, 0.1, 0.01 µg/ml) or the positive control diclofenac (10 µg/ml), previous to stimulation with 1 µg/ml LPS. After designated time points IL-6 levels were measured in supernatants using enzyme-linked immunosorbent assays (ELISA). Yarrow extract (0.1 µg/ml) sigificantly decreased the expression of IL-6 (23% inhibition vs. control), as did a caffeic acid (15% inhibition vs. control) and a flavonoid fraction (13% inhibition vs. control) obtained thereof (1 µg/ml, respectively); the effects of the extract were comparable to those of diclofenac (28% inhibition vs. control). The role of isolated pure compounds from yarrow on IL-6 secretion need to be further elucidated. Although additional studies are needed to clarify the mode of action of yarrow and to demonstrate a causative relationship between the inhibition of cytokine/chemokine secretion in cell culture and the reported clinical effects of the plant, our *in vitro* results offer a possible mechanism for the anti-inflammatory effects of yarrow observed in clinical use. **References:** [1] Benedek, B. (2007) Dissertation, University of Vienna. [2] Nishimoto, N., Kishimoto, T. (2006) Nat. Clin. Pract. Rheumatol. 2: 619 – 626. [3] Gabay, C. (2006) Arthritis. Res. Ther. 8 (Suppl 2): S3

P 063

Diverse effects of docetaxel on inducible nitric oxide production in alveolar macrophages

Wakabayashi I[1,2], Saito F[2], Matsusaka S[2], Takahashi Y[2], Poteser M[3], Groschner K[3]
[1]Department of Environmental and Preventive Medicine, Hyogo College of Medicine, Hyogo 663 – 8501, Japan; [2]Department of Environmental and Preventive Medicine, Yamagata University School of Medicine, Yamagata 990 – 9585, Japan; [3]Institute of Pharmaceutical Sciences, Pharmacology and Toxicology, University of Graz, Austria

Docetaxel is a derivative of paclitaxel (taxol), a diterpine plant compound that was isolated initially from the bark of the western yew tree, *Taxus brevifolia*, and is currently used as a fundamental drug in the treatment of a variety of solid cancers. The anti-tumor efficacy of paclitaxel has been attributed in part to modulatory effects on the generation of inflammatory mediators, in particular NO, in macrophages and tumor cells. The purpose of this study was to investigate the effects of docetaxel on lipopolysaccharide (LPS)-induced nitric oxide (NO) synthesis in alveolar macrophages isolated from rats. LPS-induced NO production and inducible NO synthase (iNOS) expression were significantly enhanced in the macrophages isolated from rats injected intraperitoneally with docetaxel (4 mg/kg body weight per day for 5 consecutive days) compared with those in macrophages from control rats (vehicle administration). I*n vivo* administration of docetaxel augmented LPS-induced p38 activation but not extracellular signal-related kinase (ERK) activation in isolated macrophages. By contrast, *in vitro* treatment of macrophages with docetaxel (5 and 10 microg/ml) inhibited LPS-induced NO production and iNOS expression. Thus, *in vitro* effects of docetaxel on NO generation of macrophages do not explain its *in vivo* effects. Release of lactate dehydrogenase (LDH) from macrophages was neither affected by *in vitro* treatment with docetaxel (up to 10 microg/ml) nor by its *in vivo* administration. These results suggest that docetaxel exerts diverse actions on LPS-induced iNOS expression in alveolar macrophages. We suggest that the *in vivo* action of docetaxel is mediated by stimulation of p38 activity.

P 064

Exploration of natural and synthetic *N*-alkyl amides as source for new cannabinoid receptor type-2 (CB_2) selective ligands

Gertsch J, Raduner S, Chicca A, Feyen F, Altmann KH
Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland

We recently reported that certain anti-inflammatory *N*-alkyl amides from purple coneflower (*Echinacea* spp.) constitute a new class of cannabinomimetics, which selectively bind to and activate the cannabinoid type-2 (CB_2) receptor [1]. In the present study, we have investigated whether chain length and substitution of the headgroup of this class of natural products can result in new compounds with nM affinities to CB2 receptors. More than 30 *N*-alkyl amide derivatives were synthesized. A comparison of the preliminary structure-activity relationship of *N*-alkyl amides with the endogenous cannabinoid arachidonoyl ethanolamine (anandamide) clearly indicates that these compounds are different pharmacophores. Moreover, unlike anandamide, *N*-alkyl amides and 2-arachidonoyl glycerol (2-AG) trigger CB2-receptor dependent intracellular calcium transients in myelo-monocytic cells. In dodecanoic acid derivatives, the 2*E*,4*E* double bonds were found to be crucial for optimal binding to CB2 while only the 2*E* double bond appears to be required for the moderate CB1 affinity. The most active compounds were isobutylamides (Ki ~ 60 nM). We show that certain derivatives segregate and form micelles, which are no longer able to interact with the receptor. Thus, the self-assembling of these compounds directly influences CB2 affinity. Based on the dodeca-2*E*,4*E*-diene chain a fluorescent nitrobenzooxadiazole ligand was synthesized, which selectively binds to CB2 (Ki ~ 1.5 µM). Biological characterization suggests that this compound could be a valuable tool to study CB2 receptor localization and as fluorescent ligand for displacement studies. Since analogs of palmitoylethanolamide have been shown to inhibit the uptake and degradation of [^{3}H]-anandamide we further tested the *N*-alkyl amide derivatives on [^{3}H]-anandamide uptake into HL60 cells and metabolism by fatty acid amide hydrolase (FAAH). Overall, *N*-isobutyl amides represent interesting lead structures for the development of anti-inflammatory drugs and may also serve as tool compounds for cannabinoid research. **References:** [1] Raduner, S. et al. (2006) J. Biol. Chem. 281, 14192 – 206.

P 065

Gingerol derivatives modulate T-cell receptor mediated effects in a whole blood assay and in T-cells via 5-HT1A receptor-dependent mechanisms

Nievergelt A[1], Schoop R[2], Altmann KH[1], Gertsch J[1]
[1]Department of Chemistry and Applied Biosciences, ETH Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland; [2]A.Vogel Bioforce AG, CH-9325 Roggwil, Switzerland

The rhizome of ginger (*Zingiber officinale* Roscoe) is widely used for digestive complaints, emesis, as well as inflammatory diseases, such as arthritis. Gingerol-derivatives have been reported to inhibit prostaglandin production, to indirectly modulate 5-HT3 receptor signalling, and also to influence calcium homeostasis. In order to assess the immunomodulatory potential of ginger we have profiled the effects of different ginger extracts in differentially stimulated whole blood. The model is more stable than assays with purified cells and the read-out is meaningful with regard to physiological function. Our experiments show that different ginger extracts (10 – 50 µg/ml) and isolated pungent compounds specifically inhibit cytokine pro-

duction in human whole blood after treatment with CD3 antibody, indicating a T-cell mediated process. Ethanolic and CO_2 ginger extracts (10 – 50 μg/ml) significantly inhibited CD3-stimulated Ca^{2+} release from intracellular stores in Jurkat T-cells, whereas a series of pure compounds showed similar, stimulatory, or no effects. To reduce these findings to a possible common denominator we postulated an effect on serotonin receptors as one possible mechanism. Serotonin can reach μM concentrations in whole blood and is known to be an important modulator of the immune response. Binding studies on the human 5-HT1A and 5-HT3 receptors led to the identification of nine compounds (8-; 10-gingerol, 6-; 8-; 10-shogaol, 1-dehydro-6-, 8-; and 10-gingerdione, as well as 6-dihydroparadol) which showed selective binding affinity to the serotonin binding site in 5HT1A receptors (K_i values = 3 to 20 μM). Moreover, ginger extracts inhibited the stimulatory effect of serotonin on CD3-stimulated calcium release in Jurkat cells. This is the first report on the immunomodulatory effects of gingerol-derivatives mediated, at least in part, via 5HT1A receptor interaction.

P 066

Comparative antioxidant evaluation of the essential oils from chamomile's commercial samples

Cruz C[1], Dandlen SA[1], Miguel MG[1], Simões MTF[2], Figueiredo AC[2], Barroso JG[2], Pedro LG[2]
[1]Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8005 – 139 Faro, Potugal; [2]Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749 – 016 Lisbon, Portugal

Aromatic plants such as *Matricaria recutita* L. (Syn. *Chamomilla recutita* L. Rauch) and *Anthemis nobilis* L. (Syn. *Chamaemelum nobile* L. All.) are popular medicinal agents used as anti-inflammatories, mild sedatives and anti-ulcer remedies [1]. Both species belong to the Asteraceae family and are known as German chamomile and Roman chamomile, respectively, and many times sold under the generic name chamomile, together with other related species. In the present work, the essential oils, isolated from chamomile commercial samples (*Matricaria recutita* or mixture of *Chamaemelum mixtum and C. fuscatum*), were evaluated for prevention of lipid peroxidation, using the TBARS method, scavenging the hydroxyl radical and the stable radical 2,2-diphenyl-1-picryl-hydrazyl and for inhibition of 5-lipoxygenase. The percentage of antioxidant activity was calculated using methanol as negative control. α-Tocopherol was used as positive control, for TBARS and DPPH, manitol for scavenging the hydroxyl radical method and nordihydroguaiaretic acid (NDGA) for inhibitory activity of 5-lipoxygenase. The chemical composition of these oils was analysed by GC and GC/MS. Apart from the difference in oil colour obtained from the *M. recutita* sample (blue) and mixture of *Chamaemelum mixtum and C. fuscatum* (yellow), these oils showed also marked differences in their composition. The main component of *Chamaemelum* mixture oils was a yet unidentified component whereas α-bisabolol oxide dominated *M. recutita* oils. These oils possess a low ability from preventing lipid peroxidation. The maximum percentage found for *Matricaria* and the *Chamaemelum* oils was 49 % at 50 mg/L and 20 % at 250 mg/L, respectively. At higher concentrations these oils seemed to possess a pro-oxidant activity. The IC_{50} found, with the DPPH method, for *M. recutita* oil was 950 mg/L, while for the *Chamaemelum* mixture the parameter was not determined because the highest percentage was only 2 % at the maximum concentration tested (1500 mg/L). The oils' best ability to scavenge OH radicals was 51 %, at 50 mg/L, for *M. recutita* oil and 60 %, at 25 mg/L, for the *Chamaemelum* mixture oil. Considering the concentration tested (500 mg/L), the inhibitory activity of 5-lipoxygenase of *M. recutita* oil (42 %) was significantly better than that of the *Chamaemelum* mixture (19 %). *Acknowledgements:* This study was partially funded by IFADAP under research contract AGRO 800. **References:** [1] Wang, Y. et al. (2005) J. Agric. Food Chem. 53: 191 – 196. [2] Ma, C.M. et al. (2007) Phytochem. Anal. 18: 42 – 49.

P 067

Screening of Chinese herbal drugs for inhibition of NF-κB1 expression

Gusenleitner S, Bauer R
Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens University, Universitätsplatz 4/I, 8010 Graz, Austria

Nuclear factor kappa B (NF-κB) is an inducible and ubiquitously expressed transcription factor for genes involved in cell survival, inflammation, differentiation, cell adhesion and growth. As an activator of many pro-inflammatory cytokines and inflammatory processes the inhibition of NF-κB is a principle target to alleviate the symptoms of such diseases as arthritis, inflammatory bowel disease and asthma [1]. We now tested the activity of 18 Chinese herbal drugs for inhibition of NF-κB1 expression. n-Hexane, dichloromethane, methanol and water extracts were prepared with Accelerated Solvent Extraction (ASE 200, Dionex, Austria). For *in vitro* screening THP-1 cells were incubated with 20 μg/ml of extract and 100 ng/ml LPS. After the incubation total RNA was extracted and reverse transcribed into cDNA. The expression of mRNA of NF-κB1 was then determined with quantitative real-time PCR using the ΔΔCt-method. As a positive control 15 μM Parthenolide and 20 μg/ml n-hexane extract of *Tanacetum parthenium* L. Schultz-Bip. were applied. The average inhibition of the expression of NF-κB1 by parthenolide was 86% and by *Tanacetum parthenium* 37%. The hexane extract of *Equisetum hiemale* L., the dichloromethane extracts of *Cinnamomum cassia* Presl & Blume, *Rubia cordifolia* L. and *Tribulus terrestris* L. as well as the methanol extracts of *Notopterygium incisum* Ting ex Ho-t.Chang and *Tribulus terrestris* showed approximately the same or a slightly higher activity as the extract of *Tanacetum parthenium*. Only the hexane extract of *Cinnamomum cassia* possessed a higher activity of 70% inhibition of the expression of NF-κB1. **References:** [1] Bremner, P., Heinrich, M. (2002) J. Pharm. Pharmacol. 54: 453 – 472

P 068

In vitro COX-1, COX-2 and 5-LOX inhibitory activity of rose hips (Rosae pseudofructus sine fructibus)

Wenzig EM[1], Widowitz U[1], Kunert O[2], Bauer R[1], Chrubasik S[3]
[1]Institute of Pharmaceutical Sciences, Dept. Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria; [2]Institute of Pharmaceutical Sciences, Dept. Pharmaceutical Chemistry, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria; [3]Institute of Forensic Medicine, University of Freiburg i. Br., Albertstr.9, 79104 Freiburg i. Br., Germany

Rose hip with or without fruits (Rosae pseudofructus cum/sine fructibus, *Rosa canina* L., Rosaceae) is traditionally used for the prevention and therapy of common cold and other infections, as a diuretic agent, for the treatment of gout and rheumatic diseases and as a vitamin C source. For none of these indications clinical evidence of effectiveness has been demonstrated except for osteoarthritis: A proprietary rose hip and seed powder had moderate evidence of effectiveness in alleviating osteoarthritic complaints [1]. Little is known about the rose hip mechanism of action and active constituents. Aim of this study was to get information on the constituents responsible for the anti-inflammatory potential of rose hip peel. The rose hip fine powder (batch 119372, supplier Martin Bauer GmbH & Co. KG, Germany) was subsequently extracted with hexane, dichloromethane, methanol and water. The extracts were screened for *in vitro* inhibition of cyclooxygenase (COX)-1, COX-2 and 5-lipoxygenase (5-LOX), three key enzymes of the arachidonic acid metabolism, which play an important role in inflammatory processes [2,3]. While the aqueous and the methanolic extract turned out to be inactive, the hexane and the dichloromethane extract showed to possess inhibitory activity against all three enzymes. Phytochemical analysis of the lipophilic extracts revealed the presence of triterpene acids (ursolic acid, oleanolic acid, betulinic acid) along with fatty acids and glycolipids. One of these glycolipids from rose hip has already been described as a chemotaxis inhibitor in human neutro-

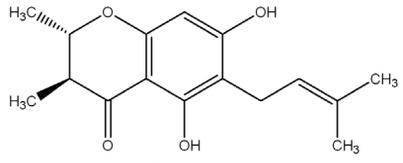
phils [4], and the triterpene acids are known to possess COX- and LOX- inhibitory potential [5]. Our results confirm that rose hip peels contain an anti-inflammatory principle. It remains to be established to what extent individual constituents contribute to the overall anti-inflammatory effect. **References:** [1] Chrubasik, C. et al. (2006) Phytother. Res. 20: 1 – 3. [2] Fiebich, B.L. et al. (2005) Planta Med. 71: 12 – 19. [3] Adams, M. et al. (2004) Planta Med. 70: 904 – 908. [4] Larsen, E. et al. (2003). J. Nat. Prod. 66, 994.995. [5] Diaz, AM et al. (2000) Biol. Pharm. Bull. 11, 1307 – 1313.

P 069

Anti-inflammatory Phloroglucinol and Terpenoid Derivatives from Clusiaceae

Crockett SL[1], Wenzig EM[1], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, 8010 Graz, Austria; [2]Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-University Graz, 8010 Graz, Austria

During a Lise-Meitner-Program research project, bioassay-guided fractionation of species belonging to Clusiaceae (Mammey Apple Family) was conducted to isolate new bioactive substances. Several species of *Hypericum* L. (St. John's Wort) have records of traditional use in topical applications for reducing inflammation and wound-healing [1]. Therefore, lipophilic extracts of 9 species of *Hypericum* (all belonging to tribe Hypericeae) were examined. In addition, lipophilic extracts from *Triadenum walterii* (tribe Hypericeae) and *Clusia valerioi* (tribe Clusieae) were studied. Results of anti-inflammatory bioassays for several isolated compounds against COX-1, COX-2, and 5-LOX, enzymes implicated in inflammation response, indicated that a new phloroglucinol derivative (**1**) from *H. lissophloeus* had the highest activity against COX-1 (IC_{50} 9.5 uM) and a known xanthone (**2**) and phloroglucinol derivative (**3**) from *H. canariense* displayed the highest activity against COX-2 (IC_{50} 16.9 uM) and 5-LOX (IC_{50} 0.3 uM), respectively.



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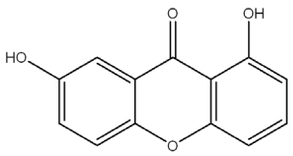

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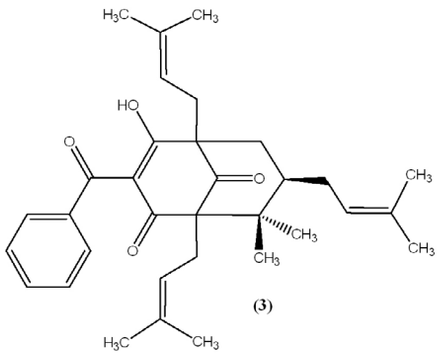

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Acknowledgements: Thanks go to Dr. Wolfgang M. Schühly for help collecting material of several *Hypericum* species in the field in the southeastern United States. Katrina Dlugosch (University of California, Santa Cruz) is thanked for *H. canariense* material. The Austrian Science Foundation (FWF) is sincerely thanked for their financial support (Project M844-B05). **References:** [1] Ernst, E. (Ed.) (2003) Hypericum: The Genus Hypericum, Taylor & Francis, London/New York.

P 070

Screening of Herbal Drug extracts for inhibitory activity on inducible nitric oxide synthase (iNOS)

Blunder M[1], Schühly W[1], Schmidt K[2], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University, Universitätsplatz 4, 8010 Graz, Austria; [2]Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, Karl-Franzens-University, Universitätsplatz 2, 8010 Graz, Austria

The inhibition of the inducible nitric oxide synthase (iNOS) turned out to be an important aspect in the field of anti-inflammatory research. Overproduction of nitric oxide (NO) induced by the enzymatic activity of iNOS in various cell types plays a role in several inflammatory and also immunoregulatory processes. [1] In continuation of our work on anti-inflammatory herbal drugs, we herein present the results of a screening of a series of plant extracts. The plants were selected particularly with regard to their use in Traditional Chinese Medicine (TCM) as anti-inflammatory agents. The assay to elucidate the effects on inducible nitric oxide synthase (iNOS) involves the macrophage cell line RAW 264.7. Cells were stimulated with lipopolysaccharides (LPS) and interferon-gamma in presence or absence of the respective n-hexane, dichloromethane, acetone/diethylether, methanol and water extracts. The iNOS effects were determined by measuring nitrite release which is an indicator for the activity of iNOS in the cell culture supernatants by utilization of the Griess assay method. [2, 3] Within our screening, several extracts showed promising results. The acetone/diethylether extracts from *Asarum europaeum* L. (Aristolochiaceae) and *Minquartia guianensis* Aubl. (Olacaceae) inhibited the NO production with an IC_{50} value of ~10 µM and the acetone/diethylether extract from *Cynara scolymus* L. (Asteraceae) with an IC_{50} in the range of ~3 µM. Extracts with similar effects were selected for further investigations. *Acknowledgements:* The authors thank Margit Rehn (Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, Karl-Franzens-University) for the help in establishing the test sytem. **References:** [1] Clancy, R.M. et al. (1998) Arthritis & Rheumatism 41: 1141 – 1151. [2] Baer, H.P., Schmidt, K. et al. (1995) Life Sci. 57: 1973 – 1980. [3] Dirsch, V.M. et al. (1998) Planta Med. 64: 423 – 426.

P 071

Cytotoxic and anti-inflammatory activities of *Luffa cylindrica (loofa sponge)*

El-Gengaihi SE, Abd El-Hamid SR, Kamel AM
Department of Medicinal and Aromatic Plants, National Research Center, Dokki, postal code: 12311, Cairo, Egypt

Luffa cylindrica (L.) Roem. cultivated in Egypt is known to contain cucurbitacins and saponins. The cucurbitacin content was determined in *L. cylindrica*, herb and fruits and found to be 3.9% and 0.92% respectively. Different extracts were evaluated for their cytotoxic and anti-inflammatory actions. Petrolether and alcohol extracts of *Luffa* were tested for their inhibitory effects on growth of human (MCF7) breast, (H2Pg2) liver and (H460) lung cancer cell lines. It was found that both extracts of herb and fruits showed the highest activity against MCF7, with an IC_{50} value of 8.2 µg/ml for petrolether extract of herb and fruits. On the other hand, the alcohol extract of *Luffa* herb and fruits revealed an IC_{50} value of 7.4 µg/ml and 7.5 µg/ml respectively. In addition, the petrolether extract of *Luffa* fruits exhibits a significant inhibition of carrageenan- induced paw edema in rats which reached 13.7, 62, 63.1 and 59.9% at 1, 2, 3 and 4 h after carrageenan injection.

P 072

Pelargonium sidoides root extract EPs® 7630 stimulates release of antimicrobial peptides from neutrophil granulocytes in human whole blood

Koch E, Wohn C
Preclinical Research, Dr. Willmar Schwabe GmbH & Co. KG, PO Box 410925, 76209 Karlsruhe, Germany

EPs® 7630 is an aequous-ethanolic extract from roots of *P. sidoides* DC. widely used for the treatment of acute bronchitis as well as other ear, nose and throat infections. The pharmacological action of EPs® 7630 includes antimicrobial effects, although the antibacterial potential of EPs® 7630 is weak compared with antibiotics. Thus, it is considered that its rapid therapeutic efficacy is primarily mediated via stimulation of innate immune mechanisms rather than direct microbiocidal properties. A key cellular component of the innate immune response is the neutrophil granulocyte, whose cytoplasmic granules contain a variety of antimicrobial proteins and peptides. Among these is the bactericidal/permeability-increasing protein (BPI) and the defensins. These peptides possess broad antimicrobial activity against bacteria, fungi and some viruses, but also exert chemotactic, immunomodulating and wound healing action. The three principal human α-defensins, human neutrophil peptide (HNP) 1 – 3, account for about 99% of the total defensin content of these cells. Because of the great importance of BPI and HNP for the host defence against infections, it was the aim of the present study to evaluate if EPs® 7630 has an effect on the release of antimicrobial peptides from neutrophils. Investigations were performed with heparinized whole human blood from each 2 male and female donors. Following addition of EPs® 7630 at concentrations between 0.3 and 30 µg/ml samples were incubated for 5 h, then the plasma was collected and the content of BPI and HNP 1 – 3 was analyzed using commercial ELISA kits. EPs® 7630 concentration-dependently increased the release of HNP 1 – 3 by up to 150% (30 µg/ml) displaying a higher efficacy as lipopolysaccharide (LPS) (+82%, 10 ng/ml). In contrast, release of BPI was much stronger stimulated by LPS (+356% at 10 ng/ml) than by EPs® 7630 (+127% at 30 µg/ml). If a combination of LPS (10 ng/ml) and EPs® 7630 (30 µg/ml) was applied, release of both groups of antimicrobial peptides was enhanced in an overadditive manner by up to 531 and 294% for BPI and HNP 1 – 3, respectively. These results demonstrate that EPs® 7630 stimulates the innate host defence by an enhanced release of antimicrobial peptides providing a further rational for its use in the treatment of respiratory tract infections.

P 073

Impact of Selected Botanicals on HT-29 Cell Proliferation & Cyclooxygenase II

Luther M[1], Moore J[1], Cheng Z[1], Yu L[1], Charles DJ[2]
[1]Department of Nutrition and Food Science, University of Maryland, Room 0112 Skinner Bldg., College Park, MD 20742; [2]Department of Research and Development, Frontier Natural Products Co-op, 3021 78th Street, Norway, IA 52318

The aim of this study was to evaluate black peppercorn, nutmeg, rosehip, cinnamon, and oregano leaf for their potential antiproliferative activities and cyclooxygenase-II (COX-II) inhibitory capacities, and total phenolic contents (TPC). The 50% acetone extracts of all the tested botanicals were able to dose dependently suppress HT-29 human colon cancer cell proliferation over a four day period. The 50% acetone extract of cinnamon exhibited the strongest antiproliferative activity against HT-29 colon cancer cells under the experimental conditions, and was followed by the 50% acetone extracts of nutmeg, oregano, black peppercorn and rosehips. At a final concentration of 0.38, 0.75, and 1.5 mg botanical equivalents/ml, cinnamom extract resulted in 47, 84, and 97% inhibition on cell proliferation after a four day treatment, respectively. To assess the possible anti-inflammatory actvity of the botanicals their COX-II-inhibitory actvities were also examined. Black peppercorn had the highest COX-II inhibitory capacity at 2063.3 mg aspirin per g botanical followed by oregano, rosehips, cinnamon, and nutmeg. Cinnamon extract exhibited the highest TPC value of 18.6 mg gallic acid equivalents (GAE) per gram of botanical followed by that of 5.5, 5.1, 2.6 and 1.3 GAE/g for oregano, leaf, rosehips, nutmeg, and peppercorn, respectively. These data indicate that these botanicals contain significant levels of potential antiproliferative and anti-inflammatory components, suggesting their possible utilization in improving human health.

P 074

In vivo and *in vitro* evaluation of anti-inflammatory activity and cytotoxicity of extracts of seven *Plectranthus* species

Minker C[1,2], Sheridan H[1], O'Meara J[1], Visdal Johnse L[1], Hook I[1], Lobstein A[2], Frankish N[1]
[1]School of Pharmacy and Pharmaceutical Sciences Trinity College Dublin, Dublin, Ireland; [2]Pharmacochimie de la communication cellulaire et moléculaire (UMR 7175), Faculté de Pharmacie, Université Louis Pasteur, Strasbourg, France

Plectranthus (Lamiaceae) is a genus of economic and medicinal interest distributed in tropical regions throughout the world [1]. The first phytochemical review of *Plectranthus* [2] showed that the main constituents of the genus are diterpenoids (abietane, kaurane and labdane) and essential oils [2]. As part of an ongoing search for novel therapeutic agents from plants, seven species of *Plectranthus*; P. *ornatus,* P. *amboinicus,* P. *argentatus,* P. *cilatus,* P. *hadiensis,* P. *zuluenesis* and P. *fructicosus* are currently under investigation in our laboratories. Anti-inflammatory activity of the acetone extracts of the tested species has been assessed using *in vivo* and *in vitro* assays. The *in vivo* arachidonic acid mouse ear assay showed *P.ciliatus* as the most anti-inflammatory species demonstrating dose-dependent behaviour. In the *in vitro* assay using RBL-2H3 cells we have shown that several species inhibit release of β-hexosaminidase which in this cell line occurs in parallel with histamine release. Several *Plectranthus* species were inactive and others appear to be pro-inflammatory. Potential anti-cancer activity of the test species has been evaluated in an *in vitro* screen using the RBL-2H3 cell line. This assay is based on the alteration of plasma membrane permeability by cytotoxic substrates and subsequent quantification of L-lactate dehydrogenase (LDH) released from damaged cells. Our results show that out of the seven species *P. ciliatus* has the greatest cytotoxicity. *P. ciliatus* has been successfully transferred into liquid cell suspension culture, extracts appear to be inactive. **References:** [1] Lukhoba, C. et al. (2006) J. Ethnopharmacol. 103: 1 – 24. [2] Abdel-Mogib, M. et al. (2002) Molecules 7: 271 – 301

P 075

Chemopreventive principles from *Puerararia lobata* flowers: *In Vitro* Evaluation of the Inhibition of the NF kappa B activating signaling pathway

Bebrevska L[1], De Bosscher K[2], Haegeman G[2], Merfort I[3], Apers S[1], Pieters L[1], Vlietinck A[1]
[1]Laboratory of Pharmacognosy, University of Antwerp, B-2610 Wilrijk, Belgium; [2]Laboratory of Molecular Biology, Flanders Interuniversity Institute for Biotechnology and University of Ghent, B-9000 Ghent, Belgium; [3]Institut für Pharmaceutische Wissenschaften, Lehrstuhl für Pharmazeutische Biologie, Universitat Freiburg, 79104 Freiburg, Germany

Research over the last years has revealed that transcription factor NF kappa B has a pivotal function in the regulation of genes from which the resulting products are involved in the processes of cell proliferation and differentiation, apoptosis and inflammation. The activation of this nuclear factor has been linked with the promotion of tumorigenesis, angiogenesis and metastasis since NF kappa B is activated by carcinogens, inflammatory cytokines and chemotherapeutic agents. Consequently, its activation is inhibited by many agents which are known to have chemopreventive potency. The

aim of the present work is to search molecular evidence for the chemopreventive activity shown by the flowers of *Pueraria lobata* (Willd.) Ohwi (*Fabaceae*). Pueraria is a medicinal plant widely used in the traditional Chinese medicine and it is known to produce large amounts of isoflavones. The main constituents of the 80% ethanol extract were identified as the isoflavones tectorigenin-7-O-[β-D-xylopyranosyl-(1 – 6)-β-D-glucopyranoside], tectorigenin-7-O-β-D- glucopyranoside and tectorigenin and quantified using a validated HPLC-UV method [1]. The total extract, subfractions and pure compounds isolated from these flowers were evaluated for their effect on the NF kappa B activating signaling pathway (Luciferase luciferase reporter gene assay and Electrophoretic electrophoretic mobility shift assay). The results revealed that the extract of the flowers of this medicinal plant exhibit moderate inhibitory activity, which is mainly due to the aglycon tectorigenin showing the highest activity in comparison to the other evaluated pure compounds and fractions. **References:** [1] Bebrevska, L. et al. (submitted) Planta Medica

P 076

Inhibitions of acute and chronic inflammations by *Bixa orellana* leaves extract

Zuraini A[1], Somchit MN[1,2], Abdul Hamid R[1], Sukardi S[1], Siti Erli Fazira AJ[1], Yong YK[1], Lee HK[1], Cheng XQ[1]

[1]Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; [2]Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Bixa orellana or *anatto* is a fruiting shrub that belongs to *Bixacea* family. The leaves extracts have been shown to have anti-gonorrhoeal [1] and anti-microbial properties [2]. Recently, it was also proven to possess neuropharmacological, anticonvulsant, analgesic, antidiarrhoeal and antibacterial activities [3]. The objective of this study is to investigate the anti inflammatory activities of *Bixa orellana* leaves. Acute inflammation was induced in adult *Sprague-Dawley* rats using various inflammatory mediators. The paw edema model was employed for this study, whereby mediators such as carageenan, histamine, serotonin and bradykinin were injected into the rat hind paws to induce inflammation. 96 *Sprague-Dawley* rats were divided into 16 groups (4 groups per mediator) consisting of six rats per group respectively. Example of groupings is as follows: e.g. for carageenan, Group 1A (positive control group) was given 10 mg/kg mefenamic acid orally, whereas group 1B was not treated but fed orally with distilled water as vehicle. The treated rats (group 1C & 1D) were treated respectively with 50 and 150 mg/kg of aqueous extract of *Bixa orellana* (BOE) via oral administration. The treatments mentioned earlier were given once daily to all rats from every groups for 4 days. On the fourth day, the treatment was given orally 1 hour prior to injection of Carageenan (0.1 ml of a 1% solution in 0.9% saline solution) into the plantar region of the right hind paw of each rat. For other mediators, specific antagonists were given to the positive control group, but the rest were treated similarly to the carageenan group. The paw volume was measured before the injection and each hour after for a period of 5 hours by means of volume displacement method by using plethysmometer. The average increase in paw volume of each group was calculated at every time point and compared statistically using one-way ANOVA. For carageenan, the paw volume of negative control rats had increased starting from 0hr and peaked at the second hour and then gradually declined during the 3 rd to 5th hours. Significant inhibitions were seen in rats treated with *Bixa orellana,* group 1C &1D, at where the peak should be ($p < 0.05$). Other mediators also induced paw swelling with various peaks. Histamine in group 2 caused maximum paw volume to be reached during the first hour and all treatments, histamine antagonist, Loratadine (group 2A), 50 mg/kg BOE (2C) and 150 mg/kg BOE (2D) significantly reduced their paw volumes at that hour ($p < 0.05$). Serotonin also induced a peak at 1hour and the peak was abolished when treated with 5HT antagonist and BOE (group 3A, C & D) ($p < 0.05$). Bradykinin similarly followed by inducing a peak at 30 mins and significant inhibitions were seen in groups treated with Ketoprofen and BOE (group 4A, C & D) ($p < 0.05$). Another 4 groups of rats ($n = 6$ per group) were used in the cotton-pellet granuloma test to induce chronic inflammation. Under light anaesthesia, a small cut was made onto the dorsal part on the right and left sides of each rats by using a sterile surgical blade. Upon incision, two sterile cotton pellet weighing ± 30 mg were implanted inside the wound in the right and left sides of the dorsal part of each rat. The parted skin is further being sutured using a surgical thread and curved needle. The wound is left undressed for eight days to produce chronic inflammation and within that period of time each rats were treated similarly as the acute inflammation study. As sutures being removed on the eighth day, the degree of inflammation is measured by weighing the cotton wet weight and compared with the cotton dry weight. The cotton inside the wound may contain dead cells as well as transudates as the product of prolonged inflammation. The percentage of inhibition (anti-transudate and anti-proliferative) is highest in the rats treated with 150 mg/kg of *Bixa orellana* extract and compared to non-treated rats. As a conclusion, this preliminary study has shown that *Bixa orellana* has proved to be an excellent anti-inflammatory agent not only in acute but chronic inflammation model as well. **References:** [1] C Armando, M Herlinda, M Emilia and C Guillermo (1995) J Ethnopharmacol 48: 85 – 88. [2] TC Fleischer, EPK Ameade, MLK. Mensah and IK. Sawer (2003) Fitoterapia 74: 136 – 138. [3] JA Shilpia, M Taufiq-Ur-Rahmanb, SJ Uddinc, MS Alamc, SK Sadhud and V Seidela (2006) J Ethnopharmacol. 108 (2): 264 – 271.

P 077

Effects of *Cryptolepis sanguinoleta* root extract in lipopolysaccharide – stimulated human primary monocytes

Olajide OA[1,2,4], Wright CW[3], Fiebich BL[1]

[1]Neurochemistry Research Laboratory, Department of Psychiatry and Psychotherapy, University of Freiburg Medical School, 79104 Freiburg, Germany; [2]Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria; [3]School of Pharmacy, University of Bradford, West Yorkshire BD7 1DP, UK; [4]Department of Pharmacology, Kampala International University, Western Campus, Ishaka, Uganda

Cryptolepis sanguinoleta is a shrub used in West Africa for the treatment of fevers, and inflammatory conditions. In the present study the effect of the crude methanolic root extract of *C. sanguinoleta* on prostaglandin E2 (PGE2) release from LPS-stimulated human primary monocytes was investigated using an enzyme immunoassay. The effects of the extract on COX – 2, I kappa Bα and p38 MAP Kinase proteins were also investigated. The extract (2.5 – 10 μg/ml) produced a dose-dependent inhibition of LPS – induced PGE2 release in human primary monocytes. Western blot experiments showed that the extract inhibited LPS – induced COX – 2 expression, as well as LPS – induced activation of p38 MAP kinase. However, the extract did not prevent LPS-induced I kappa Bα degradation in these cells. This study has therefore established *in vitro* potential anti – inflammatory properties of the root of *C. sanguinoleta* in LPS – stimulated human primary monocytes. It is suggested that the inhibition of LPS – induced PGE production in these cells by the root extract of *C. sanguinoleta* is mediated through inhibition of COX – 2 protein. We further postulate that the observed effects may be dependent on the inhibition of p38 MAP kinase activation.

2. Natural products with antimicrobial activity

P 078

Susceptibility testing of *Listeria monocytogenes* using the disk method and micro titre plate (micro broth dilution) method

Nyila MA[1,2], Lall N[2], Leonard C[3], Weyer B[3], Meyer JJM[2]
[1]Department of Life and Consumer Sciences, University of South Africa, P O Box 392 Unisa, 0003, South Africa; [2]Department of Botany, University of Pretoria, Pretoria, 0002, South Africa; [3]School of Pharmacy, Tshwane University of Technology, Pretoria, 0002, South Africa

Twelve South African medicinal plants which are used traditionally to treat symptoms associated with *Listeria monocytogenes* infections were screened for activity against the pathogen. Different plant parts were extracted separately with ethyl acetate and chloroform. All the extracts were first screened against the bacteria using disk method diffusion method. Zones of inhibition observed in the presence of chloroform extracts of *Eucomis autumnalis* and ethyl acetate extracts of *Acacia karroo* (50 mg/ml) were 12 and 14 mm, respectively. Active extracts were further tested against the bacteria for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination using microtitre dilution method. Ethyl acetate extract of *A. karroo* and chloroform extract of *E. autumnalis* exhibited MIC of 3.1 mg/ml and 12.5 mg/ml respectively. Four samples namely *Acacia karroo* (ethyl acetate extract), *Senecio inornatus* ethyl acetate extract, *S. inornatus* (chloroform extract) and *Aloe arborescens* (ethyl acetate extract) showed good minimum bactericidal activity against *L. monocytogenes* MBC ranging from 3.1 to 12.5 mg/ml. These extracts were further tested for cytotoxicity against Vero cells (XTT) using the Cell Proliferation Kit II. Fifty percent inhibition concentration (IC_{50}) of the extracts were as follows: *A. karroo* 45.5 ± 7.9 μg/ml, *Senecio inonartus* (ethyl acetate extract), 108.4 ± 0995 μg/ml, *S. inonartus* (chloroform extract) 99.9 ± 4.2 μg/ml and *A. arborescens* (ethyl acetate extract) > 400 μg/ml. *Acknowledgements:* University of Pretoria, University of South Africa, Tshwane University of Technology, Reginald Mayekiso, Clement Letsoela, Ms Audrey Dhlamini.

P 079

Modulation of isoniazid susceptibility of mycobacterial strains by different flavonoids

Lechner D[1], Gibbons S[2], Bucar F[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University, Universitätsplatz 4/I, 8010 Graz, Austria; [2]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, UK

The increasing emergence of multidrug-resistant (MDR) tuberculosis requests the development of new drugs to combat infections associated with mycobacteria. Poor patient compliance due to significant side effects of long TB therapies often lead to deadly MDR bacteria [1]. In the course of this project, several flavonoids without significant antimycobacterial activity at the tested concentrations were screened for their ability to decrease the minimum inhibitory concentrations (MICs) of the first-line anti-TB drug isoniazid (INH) against different mycobacterial strains. Flavonoids with different substitution patterns were tested to examine structure-activity-relationships (SARs) of these compounds. To find out more about the variations between different mycobacterial strains, we used *Mycobacterium smegmatis* (ATCC 14468), *M. smegmatis* mc^2155 (ATCC 700084), *M. smegmatis* $mc^2$2700 and *M. fortuitum* (ATCC 6841) for our assays. We could observe the most intense synergistic effects in *M. smegmatis* mc^2155 whereas the tendency of INH-potentialization by certain flavonoids stayed the same with each strain. Myricetin turned out to be the most efficient intensifier of INH susceptibility in all tested strains, followed by quercetin. SARs of flavonoids as INH activity modulators indicate that they overlap with SARs for their antioxidant properties, however, the potentialization of INH cannot only be explained by antioxidative activity. *Acknowledgements:* Funded by Doc-Fforte 22076 (Austrian Academy of Sciences), Dr. Jose A. Ainsa (Universidad de Zaragoza) for scientific advice. **References:** [1] Zhang, Y. et al. (2006) Drug Discov Today 11: 21 – 27.

P 080

Investigation of flavonoids and antimicrobial activity of *Ballota andreuzziana*

Abdelshafeek KA[1,2], Doboob AA[3], Zarkoon AH[1], Bashir HS[3]
[1]Altahady University, Faculty of Science, Chemistry Dept., Sirt, Libya, P. O 674.; [2]National Research Center, Chem. of Med. Plants Dept., Dokki, Cairo, Egypt; [3]Altahady University, Fac. of Sci., Biology Dept., Sirt, Libya

Ballota andreuzziana (Family *Labiatae*) is an endemic Libyan medicinal plant growing in the Gabal Alakhder region [1]. plants of the genus *Ballota* are used widely in the treatment of many ailments like nausea, vomiting, gastric ulcer, upper respiratory inflammation and as antimicrobials [2 – 3]. This is the first investigation of flavonoids which led to identification of two aglycones named 7-methoxy luteolin and 6,7-dimethoxy scutellarein in addition to three glycosides known as luteolin-7-*O*-glucoside, 6, 4′-dimethoxy scutellarein- 7-*O*-glucoside and quercetin-7-*O*-rhamnoglucoside. The structures of these compounds were established using different spectroscopic techniques (UV, MS & NMR). The study of antimicrobial activity of different extracts of the plant using disc diffusion method and measuring diameter of the inhibition zone against some Gram-pos, Gram-neg bacteria and fungi (*M. phlei, S. aureus, B.subtilis, E. colaceae* and *C. albicans*) revealed that the acetone extract exhibited the strongest activity against *Mycobacterium phlei* (I. Z.= 16.33 mm, conc.= 150 mg/ml), while butanol and chloroform extracts showed moderate activity against *Staphylococcus aureus* and *Bacillus subtilis* (I.Z.= 11.4&11.0 mm, conc.= 150 mg/ml) respectively. **Reference**: [1] Jafri, S. and Gadi, A.(1985)*"Flora of Libya"* Al Fateh univ., Fac. Of Sci., dept. of Bot., Tripoli, Libya. [2] Couladis, M.; Chinon, I. B. and Tzakon, A. (2002) *Phytother. Res.,* 16, 732 – 26. [3] Citoglu, G. S.; Yitmaz, B. S.; Tarikahya, B. and Tipirdamaz. R.(2005) *Chem. Nat. Compd.,* 14, 3.

P 081

Thermal stability of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link.: evaluation of chemical composition, antioxidant and antibacterial activities

Bounatirou S[1], Smiti S[1], Miguel MG[2], Faleiro L[2], Rejeb MN[3], Neffati M[4], Costa MM[5], Figueiredo AC[5], Barroso JG[5], Pedro LG[5]
[1]Faculté des Sciences de Tunis, Université Tunis el Manar, Campus Universitaire, 2092 Tunis, Tunisia; [2]Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8005 – 139 Faro, Portugal; [3]Institut National de Recherche en Génie Rural, Eaux et Forêts, 2080 Tunis, Tunisia; [4]Institut des Régions Arides, 4119 Mednine, Tunisia; [5]Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749 – 016 Lisbon, Portugal

Plant volatile oils have been recognized since antiquity to possess biological activities. Chief amongst these are their antibacterial, antifungal and antioxidant properties. Thyme essential oils have been previously reported to have antioxidant activity mainly mediated by the phenolic fraction of the oils (1, 2). Tunisian thyme (*Thymus capitatus* Hoff. et Link.) is a perennial, herbaceous shrub belonging to the Lamiaceae family commonly used in Tunisia for culinary purposes. The chemical composition, antioxidant and antibacterial activities of essential oils isolated from the aerial parts of Tunisian *T. capitatus* during the flowering phase were stored under dark conditions at 60° during 37 days were evaluated. Samples taken periodically were used to evaluate the chemical composition and the antioxidant and the antibacterial activities. The chemical composition was analysed by GC and GC/MS. The antioxidant activity of the oils (100, 500 and 1000 $mg.l^{-1}$) was assessed by free radical scavenging

(DPPH) and by TBARS assays. Antibacterial ability of *T. capitatus* essential oils was tested by the disc agar diffusion against three different strains of *Staphylococus aureus* (C15, ATCC6538 and ATCC25923). With some fluctuations, carvacrol (48 – 74%) remained as the major component of the oil independent of the storage period. Terpinen-4-ol was the only oil component whose relative amount increased whereas that of carvacrol decreased. α-Terpinene and γ-terpinene decreased over time whereas *p*-cymene increased in the same period. Despite the thirty-seven days of storage at 60 °C, *T. capitatus* essential oil still showed a high antioxidant activity and a stable antimicrobial activity. **References:** [1] Miguel, G., Simões, M., Figueiredo, A.C., Barroso, J.C., Pedro, L.G. and Carvalho L. 2004. Composition and antioxidant activities of the essential oils of Thymus caespititus, Thymus camphoratus and Thymus mastichina. Food Chem. 86: 183 – 188. [2] Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G. 2007. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et Link.. Food Chem. (in press).

P 082

Evaluation of essential oil from tulsi (*Ocimum sanctum L*) grown in Egypt: Chemical changes and antimicrobial activity during storage of tulsi oil

Ibrahim ME[1], Youssef AA[1], Ibrahim F[1], El Hady S[2], Abdel-Hamid MF[2]
[1]Cultivation and Production of Medicinal and Aromatic Plants Department, National Research Center, Dokki, (12622), Cairo, Egypt; [2]Faculty of Agriculture, Ain Shams University, Shobra El-Khama, Cairo, Egypt

In order to introduce new species of medicinal and aromatic plants into Egypt, the seeds of tulsi (*Ocimum sanctum* L.) (origin: USA) were subjected to propagation and cultivation in Egypt. The essential oil was obtained by hydrodistillation of the herb during the full blooming stage. The oil was subjected to different storage conditions. It was stored at three different temperature conditions, i.e. room temperature, refrigerating (4 – 0 °C) and deep freezing (-17 °C). The chemical composition of the oil was analyzed by means of GC and GC/MS [1]. In addition to the already known Eugenol (24.9%) and β-bisabolene (16.3%) as the main constituents of the oil [2], other compounds were identified. Terpenic constituents showed variable responses to storage conditions, some terpens increased while others decreased. The profound deterioration was obtained when the oil was stored at room temperature. The tulsi oils were subjected to biological assays [3] (three bacteria, two fungi and two yeasts). The antimicrobial activities of the essential oils were evaluated out using the inverted petriplate method [3]. The most important finding is the high potency of tulsi oils against fungi compared with Gram (±) bacteria and yeast strains. **References:** [1] Adams, R. P..(1989) Identification of Essential oils by Ion Trap Mass Spectroscopy. Academic Press, New York. [2] Ibrahim M. E. (1999) Physiological and Chemical Studies on Tulsi Plant (Ocimu sanctum). Egypt. J. Hort 26 (2), pp.147 – 165. [3] Bauer, J.D. (1982). Clinical Laboratory methods. P. 832. 9th Ed. The C.V. Mosby Co. St. Louis Toronto Landon. [3] Collins CT, & Lyne PM. (1985). Micrbiological Methods. 5th ed, (pp 167 – 181), Butterworth and Co Pub Ltd, London and Toronto.

P 083

The effects of three Thai herbal essential oils against *Candida* biofilms

Taweechaisupapong S[1], Khotphat P[1], Hoysang T[1], Chitropas P[2], Khunkitti W[2]
[1]Faculty of Dentistry, Khon kaen University, Khon kaen, 40002, Thailand; [2]Faculty of Pharmaceutical Sciences, Khon kaen University, Khon kaen, 40002, Thailand

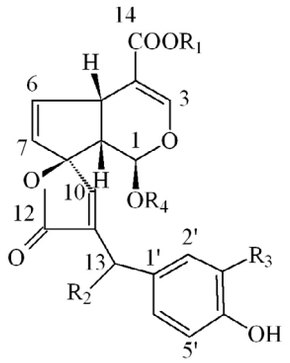
The purpose of this study was to determine the effects of three Thai herbal essential oils used in aromatherapy, namely lemongrass (*Cymbopogon citratus* DC), holy basil (*Ocimum sanctum* L.) and kaffir lime (*Citrus hystrix* DC), against *Candida* biofilms and compare those effects with nystatin. Employing a formazan salt reduction assay, the results showed that among the three essential oils, kaffir lime oil exhibited the lowest sessile minimum inhibitory concentration (SMIC), followed by lemongrass oil and holy basil oil, respectively. The SMIC of nystatin, kaffir lime, lemongrass and holy basil oil at 50% inhibition ($SMIC_{50}$) on *Candida albicans* were 0.032, > 0.93 – < 1.86, 3.46 and 7.93 mg/ml, respectively, while the SMIC at 90% inhibition ($SMIC_{90}$) were 0.5, 1.8, 13.8 and 31.7 mg/ml, respectively. The $SMIC_{50}$ of nystatin, kaffir lime, lemongrass and holy basil oil on *Candida krusei* were 0.02, 0.9, 1.7 and 4.0 mg/ml, respectively, while the $SMIC_{90}$ were 0.5, 1.9, 6.9 and 31.7 mg/ml, respectively. Overall, the data demonstrated that the three essential oils can inhibit the growth of *C. albicans* and *C. krusei* biofilms although the $SMIC_{50}$ and $SMIC_{90}$ of those three essential oils were higher than nystatin. *Acknowledgements:* This work was supported by grants from the Faculty of Dentistry, Khon Kaen University and the Thailand Research Funds (grant No RDG 5020022).

P 084

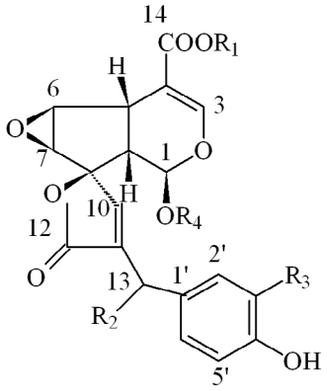
Antiamoebic activity of iridoids from *Morinda morindoides* leaves

Cimanga RK[1,2], Kambu OK[2], Tona GL[2], Apers S[1], Pieters L[1], Vlietinck AJ[1]
[1]Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium; [2]Faculty of Pharmacy, University of Kinshasa, P.O. Box 212, Kinshasa XI, Democratic Republic of Congo

An aqueous decotion (AD), an 80% methanolic extract (ME) from the leaves of *Morinda morindoides* (Rubiaceae) together with five iridoid lactones isolated from the ME extract were evaluated *in vitro* for their activity against *Entamoeba histolytica* [1] and their cytotoxicity [2]. AD and ME extracts exhibited a promising antiamoebic activity with IC_{50} values of 3.1 ± 1.7 and 1.7 ± 0.6 μg/ml, respectively. All tested iridoids displayed antiamoebic activity, the most active being epoxygaertneroside (IC_{50} 1.7 ± 0.4 μg/ml) and methoxygaertneroside (IC_{50}: 2.3 ± 0.7 μg/ml) [3]. Gaertneroside, acetylgaertneroside and gaertneric acid also showed a good activity with IC_{50} values of 4.3 ± 1.8, 5.4 ± 1.4 and 7.1 ± 1.4 μg/ml, respectively. Synergistic effects between the iridoids tested, or with other constituents, may explain the high activity of the extracts. All extracts and iridoids were devoid of any cytotoxic effect against MT-4 cells at the highest test concentration of 250 μg/ml. These findings support at least in part the traditional use of *Morinda morindoides* leaves for the treatment of amoebiasis in traditional medicine in the Democratic Republic of Congo.



	R_1	R_2	R_3	R_4
1	CH_3	OH	H	gluc.
2	CH_3	OH	H	6-acetylgluc.
3	H	OH	H	gluc.
4	CH_3	OH	OCH_3	gluc.



	R_1	R_2	R_3	R_4
5	CH_3	OH	H	gluc.

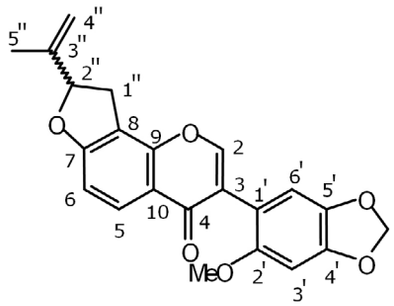
References: 1. Tona et al. (1998) J. Ethnopharmacol. 61: 57 – 65. 2. Pauwels et al. (1988) J. Virol. Methods 20: 309 – 321. 3. Cimanga et al. (2006) *Planta Med*. 72: 751 – 753.

P 085

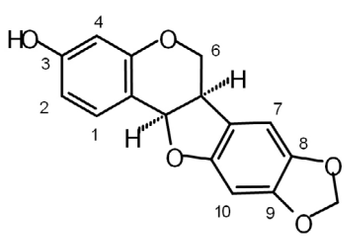
A novel isoflavonoid from *Millettia puguensis*

Kapingu MC[1], Mbwambo ZH[1], Moshi MJ[1], Magadula JJ[1], Cos P[2], Vanden Berghe D[2], Maes L[3], Theunis M[2], Apers S[2], Pieters L[2], Vlietinck A[2]
[1]Institute of Traditional Medicine, Muhimbili University College of Health Sciences, P.O. Box 65001, Dar es Salaam, Tanzania; [2]Departments of Pharmaceutical and [3]Biomedical Sciences, University of Antwerp, B-2610 Antwerp, Belgium

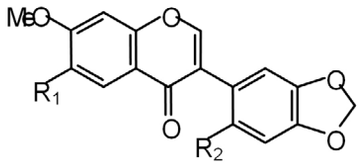
From the roots of *Millettia puguensis* (Leguminosae), a novel isoflavonoid (**1**), 2'-methoxy-4',5'-methylenedioxy-7,8-[2-(1-methylethenyl)furo]isoflavone, and four known compounds, i.e., lupeol, (-)-maackiain (**2**), 6,7-dimethoxy-3',4'-methylenedioxyisoflavone (**3**) and 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone were isolated and identified by ^{1}H-, ^{13}C-NMR and mass spectroscopy. All compounds were evaluated for their antiprotozoal and cytotoxic activities, but only a moderate antileishmanial activity was observed for compound **3** (IC_{50} 32 μM against *Leishmania infantum*), and a moderate cytotoxicity for compound **2** (IC_{50} 43 μM on MRC-5 cells) [1].



1



2



3 R_1 = OMe, R_2 = H

References: 1. Kapingu et al. (2006) Planta Med. 72: 1341 – 1343

P 086

Anti-protozoan activities of *Harungana madagascariensis* stem bark extract on trichomonads and malaria

Iwalewa EO[1,5], Omisore NO[1], Adewunmi CO[2], Gbolade AA[3], Ademowo OG[4], Nneji C[4], Agboola OI[3], Daniyan OM[1]
[1]Department of Pharmacology, [2]Department of Drug Research and Production Unit, [3]Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria; [4]Institute of Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria; [5]Department of Paraclincal Sciences (Phytomedicine Programme), Faculty of Veterinary Sciences, University of Pretoria, PB 0X4, Onderstepoort 0110, Pretoria, South Africa

The ethanolic stem bark extract of *Harungana madagascariensis* (Choisy) Poir, Clusiaceae, was evaluated for their activities on *Trichomonas gallinae* (Rivolta) Stabler isolated from the pigeon (*Columba livia*). It was also tested for their antimalarial activity on N67 *Plasmodium yoelii nigeriensis* (in-vivo) in mice and on *Plasmodium falciparum* isolates in-vitro. The IC_{50} of the extract and MDZ (flagyl) on *T. gallinae* at 48 hours are 187 and 1.6 μg/ml. The IC_{50} of the extract, CQ and ART on *P. falciparum* are between 0.05 and 0.52 μg/ml for the extract and 0.02and 0.041 μg/ml for ART and CQ, respectively. The actions of the extract in *in vivo* study on *P.y.nigeriensis* showed that in both suppressive and prophylactic tests the percentage of chemo-suppressive was between 28.6 – 44.8% and 30.2 – 78.2%, respectively, while only 80 mg/kg of the extract reduced the parasitaemia level as compared to the control and the standard drugs in curative test. *H. madagascariensis* stem bark extract therefore exhibited potent antiprotozoan effects against *Trichomonas* and *Plasmodium* both *in vivo* and *in vitro.*

P 087

Large molecules as antiadhesive compounds against pathogens

Wittschier N[1], Lengsfeld C[1], Vorthems S[1], Hensel A[1]
University of Münster, Institute for Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

Within development of alternative treatment regimes against bacterial infections the use of antiadhesive compounds as prophylactic tools is increasingly under discussion. Because bacterial adhesion is commonly mediated by carbohydrate-protein interactions between surface adhesins of the microorganism and the host cell the use of exogenous polyvalent, high-molecular carbohydrates and tannin-like plant-derived compounds should antagonize the adhesive interaction. Within a broad screening carbohydrates, carbohydrate- and proanthocyanidin-enriched plant extracts were screened on potential antiadhesive effects against *Helicobacter pylori, Campylobacter jejuni, Porphyromonas gingivalis* and *Candida albicans* in different *in situ* assays on primary tissue; thereby the adhesion of fluorescent-labelled microorganisms on the intact tissue was quantified by cell-counting and imaging systems [1]. The adhesion of *H. pylori* on human stomach tissue was effectively blocked by polysaccharides from immature okra fruits (*Abelmoschus esculentus* (L.) MOENCH). These extracts additionally had strong *in vitro* effects against *C. jejuni*, but failed to be effective within an *in vivo* study in infected chicken broilers when feeded at 5 and 10%. Polysaccharides from

Glycyrrhiza glabra L. showed strong antiadhesive properties against *H. pylori* and *P. gingivalis* at 0.1 mg/ml. *Pelargonium sidoides* DC extract, containing mainly polymeric proanthocyanidins was dose-dependently effective against *H. pylori.* Due to the multifunctional adhesive strategy of *C. albicans* no effective compounds could be detected against this yeast. Structure-activity relations are presented and the potential *in vivo* use of carbohydrate-based antiadhesives are discussed critically. **References:** [1] Lengsfeld C, Faller G, Hensel A (2007) Okra polysaccharides inhibit adhesion of Campylobacter jejuni to mucosa isolated from poultry in vitro but not in vivo. Animal Feed Science and Technology, 135, 113 – 125.

P 088

Bioassay-guided isolation of saponins from *Apodytes dimidiata*

Fouber K[1], Theunis M[1], Vermeersch M[2], Apers S[1], Pieters L[1], Maes L[2]
University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium; [1]Lab of Pharmacognosy; [2]Lab of Microbiology and Parasitology

In an *in vitro* screening programme for antiprotozoal activity, a crude extract of *Apodytes dimidiata* (Icacinaceae) showed promising activity against *Leishmania infantum* [1]. Because of the reported molluscicidal activity, the presence of saponins was suspected, and subsequently confirmed by phytochemical screening. The anti-leishmanial constituents were isolated by bioassay-guided isolation. Dried and mixed leaves of *Apodytes dimidiata* were extracted with 80% methanol. The crude extract was extracted consecutively with petroleumether, dichloromethane and *n*-butanol (*n*-BuOH). The *n*-BuOH extract showed a high *in vitro* activity against *Leishmania infantum* (IC_{50}= 0.4 µg/mL) and was also active against *Trypanosoma brucei* (IC_{50}= 1 µg/mL). In order to isolate the active compounds, further fractionation was performed on the *n*-BuOH extract by silicagel column chromatography. Elution of the column started with EtOAc, and proceeded with mixtures of EtOAc and the organic phase of *n*-BuOH – HAc – H_2O 4:1:5. Fractions were evaluated by TLC sprayed with anisaldehyde – sulphuric acid, and combined according to their chromatographic pattern. Fractions 67 – 96 showed activity against *Leishmania infantum* and *Trypanosoma brucei*. The active fraction was separated by repeated semi-preparative HPLC (C 18 Vydac Column 5 µm(250 x 10 mm)) using gradient elution (32 to 56% B in 45 min (A = 0.05% HAc; B = ACN + 0.05% HAc), 3 mL/min, 210 nm detection). The first fraction resulting from this separation was again separated by semi-preparative HPLC employing a modified gradient (32% to 34% B in 60 min. (A = 0.05% HAc; B = ACN + 0.05% HAc), 3 mL/min, 210 nm detection). Six saponins from the oleanane type were isolated and structure elucidation was performed using mass spectrometry and NMR spectroscopy. **References:** [1] Maes, L. (2003) Ph.D. Thesis, Lead Identification and Development of a New Anti-leishmanial saponin PX-6518, isolated from the Vietnamese plant Maesa balansae, University of Antwerp, Belgium

P 089

Antimicrobial activity of extracts isolated from *Plantago* sp.

Radu N[1], Chirvase A[1], Ghita I[2], Coman O[2], Stamatin L[2], Corobea C[1], Pintilie G[3]
[1]I.N.C.D.C.P. ICECHIM, Biotechnology Departement, Splaiul Indepdendentei street, no. 202, District 6, postal code 060021, Bucharest, Romania; [2]U.M.F. Carol Davila, Dionisie Lupu street, no. 37, postal code: 020021 Bucharest, Romania

The aim of the paper is to present the results of research studies concerning the effect of three main solid extracts E1, E2, E3, isolated from *Plantago sp.* from alcoholic and aqueous media on some pathogenic bacteria. In the first step, the disk diffusion Kirby Bauer method was applied for *in vitro* testing of the biological effects of the three extracts by using the following pathogenic microorganisms: *Staphyloccocus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* [1 – 3]. The obtained results were quantified in comparison with the effect generated by the usual antibiotics on the same pathogenic microorganisms. Each biological test was made by more repetitions and the Student method was applied for results' validation. The biological tests results showed that E1 had no biological effect, E2 had a moderate bactericidal effect and E3 presented a significant bactericidal effect. In the second step the solid extracts E1, E2, E3 were characterised by physico-chemical analyses. For this purpose, qualitative and quantitative analyses were performed by energy dispersive X-ray fluorescence and by atomic emmission with inductively coupled plasma, respectively. The obtained results indicated the presence of C, H, P, and some microelements in all the solid extracts as well as the presence of K and Cl in extracts E2 and E3. The physical properties investigated by thermogravimetric analyses and differential thermal analysis showed the thermolability of all extracts and the presence of organic compounds in all extracts E1, E2, E3. The infrared spectrum analysis performed for all the extracts indicated the presence of polysaccharide chains in E1, the existence of flavonoides in E2 and the presence of iridoid compounds in E3. In conclusion, the biological and physico-chemical tests performed for the solid extracts E1, E2, E3 isolated from *Plantago sp.* indicate a significant bactericidal effect of E3 which contains iridoid compounds. **References:** [1] Samuelsen A.B., 2000, J.f Ethnopharmacol. 71: 1 – 21. [2] Tuner Robert A., Hebborn Peter, 1971, Screening Methods in Pharmacology, Academic Press New York, London, v: II. [3] Yesilada E, Sezik E, 1995, J. Ethnopharmacol. 46: 133 – 52.

P 090

Antiparasitic activity against headlice and headlouse nits of *Annona squamosa* seed preparation

Gritsanapan W, Klaymongkol C, Wannasawage C
Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudthaya Rd, Ratchatewi, Bangkok 10400, Thailand

Seeds of *Annona squamosa* L., Annonaceae have long been used as anti-headlice agents in humans. An active component against headlice is known to be a triglyceride with one oleate ester [1]. A petroleum ether extract of the seeds prepared as 20% w/w oil in water cream preparation is effective and biological stable formulation against headlice [2, 3]. However, the efficacy of *Annona squamosa* seed cream on headlouse nits has not been reported. The present study is focused on testing for the efficacy of freshly prepared 20% w/w *Annona squamosa* seed cream against human headlice and their nits in school girls. After 3 hours of hair exposure to 20 g of the 20% *Annona* cream containing 9.4% w/w of the active component, triglyceride with one oleate ester, 92.4 ± 12.3% of headlice in school girls were killed. For evaluation of the killing effect of *Annona* cream on headlouse nits, 20 g of the cream was applied onto hair of each of 27 school girls for 3 hours, then shampooed off. After a week, subjects were investigated for amount of headlice and alive nits and found that 66.7% of the subjects still had young headlice (< 26%) and medium amount of alive nits. After second treatment of the cream, 40.7% of the subjects contained < 17% of young headlice and small amount of alive nits, while the third treatment provided 3.7% of the subjects who still have < 7% of young headlice and none of alive nits. The results demonstrate that single treatment of 20% w/w freshly prepared *Annona squamosa* seed cream could kill 92% of headlice in school girls but could not kill their nits. The girls should be treated with the cream once a week for 3 weeks to kill young headlice developing from alive nits. *Acknowledgements:* The author would like to thank The Royal Golden Jubilee PhD Program of The Thailand Research Fund for a contribution to the travel grant to present this work. **References:** [1] Gritsanapan, W. et al. (2006) Planta Med. 72: 966. [2] Gritsanapan, W. et al. (1998) Abstract Book of 50th IPC and 17th FAPA Congress, Mumbai, India, p 1. [3] Tiengda, CH. et al. (2000) Southeast Asian J Trop Med Public Health 31 (Suppl 1): 174 – 7.

P 091

Vismiaturucallone, a new Antimalarial tetracyclic triterpene from *Vismia laurentii* (Clusiaceae)

Noungoue DT[1], Ngom S[3], Ngoupayo J[1], Chaabi M[3], Ndjakou BL[1], Ngouela S[1], Antheaume C[3], Boyom FF[2], Gut J[4], Rosenthal PJ[4], Lobstein A[3], Tsamo E[1]

[1]Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon; [2]Department of Biochemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon; [3]Laboratory of Pharmacognosy, LC1 UMR 7175, Faculty of Pharmacy, Strasbourg, France; [4]Division of Infectious Diseases, Department of Medicine, University of California, United States

In the framework of our interest in antimalarial natural products from Clusiaceae family [1,2], the apolar extract of the stem bark of *Vismia laurentii* showed good antimalarial activity (IC_{50}= 4.6 μg/ml). Thus, the isolation of its constituents led to the identification of a new tetracyclic triterpene, (20-ethylnortirucalla-7,24-dien-3-one) vismiaturucallone (**1**) (Fig. 1) along with eight known compounds namely 3-geranyloxyemodin, vismiaquinone A (**3**), vismiaquinone B, bivismiaquinone, epifriedelinol, betulinic acid, tirucalla-7,24-dien-3-one, and stigmasta-7,22-dien-3-ol. Compounds (**1**) and (**3**) showed antiplasmodial activity against chloroquine-resistant W2 strain of *Plasmodium falciparum* with IC_{50} of 1.17 μM and 1.42 μM, respectively.

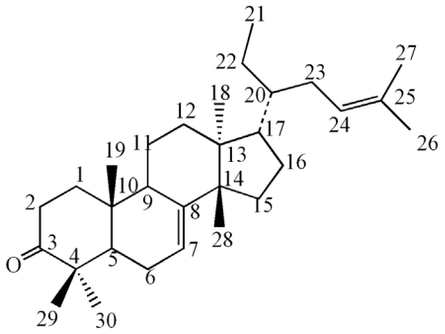


Fig. 1

References: [1] Ngouela, S. et al. (2005) Nat Prod Res 19(7):685 – 8. [2] Ngouela, S. et al. (2006) Phytochemistry 67(3):302 – 6.

P 092

Phenolic content, antioxidant and antibacterial activities of *Hymenocardia acida*

Sofidiya MO[1,2], Odukoya OA[1], Afolayan AJ[2], Familoni OB[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria; [2]Department of Botany, University of Fort Hare, Alice 5700, South Africa; [3]Department of Chemistry, Faculty of Science, University of Lagos, Nigeria

The antioxidant and antibacterial activities of the aqueous and methanolic extracts obtained from *Hymenocardia acida* Tul., Hymenocardiaceae (Euphorbiaceae), were investigated in this study. The inhibition values of extracts and quercetin were found to be very close with no significant difference in the antioxidant activity at 0.05 mg/ml concentration evaluated by DPPH method. Total proanthocyanidins for both water and methanol extracts were 20.22 ± 0.01 and 30.62 ± 0.51 mg/g (catechin equivalent) while the total phenol contents were 20.0 ± 0.5 and 35.6 ± 1.4 mg/ml (tannic acid equivalent) respectively. Total flavonoid 1.6 ± 0.1 and 5.4 ± 0.2 for aqueous and methanolic extracts respectively. In the DPPH assay, antioxidant activity of the samples was in the order of quercetin > methanol extract > water extract. At 0.02 mg/ml, quercetin, methanol and water extracts of *H. acida* exhibited 93.4, 85.5 and 55.4% scavenging activity, respectively. There was no significant difference ($P < 0.01$) in scavenging activity between the extracts and quercetin at 0.05 mg/ml. Activity of quercetin was found to be significantly more pronounced than ascorbic acid, methanol and water extracts. These results revealed that extracts of *H. acida* were electron donors and could react with free radicals, converting them to more stable products and terminating the radical chain reaction. The antioxidant activity trend obtained in this result corresponds directly with the content of total phenolics in the extracts, with a correlation coefficient of R^2 = 0.85; R^2= 0.94; R^2= 0.97 for DPPH, reducing power and ABTS respectively. Linear regression analysis also produced a high correlation coefficient with total proanthocyanidins (DPPH, R^2= 0.69; ABTS, R^2 = 0.94). *H. acida* extracts showed low antibacterial activity (MIC value ≥ 5.0 mg/ml) against Gram-negative bacteria but significantly (MIC value ≤ 2.5 mg/ml) inhibited the growth of the Gram-pos strains tested. *Acknowledgements: NRF, South Africa, and University of Lagos, Nigeria.*

P 093

Antibacterial activity of *Piqueria trinervia* Cav. against several enteropathogenic bacteria

Goldhaber-Pasillas D[1], Ruiz de Esparza Villarreal R[2], Avila-Acevedo JG[1], Jiménez-Estrada M[2]

[1]FES-Iztacala, Universidad Nacional Autónoma de México, Av. de los Barrios # 1 Los Reyes Iztacala, 54090, Tlalnepantla, Estado de México; [2]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior Cd. Universitaria, 04210, México D.F

Piqueria trinervia Cav. (Asteraceae) has been known since the 16th century as a remedy in the treatment of digestive disorders including fevers, intestinal infections, indigestion, diarrhea, dysentery and of wounds. Nevertheless, there are no pharmacological studies to date that establish its biological activity [1]. Using plants collected in October 2001 and 2002 in the Ajusco, México City, nine extracts of different polarity were obtained from 3 kg of dried roots, stems and leaves-inflorescences. Antibacterial activity of the extracts was determined by agar diffusion method (Kirby Bauer). The hexane extract of stems and leaves-inflorescence showed the highest antibacterial activity, especially, against Gram-negative bacteria and their minimum bactericidal concentration (CMB) as well as minimum inhibitory concentration (MIC) were determined against *Shigella boydii, Salmonella typhi, Escherichia coli, Vibrio cholerae, Bacillus subtilis* and *Staphylococcus aureus*. Bioactive fractions derived from these extracts and separated by column chromatography and thin layer chromatography were obtained from stems (5) and leaves-inflorescence (9) against *S. boydii, V. cholerae* and *S. aureus*. According to data from NMR ^{13}C, ^{1}H, IR, HETCOR, COSY and DEPT, the most active fraction, is composed of a mixture of waxes and the alcohol 3-methyl-2-buten-1-ol. The latter is toxic causing skin irritation in rats (LD_{50}= 810 mg/kg) and rabbits (LD_{50}= 3900 mg/kg) [2] and the antibacterial activity may be due to its cellular toxicity. These results suggest that *Piqueria trinervia* is useful in treating gastrointestinal disorders caused by bacteria as well as skin and ear infections, which confirms its ethnobotanical uses. **References:** [1] Argueta, V.A., Cano, A.L. y Rodarte, M.E. 1994. Atlas de las Plantas de la Medicina Tradicional Mexicana. Tomo II. Instituto Nacional Indigenista. México. [2] Chapman and Hall, 1982 – 2004. Dictionary of Natural Products. CD-ROM v.12: 1

P 094

The chemical composition and *in vitro* antibacterial activities of the oil of *Ziziphora clinopodioides* Lam. from Iran

Tabatabaei-Anaraki M[1], Chalabian F[1], Masoudi S[2], Rustaiyan A[3]

[1]Department of Biology and Chemistry, North Tehran Branch, I. A. University, Tehran, Iran; [2]Department of Chemistry, Central Tehran Branch, I. A. University, Tehran, Iran; [3]Department of Chemistry, Science & Research Campus, I. A.University, P.O.Box 14515 – 775, Tehran, Iran

The genus *Ziziphora* is represented in the flora of Iran by four species, *Z.capitata* L. subsp. *capatita*, *Z.capitata* L. subsp. *orientalis* Samuelsson ex Rech.f., *Z.clinopodioides* Lam., *Z. persica* Bunge. and

Z.tenuir L. *Z.clinopodioides* Lam. with the common persian name kakuti-e kuhi comprised nine subspecies native to Iran. In Iranian and Turkish folk medicine, *Ziziphora* species have been used as sedative, against stomachache and as carminative. In Iranian folk medicine and folklore, the dried aerial parts of this plant have been frequently used as culinary and also in cold and cough treatments. The composition of the essential oils of *Ziziphora clinopodioides* Lam. (two samples from two different locations) as well as the *in vitro* antibacterial activity of their essential oils were studied. The water distilled essential oils from aerial parts of *Z. clinopodioides* were analyzed by GC and GC/MS. The main compounds of the sample A of *Z. clinopodioides* were thymol (53.6%), p-cymene (10.5%) and carvacrol (8.7%), while in the sample B, 1,8- cineole (21.6%) and terpinen-4-ol (18.2%), linalool (7.9%) and pulegone (7.7%) were the most abundant constituents. The in vitro antibacterial activity test was carried out using the well diffusion method. *Z.clinopodioides* oil, sample A, was strongly active against the Gram-pos bacteria: *Staphylococcus epidermidis, Staphylococcus aureus* and *Staphylococcus saprophyticus* with MIC value of 0.3, 1.15 and 1.2 (mg/ml) respectively and also against Gram-negative bacteria: *Escherichia coli*, *Shigella flexneri* and *Salmonella typhi*, again with MIC values of 0.3, 0.3, 0.6 (mg/ml) respectively. While, the oil of sample B had moderate inhibitory against the Gram- positive and Gram- negative bacteria comparing to sample A; the inhibitory activity against three Gram-positive, *St. aureus, St. epidermidis, St. saprophyticus* was shown by the 1.9, 1.2, 1.1 (mg/ml) of MIC value and against Gram- negative bacteria *Sh. flexneri*, *S. typhi and E. coli* was shown by 2.2, 1.2 and 1.0 (mg/ml) of the MIC value. Moderate inhibitory activity against *Ps. aeruginosa* was obtained for both sample A and B.

P 095

Phytochemical and antimicrobial studies on *Artemisia monosperma*

El-Toumy SAA[1], Hawas UW[2], Hussein AA[3], Abd El-Nasser NH[4]
[1]Chemistry of Tannins Department, [2]Phytochemistry and Plant systematic Department, [3]Chemistry of Medicinal Plants Department, [4]Microbial Chemistry Department, National Research Center, El-Tahrir Street, Dokki, Cairo, Egypt

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main source of drugs. Herbal medicines used to treat many diseases including several infections. The present study deals with the isolation and identification of flavonoids from the aerial part of *Artemisia monosperma* and evaluation of the antimicrobial effects of a methanol-water (70:30) extract. This extract of *A. monosperma* was subjected to repeated chromatography on polyamide, cellulose and Sephadex LH-20 resulting in, quercetin 3-*O*-β-glucopyranoside, quercetin 5-*O*- β -glucopyranoside, isorhamnetin 5- *O*-β-glucopyranoside, 5,4′-dihydroxy 6,7-dimethoxy flavone, 5,4-dihydroxy 6,7, 3′-trimethoxy flavone, quercetin and isorhamnetin. The structures of the isolated compounds was elucidated on the basis of spectral analysis. The hydroalcoholic extract of the aerial part of *A. monosperma* exhibited variable degrees of antimicrobial activity (diffusion method). The extract shows a moderate activity against *Escherichia coli* and *Bacillus cereus* (20 mm diameter) and low activity against *Bacillus subtilis* (16 mm diameter) compared with that exerted by antibiotics (MIC,1.020 – 3.05 mg/ml). It was found inactive against *Staphylococcus aureus* and *Streptococcus pyogens* [1,2]. **References:** [1] Zhu X, Zhang H, Lo R. (2004) J. Agric. Food Chem. 52: 7272 – 7278, [2] Chacha M, Bojase-Moleta G, Majinda RRT. (2005) Phyt. 66: 99 – 104.

P 096

Composition and antimicrobial activity of *Laserpitium zernyi* Hayek essential oils

Petrović S[1], Pavlović M[1], Couladis M[2], Tzakou O[2], Milenković M[3], Pavlović I[1], Niketić M[4]
[1]Institute of Pharmacognosy, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade, Serbia; [2]Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, Panepistimioupoli Zographou, 15771 Athens, Greece; [3]Institute of Microbiology and Immunology, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade, Serbia; [4]Natural History Museum, Njegoževa 51, 11000 Belgrade, Serbia

Laserpitium zernyi Hayek (Umbelliferae) [1] is an aromatic perennial plant distributed in the mountain regions of the C. Balkan (S. Serbia, W. FYROM, E. Albania, N. W. Greece). It was treated earlier as a subspecies of the closely related *L. siler* L., subsp. *zernyi* (Hayek) Tutin [2]. The essential oils of flowers (sample 1) and leaves (sample 2), isolated from air-dried plant material by hydrodistillation, according to the procedure of Ph. Eur. 4 were investigated. Essential oil yields were 0.22% and 0.14% (w/w), respectively. The chemical analysis of the oils was performed using GC-FID and GC-MS. Fifty-five compounds (96.8% of the total oil) and fifty-eight compounds (89.4% of the total oil) were identified in sample 1 and 2, respectively. The content of monoterpenes was 75.6% in sample 1 and 59.1% in sample 2, while sesquiterpenes were present in smaller quantity (21.2% and 29.2%, respectively). In flower oil the major components were sabinene (18.5%), limonene (12.0%), β-phellandrene (12.0%) and terpinen-4-ol (10.6%). In leaf oil the most abundant constituents were β-pinene (20.0%), terpinen-4-ol (12.0%) and α-bisabolol (6.4%). Antimicrobial activity of the oils was tested using the agar diffusion [3] and broth microdilution methods [4] against six bacterial strains (*Staphylococcus epidermidis* ATCC 12228, *S. aureus* ATCC 25923, *Micrococcus luteus* ATCC 10240, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* NCIMB 9111*)* and a yeast *Candida albicans* ATCC 10259. The best inhibitory effect (MIC 80 µl/ml) exhibited sample 1 against *S. epidermidis* and *K. pneumoniae*, sample 2 against *E. coli*, and both oils against *M. luteus.* **References:** [1] Micevski, K. (2005) Flora na Republika Makedonija 1(6). Makedonska Akademija na Naukite i Umetnostite. Skopje. [2] Tutin, T.G. (1968): Laserpitium L. In: Tutin, T.G. et al. (eds). Flora Europaea 2. University Press. Cambridge. [3] Acar, J.F., Goldstein, F.W. (1996) In: Lorian, V. (ed) 4th Ed. Williams & Wilkins. Baltimore. [4] Candan, F., Unlu, M. et al. (2003) J. Ethnopharmacol.: 87, 215 – 220.

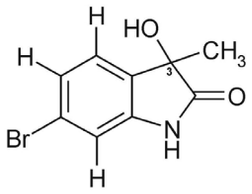
P 097

Bromoanaindolone – a new antimicrobial active metabolite excreted by the cyanobacterium *Anabaena constricta*

Volk RB[1], Girreser U[2], Al-Refai M[3], Laatsch H[3], Blaschek W[1]
[1]Pharmaceutical Institute, Department of Pharmaceutical Biology, University of Kiel, Gutenbergstraße 76, D-24118 Kiel, Germany; [2]Pharmaceutical Institute, Department of Pharmaceutical Chemistry, University of Kiel, Gutenbergstraße 76, D-24118 Kiel, Germany; [3]Institute for Organic and Biomolecular Chemistry, University of Göttingen, Tammannstraße 2, D-37077 Göttingen, Germany

Cyanobacteria have been increasingly recognized as an excellent source of biologically active metabolites, fine chemicals (e.g. vitamins, polyunsaturated fatty acids, proteins) and other useful natural products [1]. Within our own research activities in this field of interest, a new brominated indole alkaloid, designated as bromoanaindolone, was isolated from culture media of the cyanobacterium *Anabaena constricta*. By means of IR, MS and NMR data, the molecular structure of this cyanobacterial exometabolite was identified as 6-bromo-3-hydroxy-3-methyl-indol-2-one (**1**) with a slight excess of the (3R) enantiomer. For the isolated and purified compound an antimicrobial activity in different test systems such as suspension and porous matrix tests was quantifiable (minimum toxic quantities against cyanobacteria: 8 – 20 µg; minimum inhibitory concen-

tration against bacteria: 128 μg ml^{-1}). Thus, these results point to a natural function of bromoana-indolone as an allelopathic substance as outlined for other antimicrobial exometabolites of cyanobacteria earlier [2,3,4].



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P 098

Anti-inflammatory and antimicrobial activity of *Sideritis scardica* extracts

Tadić VM[1], Djordjević S[1], Arsić I[1], Dobrić S[2], Milenković M[3], Antić-Stanković J[3]

[1]Institute for Medicinal Plant Research "Dr. Josif Panèić", 11000 Belgrade, Serbia; [2]Institute for scientific information, Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia; [3]University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia

Sideritis scardica Griseb., Lamiaceae (mountain tea) is an endemic plant of the Balcan peninsula. Traditionally, mountain tea has been used as descongestant of the respiratory tract, analgesic, wound healing and astringent agent, known as well for its anti-inflammatory and gastroprotective properties [1]. In the present study we evaluated anti-inflammatory and antimicrobial effects of fractions prepared from an ethanolic extract of aerial plant material (ether, ethyl acetate and 1-butanol fractions). Anti-inflammatory activity was tested by the carrageenan-induced rat's paw oedema test. The extracts, dissolved in DMSO and applied in concentrations of 200, 100 and 50 mg/kg, showed significant anti-inflammatory effect. Compared to the effect of the positive control anti-inflammatory drug indomethacine (4 mg/kg) which produced 50% decrease of inflammation, ether and 1-butanol extracts exhibited about the same effect in doses 200 and 100 mg/kg (53.6 and 48.7%; 48.4 and 49.9%, respectively). Data were statistically evaluted applying Tukey test as a post hoc multiple comparisons. The ethanol extract and its ether, ethyl acetate and 1-butanol fractions have been examined as well for their antimicrobial activity using agar diffusion and broth microdilution methods [2,3]. The antimicrobial activity was evaluated using the following laboratory strains of microorganisms: *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCIMB9111), *Pseudomonas aeruginosa* (ATCC 27853) and yeast *Candida albicans* (ATCC 10259). The investigated samples exhibited antimicrobial activity to varying degree against all tested strains. The maximum activity was observed against *S. epidermidis*, *M. luteus, E. coli* and *P. aeruginosa,* moderate against *K. pneumoniae*. The n-ButOH fraction was the most active (50 mg/ml ethanol solution exhibited 206.7% of ampicillin activity against *S. epidermidis*). **References:** [1] Gabrieli, C.N. et al. (2005) J. Ethnopharnacol 96: 423 – 428. [2] Hili, P. et al. (1997) Lett Applied Microb 24: 269 – 275. [3] Candan, F. et al. (2003) J Ethnopharmacol 87: 215 – 220.

P 099

Antilisterial activity of *Euclea natalensis* and its naphthoquinones

Lall N, Meyer JJM, van der Kooy F

University of Pretoria, Medicinal Plant Sciences, 0002, South Africa

The roots of *Euclea natalensis* DC (Ebenaceae) are used by the indigenous people of Southern Africa against various bacterial infections [1]. Napthoquinones (NQs) isolated from its roots (diospyrin, neodisopyrin, isodiospyrin, 7-methyljuglone), a few synthesized napthoquinones and its root extracts were investigated against the foodborne pathogen, *Listeria monocytogenes* (ATCC 7644). Extract and compounds were tested at concentrations ranging from 500.0 to 0.12 μg/ml for the determination of the minimum inhibitory concentration (MIC) against *L. monocytogenes*. The optical density of the culture was adjusted to 0.1 using fresh broth to give a standard inoculum of ca. 10^6 colony forming units. The minimum bactericidal concentration (MBC), defined as the concentration producing a 99.9% reduction in CFU in the initial inoculum was also determined. The chloroform extract of the roots of *E. natalensis* exhibited an MIC of 250.0 μg/ml. A complete inhibition of the bacteria was observed at 500 μg/ml indicating its bactericidal effect. Among naphthoquinones tested, 7- methyljuglone was found to be the most potent naphthoquinone, exhibiting an MIC and MBC of 125 μg/ml followed by its dimer, diospyrin. The MIC's of the naphthoquinones indicates that the keto groups at C1 and C4 are important for antibacterial activity. This can be seen by comparing the activity of 7-methyljuglone (MIC = 125.0 μg/ml) with shinanolone (MIC => 1000.0 μg/ml). The keto group at carbon 1 of shinanolone is reduced to the corresponding hydroxyl group. The MICs of other naphthoquinones, neodiospyrin, isodiospyrin, chloro-methyljuglone, ranged from 125 – 500 μg/ml. In conclusion, the most potent antilisterial compound, 7-methyljuglone, merits further studies to determine its pharmacological properties *in vivo* for antilisterial therapy. *Acknowledgements:* National Research Foundation, University of Pretoria, CNRS, Orleans France. **Reference:** [1] Van Wyk, B et al (1997). Field Guide to Trees of Southern Africa, McKenzie, Cape Town.

P 100

Screening of antimicrobial activity of aqueous extracts of selected *Potentilla* L. species

Tomczyk M[1], Leszczyńska K[2], Tomczykowa M[1], Jakoniuk P[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Białystok, ul. Mickiewicza 2a, 15 – 230 Białystok, Poland; [2]Department of Microbiological Diagnostics, Faculty of Pharmacy, Medical University of Białystok, ul. Waszyngtona 15A, 15 – 274 Białystok, Poland

Potentilla species (Rosaceae) are widely distributed in regions of the northern hemisphere. Many of them are cultivated in gardens for ornament. *Potentilla* species are known for their therapeutic properties and are considered to be one of the safest astringents in the treatment of diarrhoea, dysentery and sore throats. Recent investigations have also shown that some extracts of different parts of plant from *Potentilla* species exhibit antioxidant, hypoglycemic, anti-inflammatory, antitumor and anti-ulcerogenic potential properties [1,2]. The aim of our study was to compare the antimicrobial activities of nine aqueous extracts obtained from aerial parts of *Potentilla* species: *P. anserina*, *P. argentea*, *P. erecta*, *P. fruticosa*, *P. grandiflora*, *P. nepalensis, P. recta, P. rupestris* and *P. thuringiaca*. These extracts were tested for their antimicrobial activity against eight microorganisms: *Helicobacter pylori* ATCC 43504, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 using a broth microdilution method [3,4]. The aqueous extracts of *Potentilla* species showed the strongest antimicrobial activity against *H. pylori* (MIC = 0.1÷0.5 mg/ml) and almost none in case of Gram-negative bacteria. The investigated extracts were moderately active against Gram-positive

bacteria, as well as against *C. albicans*. *Acknowledgements:* This study is financially supported by Medical University of Białystok (grant No. 4 – 12670 F) **References:** [1] Goswami, D. A. et al. (1975) New Phytol. 75: 135 – 146. [2] Tomczyk, M. (2006) Biochem. Syst. Ecol. 34: 770 – 773. [3] Jorgensen, J.H. et al. (1999) Manual of Clinical Microbiology, Murray, P.R. et al. (Eds.), American Society for Microbiology, Washington DC. [4] Espinel-Ingroff, A. et al. (1999) Manual of Clinical Microbiology, Murray, P.R. et al. (Eds.), American Society for Microbiology, Washington DC.

P 101

In vitro inhibitory activity of essential oil of *Apium nodiflorum* against *Helicobacter pylori*

Menghini L[1], Epifano F[1], Leporini L[1], Pagiotti R[2], Angelini P[2], Genovese S[3]
[1]*Dipartimento di Scienze del Farmaco, Via dei Vestini, 31, 66013, Chieti Scalo (CH), Italy;* [2]*Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Borgo XX Giugno, 74, 06126 Perugia, Italy;* [3]*Dipartimento di Chimica e Tecnologia del Farmaco, Sezione di Chimica Organica, Via del Liceo, 06123 Perugia, Italy*

Apium nodiflorum (L.) Lag. (syn. *Helosciadium nodiflorum* Koch.) (Fam. Umbelliferae), commonly known as "Fools watercress" but also known under several locally common names such "sedanina d'acqua", "cannole" or "cannizzelle" is a glabrous perennial herb which typically grows in shallow streams, lakes, ponds and marshes. It can be found throughout the Mediterranean region. *A. nodiflorum* is used in the ethnomedical traditions of people from the Abruzzo region (Central Italy) as culinary herb, mixed with fried garlic and capsicum. Extracts from hypogeous parts are used as diuretic and finally decoction of leaves is used against stomach ache [1]. In the present communication we wish to report results on the qualitative and quantitative analysis of essential oil from fresh leaves of *A. nodiflorum* and its activity against growth of *Helicobacter pylori in vitro*. The essential oil was obtained by steam distillation using a Clevenger apparatus and analyzed by gas chromatography and mass spectrometry. The major components found in the essential oil were limonene (27.72%), *p*-cymene (23.06%), myristicine (18.51%) and β-pinene (6.62%). The essential oil of *A. nodiflorum* exhibited an appreciable activity as inhibitor of growth of *Helicobacter pylori* (strain DSMZ 4867, originated from human gastric samples) *in vitro* (MIC = 12.5 µg/mL). *Acknowledgements:* Regione Abruzzo (L.R. 35/97) Project 'Tutela della Biodiversita', University "G. d'Annunzio", Ufficio Relazioni Internazionali". **References:** [1] Tammaro, F. (1985) Flora Officinale d'Abruzzo. Regione Abruzzo. Chieti.

P 102

Antimicrobial activities of various essential oils and their main aromatic volatile constituents

Höferl M[1], Jirovetz L[1], Buchbauer G[1], Schmidt E[2], Stoyanova A[3], Denkova Z[4], Slavchev A[4], Geissler M[5]
[1]*Department of Clinical Pharmacy and Diagnostics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria;* [2]*Kurt Kitzing GmbH, Hinterm Alten Schloss 21, D-86757 Wallerstein, Germany;* [3]*Department of Essential Oils, University of Food Technology, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria;* [4]*Department of Microbiology, University of Food Technology, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria;* [5]*SHIMADZU Germany, Department of GC and GC-MS, Albert-Hahn-Strasse 6 – 10, D-47269 Duisburg, Germany*

In continuation of our effort on data correlation of antimicrobial activities of aroma compounds among various essential oils and plant extracts [1], the aromatic volatiles *p*-cymene, carvacrol, eugenol and thymol as well as the essential oils of *Cinnamomum zeylanicum*, *Eugenia caryophyllus*, *Origanum vulgare*, *Pimenta dioica*, *Pimenta racemosa*, *Satureja hortensis*, *Trachyspermum ammi* and *Thymus vulgaris* were investigated. Therefore, these samples and as reference substances synthetic antibiotics as well as the natural antimicrobial components carveol, *m*-cresol, *o*-cresol and *p*-cresol were tested against strains of two Gram-(+) and five Gram-(-) bacteria and the fungus *Candida albicans* using agar dilution and agar diffusion methods. The analysis of the chemical composition of the essential oils by means of GC and GC-MS focusing on aromatic volatiles resulted as follows: *C. zeylanicum*: eugenol (74.9%); *E. caryophyllus*: eugenol (76.8%); *O. vulgare*: carvacrol (66.1%); *p*-cymene (9.2%); *P. dioica*: eugenol (76.0%); *P. racemosa*: eugenol (45.6%); *S. hortensis*: carvacrol (41.5%), *p*-cymene (10.7%), thymol (8.7%); *T. ammi*: thymol (43.7%), *p*-cymene (17.7%); *T. vulgare*: thymol (43.4%), *p*-cymene (23.5%) and carvacrol (4.1%). All investigated aromatic volatiles and essential oils exhibited a medium to strong antimicrobial activity against the Gram-(+) bacteria *Staphylococcus aureus* and *Enterococcus faecalis* and the Gram-(-) bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella* sp., whereas merely weak to medium effects were observed against *Candida albicans*. **References:** [1] Jirovetz, L. et al. (2006) Recent Progress in Medicinal Plants, Vol. 13: Search for Natural Drugs (Govil, J.N., Singh, V.K., Arunachalam, C., eds.), Studium Press LLC, Houston (Tx), USA.

P 103

Molecular mechanism of action of the flavanone pinostrobin from *Cajanus cajan* leaves in cancer cells

Ashidi JS[1], Houghton PJ[1], Hylands PJ[1], Sieber S[2], Efferth T[2]
[1]*Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom;* [2]*Pharmaceutical Biology of Natural Products Group (C015), German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany*

We have earlier reported the *in-vitro* cytotoxic effect of the methanol extract of *Cajanus cajan* (L.) Millsp. (Leguminosae) and three of its constituents on some solid tumour cell lines.[1] Pinostrobin, one of these constituents, is now reported to show significant inhibition in a cell proliferation assay using human CCRF-CEM leukaemia cells. The IC_{50} value obtained in an XTT assay was 5.0 µM. Additionally, the compound was tested against the multi-drug resistant subline, CEM/ADR5000, where we observed a cross-resistance with the MDR cells. It arrested the migration of cells into the G_1 phase of the cell cycle which could be demonstrated in a flow cytometry study. The compound also generated reactive oxygen species (ROS) in the ROS assay. The damage of the mitochondria membrane has been known to play a key role in apoptosis, hence we measured the effect of pinostrobin on its membrane potential ($\Delta\Psi m$): it was intact. We further confirmed the mechanism behind the observed massive apoptosis by measuring its impact on the Fas receptors (Apo 1 or CD95), an important molecule of the extrinsic pathway of apoptosis. There was significant dose-dependent upregulation of the Fas receptors which explained the observed apoptosis. Significant accumulation of the compound in the cytosol after 2 h incubation when assessed by auto-fluorescence assay was also found. Our findings lend support to the local use of *C. cajan* in prevention and therapy of cancer. An *in-vivo* study of pinostrobin in leukaemia subjects is suggested. *Acknowledgements:* JSA thanks the Commonwealth Scholarship Commission and King's College London, United Kingdom for their financial support. **Reference:** 1. Ashidi J.S. *et al.* (2006), Planta Med. **72**:P016.

P 104

Antibacterial activity of liquorice, glycyrrhetinic acid and derivatives against *Helicobacter pylori*

Krausse R[1], Bielenberg J[2], Blaschek W[3]
[1]Institute for Infection Medicine, University Medical Center Schleswig-Holstein Campus Kiel (UKSH) and Christian-Albrechts University of Kiel, Brunswiker Straße 4, 24105 Kiel, Germany; [2]Raphael-Pharmacy, Bahnhofstr. 53, 25364 Westerhorn, Germany; [3]Institute of Pharmacy, Department of Pharmaceutical Biology, Christian-Albrechts University of Kiel, Gutenbergstrasse 76, 24118 Kiel, Germany

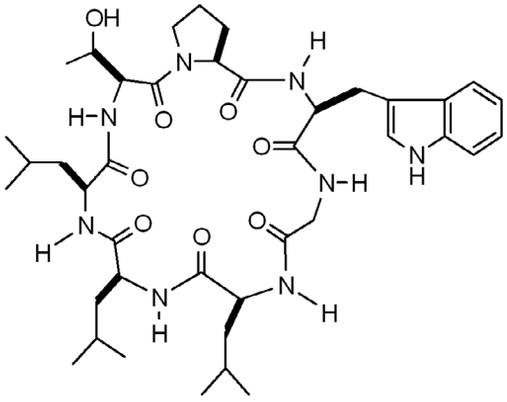
The medicinal use of Liquorice and its main constituent, glycyrrhizin (GL) on peptic ulcers has a long tradition [1]. The beneficial effects might also be, in part, due to their bactericidal effect on *H. pylori* (*Hp*) [2,3,4]. Therefore, the *in vitro* activity of liquorice (*Extractum Liquiritiae* [EL], GL content 6.4%), glycyrrhizic acid (GL), the aglycone of GL, glycyrrhetinic acid (GA) and a novel lipophilic derivative of glycyrrhetinic acid monoglucuronide (GAMG), acetylated GAMG (aGAMG) against several *Hp* strains has been investigated. 29 *Hp* strains (two clarithromycin [CLR]- and five metronidazol [MET]-resistant) were tested. The minimal inhibitory concentrations (MICs) were determined by the agar dilution method on BHI-agar, containing two fold serial dilutions (range [μg/ml]: EL 3.15 – 400, GA and GL 3.5 – 400, aGAMG 0.78 – 400). The killing kinetics of *Hp* strains were monitored in BHI-broth (~ 10^{6-7} cfu/ml), after 0, 4, 24, 48, 72 and 96 h. GA was the most potent compound ($MIC_{50/90}$= 50/100 μg/ml). 23 of the tested 29 *Hp* strains were inhibited at MIC ≤ 50 μg/ml. CLR-resistant strains were susceptible at 12.5 and 25 μg/ml, 4 MET-resistant strains at 25 – 50 and one at 200 μg/ml. The MIC (μg/ml) of aGAMG tested with 24 strains was ≤ 6.25 (7 strains), 50 (1 strain), 100 – 200 (3 strains) and ≥ 400 (13 strains). EL and GL were less active (MICs > 400 μg/ml). GA exhibited rapid, concentration and strain-dependent bactericidal activity. The potent *in vitro* activity of GA against different susceptible and antibiotic-resistant strains of *H. pylori* provides an extended interpretation of its beneficial effect on peptic ulcers. **References:** [1] Armanini, D. et al. (2000) Exp. Clin. Endocrinol. Diabetes 110: 257 – 261. [2] Kim, D.H. et al. (2000) Arch. Pharm. Res. 23: 172 – 177. [3] Dunn, B.E. et al. (1997) Clin. Microbiol. Rev. 10:720 – 741. [4] Krausse, R. et al. (2004) J. Antimicrob. Chemother. 54:243 – 246.

P 105

Cyclic Heptapeptides from the latex of *Jatropha integerrima*

Conrad J[1], Mongkolvisut W[2], Sutthivaiyakit S[2], Leutbecher H[1], Mika S[1], Klaiber I[3], Möller W[4], Rösner H[4], Beifuß U[1]
[1]Institute of Chemistry, Hohenheim University, Garbenstraße 30, 70599 Stuttgart, Germany; [2]Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand; [3]Institute of Physiology, Hohenheim University, August-von-Hartmann-Straße 2, 70599 Stuttgart, Germany; [4]Institute of Zoology, Hohenheim University, Garbenstraße 30, 70599 Stuttgart, Germany

Jatropha species (Euphorbiaceae) are known to be a rich source of bioactive diterpenes and cyclic peptides. [1] In the course of our phytochemical investigations of *J. integerrima* we isolated two new cylic heptapeptides, namely integerrimide A (**1**) and B (**2**), from the latex of *J. integerrima* [2]. The structures of **1** and **2** (both existing as equilibrium mixtures of different conformers) were elucidated by extensive 1D, sel-1D, and 2D NMR studies, MS, as well as chemical degradation. In a screening on basic cytoskeleton- dependent cellular processes (neurite outgrowth, cell proliferation, cell migration) both compounds (50 μM each) showed a significant inhibition of neurite outgrowth of E7 chicken spinal cord neurons as well as an antiproliferation (up to 40% lower cell densities after 2 days) but no cytotoxic activity on human melanoma cells (IPC-298). Standard cell migration assay (SMA) with confluent human pancreatic carcinoma cells (Capan II) revealed an inhibition of cell migration by about 30 and 20% of **1** and **2** (50 μM each), respectively.



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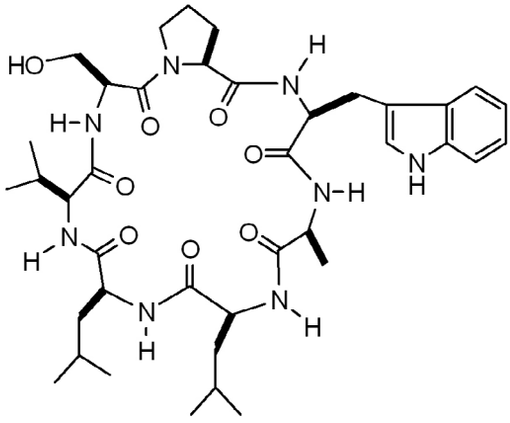

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P 106

Analysis and stability of the constituents of St. John's wort oils prepared with different methods

Isacchi B[1], Bergonzi MC[1], Carnevali F[2], van der Esch SA[2], Vincieri FF[1], Bilia AR[1]
[1]Department of Pharmaceutical Sciences, University of Florence, 50019 Sesto Fiorentino, Florence, Italy; [2]BIOTEC – AGRO ENEA C.R. Casaccia, V. Anguillarese, 301 – 00123 S. M. Galeria, Rome, Italy

St John's wort (SJW) is one of the most interesting medicinal plants with a long history of use in traditional medicine all over Europe [1]. Traditional preparations and in particular the oil from SJW flowers [3] remain one of the most popular and curative topical remedy against ulcerations and burns. In this study we tried to rationalize the production system of the oily preparation in order to obtain the highest concentration and stability of phloroglucinols. Five different samples of SJW oils were evaluated to verify the variability and stability of the constituents according to the following factors: different harvesting time, fresh or dried plant material, use of sunlight or heating during extraction. The stability of these oils during was tested over one year. Only constituents can be extracted during maceration and their variability, especially phoroglucinol content in the various oils is great. Extractability of hyperforin ranged from 19.5 to 2.4% and maximum extraction was obtained with fresh plant material exposed to sunlight during maceration. The drying process and the use of artificial light during maceration affected the extraction of hyperforin, producing a very low percentage of this compound. Adhyperforin showed a better extractability if fresh plant material was used. Furohyperforin was identified only in one sample

(12%), probably as oxidation product derived from the phoroglucinol under heat. No naphthodianthrones neither flavonoids, with the exception of I3, II8-biapigenin were found in the oils. However, its content had a wide variability: the highest content was 3.51 ppm, while it was not detected in the samples stressed with heat and/or light. Concerning the stability study during one year, the results evidenced that the traditional oil and the oil obtained by extraction of fresh material represent the best procedures to obtain high phloroglucinol contents which seems to be responsible for the oil's activity. **References:** [1] Bradley P. St. John's wort. British Herbal Compendium (2006) 363 – 368. vol. 2. British Herbal Medicine Association. Bournemouth UK. [2] ESCOP "Hyperici Herba" Monographs on the Medicinal Uses of Plant Drugs. Exeter, U.K.: European Scientific Cooperative on Phytotherapy, 1997. [3] DAB6, EB 6, 1941.

P 107

Antimicrobial activity of selected plants used in Ethnomedicine in South-Western Nigeria

Sowemimo A[1], Idika N[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, 101017, Nigeria; [2]National Institute for Medical Research, Edmond crescent, Lagos, 100001, Nigeria

Nine plants [*Aframomum melegueta* K. Schum. (Zingiberaceae), *Alstonia boonei* De Wild. (Apocynaceae), *Annona senegalensis* Pers. (Annonaceae), *Bixa orellana* L. (Bixaceae), *Cassia podocarpa* Guill. & Perr. (Leguminosae), *Combretum racemosum* P. Beauv. (Combretaceae), *Gardenia ternifolia* Schum & Thonn. (Rubiaceae), *Heliotropium indicum* L. (Boraginaceae) and *Mucuna pruriens* (Linn) DC (Leguminosae)] are used in herbal remedy to treat diarrhoea, dysentery and skin diseases [1]. The methanolic extracts of the plants were evaluated for *in vitro* antimicrobial activity at concentrations ranging from 25 – 125 mg/ml using the agar diffusion method to validate the ethnobotanical uses of the plants and compare activities. The root of *Combretum* and the *Annona* stem bark showed significant activity against the local and standard strains of *Staphyloccocus aureus* (MIC 25 – 50 mg/ml) and *Shigella dysenterae* (MIC 25 – 50 mg/ml) while the root of *Combretum* was also active against *Escherichia coli (*MIC 25 mg/ml*)* and *Salmonella paratyphi* (MIC 25 mg/ml*). Aframomum* root, *Cassia* root and *Heliotropium* whole plant were active against *Salmonella paratyphi*, *S. aureus* and *E. coli* respectively (MIC 100 mg/ml). *Alstonia* bark, *Bixa* fruit, *Gardenia* and *Mucuna* root showed no antimicrobial activity. None of the plant extracts exhibited activity comparable to the reference compound tetracycline. However, *Combretum* afforded greater antimicrobial activity than *Annona, Aframomum, Cassia and Heliotropium. S. aureus* was most susceptible to the plant extracts. **References:** [1] Burkill, H.M. (1994). Royal Botanic Gardens. Surrey. [2] Bauer, A. et al. (1966) Amer. J. Clin. Path. 45: 493 – 496.

P 108

Isolation and Characterisation of Antiviral and Immunomodulatory Constituents from Nigerian mistletoe, *Loranthus micranthus*

Osadebe PO[1], Omeje EO[1]
[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.41001, Enugu, Nigeria

The avalanche of data on the immunomodulatory and anti-cancer activites of the European mistletoe, *Viscum album* especially with respect to its lectin constituents have been well documented [1]. These activities are always dependent on the season and host tree from which the plant material was harvested. The antimicrobial, antidiabetic and antimotility activities of the Eastern Nigeria mistletoe, *Loranthus micranthus* Hook. f. (Loranthaceae) have been reported [2,3,4]. Preliminary phytochemical evaluation showed presence of tannins, flavonoids, alkaloids, terpenoids, proteins, carbohydrates and saponins. Preliminary investigation with the crude aqueous (hot and cold extraction), methanolic (hot and cold extraction) and n-hexane (hot and cold extraction) extract of the mistletoe extract harvested in early January 2007 showed marked immunostimulatory activity using the total and differential leucocyte count, the delayed hypersensitivity reaction (DTH) and antibody titration models at three different dose levels of 50, 100, and 200 mg/kg body weight in mice and rats. We observed a dose dependent increase in total and differential leukocyte count by well over 90% compared to the control ($p < 0.05$, one-way ANOVA and Student t-test). Higher doses did not show any further benefit. The average increase in indurations in mice footpads in the presence of the extracts by 13, 47.8 and 56% followed similar trends as above. The mean antibody titre values increased by 100, 120, and 185% ($p < 0.05$ compared to control) respectively at the tested dose levels of the extract. The results were all subjected to statistical analysis and no significant differences in respect to host tree variation were observed ($P < 0.001$). Acknowlegement: The authors are thankful to Mr. Ozioko of Bio resource Development and Conservation Centre, Nsukka, Enugu state for collection and authentication of the plant material from different host trees. **References:** [1] Becker H, (1986) Oncology 43: 2 – 7. [2] Osadebe PO and Akabogu IC (2006) Fitoterapia 7; 54 – 56 3. Osadebe PO, Okide GB and Akabogu IC, (2004), J. Ethnopharmacol. 95: 133 – 138. 4. Osadebe PO and Uzochukwu IC (2006), J. Pharm. Allied Sci. 3: 263 – 268.

P 109

Successful *in silico* discovery of natural inhibitors for human rhinovirus coat protein

Rollinger JM[1], Steindl TM[2], Anrain K[1], Ellmerer EP[3], Schmidtke M[4], Langer T[2], Stuppner H[1]
[1]Institute of Pharmacy/Pharmacognosy; [2]Institute of Pharmacy/Pharmaceutical Chemistry, [3]Institute of Organic Chemistry; LFUI, Innrain 52, A-6020-Innsbruck, Austria; [4]Institute of Virology and Antiviral Therapy, Friedrich Schiller University, Hans-Knoell-Str. 2, D-07745 Jena, Germany

Human rhinoviruses (HRVs) are the major cause of mild respiratory diseases generally known as the common cold. These illnesses still lack effective antiviral treatment. A promising strategy deals with the inhibition of the structurally identified HRV coat protein, thus blocking the uncoating of the viral particles and preventing cell attachment [1]. Based on this target information, the aim of this study was to apply an in silico approach to (i) identify virtually active ligands from nature, (ii) detect promising antiviral herbal products, and (iii) validate this strategy by the isolation and experimental investigation of the predicted virtual hits. A recently generated pharmacophore model [2] was used to virtually screen our in-house generated database DIOS consisting of > 9 000 natural products [3]. Different sesquiterpene coumarins from asafetida revealed as virtual hits from the screening filtering experiment with Catalyst (Vers. 4.11). Four sesquiterpene umbelliferone ethers were isolated and identified as microlobiden, kellerine, farnesiferol B and C by 1D and 2D NMR experiments. Their antiviral activity has been determined in cytopathic effect inhibitory assays [4] with different picornavirus species. All compounds were tested using non-cytotoxic sample concentrations. Results were compared with the capsid-binding activity of pleconaril. The virtual hits farnesiferol B and C showed selective inhibitory effects against pleconaril-sensitive HRV-2 with IC_{50} of 1.25 (0.10 – 4.26; CI_{95}) μM and 3.79 (2.46 – 8.23; CI_{95}) μM, respectively. By contrast, microlobiden and kellerine, which did not map with the pharmacophore's features, showed no antiviral potential. The correlation of experimental results with virtually predicted activities obtained from this study underlines the power of the virtual screening approach for the rational discovery of bioactive natural products [5]. *Acknowledgements:* This work was granted by the 'Nachwuchsförderung 2005' of the LFU Innsbruck (J.M.R.). **References:** [1] Hadfield, A.T. et al. (1999) Proc Natl Acad Sci USA 96: 14730 – 5. [2] Steindl, T.M. et al. (2005) J Med Chem 48: 6250 – 60. [3] Rollinger, J.M. et al. (2004) J Chem Inf Comput Sci 44: 480 – 8. [4] Schmidtke M. et al. (2001) J

Virol Meth 95: 133 – 43. [5] Rollinger, J.M. et al. (2006) Planta Med. 72: 671 – 8.

P 110

Constituents from *Morus* root bark against *Venturia inaequalis* – the causal agent of apple scab

Rollinger JM[1], *Spitaler R*[1], *Menz M*[1], *Schneider P*[1], *Ellmerer EP*[2], *Marschall K*[3], *Zelger R*[3], *Stuppner H*[1]

[1]*Institute of Pharmacy/Pharmacognosy;* [2]*Institute of Organic Chemistry; Leopold-Franzens University of Innsbruck, Innrain 52, A-6020-Innsbruck, Austria;* [3]*Research Centre for Agriculture and Forestry Laimburg, 39040 Post Auer, Italy*

Apple scab, which is caused by infection of *Venturia inaequalis* (Cooke) Winter, is the most serious disease in the cultivation of apples. It causes yield decrease and loss of fruit quality. Especially in organic apple orcharding effective organic options for synthetic pesticides are being called for [1], of which the use of plant extracts exhibiting antifungal activity seems most promising. In this study an *in vitro* assay is presented derived by modification of protocols by Olaya and Köller [2] and Gilliver [3]. It allows for quantitative and qualitative discrimination of the germination inhibitory potential of extracts and pure compounds; it provides cheapness, a minimum requirement for technical equipment, and minute sample amounts. From a screening of plant extracts, the methanol extract of *Morus* root bark, which previously showed pronounced cyclooxygenase-1 and -2 inhibitory potential [4], revealed distinct qualities against *V. inaequalis*. Based on the established assay, a bio-guided fractionation was performed to isolate the active constituents applying different chromatographic methods. Using this approach, the aim of this study was to identity the antifungal components within the active extract and to determine the germination inhibiting effect of the isolated single chemical entities. A further goal was to verify the *in vitro* activity by application and evaluation of the *V. inaequalis* inhibiting plant material in vivo on inoculated seedlings. Among the isolated metabolites from Morus root bark [moracin M, O/P, kuwanon L, sanggenons D, B, G, O, E, and C] all the Diels-Alder adducts, showed an antifungal activity with IC_{50} values between 10 and 123 μM. The *in vitro* activity of the most active fraction (A5, IC_{50} 39.0 ± 4.2 μg/mL) was tested on inoculated Golden Delicious seedlings, confirming a distinct antifungal activity against *V. inaequalis* for the tested natural material [5]. **References:** [1] Holb, I. J., Heijne, B. (2001) Gartenbauwissenschaft 66: 254 – 61. [2] Olaya, G., Köller, W. (1999) Pestic Sci: 55, 1083 – 8. [3] Gilliver, K. (1947) Ann Appl Biol 34: 136 – 43. [4] Rollinger et al. (2005) Planta Med. 71: 399 – 405. [5] Rollinger et al. (2006) J Agric Food Chem 54: 8432 – 6.

P 111

Studies on anti-inflammatory and antimicrobial activities of crude methanol extracts of *Zapoteca portoricensis*

Nwodo NJ, Uzochukwu IC

Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

In Eastern Nigeria the aqueous and alcoholic extract of *Zapoteca portoricensis* are used traditionally for the treatment of tonsillitis, gastrointestinal disorders, as antidiarrhoic, anticonvulsant and antispasmodic. In this study, the anti-inflammatory and antimicrobial activities of the methanol extracts of the root of *Zapoteca portoricensis* (Fabaceae) were examined. The effects of the extract on the acute and chronic inflammation were evaluated by egg-albumin-induced hind paw oedema and formaldehyde induced arthritis in rats (n = 3). Phytochemical tests on the whole root and methanol extract were done by standard procedures while the acute toxicity tests were carried out by Lorke's method [1]. The oral LD_{50} of the methanol extract was found to be 470 mg/kg. The MIC and MBC for the crude methanol extract ranged between 0.57 to 4.55 mg/ml. The killing rate constant of the crude methanol extract on clinical isolates ranged between 1.5474 to 3.054 s^{-1} depending on the isolate. The methanol extract of *Z. portoricensis* also showed significant ($P < 0.05$) acute at the dose of 250 mg/kg and chronic at the doses of 150 and 250 mg/kg anti-inflammatory activities in rats. Several medicinal plants used in folkloric medicine have been scientifically proven to possess anti-inflammatory activities [2]. The plant may prove beneficial in the management of different inflammatory conditions with concomitant bacterial infection. **References:** [1] Lorke, D.(1983).Archives of Toxicology 54, 275 – 287. [2]. Akah, P. A. and Nwambe, I. A. (1994) Ethnopharmacology 42, 179 – 182.

P 112

The Seasonal Variations of Lignan Profiles in *Anthriscus sylvestris* (L.) Hoffm

Koulman A[1,2], *Hendrawati O*[2], *Batterman S*[2], *Van Putten FMS*[2], *Bos R*[2], *Kayser O*[2]

[1]*Grasslands Research Centre, AgResearch, Palmerston North 4410, New Zealand;* [2]*Dept. of Pharmaceutical Biology, University of Groningen, A. Deusinglaan 1, 9713 AV, Groningen, The Netherlands*

Anthriscus sylvestris (L.) Hoffm. (Apiaceae) is a common wild plants in Northwest Europe that accumulate considerable amounts of lignans. Deoxypodophyllotoxin as the main attractive constituent can be used as a precursor for the production of podophyllotoxin but is not yet applied on an industrial scale. In previous studies we showed that different populations of *A. sylvestris* yielded significantly different phenylpropanoid profiles [1] and that there is no clear genetic factor determining the lignan profile when seed material from different locations is grown under identical conditions [2]. It was assumed that environmental factors determine the lignan profile. To test this hypothesis we collected 5 plants weekly during one year from 2 different locations. The roots and the aerial parts were profiled by GC-MS [1]. Large variation in lignan profiles were shown between individual plants collected at the same time and location. The aerial parts contain equal amount of lignans as the roots in early spring, but during the season the concentration of lignans in the aerial parts decreased. Especially the lignans present in lower amounts (like hinokinin and angeloyl podophyllotoxin) varied highly during the season. There is however a clear correlation between root mass and Deoxypodophyllotoxin content. Plants that were mowed several days before harvesting showed significant increase in their lignan content. The large variations in lignan contents could be to the advantage of the plant against herbivores. **References:** [1] Koulman, A. et al. 2001 Planta Med. 67: 858 – 62. [2] Koulman, A. et al. 2003 Planta Med. 69: 733 – 738.

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Antimicrobial activity of Ugandan Medicinal Plants

Kuglerova M[1], *Halamova K*[1], *Kokoska L*[1], *Van Damme P*[2], *Grade J*[2]

[1]*Department of Crop Science and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic;* [2]*Laboratory for Tropical and Subtropical Agriculture and Ethnobotany, Department of Plant Production, Faculty of Bio-Science Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium*

Different methods of traditional medicine are still used by local healers in lots of developing countries. For the specific situation of Uganda, there is little to no information on medicinal plant use by Karamojong healers. Moreover, even though there are some reports on pharmacology of Ugandan medicinal plants [1, 2], their antimicrobial activities are still poorly documented [3]. Thus, we decided to investigate the antimicrobial activity of five Ugandan tree species selected based on the information on their use in traditional medicine as evidenced by ethnobotanical research. Five ethanol extracts obtained by maceration of barks from *Dregea rubicunda* K.Schum., *Trichilia prieureana* A. Juss., *Turraea floribunda* Hochst., *Warburgia ugandensis* Sprague, and *Zanthoxylum chalybeum* Engl. were studied

for potential antimicrobial activity against *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis,* and *Candida albicans* using broth microdilution method [4]. With the exception of *D. rubicunda*, results show that all plant species possess antimicrobial activity against one or more micro-organisms tested in this study at concentrations of 16 mg/ml or below. Among the plants tested, *T. prieureana* and *T. floribunda* showed the most promising antimicrobial properties, inhibiting both stains of Gram-positive bacteria with minimum inhibitory concentrations 0.25 and 0.5 mg/ml, respectively. The extract from *W. ugandensis* exhibited the broadest spectrum of inhibitory activity against all microbial strains tested at concentrations of 8 mg/ml or below. **References:** [1] Katuura, E. et al. (2007) Afr J Ecol 45: 48 – 51. [2] Freiburghaus, F. et al. (1996) Trop Med Int Health 1(6):765 – 771. [3] Hamill, F.A. et al. (2003) J Ethnopharmacol 87 (1):15 – 19 2. [4] Jorgensen J.H. et al. (1999). In: Murray P.R. (ed.) Manual of Clinical Microbiology. ASM Press. Washington, DC.

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Anti-staphylococcal activity of rosemary extract

Zurak I
University Hospital, Kosorova 13, HR-10000 Zagreb, Croatia

The infection caused by *Staphylococcus* spp. and resistance against commonly used antibiotics is common in clinical practice. The use of creams, soaps, gels with antimicrobials could decrease level of contamination of the skin or mucous membranes. The aim of this work was to check sensitivity of staphylococci using rosemary extract. We have used more than 100 freshly isolated strains of staphylococci belonging to three main species: *Staphylococcus aureus, S. epidermidis,* and *S. saprophyticus* (some of them were MRSA positive and multiple-antibiotics resistant strains). Rosemary extract was prepared from air-dried leaves (Rosmarini folium) collected in Dalmatian (Croatian coast), and extracted with 60% (V/V) ethanol. Hole-plate diffusion antibacterial assay was performed with 50 μL of alcoholic rosemary extract in concentration range from 10 to 60 mg/mL. Ethanol and standard antibiotics were used as control. All investigated staphylococcal strains were sensitive to rosemary extract at 10 mg/mL with inhibition zones of growth between 15 and 17 mm. Higher concentration of rosemary extract (from 20 to 60 mg/mL) exhibited higher inhibition zones of growth, and correlation between concentration of rosemary extract and inhibition zones was found. Inhibition zones of rosemary extracts were found bigger or equal to standard antibiotics at 10 μg/mL (β-lactams, macrolides and glycopeptides). Equal sensitivity of staphylococcal species (MRSA and multiple-resistant) gives a possibility to use rosemary extract in topical preparations. Differences in antimicrobial activity between staphylococcal species and strains as well as between resistant profiles were not found, indicating uniform anti-staphylococcal activity of ethanol rosemary extract.

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Bioassay-guided fractionation for antifungal effects of *Centaurea virgata* subsp. *sqaurrosa* and *Centaurea iberica*

Asgari T[1], Amin G[1], Darvish M[2], Shidfar MR[1], Asgarpanah G[2], Mohammadi S[2], Badiozzaman M[2], Mazdiyasn M
[1]*Faculty of Pharmacy, Tehran University of Medical Sciences, Pursina Ave, Tehran, Iran 14155 – 3451;* [2]*and Faculty of Pharmacy, Azad university, Tehran, Iran*

Centaurea iberica Tren. et Soreng and *Centaurea virgata* Lam. subsp. squarrosa (Wild) Gugler were collected from Taleghan 80 km NW of Tehran – Iran, voucher specimens were identified and deposited at the herbarium of Faculty Pharmacy, Tehran University of Medical Sciences, on the basis of previous antifungal screening in August 2005. Their aerial parts were air dried and hydro-alcoholic extractions were prepared by percolation and dried by evaporation of solvents. Anti-fungal studies were done on seven pathogenic fungi including *Aspergillus niger, Microsporum canis, Microsporum gypseum, Tricophyton mentogrophytes, Epidermophyton floccossum* using cylinder tube test method and Sabourau dextrose agar as culture medium for total extract and fractions. The growth of fungi was achieved at 25 – 35^0 C after 72 – 168 hr of incubation [1]. The total extract was fractionated via solvent to solvent extraction with petroleum ether, chloroform, ethyl acetate and water. The most effective fraction was the petroleum ether extract with 0.5%, 1% of dilution for two species respectively. Pre-phytochemical tests showed flavonoids, sterols, terpenoides and saponins for this effective fraction. Primary spectrometry, IR and ^{1}H NMR, showed that the major component of the effective fraction could have a sesquiterpene lactone structure. **References:** [1] Mitscher, L.A. et al. (1972) Loydia. 35: 157 – 16.2.

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In vitro evidence of synergism for plant part combinations of *Croton gratissimus* (Euphorbiaceae) used in African traditional healing

van Vuuren SF[1], Viljoen AM[2]
[1]*Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa;* [2]*School of Pharmacy, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa*

Croton gratissimus Burch. var *gratissimus* is a tree traditionally used medicinally to treat abdominal and uterine disorders, inflammation and swelling, fevers, coughs, colds and inflammation of the lungs. The hydro-distilled leaf essential oil, extracts of bark, root and leaf are comparatively assessed with results indicating higher efficacies for extracts than the oils. *Croton gratissimus* is one of many plants used in African traditional healing in combination i.e. roots and leaves are combined and used in therapeutic regimens. The root, leaf and bark extracts were investigated singularly and combined in various ratios to establish possible interaction. The minimum inhibitory concentration (MIC) and fractional inhibitory concentration (FIC) results indicated variable efficacies for the plant combinations, the greatest of which was noted for *C. neoformans* in the root and leaf combination (MIC 0.4 mg/mL and FIC of 0.4). Further combination studies were thereafter conducted on varying ratios of root and leaf extracts based on the traditional usage of the plant. Isobolograms indicated greatest synergy for *Bacillus cereus* and *Cryptococcus neoformans*.

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Antifungal activity of 2-phenylethanol and levomenthol against molds from indoor air and damp dwellings

Kosalec I, Šegvić Klarić M, Pepeljnjak S
Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39, HR-10000 Zagreb, Croatia

Molds are widespread microbes which could be found in indoor and outdoor environment. They have been recognized as possible causative agents of the indirect (noninfectious) pathologic changes in humans. Possible mechanisms of these pathologic events include immune (IgE)-mediated (hypersensitivity pneumonitis, allergic rhinitis and asthma), toxic (mucosal irritation) and carcinogenic (mycotoxins) mechanisms [1]. Prevention of those toxic effects includes decrease and elimination of molds conidia (in air) and mycelium (in walls) in indoor environment using fungicides, and mechanical methods, as well as heating/moisture control. Since some chemical fungicides exert adverse effects, the aim of presented study was to evaluate antifungal activity of low-molecular-weight natural meta-

bolites from the class of alcohols: 2-phenyl-ethanol (2-PE) and levomenthol (LM) using serial broth twofold dilution assay [2] and vaporous microathmospheric contact assay [3] against twenty-two allergenic and/or toxigenic strains of ten mold species (*Aspergillus, Penicillium, Absidia, Chaetomium,* and *Trichoderma*) isolated from indoor air and from damp dwellings. 2-PE inhibited growth of molds with MIC values between 0.65 and 7.67 mg/mL, and LM between 0.78 and 13.21 mg/mL. Also, 2-PE exhibited stronger antifungal activity against *Aspergillus* spp. and *Penicillium* spp. (MIC_{mean} 7.67 ± 4.04 mg/mL; 5.78 ± 4.41 mg/mL, respectively) than LM (MIC_{mean} 13.21 ± 10.06 mg/mL; 13.20 ± 10.17 mg/mL, respectively). The most sensitive were *Chaetomium globosum* and *Absidia* spp. for which MICs of 2-PE were below 2.6 mg/mL. In addition, 2-PE exhibited stronger activity with significantly lower MIC values against all tested molds, comparing to LM (MIC_{mean} 2-PE 5.59 ± 4.32 mg/mL; MIC_{mean} LM 10.15 ± 9.98 mg/mL, $p < 0.05$). Contrary to the results of the dilution assay, a vaporous microathmospheric contact assay showed that 2-PE was less effective (inhibition of mycelium development in 5 mg/100 cm^3) than LM (inhibition of mycelium development in 0.1 mg/100 cm^3). These results indicate that fungicidal activity depends on nature of contact of 2-PE/LM with mycelium and/or conidia of molds, as well as volatility of tested substances. **References:** [1] Chapman, M. D. (2006) Med Mycol 44:S29-S32. [2] Šegvić Klarić, M. et al. (2006) Lett Appl Microbiol 44: 36 – 42. [3] Inouye, S. et al. (1998) Mycoses 41: 403 – 410.

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Anticandidal activity of essential oils from commercial herbal spices from Turkey

Demirci F, İşcan G, Demirci B, Baser KHC
Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, TR-26470, Eskişehir, Turkey

Herbs such as *Laurus nobilis* L., *Rosmarinus officinalis* L., *Ocimum basilicum* L., *Salvia fruticosa* Mill., and *Thymbra spicata* L. grown in Turkey are important export crops as whole dried plant material or as value added products such as essential oils [1]. The essential oils of herbal species (spices) were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography – mass spectroscopy (GC-MS), simultaneously. The major constituents were identified as 1,8-cineole (47%), α-terpinyl acetate (11%) for *L. nobilis* L., 1,8-cineole (53%), α-pinene (11%) for *R. officinalis* L., linalool (43%), 1,8-cineole (15%) for *O. basilicum* L., 1,8-cineole (44%), camphor (12%) for *S. fruticosa* Mill., and carvacrol (65%), *p*-cymene (18%) for *T. spicata* L., respectively. All essential oils and their major constituents were evaluated *in vitro* for their anticandidal properties against pathogenic yeast using agar well diffusion, microdilution and TLC bioauthographic methods in combination [2]. *Candida albicans*, *C. utilis*, *C. tropicalis*, *C. krusei*, *C. zeylanoides*, *C. parapsilosis*, *C. glabrata* were used in the assays. Minimum inhibitory concentrations (MIC) were observed as 0.125-> 1.0 mg/ml and active compounds were identified in comparison to standard antifungal agents. Qualitative and quantitative preliminary radical scavenging activity ($DPPH^{\cdot}$) [3] of the oils and their active components eugenol, carvacrol, methyl eugenol were determined. The results obtained suggest that essential oils and their components are invaluable sources for inhibition of *Candida* sp. and the DPPH radical. *Acknowledgements:* The authors would like to thank TAMSAN, İzmir for providing the plant material. Pharm. E. Civisov for his assistance. TUBITAK TBAG 106T117 for partial financial support. **References:** [1] Anonymous (2004) EU Market Survey 2004: Natural ingredients for pharmaceuticals. CBI, http://www.cbi.nl/. [2] Iscan, G. et al. (2002) J. Agric. Food Chem. 50, 3943 – 6. [3] Kumarasamy, M.E. et al. (2002), Pharm. Biol. 40, 307 – 310.

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Antimicrobial activity of copaiba oils obtained from different species of *Copaifera* in Brazil

Oliveira dos Santos A[1], Ueda-Nakamura T[2], Prado Dias Filho B[1,2], Florêncio da Veiga Junior V[3], da Cunha Pinto Â[4], Vataru Nakamura C[1,2]
[1]Universidade Estadual de Londrina; [2]Laboratório de Microbiologia Aplicada aos Produtos Naturais e Sintéticos, Universidade Estadual de Maringá, PR; [3]Universidade Federal do Amazonas, AM; [4]Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Copaiba oils are extracted from the trunks of trees of the genus *Copaifera* [1]. The effects attributed to Copaiba oil in folk medicine are anti-inflammatory, antitumor, antiblenorrhagea, antisyphilis, urinary antiseptic, anti-ulcer, and for healing wounds [2]. In Brazil there are more than 20 species of *Copaifera*, which show some differences in chemical composition [3]. We assessed the antimicrobial activity of 8 species of *Copaifera* (*C. reticulata, C. multijuga, C. martii, C. cearensis, C. paupera, C. langsdorffii, C. officinalis,* and *C. lucens*). The test organisms included Gram-positive bacteria: *Staphylococus aureus, Bacillus subtilis, Staphylococcus epidermidis,* and *Enterococcus faecalis;* Gram-negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Shigella flexneri,* and *Enterobacter cloacaea;* Yeasts: *Candida albicans, C. tropicalis,* and *C. parapsilosis*; and dermatophytes: *Trichophyton rubrum, T. mentagrophytes, Microsporum canis,* and *M. gypseum*. The minimum inhibitory concentrations (MICs) of oils were determined by microdilution techniques. *S. aureus* treated with oil from *C. martii* was fixed, dehydrated, critical-point dried, and observed by scanning electron microscopy. Copaiba oils showed pronounced antibacterial activity against Gram-positive bacteria. *Copaifera martii* was the most effective oil, with activity against *S. aureus, S. epidermidis,* and *E. faecalis* with MIC of 62.5 μg/ml. Against *B.subtilis* it showed a MIC of 12.5 μg/ml. No activity was exhibited by copaiba oils against Gram-negative bacteria, yeasts or dermatophytes. *Staphylococcus aureus* treated with oil showed alterations in the cell wall, observed by scanning electron microscopy. These results can contribute to opening perspectives for finding new treatments of diseases caused by bacteria. *Acknowledgements:* This study was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Capacitação de Aperfeiçoamento de Pessoal de Nível Superior – Capes, Fundação Araucária, Financiadora de Estudos e Projetos – FINEP, and Post-Graduate Program in Microbiology of the Universidade Estadual de Londrina-PR, Brazil. **References:** [1] Veiga-Jr, V.F., et al. (2001) Phytother. Res. 15: 476 – 480. [2] Gomes, N.M., et al. (2007) J. Ethnopharmacol. 109: 486 – 492. [3] Cascon, V., et al. (2000) Phytochemistry 55: 773 – 778.

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Screening of selected essential oils for antibacterial activity in vapour phase

Nedorostova L[1], Kloucek P[1], Stolcova M[1], Kokoska L[2]
[1]Department of crop production, Faculty of Food and Natural Recourses, Czech University of Life Sciences Prague, Kamycka 129, Prague, 165 21, Czech Republic; [2]Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, Prague, 165 21, Czech Republic

The aim of this study was to identify antimicrobial properties of essential oils in vapour phase from selected species of spices, medicinal and aromatic plants. The essential oils, obtained from fresh plant materials by hydrodistillation, were selected either according to etnobotanical data or based on a chemotaxonomic approach. The tests for antimicrobial properties were carried out by the modified diffusion method for testing of essential oils in vapour phase [1]. The tests were performed against two Gram-positive (*Listeria monocytogenes, Staphylococcus aureus*) and 3 Gram-negative (*Eschericia coli, Pseudomonas aeruginosa, Salmonella enteritids*) food-borne bacteria. The results were expressed as minimum inhibitory dose (MID) in μl/cm^3 of air. Thirteen of the 28 essential oils were active at

least against one bacterial strain. The best results were exhibited by horse-radish (*Armoracia rusticana,* MID 0.0083 µl/cm^3) against all of the strains, garlic (*Allium sativum,* 0.0083 – 0.53 µl/cm^3) against all of the strains, oregano (*Origanum vulgare,* 0.066 – 0.13 µl/cm^3) against all of the strains except *P. aeruginosa,* which was not inhibited and thyme (*Thymus vulgaris,* 0.017 – 0.26 µl/cm^3) except *P. aeruginosa.* Among the others species interesting results were shown *Nepeta x faassenii* and *Caryopteris x clandonensis* (MID 0.53 µl/cm^3 against *S. aureus*), for which this is the first report of antibacterial activity. In conclusion, certain essential oils are highly effective in vapour phase and can be used in fight against food-borne bacterial pathogens. *Acknowledgements:* Ministry of Education of the Czech Republic MSM 6046137305 **References:** [1] Lopez, P. et al. (2005) J. Agr. Food Chem. 53: 6939 – 6946.

P 122

Antibacterial geranylflavonoids from *Paulownia tomentosa* (Scrophulariaceae) fruits

Kloucek P[1], Smejkal K[2], Chudik S[3], Marek R[3], Kokoska L[4], Urbanova M[5], Julinek O[5], Slapetova T[2], Holubova P[2], Zima A[2], Dvorska M[2]
[1]Department of Crop Production, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, Prague, 165 21, Czech Republic; [2]Department of Natural Drugs, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho 1 – 3, 612 42, Czech Republic; [3]National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5/A4, 625 00, Czech Republic; [4]Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21, Prague, Czech Republic; [5]Department of Physics and Measurements, Institute of Chemical Technology, Technicka 3, Prague, 166 28, Czech Republic

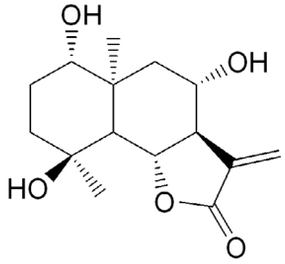
Paulownia tomentosa (Thunb.) Steud. has been used as a traditional Chinese herbal medicine for the treatment of enteritis, tonsillitis, bronchitis and dysentery [1]. Previous phytochemical studies of this species identified several compounds, falling within iridoids, flavonoids, phenolic glycosides, furanofuran lignans and furanoquinones [2]. Our study was focused on isolation and identification of partly polar and non-polar compounds and aims to find bioactive substances with antibacterial activity. Eight geranylflavanones were isolated from ethanol extract of *P. tomentosa* fruit, four of which are described for the first time from a natural source. For isolation, liquid-liquid fractionation, column chromatography, flash chromatography and preparative HPLC were used. The compounds were described by spectrophotometric methods, and the structures of compounds were determined by mass spectrometry including HR-MS, and 1D and 2D NMR spectroscopy, absolute configuration was deduced from circular dichroism. Antibacterial activity against 6 G+, 3 G- human pathogenic bacteria and one yeast was determined by microdilution method. Seven compounds exhibited some degree of activity; however, any of the compounds was able to inhibit G- bacteria or the yeast. Six compounds were active against all G+ bacteria with MICs from 2 to 8 µg/ml. Isolated antibacterial compounds from *P. tomentosa* may help with treatment of certain bacterial diseases. *Acknowledgements:* IGA VFU 23/2004, Ministry of Education of the Czech Republic MSM0021622413, MSM 6046137305 and LC06030, Ministry of Health 1A8666. **References:** [1] Jiang, T. et al. (2004) Chromatographia 59: 255 – 258. [2] Kang, H.K. et al. (1994) Arch. Pharm. Res. 17: 470 – 475

P 123

Sesquiterpene lactones from the aerial parts of *Anthemis melanolepis* L

Saroglou V, Karioti A, Skaltsa H
Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, GR-15771 Athens, Greece

Continuing our research on the chemical constituents from the aerial parts of Greek *Anthemis melanolepis* L., a species belonging to the section Cota [1], we report here the isolation and identification of six sesquiterpene lactones, luteolin, 3-hydroxy-4-methoxybenzoic acid, loliolide and taraxasterol. The isolation was proceeded according to the Bohlmann isolation method [2]. The structures of the isolated compounds were elucidated by spectroscopic methods, particularly high-field NMR spectroscopy. Compounds **1**, **2** and **3** namely melanolepin B, anthemin A and anthemin B are three new naturally occurring sesquiterpene lactones. Besides compounds **1**-**3**, three known sesquiterpene lactones were isolated, namely dentatin A, 1β,4α,6α-trihydroxy-eudesm-11-en-8α-12-olide and desacetyllaurenobiolide.



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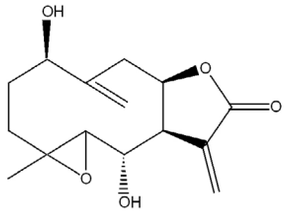

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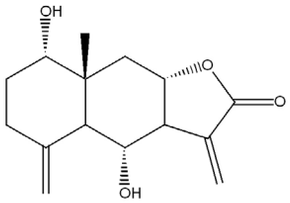


3

Acknowledgments: This project is co-financed within Op. Education by the ESF (European Social Fund) and National Resources. **References:** [1] Davis P. H. (1975), Flora of Turkey and the East Aegean islands, Edinburgh, 5: 174 – 221. [2] Bohlmann F. et al. (1984), Phytochemistry 23: 1979 – 88.

P 124

Tanacetum vulgare: anti-herpes virus activity of crude extract and the purified compound parthenolide

Onozato T, Vataru Nakamura C, Aparício Garcia Cortez D, Prado Dias Filho B, Ueda-Nakamura T
Universidade Estadual de Maringá, Av. Colombo, 5790, 87020 – 900 Maringá-PR, Brazil

Herpes simplex virus type 1 is a common human pathogen which causes several infectious diseases. In immunocompetent hosts these clinical symptoms are usually benign, but in immunocompromised patients they become severe [1]. Nucleoside analogues, such as acyclovir, have been successfully used to treat herpetic infections. However, resistant strains and drug toxic side effects encourage the search for new compounds to improve the current antiviral arsenal [2]. The present study demonstrated that the ethyl-acetate extract and the compound parthenolide, isolated from aerial parts of *Tanacetum vulgare* L. (Asteraceae), were able to protect Vero cells from herpes simplex virus (HSV-1) infection *in vitro*. The extract and parthenolide were assayed against HSV-1 by sulforhodamine B colorimetric assay, and exhibited anti-HSV-1 activity with an EC_{50} of 40 µg/ml and 0.3 µg/ml, respectively. In order to determine which step of the virus-cell interaction was affected by parthenolide, the

pure compound from a commercial source was used. No effect was observed when both viruses and cells were pre-treated, suggesting that parthenolide neither inactivated virus particles directly nor protected the cell from infection, but interfered with the virus replication after the penetration step, inhibiting approximately 80% of plaques formed at a concentration of 2.5 µg/ml when compared with an untreated control. These results demonstrate that parthenolide is one of the compounds responsible for the antiviral activity of *T. vulgare*, contributing to its validation as a medicinal plant useful in the treatment of diseases caused by HSV-1. *Acknowledgements:* Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP), Fundação Araucária and Post-Graduate Program in Pharmaceutical Sciences of the Universidade Estadual de Maringá-PR, Brazil. **References:** [1] Bacon, T. H., et al. (2003). Clin Microbiol Rev. 16(1), 114 – 128. [2] Chibo, D., et al. (2004) Antiviral Res. 61, 83 – 91

P 125

Mode of action of 6-oxophenolic triterpenoids against *Bacillus subtilis*

Moujir L[1], de León L[1], Bazzocchi IL[2]
[1]*Departamento de Microbiología, Facultad de Farmacia, Universidad de La Laguna, 38206, Tenerife, Canary Islands, Spain;* [2]*Instituto Universitario "Antonio Gónzález", Avda Astrofisico Fco Sánchez n 1 La Laguna 38206, Tenerife, Canary islands, Spain*

Four 6-oxophenolic triterpenoids were isolated from *Maytenus blepharodes* Lundell, with activity against Gram positive bacteria, especially on spore forming bacteria, and the yeast *Candida albicans* [1]. Chemically, these triterpenoids differ in the functional group in ring A and E (Figure 1). The MIC values against *B. subtilis* suggest that the increase of the electronegativity produces a decrease of the antibacterial potency (**1** zeylasterone 3 µg/ml < **2** demethylzeylasterone 13 µg/ml and **3** zeylasteral 10 µg/ml < **4** demethylzeylasteral 13 µg/ml).

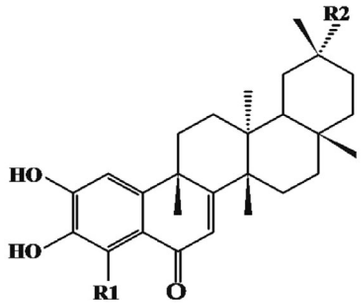


Fig. 1: Structure of 6-oxophenolic triterpenoids

	R1	R2	MIC (µg/ml)
1.	COOH	$COOCH_3$	3
2.	COOH	COOH	13
3.	COH	$COOCH_3$	10
4.	COH	COOH	13

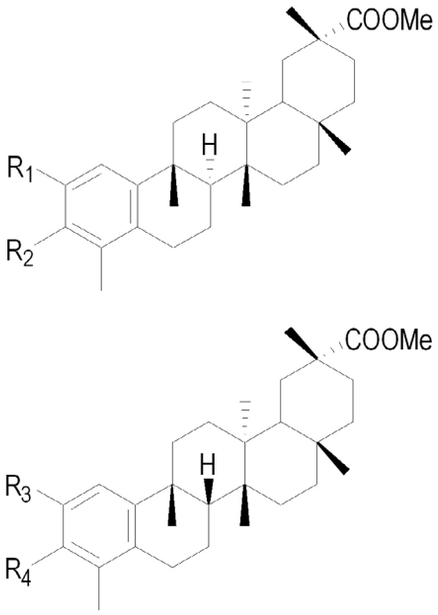
The activity of the compounds (between 2 and 3 x MIC) on *B. subtilis* cultures varies according to the bacteria concentrations, showing bactericidal activity (≥ 3 $\log_{10}$ in CFU/ml reduction) at lowest inocula size (10^5-10^4 CFU/ml), except for demethylzeylasterone and demethylzeylasteral. At highest inocula (10^7-10^6 CFU/ml), the phenols showed bacteriostatic activity (< 3 $\log_{10}$ in CFU/ml reduction), except demethylzeylasteral. Treatments of *B. subtilis* cultures with the phenolic compounds revealed a blockage of biosynthetic pathways as DNA, RNA, protein and cell wall synthesis. However, inhibition of all processes did not occur simultaneously. The macromolecular synthesis of peptidoglycan was stopped 2 min after addition of the phenols, and later DNA, RNA and protein synthesis. These results suggest that the compounds could act on multiple targets on *B. subtilis* cells. *Acknowledgements:* This study was supported by the Spanish Grant CTQ2006 – 13376/BQU. **References:** 1. De León, L., Beltrán, B., Moujir, L. (2005) Plant. Med. 71: 313 – 319.

P 126

Antimicrobial activity of *D:A*-friedo-*nor*-oleanane triterpenoids

López MR[1], Jiménez IA[2], Bazzocchi IL[2], Moujir L[1]
[1]*Departamento de Microbiología y Biología Celular, Facultad de Farmacia. Avda Astrofísico F[co]Sánchez s/n. Universidad de La Laguna, 38206, La Laguna, Tenerife, Canary Islands, Spain;* [2]*Instituto Universitario de Bio-Orgánica "Antonio González". Universidad de La Laguna, 38206, La Laguna, Tenerife, Canary Islands, Spain*

The study of the antimicrobial activity (MIC and MBC) of two natural triterpenophenols: 6-deoxoblepharodol (**1**), 8-*epi*-6-deoxoblepharodol (**2**) and two derivatives: 2,3-diacetoxy-6-deoxoblepharodol (**3**) and 2,3-diacetoxy-8-*epi*-6-deoxoblepharodol (**4**) obtained after acetylation, were determined. The natural products showed antimicrobial activity against Gram positive bacteria. The relation structure-activity demonstrates that the presence of the hydroxyl groups in C2 and C3 is necessary for antimicrobial activity. We studied the mode of action of **1** and **2** against *Bacillus subtilis*. The time-kill curves for both compounds verified that inclusion of either compound in the log phase was bactericidal (> or = 3 $\log_{10}$ CFU/ml reduction). However, when **2** was added in the lag phase it reduced the initial inoculum by 2-$\log_{10}$ in the nine hours of treatment (bacteriostatic activity), while **1** showed bactericidal activity. The activity of **1** was bactericidal at different bacterial concentrations (10^7, 10^6, 10^5, and 10^4 UFC/ml), where **2** showed bacteriostatic activity for all inoculum densities except for 10^7 UFC/ml, in which it was inactive.



	R_1	R_2	R_3	R_4
1	OH	OH	--	--
2	--	--	OH	OH
3	AcO	AcO	--	--
4	--	--	AcO	AcO

Acknowledgements: This study was supported by Spanish Grants CTQ2006 – 13376-BQU

P 127

Antioxidant and preservative properties of *Caesalpinia sappan* L. extract

Wongkrajang Y, Manamuti C, Saraya S, Temsiririrkkul R, Peungvicha P, Cheewansirisuk C
Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhya, Rajthewi, Bangkok, 10400, Thailand

The study was conducted to evaluate the antioxidant activity, toxicity test and antimicrobial property as natural food preservative of sappan heartwood (*Caesalpinia sappan* L.) water extract. The antioxidant and antimicrobial activities were evaluated using free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 96-well microdilution broth method. The extract concentration provided 50% inhibition (IC_{50}) at 63.4 μg/ml while vitamin C and Trolox acted at 9.1 μg/ml and 11.6 μg/ml, respectively. LD_{50} of the extract given orally was greater than 5 g/kg in ICR mice and Wistar rats. The minimum inhibitory concentrations of sappan wood extract against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 13311 and *Candida albicans* ATCC 10231 were 250, 120, 500 and more than 16,000 μg/ml, respectively. The MIC chloramphenicol and ketoconazol used as positive controls ranged from 0.97 – 62.5 μg/ml for bacteria and 62.5 μg/ml for yeast. The sappan wood extract at 2, 4, 8 times of the selected maximum bacteria MIC were added to the chilli paste which is Thai favorite food in order to determine the preservative property for 3 months. Total aerobic count in Thai chilli paste preserved with 2 MIC, 4 MIC and 8 MIC of sappan wood extract were reduced at 41.9%, 40.9% and 44.6%, respectively.

P 128

Development of commercial mouthwash combined with herbal extracts for anticariogenic activity against *Streptococcus mutans*

Saraya S, Cheewansirisuk C, Wongkrajang Y, Kongsaktrakoon B, Manamuti C, Temsiririrkkul R
Faculty of pharmacy, Mahidol university, 447 Sri-Ayudhya, Rajthewi, Bangkok, 10400, Thailand

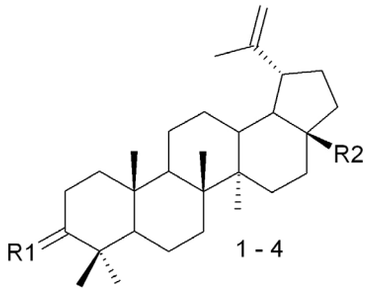
The aim of the present study was to develope the commercial mouthwash combined with herbal extracts against *Streptococcus mutans* ATCC 25175. The 50% ethanol herbal extracts were from *Cassia alata* L., *Quercus infectoria* Oliver., *Harrisonia perforata* Merr., *Camellia sinensis* L. (oolong and green tea) and *Psidium guajava* L., *H. perforata* and *P. guajava* exhibited the highest antibacterial activity of 0.625 mg/ml. However *C. alata, C. sinensis* (oolong and green tea) and *Qu. infectoria* showed antibacterial activity of 1.25 mg/ml. Evaluation of the combination effect of commercial mouthwash with herbal extracts by synergy test found that *C. alata, C. sinensis* (oolong and green tea) and *Qu. infectoria* increased antibacterial activity against *S. mutans* ATCC 25175 (FIC = 0.5) while *H. perforata* and *P. guajava* were indifference (FIC = 1.0). The amount of commercial mouthwash used to inhibit *S. mutans* ATCC 25175 growth was then reduced 16 fold when combined with *C. alata, C. sinensis* (oolong and green tea) and *Qu. infectoria*. It is concluded that the combined formulation of commercial mouthwash with herbal extracts can lead to the better treatment of dental caries caused from *S. mutans*.

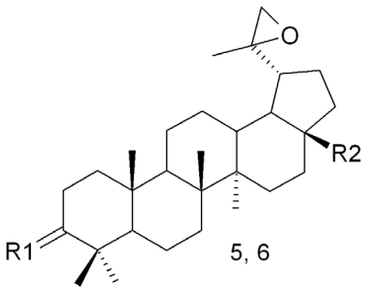
P 129

The chemical transformations of betulin

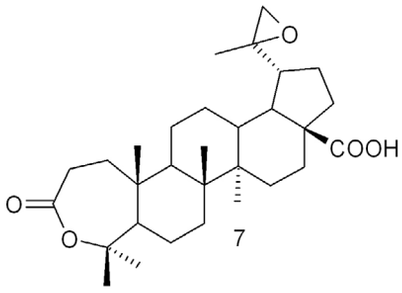
Tubek B, Wawrzeńczyk C
Department of Chemistry, Wrocław University of Environmental and Life Sciences, C.K.Norwida 25, 50 – 375 Wrocław, Poland

Betulin (1) is a naturally occurring pentacyclic lupane-type triterpenoid, isolated from the outer white birch bark. Betulin and its derivatives such as betulinic acid (2) and betulonic acid (3) are studied for their antitumor, antiviral, anti-inflammatory, hepatoprotective, antiparasitic, antibacterial and antimycotic activities [1,2,3]. Betulin is stable in a natural environment and examples of its microbial transformations are rare. The chemical transformations look to be the best way leading to structural analogs of betulin or its derivatives. We are interested in oxoanalogs of betulin. At first, we have studied the oxidation of betulin with common reagents. The reaction of betulin with Jones reagent afforded the mixture of ketoaldehyde (4), (53%) and betulonic acid (23%). We have also studied the oxidation of betulin and betulonic acid with 1.1 mol of m-chloroperbenzoic acid (m-CPBA). The reaction of betulin gave the epoxy betulin (5). Betulonic acid in the same conditions afforded also product of epoxydation of double bond. When the oxidation was carried out with 2.2 mol of m-CPBA, the mixture of epoxy betulonic acid (6) and epoxy lactone (7) was obtained.





	R1	R2
1	α-H, β-OH	CH_2OH
2	α-H, β-OH	COOH
3	O	COOH
4	O	CHO
5	α-H, β-OH	CH_2OH
6	O	COOH



References: [1] Dzubak, P. et al. (2006) Nat. Prod. Rep. 23, 394 – 411. [2] Eiznhamer, D. et al. (2004) IDrugs 7,4, 359 – 373. [3] Kim, D.S.H.L. et al. (1998) Bioorg. Med. Chem. Lett. 8, 1707 – 1712.

P 130

Antiplasmodial activity of abietane diterpenes isolated from five southern African *Plectranthus* species

van Zyl RL[1], Khan F[2], Drewes S[2], Edwards T[3]
[1]Pharmacology Division, Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, 2193, South Africa; [2]School of Chemistry, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209; [3]School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209

The emergence of drug-resistant strains of *Plasmodium falciparum* has resulted in an urgent need to develop new antimalarial chemotherapeutic agents. The rich plant diversity and long history of traditional medicine in southern Africa warrants investigation and may be a valuable source of novel compounds. *Plectranthus* species were investigated based on their association with the treatment of "fever", which could be due to a "malarial or febrile condition". Seven known abietane diterpenes have been isolated in pure form from the leaves of five indigenous *Plectranthus* species, namely *P. hadiensis*, *P. lucidus*, *P. ecklonii*, *P. purpuratus* subsp. *purpuratus* and *P. purpuratus* subsp. *tongaensis*. The seven compounds were tested for antimalarial activity against the intra-erythrocytic stage of the chloroquine-resistant strain of *P. falciparum* (FCR-3) using the [^{3}H]-hypoxanthine incorporation assay. Their ability to inhibit β-haematin formation was performed at acidic conditions as in the parasite food vacuole. The toxicity profiles were determined using the tetrazolium cell proliferation assay against human kidney epithelial cells. Overall, the compounds displayed good antiplasmodial activity (IC_{50} values ranging from 3.11 to 14.65 μM), with compound **4** (11-hydroxy-2α-(3,4-dihydroxybenzoyloxy)abieta-5,7,9(11),13-tetraene-12-one) being 62% as effective as chloroquine in inhibiting β-haematin formation. However, the cytotoxicity profile indicated a low degree of specificity towards the malaria parasite. When combined with quinine, compounds **4**, **5** (11-hydroxy-19-(methyl-buten-2-oyloxy)-abieta-5,7,9 (11),13-tetraene-12-one), and **7** (11-hydroxy-19-(3,4-dihydroxybenzoyloxy)-abieta-5,7,9(11),13-tetraene-12-one) interacted in an additive manner; while compound **1** (7α-formyloxy-6β,12-dihydroxy-abieta-8,12-diene-11,14-dione) interacted synergistically. Further chemical modifications of these naturally-derived compounds could yield more active antiprotozoal agents with decreased toxicity. *Acknowlegements*: National Research Foundation (South Africa); University Research Committee (University of KwaZulu-Natal).

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Antimicrobial activities of six a*rtemisia* species of west Anatolia

Baykan Erel Ş[1], Karabay Yavaşoğlu NÜ[2], Zeybek U[1]
[1]Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Bornova, 35100, İzmir, Turkey, [2]Ege University, Faculty of Science, Department of General Biology, Bornova, 35100, İzmir, Turkey

Artemisia L. (Asteraceae) is represented by 23 species in the Turkish flora [1]. *A. absinthium* L., *A. arborescens* L., *A. campestris* L., *A. scoparia* Waldst. & Kit., *A. santonicum* L., and *A. vulgaris* L., naturally distributed in West and South West of Turkey, were chosen as experimental material in this study. This study was conducted to investigate antimicrobial activity of the essential oils and methanol extracts of the *Artemisia* taxa mentioned above. Essential oils and methanol extracts of *Artemisia* taxa were tested for antimicrobial activities by disc diffusion method [2]. *In vitro* antimicrobial studies were carried out against eight bacteria strains, *Staphylococcus aureus* ATCC 6538/P, *St. epidermidis* ATTC 12228, *Escherichia coli* ATCC 29998, *E. coli* ATCC 11230, *Pseudomonas aeroginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Enterobacter cloacae* ATCC 13047, *Salmonella typhimurium* CCM 5445 and a fungal strain, *Candida albicans* ATTC 10239. Ketakanozole and seftacidime were used as positive controls. Essential oils of *Artemisia* taxa were showed a broad spectrum of antimicrobial activity. *A.santonicum* inhibited the growth of *S. epidermidis* ATTC 12228 (inhibition zone 21 mm) and *Enterococcus faecalis* ATCC 29212 (19 mm) more than Seftacidime (14 mm). All of the oils except *A. arborescens* were active mm againist medically important pathogen *S. aureus*. with over 14 mm zone while the inhibition zone of seftacidime was 12 mm. *A.santonicum* and *A.scoparia* oils have been shown to inhibit the growth of *C. albicans*, similar to ketokonazole as a conventional antifungal. The results indicate that the essential oils of these *Artemisia* spp. are more effective against tested microorganisms than their methanol extracts. **References:** [1] Davis PH. and Mill RR. (1988). Flora of Turkey, Volume 10, Edinburgh University Press, 163,418. [2]. Bradshaw LJ. (1992). Laboratory Microbiology. 4th edn. Emeritus California State University, Fullerton, Saunders College Publishing: New York, USA. 435 p.

P 132

Synergistic action of extracts from carnivorous plants and synthetic peptides against plant pathogenic bacteria

Królicka A[1], Szpitter A[1], Lotkowska M[1], Plewko D[1], Gilgenast E[2], Romanik G[2], Kamiński M[2], Lojkowska E[1]
[1]Department of Biotechnology UG & AMG, Kladki 24, 80 – 822 Gdansk, Poland; [2]Technical University of Gdansk, Chemical Faculty, Analytical Chemistry Department, Narutowicza 11/12, 80 – 952 Gdansk, Poland

Preparations from carnivorous plants e.g. *Drosera* spp. and *Dionaea* sp. (Droseraceae) were shown to possess antimicrobial properties [1]. The aim of the study was to check the bactericidal effectivity of extracts from *Drosera capensis* and *Dionaea muscipula* in combination with two synthetic peptides CAMEL (cecropin – melittin hybrid peptide) and pexiganan (magainin 2 analogue) and evaluation of a possible synergistic action. Antimicrobial activity was examined against *Pectobacterium carotovorum* subsp. *artrosepticum* (Pca), *Pc* subsp. *carotovorum* (Pcc) and *Dickeya* sp. (*D*. sp.). These are plant pathogenic bacteria responsible for significant crop losses causing e.g. soft rot and blackleg. Cultures of plants were grown on ½ MS medium (2% sucrose, pH 5.6). Quantitative and qualitative determination of naphthoquinones and flavonoids in $CHCl_3$ and MeOH extracts was performed using NP-HPLC/UV-DAD. Plant preparations and peptides were tested for antimicrobial activity and their minimal bactericidal concentration (MBC). Values against three strains of Pca, 3 of Pcc and three strains of *D.* sp. were established using broth microdilution method. Extracts from both plant species with the lowest MBC values were used in combinations with peptides by a checkerboard titration method. The fractionary bactericidal concentration (FBC) index for mixtures of 2 antimicrobials was calculated according to the equation: FBC index $=A/MBC_A + B/MBC_B$, where MBC_A and MBC_B are the MBCs of peptide and extract separately while *A* and *B* are values for the combination of 2 compounds. The FBC indexes were interpreted as follows: < 0.5, synergy; 0.5 – 4.0, indifferent; and > 4.0, antagonism. Plant extracts tested separately were effective against all bacterial pathogens with MBC values in the range 10 – 50 μg FW ml^{-1}. Synergistic action of plant preparations with CAMEL was proven. The most effective combination of tested compounds was a $CHCl_3$ extract from *D. muscipula* with CAMEL against Pca (FBC = 0.37) and $CHCl_3$ preparations from *D. capensis* with CAMEL against *D*.sp. (FBC = 0.45).

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Broad spectrum antiviral fraction from the lichen *Ramalina farinacea*

Esimone CO[1,3], Grunwald T[1], Kuate S[1], Tippler B[1], Proksch P[2], Überla K[1]
[1]Department of Molecular and Medical Virology, Ruhr-University, Bochum, Germany; [2]Department of Pharmaceutical Biology, Heinrich-Heine University, Düsseldorf, Germany; [3]Department of Pharmaceutics, University of Nigeria, 410001 Nsukka, Enugu State, Nigeria

Extracts from the lichen *Ramalina farinacea (RF)* have previously been shown to be antibacterial, antifungal and most recently, inhibitory to lentiviral and adenoviral vectors. We now determined the antiviral activity of subfractions of the ethylacetate-soluble fraction of RF against a broad spectrum of wild-type viruses, including human immunodeficiency virus type1 (HIV-1), herpes simplex type1 (HSV-1) and respiratory syncytial virus (RSV). Wild-type HIV-1, HSV-1 or RSV were pre-incubated with various concentrations of the ET4 subfraction for 30 minutes at 37 °C before adding to P4CCR5 indicator cell line (HIV-1), ELVIS TM indicator cell line (HSV-1) or Hep2 cell line (RSV) in 96-well microtitre plates. Controls contain virus alone without ET4. The anti-HIV and anti-HSV activities were quantified by estimating the beta-galactosidase expression of the respective indicator cell lines in single round infection assays while the anti-RSV activity was determined via an immunoflourescent technique, employing monoclonal mouse antibody against the P-protein of RSV (as 1 ° antibody) and a peroxidase-conjugated rabbit antimouse IgG (as 2 ° antibody). Toxicity of ET4 to cell lines was evaluated in parallel using either the BrdU incorporation method or the MTT method. The effect of ET4 on the enzymatic activity of HIV-1 reverse transcriptase was also evaluated using a chemiluminescent RT assay. ET4 has previously been shown to strongly inhibit the infectivity of lentiviral, retroviral and adenoviral vectors. The present result also shows very strong inhibition of HIV-1, HSV-1 and RSV, with the 50% antiviral concentrations (IC_{50}) of 0.33, 6.09 and 3.65 μg/ml respectively. Time-of-addition studies suggest that both entry and post-entry steps of the HIV-1 replication cycle are targeted. Furthermore, ET4 inhibited HIV-1 RT with an IC_{50} of 0.022 μg/ml. ET4 may represent a novel source for lead phyto-antiviral agents. Further fractionation will reveal whether the broad spectrum antiviral activity results from a single compound or from different compounds present in ET4. *Acknowledgements:* The support of the Alexander von Humboldt Foundation to ECO is gratefully acknowledged. State Committee for Scientific Research, Grant No PBZ-KBN 112/P06/2005 **References:** [1] Juniper, BE. et al. (1989) The carnivorous plants. Academic Press, inc. Harcourt Brace Jovanovich, Publishers.

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Antimicrobial activity of some Yemeni medicinal plants

Taleb M[1], Svobodova B[1], Langrova I[2], Kokoska L[1]
[1]Department of Crop Science and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic; [2]Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic

Herbal medicine represents one of the most important fields of traditional medicine in Yemen especially in rural areas. Despite this rich tradition in use of medicinal plants e.g. for the treatment of various infectious diseases, there are still only few reports on antimicrobial activity of Yemeni species [1,2,3]. Therefore, it is of great interest to carry out an antimicrobial screening of these plants in order to validate their ethnopharmacological use and to reveal their active constituents. The ethanol extracts obtained by maceration of different plant parts from *Catha edulis* Forssk., *Dracaena cinnabari* Balf.f, *Lawsonia inermis* L., *Mallotus philippinensis* Müll.Arg., *Nepeta densiflora* Kar. & Kir, *Salvadora persica* L., and *Ziziphus spina-christi* Willd. were evaluated for potential antimicrobial activity against *Bacillus cereus*, *B. subtilis*, *Bacteroides fragilis*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus pyogenes* using the broth microdilution method [4]. The results showed that all extracts exhibited antimicrobial activity against at least one of the microorganisms tested at concentrations of 8 mg/ml or below. The extract from *M. philippinensis* showed the strongest activity inhibiting all microbial strains with minimum inhibitory concentrations (MICs) ranging from 0.125 to 0.250 mg/ml. The extracts from *C. edulis* and *L. inermis* were significantly more active against Gram-positive (MICs ranging from 0.125 to 2 mg/ml) than against Gram-negative bacteria (MICs ≥ 4 mg/ml). *Acknowledgements:* This research was supported by projects research project MSM 6046070901. **References:** [1] Ali, N.A.A. et al. (2001) J Ethnopharmacol 74: 173 – 179. [2] Mothana, R.A.A., Lindequist, U. (2005) J Ethnopharmacol 96: 177 – 181. [3] Al-Fatimi, M. et al. (2006) Planta Med. 72: 1063 – 1063. [4] Jorgensen, J.H. et al. (1999). In: Murray P.R. (ed.) Manual of Clinical Microbiology. ASM Press. Washington, DC.

P 135

Modulation of the *in vitro* antimalarial effects of artemisinin by selected flavonoids and by reducing agents

Sannella AR[1], Messori L[2], Casini A[2], Vincieri FF[3], Maiori G[1], Severini C[1], Bilia AR[3]
[1]Department of Infectious, Parasitic and Immunomediated Diseases, Vector-Borne Diseases and International Health Section, Istituto Superiore di Sanità, Viale Regina Elena 299, I-00161 Rome, Italy; [2]Department of Chemistry, University of Florence, Via della Lastruccia 3, I-50019 Sesto Fiorentino, Florence, Italy; [3]Department of Pharmaceutical Sciences, University of Florence, Via U. Schiff 6,50019 Sesto Fiorentino, Florence, Italy

Within the framework of a larger research project [1] aimed at evaluating the possible synergistic effects in malaria treatment between artemisinin and a variety of natural substances commonly present in plant extracts, the specific antiplasmodial properties of some flavonoids (catechin, epicatechin, quercetin, rutin, eryodictiol and eriodictyol chalcone) and of two reducing agents (ascorbic acid and ascorbyl octanoate) in combination with artemisinin were evaluted against 3D7 *P. falciparum* strains *in vitro*. The possible synergism between artemisinin and the single constituents on the parasite growth was investigated adding artemisinin at sublethal doses ranging from 0.625 to 20 nM, in presence or absence) of 10 μM or 1 mM concentrations of the individual compounds. The effect of artemisinin tested at different concentrations and 10 μM quercetin are more than additive, and this effect greatly increased when quercetin was tested at 1mM concentrations, implying a moderate synergism between these two substances. The other flavonoids did not show any significant synergistic activity. Ascorbic acid did not show any synergistic effect while ascorbyl octanoate tested at 1mM concentrations either alone or in combination with artemisinin determined a dramatic increase of the parasitemia. The observed synergism between quercetin and artemisinin might be conveniently exploited to design new and/or more effective combination therapies. *Acknowledgments*: The Ente Cassa di Risparmio di Firenze is gratefully acknowleged for generous financial support. **References:** [1] Sannella, A.R. et al. (2007) Biochem Biophys Res Commun 353: 177 – 181.

P 136

Development of a suitable culture medium for TLC-bioautography. Application to the detection of antibacterial compounds from *Cordia gilletii* extracts

Okusa PN[1], Devleeschouwer M[2], Duez P[1]
[1]Université Libre de Bruxelles, Institut de Pharmacie, Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine, CP 205/9, Bd du Triomphe, 1050 Bruxelles, Belgique; [2]Université Libre de Bruxelles, Institut de Pharmacie, Laboratoire de Microbiologie Pharmaceutique et Hygiène, CP 205/2, Bd du Triomphe, 1050 Bruxelles, Belgique

TLC-bioautography is a convenient and simple way of testing plant extracts and pure substances for their effects on pathogenic microorganisms, allowing an easy detection of active fractions [1]. Its application requires a medium fluid enough to cast microorganism suspensions but viscous enough to stick to the TLC plate and maintain sufficient humidity for bacterial growth. Mueller-Hinton (MH) agar is often used for this purpose, the microorganism suspension being prepared in molten MH agar at about 50 °C and distributed over the plate; upon solidification at ambient temperature, the plate is then incubated at 37 °C [2]. A MH broth is also frquently used, TLC plates being dipped in the broth containing the microorganisms, air dried and incubated in humidified chambers [3]. These two techniques, however, each present a major drawback; hot molten agar can induce heat shock to microorganisms [4] and broth does not conveniently adhere to the TLC plate or dies too fast. To overcome these problems, we investigated the combination of MH broth and MH agar in different proportions (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:60 and 50:50) to determine a medium fluid enough to prepare bacterial suspensions at 37 °C (temperature for optimum growth of many pathogenic bacteria) and which gets solid at ambient temperature to adhere to the TLC plate. The 90:10 mixture of MH broth: MH agar fulfilled both these requirements; higher MH agar proportions were solid at 37 °C, and lower MH agar didn't adhere conveniently to the TLC plate. The proportion 90:10 was applied to detect antimicrobial compounds of a Congolese medicinal plant, *Cordia gilletii* [5], affording superior chromatographic resolutions compared to MH agar alone or MH broth alone. *Acknowledgement:* M. Faes and O. Vaillant (Laboratoire de Pharmacognosie, ULB, Brussels). **References:** [1] Rios, JL et al. (1988) J of Ethnopharmacol 23: 127 – 149. [2] Ahmad, I and Beg, A.Z (2001) J of Ethnopharmacol 74: 113 – 123. [3] Horvath, G et al. (2002) Acta Biol Szeged 46: 145 – 146. [4] Stamm, L.V et al. (1991) Infect Immun 59 (4): 1572 – 1575. [5] Okusa, P.N et al. (2007) J. Ethnopharmacology, in press.

P 137

The antimicrobial activity of South African lichens and lichen-derived usnic acid

Seaman T, Campbell W, Lategan C, Smith P
University of Cape Town Medical School, H50 Division of Pharmacology, Old Main Building, Groote Schuur Hospital, Anzio Road, Observatory 7925, South Africa

Old Main Building, Groote Schuur Hospital, Anzio Road, Observatory 7925, South Africa Nine lichen specimens were collected from various locations in the Western and Eastern Cape provinces of South Africa with the aim of isolating compounds exhibiting antimicrobial activity. The specimens were ground and extracted using acetone. The extracts were subsequently tested against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycobacterium aurum* and *Candida albicans*. The best activity obtained against *S. aureus* was from the extract of *Telochista* sp. with a minimum inhibitory concentration (MIC) of 50 μg/ml, followed by *Usnea sp.* with and MIC of 62.5 μg/ml. None of the extracts exhibited particularly good activity against *K. pneumoniae*, with *Flavoparmelia* sp. being the most active with an MIC of 1 mg/ml. This extract also exhibited the best antifungal activity with an MIC of 31.25 μg/ml against *C. albicans*. The *Usnea* sp. exhibited potent antimycobacterial activity with an MIC of 62.5 μg/ml against *M. aurum*. HPLC analysis of the extracts revealed that the major constituent of most of the lichen extracts tested was usnic acid ($C_{18}H_{16}O_7$). This compound exhibited antimycobacterial activity against *M. aurum* with an MIC of 15.6 μg/ml and inhibited the growth of *S. aureus* at 500 μg/ml, while not affecting the growth of *K. pneumoniae* and *C. albicans*. Furthermore, usnic acid was tested for antimalarial activity against *Plasmodium falciparum* D 10. Usnic acid inhibited 50% of plasmodial growth at a concentration of 21.6 μg/ml. *Acknowledgements:* South African National Research Foundation, University of Cape Town

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Ornamental "Drumstick Onions" of *Allium* Subgenus *Melanocrommyum* Used as Medicinal Plants in Southwest and Central Asia

Fritsch RM[1], Gurushidze M[1], Jedelská J[2], Keusgen M[2]
[1]Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany; [2]University of Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany

Many members of *Allium* subg. *Melanocrommyum* are cultivated as ornamentals in European and North American gardens. Most of these species occur naturally in Southwest and Central Asia where they are collected in nature and used as vegetable, spice, and medicinal plants by native populations. Between 2002 and 2006, *Allium* samples were collected in the republics of Iran, Turkmenistan, Uzbekistan and Tajikistan. Eighteen species reportedly used as medicinal plants were morphologically and taxonomically characterized. Most of these species were chemically analyzed by means of HPLC and also screened for radical scavenger activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Our data verified relatively large amounts (> 0.25%) of cysteine sulphoxides (the main medicinally relevant sulphur compounds of garlic and common onion) to be present in ten species. However, some highly valued species showing also a very high radical scavenger activity contained only traces of cysteine sulphoxides but significant amounts of the newly detected sulphur pyrrole. This compound is apparently responsible for the high radical scavenger activity of *A. motor*, *A. komarowii*, *A. jesdianum*, *A. rosenorum*, and possibly also *A. karataviense*. Unexpectedly, some species showed high scavenger activity but did contain neither remarkable amounts of cysteine sulphoxides nor of sulphur pyrroles (e.g., *A. alaicum*, *A. chelotum*). These data underlined that another bioactive principle might be present, and more species of subg. *Melanocrommyum* than hitherto used may represent valuable medicinal plants. *Acknowledgements:* The authors are greatly indebted to organizers and local scientists supporting the field-work in Iran, Tajikistan and Uzbekistan, especially to Dr. Mehrdad Abbasi, Prof. Dr. Hikmat Hisoriev, and Dr. Furkat Khassanov. Funding by VolkswagenStiftung (Hannover, Germany) under the general funding theme "Zwischen Europa und Orient – Mittelasien/ Kaukasus im Fokus der Wissenschaft" is gratefully acknowledged.

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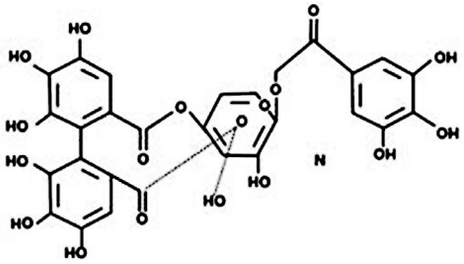
Screening of antimicrobial activity of *Kaempferia galanga* and *Quercus infectoria* against eight reference strain microorganisms

Mekseepralard C[1], Boriboonkaset S[1], Kamkaen N[2]
[1]Faculty of Medicine, Srinakharinwirot University, Klongtoei, Wattana, Bangkok 10110, Thailand; [2]Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhon-Nayok, 26120, Thailand

The rhizome of aromatic ginger, *Kaempferia galanga*, and the nutgall, *Quercus infectoria*, have been used as traditional Thai herbal medicines. Crude extracts of the herbs were screened for antibacterial activity against eight reference strain bacteria, both pathogens and normal flora. Seven bacteria associated with human infections in gastrointestinal tract were *Escherichia coli*, *Klebsiella pnuemoniae*, *Salmonella typhi*, *Serratia marcescens*, *Vibrio cholerae* non 01, non

0139, *Vibrio parahaemolyticus*, and *Enterococcus faecalis*, and one opportunistic pathogen that could cause community or hospital acquired infections was *Pseudomonas aeruginosa*. The test was carried out using modified agar dilution method [1,2] at various concentrations of the crude extracts. The MICs of *Kaempferia galanga* ranged from 1.625 mg.ml^{-1} to > 6.5 mgml^{-1} whereas the crude extract of *Quercus infectoria* showed higher antimicrobial activity with MIC values ranging from ≤ 0.4 mgml^{-1} to > 6.5 mgml^{-1}. The principal constituent of *Quercus infectoria* galls is gallotannic acid with galls containing 50 to 70 per cent [3]. These results provide evidence that *Quercus infectoria* extract may offer an alternative way for human treatment.



Gallotannic acid

Acknowledgements: National Research Council of Thailand, Faculty of Medicine and Faculty of Pharmacy, Srinakharinwirot University. **References:** [1] Forbes B. A., Sahm D. F., and Weissfeld A. S. (2002) S. Bailey & Scott's Diagnostic Microbiology, 11th ed. Andrew Allen, St. Louis, Missouri, USA, page: 229 – 250. [2] Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard M7-A6, (2005), 6th ed. In Performance Standards for Antimicrobial Susceptibility Testing, Fifteenth Informational Supplement, Clinical and Laboratory Standards Institute. [3] The British Pharmaceutical Codex. Galla B.P. (Accessed on Mar 30, 2007, at http://www.henriettesherbal.com/eclectic/bpc1911/quercus_gall.html)

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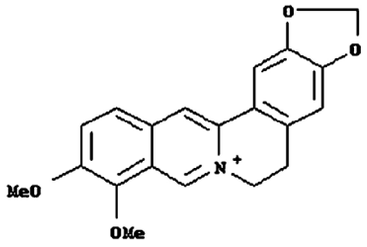
Antibacterial activities of *Coptis chinensis* and *Glycyrrhiza glabra* against seven bacteria associated with human gastrointestinal infections

Mekseepralard C[1], *Poonpon P*[1], *Kamkaen N*[2]
[1]*Faculty of Medicine, Srinakharinwirot University, Klongtoei, Wattana, Bangkok 10110, Thailand;* [2]*Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhon-Nayok, 26120, Thailand*

Minimum inhibitory concentration (MIC) of crude extracts of the rhizome of huang lian (*Coptis chinensis*) and the root of licorice (*Glycyrrhiza glabra)* were determined *in vitro* by a modified agar dilution method, at concentration levels of 0.4 – 6.5 mgml^{-1}, according to Clinical and Laboratory Standards Institute (CLSI) 2005 (1,2) against seven bacteria frequently involved in gastrointestinal infections. Tested bacteria were *Escherichia coli*, *Klebsiella pnuemoniae*, *Salmonella typhi*, *Serratia marcescens*, *Vibrio cholerae* non 01, non 0139, *Vibrio parahaemolyticus*, and *Enterococcus faecalis*. Most bacteria were susceptible to the extract of *Glycyrrhiza glabra* with the MIC values > 6.5 mgml^{-1}. MIC values of *Coptis chinensis* extract within the group of bacteria range from 3.25 to > 6.5 mgml^{-1} which did not much differ from those of *Glycyrrhiza glabra* extract. However, the crude extract of *Coptis chinensis* which contained berberine showed the best antibacterial activity against *Vibrio cholerae* non 01, non 0139. The results indicated that both natural products showed antimicrobial activity which may be useful for treating gastrointestinal infections.



Berberine

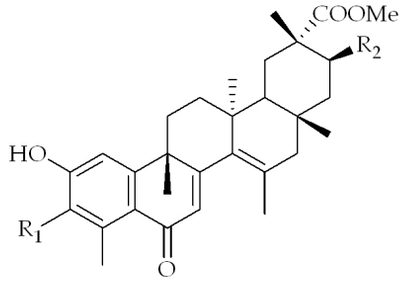
Acknowledgements: National Research council of Thailand, Faculty of Medicine and Faculty of Pharmacy, Srinakharinwirot University. **References:** [1] Forbes B. A., Sahm D. F., and Weissfeld A. S. (2002) S. Bailey & Scott's Diagnostic Microbiology, 11th ed. Andrew Allen, St. Louis, Missouri, USA, page: 229 – 250. [2] Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard M7-A6, (2005), 6th ed. In Performance Standards for Antimicrobial Susceptibility Testing, Fifteenth Informational Supplement, Clinical and Laboratory Standards Institute. [3] Yang, F. et al. (1998) J Chromatogr A, 829: 137 – 141.

P 141

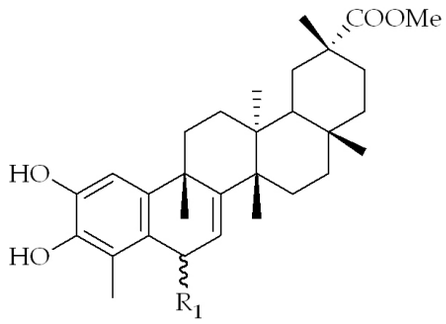
Antimicrobial activity of phenolic triterpenes from *Maytenus cuzcoina*

Bazzocchi IL[1], *Reyes CP*[1], *Jiménez IA*[1], *Moujir L*[2]
[1]*Instituto Universitario de Bio-Orgánica Antonio González, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain;* [2]*Departamento de Microbiología y Biología Celular, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez s/n, 38206 La Laguna, Tenerife, Spain*

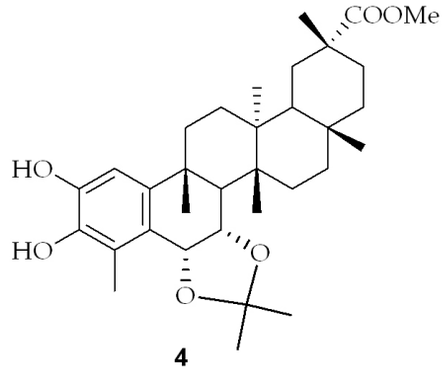
The aromatic triterpenoids constitute a small group of unsaturated and oxygenated *D:A*-friedo-*nor*-oleananes restricted to the plant family Celastraceae, and they are of medical interest since they tend to exhibit antibiotic and anticancer activities [1]. As part of our studies on medicinal plants belonging to this family, which is widely used as folk medicine in South and Central America, we had previously reported on dihydro-β-agarofuran sesquiterpenes as multidrug-resistence inhibitors and anti-tumor promoters [2] from *Maytenus cuzcoina*, a plant endemic to the Cusco region, Peru. A further search for structurally interesting and bioactive compounds from this plant resulted in the isolation of six known phenolic triterpenes in addition to the new compounds, 21β-hydroxycuzcoinol (1), cuzcoinol (2), 6β-(2-oxo-propyl)-pristimerol (3), and 6α,7α-isopropylidendioxo-blepharodol (4). Their structures were determined by spectroscopic methods, including ^{1}H-^{13}C (HSQC and HMBC), and ROESY NMR experiments.



1 R_1=OCH_3, R_2=OH
2 R_1=OH, R_2=H



3 R_1=β–C_3H_5O



4

The compounds were assayed for antimicrobial activity against Gram-positive and Gram-negative bacteria, and the yeast *Candida albicans*. Compound 3 and 6-deoxoblepharodol exhibited the highest activity against *Bacillus subtilis* (MIC 5 – 10 µg/ml), while compound 10 showed a moderate activity against *B. cereus* and *Staphylococcus epidermidis* (CMI 10 – 20 µg/ml). The structure-activity relationship established the relevance of the substituents on B ring for the antimicrobial activity. *Acknowledgments*: This work has been supported by the Spanish Grant CTQ-2006 – 13376/BQU. C.P.R. thanks to the Gobierno de Canarias for a fellowship. **References:** [1] Alveranga, N., Ferro, E. A. (2005) In Studies in Natural Products Chemistry, Bioactive Natural Products. Elsevier Science. Amsterdam, 30: 635 – 702. [2] Cortés-Selva, F., Campillo, M. et al. (2004) J. Med. Chem. 77: 576 – 587.

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Antihelicobacterial and antioxidant activities of Mongolian medicinal plants

Batkhuu J[1], Khishigbuyan B[1], Feierl G[2], Brantner AH[3]
[1]Faculty of Biology, National University of Mongolia, P.O.B-617, Ulaanbaatar-46A, Mongolia; [2]Institute of Hygiene, Medical University Graz, Universitaetsplatz 4, A-8010 Graz, Austria; [3]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Universitaetsplatz 4/1, A-8010 Graz, Austria

Biological activities of Mongolian medicinal plants are not so well investigated. Gastric ulcer created by *Helicobacter pylori* is one of the main diseases among Mongolians. Therefore, the aim of this study was the evaluation of the antihelicobacterial and antioxidant activities of 45 methanolic plant extracts belonging to 36 species of 19 families, which were selected on the basis of their traditional medicinal use. The disc diffusion method [1] and the DPPH assay [2] were used to evaluate these activities. A remarkable antihelicobacterial activity was obtained with the extract of *Thymus dahuricus* root (Table 1), which might be therefore a reasonable drug in the prevention of gastric ulcer. The strongest antioxidant activity was shown by the *Bergenia crassifolia* root extract (Table 2). Table 1. Activity against *H. pylori* lab strains

	Inhibition zone Ø [a], mm	
Plant species, parts used [b]	*H. pylori* 106.903	*H. pylori* 107.594
Thymus dahuricus, root	> 40	> 40
Artemisia changaica, leaves	28	28
Juniperus pseudosabina, stem	28	26
Artemisia pectinata, flowers	22	22
Juniperus sibirica, leaves	22	20
Ciprofloxan (ref, 5 µg/disc)	> 40	> 40

Table 2. Antioxidant activity

Plant species, parts used	DPPH radical scavenging activity IC_{50}, µg/ml
Bergaenia crassifolia, root	11.75
Saxifraga hirculis, leaves	12.06
Potentilla viscose, flowers	15.35
Ephedra equisetina, leaves	22.19
Reaumuria soongarica, flowers	25.67
Rutin (ref.)	17.05

[a] Disc Ø: 9 mm, [b] Concentration of MeOH extracts: 500 µg/disc, n = 3 *Acknowledgements:* Asia-Pacific UNINET, Austrian Exchange Service (OEAD), Asian Research Centre (ARC) at National University of Mongolia. **References:** [1] Pharmacopoeia Europaea, ed. 5 (2005) [2] Hatano, T et al. (1988) Chem. Pharm. Bull. 36(6): 2090 – 2097

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Chemical composition of volatile oils and evaluation of antioxidant and antimicrobial activities of ethnopharmacologically selected Thai medicinal plants

Wungsintaweekul J[1], Putalun W[2], Sitthithaworn W[3], Pfeifhofer HW[4], Brantner AH[5]
[1]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90112, Thailand, [2]Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand, [3]Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Srinakharinwirot University, Ongkarak, Nakornayok 26120, Thailand; [4]Institute of Plant Sciences, University of Graz, Holteigasse 6, A-8010, Graz, Austria, [5]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4/I, A-8010 Graz, Austria

Thai medicinal plants belonging to the Lamiaceae, Rutaceae and Zingiberaceae were selected regarding their traditional medicinal use. Volatile oils and methanolic extracts were evaluated for their antimicrobial and antioxidant activities as well as for their chemical compositions using GC-MS and other chromatographic methods. The antimicrobial activity was tested against, 4 gram-positive and 5 gram-negative bacterial strains including *Mycobacterium* species and 3 fungi [1] by the disc diffusion method. The MIC was estimated by the microdilution broth method [2]. The result showed that the volatile oil of *Ocimum americanum* (major components (*E*)-citral and (*Z*)-citral) exhibited an activity against gram-positive bacteria (MIC 1.2 – 1.4 mg/ml), gram-negative bacteria (MIC 1.8 – 2.0 mg/ml) and fungi (MIC 0.2 – 0.3 mg/ml). In addition, O*cimum sanctum* volatile oil exhibited a considerable activity against *Candida albicans*, *C. parapsilosis* and *C. tropicalis* (MIC 0.8, 1.3 and 1.4 mg/ml, resp.). The antioxidant activity of the volatile oils and the methanolic extracts were assessed using the DPPH and lipid peroxidation assays [3,4]. The results showed that the methanolic extracts of *Citrus hystrix* leaf (IC_{50} 30 µg/ml) and fruit peel (IC_{50} 68 µg/ml) as well as *Kaempferia parviflora* black rhizome ((IC_{50} 68 µg/ml) exhibited a remarkable scavenging activity by the DPPH method but showed less activity on lipid peroxide. *Acknowledgements:* The authors thank the Ministry of Higher Education of Thailand for traveling grant support. **References:** [1] Brantner, A.H., Grein, E. (1994) J. Ethnopharmacol. 44: 35 – 40. [2] Pharmacopoeia Europaea, ed. 5 (2005) [3] Hatano, T.

et al. (1988) Chem. Pharm. Bull. 36(6): 2090 – 2097. [4] Houghton P.J et al. (1995). Planta Med. 61, 33 – 36.

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The antioxidant and antimicrobial activities of methanolic extracts from houseleek *(Sempervivum marmoreum L.)* leaves

Stojièević S[1], Stanisavljević I[1], Velièković D[2], Veljković V[1], Lazić M[1]
[1]Faculty of Technology, Bulevar oslobođenja 124, 16000 Leskovac, Serbia; [2]Zdravlje-Actavis, Vlajkova 199, 16000 Leskovac, Serbia

This work is a comparative study of antioxidant and antimicrobial activity of extracts obtained by ultrasound-assisted extraction (UE), classical solvent extraction (CE) and Soxhlet extraction (SE) of *Sempervivum marmoreum* L. leaves, using methanol. The yield obtained by Soxhlet extraction was also taken to represent the extractive substances (ES) content in the plant material. The extractions were carried out at the room temperature with the ratio of plant material to solvent 1:10 g/ml. Two antioxidant assays, DPPH photometric assay [1] and hydroxyl radical assay [2], were used to evaluate antioxidant activities. The results are presented in Table 1. The agar well-diffusion method was employed for determination of antimicrobial activities of the extracts. Independently of the extraction technique, the methanolic extracts of *S. marmoreum* showed antibacterial activity against two of the seven tested microorganisms, the mould *Aspergillus niger* and the yeast *Candida albicans*. The results indicate that the extract obtained by classical solvent extraction from houseleek leaves, showed better antioxidant, but similar antimicrobial activities, than the extracts obtained by employing two other techniques. Table 1 Yield of ES and antioxidant activities of *S. marmoreum* extracts obtained by different extraction techniques

Extraction technique	Yield of ES[a]	DPPH, EC_{50}, mg/ml [b]	Hydroxyl radical, EC_{50}, mg/ml
CE[c]	2.4	0.09 ± 0.01	1.08
UE[c]	2.3	0.06 ± 0.01	1.26
SE[d]	2.9	0.12 ± 0.04	ND

[a] (g/100 g fresh plant material) [b] Mean of three replicates ± standard deviation [c] (1:10 g/mL, 40 min, 25 °C) [d] 150 min, ND – not determined. *Acknowledgements:* Ministry of science and environmental protection, Republic of Serbia projects OI 142073b. **References:** [1] Choi C.W. et al. (2002) Plant Sci. 163 (6): 1161 – 1168. [2] Canadanovic-Brunet J.M. et al. (2004) J Sci Food Agric 85 (2): 265 – 272.

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The antioxidant and antimicrobial activities of *Echinacea purpurea* L. methanolic extracts

Stanisavljević I[1], Stojièević S[1], Velièković D[2], Veljković V[1], Lazić M[1]
[1]Faculty of Technology, Bulevar oslobođenja 124, 16000 Leskovac, Serbia; [2]Zdravlje-Actavis, Vlajkova 199, 16000 Leskovac, Serbia

The antioxidant and antimicrobial activities of *Echinacea purpurea* L. (Asteraceae) extracts obtained by ultrasound-assisted extraction were investigated. The aerial parts of plants were extracted at room temperature with 70% ethanol at a solid:liquid ratio of 1:10 (w/v). Each of the measurements was carried out in three repeated experiments. Total contents of phenolic compounds and flavonoids were determined by Folin-Ciocalteu [1] and aluminium chloride colorimetric method [2], respectively. Total phenolic and flavonoid content were 46.8 ± 0.29 mg gallic acid equivalents (GAE)/g dry extract and 27.0 ± 0.39 mg rutin equivalents (RE)/g dry extract, respectively. The antioxidant activity of *Echinacea purpurea* L. extract was determined using the DPPH free radical method [3]. 2,2-Diphenyl-1-picril hydrazyl radical (DPPH) scavenging reached 93.6% and the EC_{50} concentration for the inhibition of 50% DPPH was 1.88 ± 0.02 μg/μg DPPH. Antimicrobial activity of *Echinacea purpurea* extract was evaluated by an agar well-diffusion method against seven microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus, Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*. The results indicate that the extract showed a considerable growth inhibition on *Candida albicans* and *Saccharomyces cerevisiae* while no growth inhibition zones were observed only for *Aspergillus niger. Acknowledgements:* Ministry of science and environmental protection, Republic of Serbia projects OI 142073b. **References:** [1] Singleton V. L., Rossi J. A., (1965) Amer. J. Enol. Viticult. 16 (3): 144 – 158. [2] Chang C. et al.(2002) J. Food Drug Anal. 10 (3): 178 – 182. [3] Choi C.W. et al. (2002) Plant Sci. 163(6): 1161 – 1168.

P 146

Anti-adhesion activity of berries and berry juices against the serious pathogens *Neisseria meningitidis*, *Streptococcus pneumoniae, Streptococcus agalactiae*, and *Streptococcus suis*

Toivanen M[1], Pyykönen S[1], Ïuricová J[1], Loimaranta V[2], Haataja S[2], Finne J[2], Lapinjoki S[1], Tikkanen-Kaukanen C[1]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland, [2]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, University of Turku, FI-20520 Turku, Finland

Anti-adhesive compounds have potential in preventing bacterial infections by blocking bacterial attachment to host mucosal surfaces [1]. In the present study, interactions of the important human or animal pathogens *Neisseria meningitidis*, *Streptococcus pneumoniae, Streptococcus agalactiae*, and *Streptococcus suis* were investigated with different berries or berry and fruit juice concentrates. The studied material was bilberry (*Vaccinium myrtillus*), cranberry (*Vaccinium oxycoccus*), lingonberry (*Vaccinium vitis-idaea*), cloudberry (*Rubus chamaemorus*), crowberry (*Empetrum nigrum*), apple (*Malus domestica*), blackcurrant (*Ribes nigrum*), pineapple (*Ananas comosus*), raspberry (*Rubus idaeus*), red grapefruit (*Citrus paradisi*), sour cherry (*Prunus cerasus*) and tomato (*Solanum lycopersicum*). Berries and juices were fractionated according to their molecular size into three different fractions (< 10 kDa, 10 – 100 kDa, > 100 kDa) by using centrifugal filter devices. A microtiter well assay for the binding of *N. meningitidis* pili was employed [2] and binding assay for *S. pneumoniae* and *S. agalactiae* bacterial cells was constructed. Adhesion inhibition of *N. meningitidis* pili to human epithelial cell line (HEC-1B) and hemagglutination inhibition of *S. suis* cells [3] was examined by employing selected berry and fruit material as inhibitors. Meningococcal pili, *S. pneumoniae* and *S. agalactiae* bacterial cells bound most effectively to the fractions prepared from *Vaccinium* berries. Bilberry, cranberry, lingonberry, crowberry and blackcurrant juice fractions of 10 – 100 kDa inhibited binding of *N. meningitidis* pili to HEC-1B cells in a dot binding assay. Hemagglutination induced by *S. suis* was effectively inhibited by cranberry. The results identify several previously unknown berry sources having inhibitory activity against the adhesion of bacterial pathogens. *Acknowledgements:* Kiantama Ltd, VIP-Juicemaker Ltd, Xavier Nassif **References:** [1] Ofek, I., Hasty, D.L. et al. (2003) FEMS Immunol Med Microbiol 38: 181 – 91. [2] Hakkarainen J., Toivanen M. et al. (2005) J Nutr 135: 2445 – 8. [3] Tikkanen, K., Haataja, S. et al. (1995) JBC 270: 28874 – 8.

P 147

Production of xanthones with antimicrobial and antioxidant activities by *Hypericum perforatum* L. cells

Franklin G[1], Conceição L[1], Kombrink E[2], Dias A[1]
[1]University of Minho, Campus de Gualtar, 4710 – 057 Braga, Portugal; [2]Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany

Hypericum perforatum L. (HP) is a well-known medicinal plant, widely used across the world. Previously we found that elicitation of HP cell cultures induces high accumulation of xanthones [1]. Xanthones are excellent antioxidants, which can act as metal chelators, free radical scavengers and inhibitors of lipid peroxidation [2]. Se-

veral pharmacological properties of xanthones such as hepatoprotective, anti-inflammatory, cancer chemopreventive and anticancer activities can be explained in part by their antioxidant properties. Other activities of xanthones such as antimycobacterial, antiparasitic, antiretroviral and antimalarial can be attributed to their antimicrobial properties [2]. We studied the potential of HP cells to produce xanthones with pharmacological activities when induced by a biotic stress: elicitation with *Agrobacterium tumefaciens*. Elicited HP cells showed the accumulation of xanthones in high concentration, up to 4 mg/g cells (dry wt), within 24 h. Those values were 12 times higher than observed on control samples. Subsequently, *A. tumefaciens* viability started to decline and reached the level of complete mortality within 12 h while the HP cells remained viable. Additionally, the methanolic extract from elicited cells showed 10 times higher antimicrobial and increased antiradical activities than the extract from non-elicited cells. The phenolic profile of the elicited HP cells showed the production of several new xanthones. Some major xanthones were isolated and fully identified. Antioxidant (DPPH reduction and inhibition of lipid peroxidation) and antimicrobial (colony forming units and growth inhibition zone) activity assays with isolated compounds revealed that the xanthones accumulated after elicitation plays two significant roles: i- acting as phytoalexins/phytoanticipins having anti-bacterial activity against *A. tumefaciens*; ii- acting as antioxidants, probably protecting the plant cells from oxidative damage (an intense oxidative burst is observed during the elicitation procedure). *Acknowledgments:* This work is supported by an FCT grant (POCTI/AGR/40283/2001). G. Franklin is supported by an FCT postdoctoral fellowship (SFRH/BPD/17102/2004). L. Conceição is supported by an FCT PhD fellowship (SFRH/BD/13318/2003). **References:** [1] Conceição, L. et al. (2006) Phytochemistry 67: 149 – 155. [2] Pinto, M. et al. (2005) Curr. Med. Chem. 12: 2517 – 2538.

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Sesquiterpene Hydrocarbons of the essential oil of *Actinolema macrolema* Boiss

Demirci B, Koltuksuz G, Baser KHC
Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University, 26470 Eskişehir, Turkey

The essential oils obtained by steam distillation in the first three hours and following three hours from crushed fruits and dried leaves of *Actinolema macrolema* Boiss. collected from its natural habitat in Konya, Turkey, were analysed by gas chromatography and gas chromatography-mass spectrometry. 68 components have been characterized representing 93% of the leaf oil. 33 components were characterized representing 95% of the first fraction and 90% of the second fraction. Identification of some of the compounds was carried out using techniques such as GC-MS, ^{1}H-NMR and ^{13}C-NMR. The sesquiterpene hydrocarbons guaia-5,7(11)-diene, selina-3,7(11)-diene and juniper camphor were isolated from the oils by column chromatography. The occurrence of guaia-5,7(11)-diene is reported for the first time in nature. Guaia-5,7(11)-diene (37% and 30%), germacrene-B (25% and 21%), selina-3,7(11)-diene (12% and 12%) and 4,6-guaiadiene (10% and 8%) were found as major components in the oil of the first three hours and the following three hours, respectively. In the leaf oil, 1-octadecanol (24%) and hexadecanoic acid (19%) were identified as the major components. Antimicrobial activities of the fruit oils on Gram negative, Gram positive microorganisms and the yeast *Candida albicans* using the microdilution method have been determined. The distillation product obtained within the first three hours showed a good inhibitory effect on *Staphylococcus epidermidis* (MIC = 62.5 μg/ml). The two fruit essential oils had the same inhibitory effect on *C. albicans* as the standard antifungal used (MIC = 125 μg/ml). *Acknowledgements: This work is part of the MSc thesis of GK.*

P 149

Biological activities of South African *Salvia* species and isolated compounds

Kamatou GPP[1], van Zy RL[1], van Vuuren SF[1], Davids H[1], Viljoen AM[2], Seaman T[3], van Heerden FR[4]
[1]*Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa;* [2]*Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa;* [3]*Division of Pharmacology, Faculty of Health Sciences, University of Cape Town, Observatory, 7925, Cape Town, South Africa;* [4]*School of Chemistry, University of KwaZulu-Natal, Pietermaritzburg, 3209, South Africa*

Salvia L. species are traditionally used in South Africa to treat various ailments. The antibacterial, antimalarial and anticancer activities of the extracts of sixteen species were investigated. The extracts exhibited moderate to good antimalarial activity against the chloroquine-resistant *Plasmodium falciparum* (FCR-3) strain (3.9 μg/ml < IC_{50} values < 26.1 μg/ml). *Salvia radula* Benth. exhibited the best activity and bioassay-guided fractionation lead to the isolation of betulafolientriol oxide and salvigenin (IC_{50} values: 4.9 and 24.6 μg/ml, respectively). The antibacterial and antimycobacterial activities were evaluated using the microdilution and BACTEC ™ 460 radiometric methods, respectively. Nearly all the solvent extracts displayed good to moderate activity against the Gram-negative and Gram-positive bacteria (0.03 mg/ml < MIC values < 8.0 mg/ml). The extracts also exhibited promising activity against *Mycobacterium tuberculosis* (MIC values < 0.50 mg/ml). Four compounds with antibacterial activity were isolated from *S. chamelaeagnea* P.J. Bergius and characterized as carnosol, ursolic acid, oleanolic acid and 7-O-methylepirosmanol). The potential of the solvent extracts to inhibit cell proliferation of three cancer cell lines (MCF-7, HT-29 and SF-268) and Graham cells was evaluated using the SRB and MTT assays, respectively. The concentration required to inhibit 50% of cell growth ranged from 9.6 to 43.7 and between 8.7 and 59.1 μg/ml against the MCF-7 and SF-268 cell lines, respectively. The IC_{50} values against the HT-29 cell line ranged from 17.1 to 57.0 μg/ml. The toxicity of the solvent extracts on Graham cells ranged from 12.1 to 53.3 μg/ml. The *in vitro* pharmacological properties of these plants support the traditional medicinal uses of these plants. However, considering the toxicity that may be associated with high doses, these plants should be used with caution. *Acknowledgements:* National research Foundation (South Africa); South Africa National Biodiversity Institute (Pretoria).

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Antimicrobial activity of some medicinal plants growing in Serbia and Montenegro

Zdunić G, Stević T, Šavikin K, Menković N, Janković T
Institute for Medicinal Plants Research, T. Kožćužka 1, 11000 Belgrade, Serbia

Antimicrobial activity of four medicinal plant species *Alnus incana* (L.) Moench (leaves, bark, cones), endemic plant species *A. viridis* (Chaix) DC. (leaves, bark, cones), *Cornus mas* L. (leaves, flowers) and *Cotinus coggygria* L. (leaves, flowers) were investigated. According to the literature and our own results, all examined species are rich in polyphenols. Plant material was collected on the mountains of Serbia and Montenegro. Air-dried powdered material was extracted with methanol in Soxhlet for 24 h and solvent was evaporated. Dry extracts were used for experiments. A variety of microorganisms [Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Enterobacter cloacae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *P. tolaasii* (NCTC 387), *Proteus mirabilis* (ATCC 14273), and Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATCC 12952), *Bacillus subtilis* (ATCC 6051), *Micrococcus luteus* (ATCC 10240), *M. flavus* (ATCC 14452), *Sarcina lutea* (ATCC 10054), *Listeria monocytogenes* (ATCC 15313) as well as human pathogen fungi *Candida albicans*] were used in antimicrobial assay.

The MIC values were determined using the broth microdilution method in 96-hole plates according to NCCLS [1]. Serial dilutions of the stock solutions of tested extracts in broth medium (Muller-Hinton broth or Sabouraud broth) were prepared in a microtiter plate. The microbial suspensions were added in the microwells at the concentration of 5×10^5 organisms/mL. MICs were determined as the lowest concentrations preventing visible growth. Streptomycin and nystatin were used as a positive control. Each assay was repeated, independently, two times. Antimicrobial activity was noticed in all examined extract with MIC values ranging from 0.117 – 0.266 mg/mL. The most active were dry extract of cones of *A. incana and A. viridis*. Antimicrobial activity was not in correlation with the amount of total phenolics. **Reference:** 1. NCCLS (2000) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Fifth Edition. NCCLSocument M7-A5. NCCLS: Wayne, PA, USA.

P 151

Antimicrobial activity of *Gentiana lutea* L. extracts and isolated compounds mangiferin, isogentisin and gentiopicrin

Šavikin K, Menković N, Zdunić G, Stević T, Janković T
Institute for Medicinal Plants Research, T. Kožćužka 1, 11000 Belgrade, Serbia

Plant material of *Gentiana lutea* L. was collected on the mountain Suvobor. Air-dried leaves and flowers were extracted with methanol in a Soxhlet apparatus for 24 h. Evaporated dry extracts were used for experiments. Mangiferin (MG), isogentisin (IG) and gentiopicrin (GP) have been isolated according to previously published procedures [1] and their structures were confirmed using UV-VIS, IR and NMR techniques. A variety of microorganisms *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Enterobacter cloacae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *P. tolaasii* (NCTC 387), *Proteus mirabilis* (ATCC 14273), *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATCC 12952), *Bacillus subtilis* (ATCC 6051), *Micrococcus luteus* (ATCC 10240), *M. flavus* (ATCC 14452), *Sarcina lutea* (ATCC 10054), *Listeria monocytogenes* (ATCC 15313) as well as human pathogen fungi *Candida albicans* were used in the antimicrobial assay. The MIC values have been determined using the broth microdilution method in 96-hole plates according to NCCLS [2]. Serial dilutions of the stock solutions of tested extracts in broth medium (Muller-Hinton broth or Sabouraud broth) were prepared in a microtiter plate. The microbial suspensions were added in the microwells at the concentration of 5 x 105 organisms/mL. MICs were determined as the lowest concentrations preventing visible growth. The standard antibiotic streptomycin was used to control the sensitivity of tested bacteria, whereas nystatin was used as a control against the fungi. Each assay was repeated two times. Leaves contained 9.57 ± 0.4 mg/g dw of MG, 12.86 ± 0.7 mg/g dw of IG and 38.85 ± 0.7 mg/g dw of GP while flowers contained 8.98 ± 0.4 mg/g dw of MG, 123.23 ± 3.1 mg/g dw of IG and 48.38 ± 1.4 mg/g dw of GP. Mangiferin, isogentisin and gentiopicrin as well as extracts of leaves and flowers showed antimicrobial activity with MIC values ranging from 117 – 310 μg/ml. **References:** [1] Menković, N (1997) Ph D. Thesis, Faculty of Pharmacy, Belgrade. [2] NCCLS (2000) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Fifth Edition. NCCLSocument M7-A5. NCCLS: Wayne, PA, USA.

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Artemisinin and flavonoid content of several *Artemisia annua* breedings from Burundi (Africa)

Lapenna S, Bergonzi MC, Vincieri FF, Bilia AR
Department of Pharmaceutical Sceinces, University of Florence, I-50019 Sesto Fiorentino, Florence, Italy

Malaria mortality continues to increase across the world and represents the most important parasitic human disease. It causes over 1 million deaths, 300 million cases of illness, and an economic loss of US$ 12 billion annually [1]. *Artemisia annua* L. (Asteraceae) has been used to treat fevers in China for over 2 millennia [2] and recently, the clinical efficacy of *A. annua* teas or decoctions has been demonstrated, using high artemisinin-yielding plants [3 – 5]. Thus, it is important to verify the artemisinin levels in local cultivations of malaria areas and to assess how different geographical and climate conditions may affect the efficacy of the traditional treatments. In this study, the analysis of an *A. annua* cultivar (ANAMED 3 hydrid) cultivated in three different locations in Burundi (Kyenzi, a rainy and cool region in central Burundi at 2,300 m altitude, J1 from Northern Burundi, near Ruanda at about 2,000 m altitude, and from Bubanza, in northwestern Burundi, a hot and dry area at 950 m altitude) were compared for their content in active principles. The n-hexane extract of dried aerial parts from each cultivation was evaluated for the quali-quantitative profiles of artemisinin and polymethoxyflavones by means of HPLC-DAD-MS [6]. Artemisinin content in plant materials ranged from 0.20 to 0.35%, flavonoids, namely artemetin, chrysoplenetin, casticin, cirsineol and eupatin ranged from 0.32 to 0.80%. The best yields of both classes of constituents were obtained in the Kyenzi cultivation. *Acknowledgements:* The financial support of Ente Cassa di Risparmio di Firenze is gratefully acknowledged. We thank Dr. Paolo Monti for sending us plant materials. **References:** [1] World Health Organization, (2000) Trans. R. Soc. Trop. Med. Hyg. 94: 1 – 90. [2] O'Neill P et al. (2004) J Med Chem 47: 2945 – 64. [3] Mueller MS et al. (2000) J Ethnopharmacol 73: 487 – 493. [4] Mueller MS et al. (2004) Trans Royal Soc Trop Med Hyg 98: 318 – 21. [5] Räth K et al. (2004) Am J Tropical Med Hygiene 70, 128 – 132. [6] Bilia AR et al. (2006) Phytomedicine 13: 487 – 493.

P 153

Chemical composition and antimicrobial activity of the essential oils of *Micromeria thymifolia*, *M. dalmatica* and *Satureja cuneifolia*

Menković N, Zdunić G, Tasić S, Ristić M, Stević T, Šavikin K
Institute for Medicinal Plants Research, T. Kožćužka 1, 11000 Belgrade, Serbia

Plant material of *Micromeria thymifolia* (Scop.) Fritsch., *M. dalmatica* Benth. and *Satureja cuneifolia* Ten. was collected on Orien mountain, Montenegro in July 2006. Essential oils were obtained by steamdistillation using a Clevenger-type apparatus, according to procedure I of the Yugoslav Pharmacopoeia IV. Light yellow oils were obtained in 1.33%, 0.75% and 0.24% yield from the aerial parts of *M. thymifolia*, *M. dalmatica* and *S. cuneifolia*, respectively. The oils were subjected to qualitative and quantitative analysis by GC and GC/MS and antimicrobial activity of the oils (diluted 1:10 in DMSO) was tested. A variety of microorganisms: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Enterobacter cloacae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *P. tolaasii* (NCTC 387), *Proteus mirabilis* (ATCC 14273), *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATCC 12952), *Bacillus subtilis* (ATCC 6051), *Micrococcus luteus* (ATCC 10240), *M. flavus* (ATCC 14452), *Sarcina lutea* (ATCC 10054), *Listeria monocytogenes* (ATCC 15313) and *Candida albicans* were used in the antimicrobial assay. The MIC (minimum inhibitory concentrations) values were determined using the broth microdilution method in 96-hole plates according to NCCLS [1]. Serial dilutions of the stock solutions of tested oils in broth medium (Muller-Hinton broth or Sabouraud broth) were prepared in a microtiter plate. The microbial suspensions were added in the microwells at the concentration of 5 x 105 organisms/mL. Streptomycin was used to control the sensitivity of tested bacteria, whereas nystatin was used as a control against the fungi. Each assay was repeated two times. Piperitenone, pipertitenone oxide and pulegone were among the dominant compounds in essential oils of *M. thymifolia and M. dalmatica* while *S. cuneifolia* oil did not contain thymol or carvacrol. Antimicrobial activity was noticed in all examined essential oils with MIC values ranging from 5.6 – 54 μg/ml. The most active was essential oil of *S. cuneifolia*. **Reference:** 1. NCCLS (2000) Methods for Dilution Antimicrobial Sus-

ceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Fifth Edition. NCCLSocument M7-A5. NCCLS: Wayne, PA, USA.

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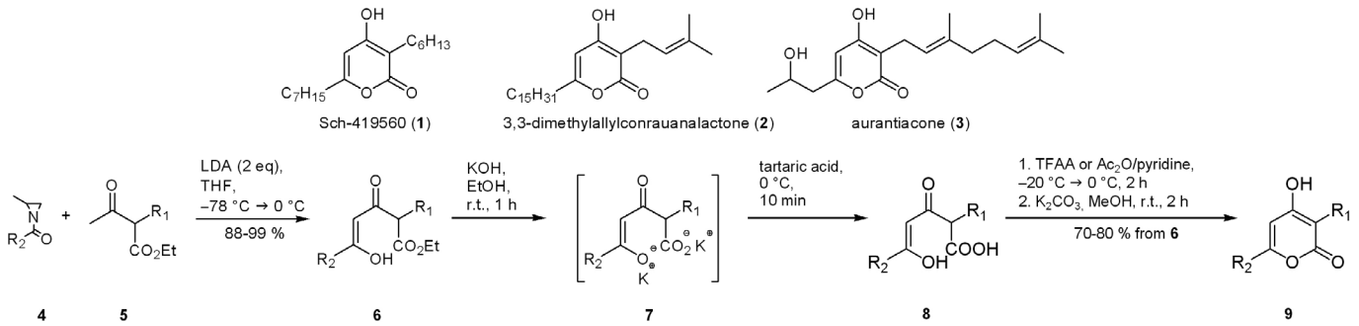
Efficient synthesis of natural products with a 3,6-disubstituted 4-hydroxy-2*H*-pyran-2-one framework

Schmidt D, Conrad J, Mika S, Klaiber I, Beifuß U
Institute of Chemistry, Section Bioorganic Chemistry, Hohenheim University, Garbenstraße 30, D-70599 Stuttgart, Germany

3,6-disubstituted 4-hydroxy-2*H*-pyran-2-ones have generated a lot of interest in medicinal chemistry since this structural scaffold is found in many biologically active natural products. Sch-419560 (**1**) has recently been isolated from the fermentation culture of *Pseudomonas fluorescens* and exhibited remarkable antibiotic properties. [1] 3,3-Dimethylallylconrauanalactone (**2**) was isolated from the bark of *Garcinia conrauana Engl.* (Guttiferae), [2] Aurantiacone (**3**) from the leaf resin of *Diplacus aurantiacus*. [3] Here we report on the total synthesis of these natural products: [4–5] The 2-alkylated 5-hydroxy-3-oxopent-4-enoic acid esters **6** were prepared according to Lygo's method [6] by reacting the dianions of 2-alkylated ethyl acetoacetates **5** under selective γ-acylation with *N*-acyl-2-methylaziridines **4**. The 5-hydroxy-3-oxopent-4-enoic acid ethyl esters **6** were transformed to produce the bispotassium salts **7**. The free 5-hydroxy-3-oxopent-4-enoic acids **8** were released with aqueous tartaric acid solution and lactonized under mild conditions with TFAA or Ac_2O-pyridine. The primarily formed *O*-acetyl derivatives were finally hydrolyzed with potassium carbonate to give the 4-hydroxy-2*H*-pyran-2-ones **9**. **References:** [1] Chan, T. A. et al. (2002) J. Antibiot. 55: 215. [2] Waterman, P. G. et al. (1982) Phytochemistry 21: 1393. [3] Wollenweber E. et al. (1989) Phytochemistry 28: 3493. [4] Schmidt, D. et al. (2006) Chem. Commun. 4732. [5] Schmidt, D. et al. (2007) Synlett 333. [6] Lygo, B. (1995) Tetrahedron 51: 128591.



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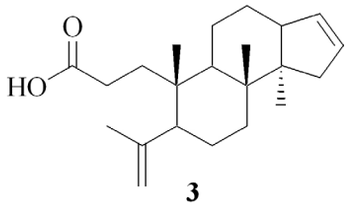
The anti-staphylococcal activity of terpenes from *Commiphora molmol* Engl

Rahman MM, Gibbons S
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, UK

Although a large number of antibiotics are available on the market, the development of resistance by bacteria is a major problem in the treatment of infectious diseases. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been headline news in the UK for the past few years with a sudden increase in death certificates (from 669 in 2000 to 1,168 in 2004) [1]. As a part of an effort to characterize new antibacterials with activity against effluxing multidrug-resistant strains of *Staphylococcus aureus*, oleo-gum resins from the stems of *Commiphora molmol* Engl. Have been extracted with chloroform and fractionated by VLC. Further sub-fractionation by SPE and purification by PTLC led to the isolation of a sesquiterpene, β-elemene (**1**) and two octanordammaranes; mansumbinone (**2**) and 3,4-seco-mansumbinoic acid (**3**). The compounds were identified by a series of spectral data, mainly 1D and 2D NMR spectra, and direct comparison to those of published data [2,3]. Compounds **2** and **3** are reported here for the first time from this species. We also unambiguously assigned all ^1H and ^{13}C NMR resonances for **3** and revised both ^1H and ^{13}C data. The antibacterial activity of these compounds was determined by broth microtitre MIC assay against a number of *Staphylococcus aureus* strains (SA1199B, ATCC25923, XU212, RN4220 and EMRSA15). MICs of **1** and **3** were found to be in the range of 4 – 256 μg/ml. The highest activity was observed by **3** (MIC = 4 – 8 μg/ml) against MDR SA1199B compared to the antibiotic, norfloxacin (MIC = 32 μg/ml).



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The anti-staphylococcal activity of *Hypericum* species

Ka Po Shiu W[1], Gibbons S[1]
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, UK

The genus *Hypericum* has attracted much attention in the past decade due to the popular use of St John's Wort (*Hypericum perforatum* L.) as an herbal remedy to treat depression. Its antibacterial action has been studied and the major anti-staphylococcal principal was found to be hyperforin, an acylphloroglucinol derivative, with minimum inhibitory concentration (MIC) ranging from 0.1 to 1 μg/ml against *Staphylococcus aureus* strains [1]. As with the ongoing project to investigate the anti-staphylococcal activity of the *Hypericum* genus [2], the aim of this study was to investigate the anti-staphylococcal activity of four different species, namely *H. beanii*

Robson, *H. revolutum* Vahl, *H. kouyhtchense* Robson and *H. choisianum* H. Léveillé. Air-dried, powered aerial parts of the plants were extracted sequentially in *n*-hexane, dichloromethane and methanol. The multidrug-resistant (MDR) strain SA1199B which over expresses the NorA efflux protein was used in the MIC assay. The MICs of these extracts ranged from 16 – 512 μg/ml, with the hexane and dichloromethane extracts being more active than the methanol extracts (Table 1). In conclusion, the genus *Hypericum* is a promising candidate which produces metabolites that show anti-staphylococcal activity. Further bioassay-guided fractionation of the above plants will allow the identification of the individual antibacterial components.

Species	*n*-Hexane	Dichloromethane	Methanol
H. beanii	16	64	256
H. revolutum	128	256	512
H. kouytchense	256	256	512
H. choisianum	64	64	512

Table 1. MICs (μg/ml) of *n*-hexane, dichloromethane and methanol extracts against MDR strain SA1199B *Acknowledgements:* Mr Chris Clennett (Royal Botanic Gardens Kew at Wakehurst Place) **References:** [1] Schempp, C.M. et al. (1999) Lancet 353: 2129. [2] Gibbons, S. et al. (2002) Fitoterapia 73: 300.

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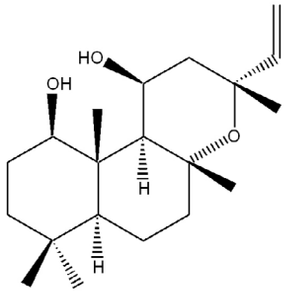
New diterpenes from *Plectranthus ernstii*

Stavri M, Gibbons S
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, WC1N 1AX, London, U.K

The Lamiaceae herb *Plectranthus ernstii* L.E.Codd was phytochemically and biologically studied as part of a project to assess plants belonging to the genus *Plectranthus* for antibacterial activity. This genus is known to be a rich source of diterpenes, particularly of the abietane and labdane class [1,2]. The phytochemical investigation of this herb led to the characterisation of a new pimarane (**1**) and a labdane diterpene with an unusual 8,13-ether moiety (**2**). The chemical structures were elucidated by 1D and 2D NMR experiments as 15,16-epoxy-7α-hydroxy-pimar-8,14-ene (**1**) and 1β,11β-dihydroxy-8,13-epoxy-labd-14-ene (**2**) and are reported here for the first time. Compound **1** also exhibited moderate antibacterial activity, using the broth microtitre dilution assay, with a minimum inhibitory concentration of 32 μg/ml recorded against a panel of *Staphylococcus aureus* strains, including methicillin-resistant and multidrug resistant strains.



[2]

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Antibacterial constituents from *Plectranthus ciliatus*

Stavri M, Gibbons S
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, WC1N 1AX, London, U.K

Plectranthus ciliatus E.Mey is an herb belonging to the family Lamiaceae and is used as an ornamental in gardens and homes. This species has not been very well studied phytochemically, although the genus has been reported to be a rich source of diterpenoids particularly of the abietane, labdane and kaurane class [1 – 3]. A total of 14 known compounds were isolated as part of the phytochemical assessment of this plant, six of which belonging to the kaurane diterpenoid class. The known labdane manool, phytol and phytyl acetate, two methoxylated flavones and two simple polyhydroxylated aromatic compounds have also been characterised from *P. ciliatus*. Bioassay-guided isolation led to the characterisation of the kaurane diterpene, kaurenic acid. This compound exhibited moderate antibacterial activity against a panel of *Staphylococcus aureus* and rapidly growing mycobacteria strains, determined using the broth microtitre dilution assay, with minimum inhibitory concentration values ranging between 8 – 32 μg/ml. The antibacterial activity of this compound is reported here for the first time against methicillin-resistant and multidrug resistant strains of *Staphylococcus aureus*. *Acknowledgement*: The University of London School of Pharmacy and EPSRC are thanked for a post-doctoral scholarship to M. Stavri **References:** [1] Adler, A.C. et al. (1984) Helv Chim Acta 1003 – 1011. [2] Gabetta, B. et al. (1989) Phytochemistry 859 – 862. [3] Gaspar-Marques, C. et al. (2003) J Nat Prod 491 – 496.

P 159

Screening for antimycobacterial activity of plants used in traditional medicine

Barata Z[1], Soares N[1], Ferreira MJU[1], Duarte A[1], Silva AN[1], Anes E[2], Mulhovo S[3], Madureira AM[1]
[1]CECF, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; [2]URIA-CPM Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; [3]Department of Medicinal Plants and Traditional Medicine, Ministry of Health, Maputo, Mozambique

Tuberculosis is a disease mainly caused by *Mycobacterium tuberculosis*. It is estimated that one-third of the world's population is infected with tuberculosis bacillus and two million people will develop an active disease per year. The increasing prevalence of the disease is due to the emergence of multidrug-resistant strains as well to the HIV pandemic which provides a large reservoir of highly susceptible individuals [1]. Since no anti-tuberculosis drugs have been introduced in the past 30 years, there is an urgent need for new effective anti-mycobacterial agents to circumvent new drug resistance cases. In this instance, the plant kingdom is undoubtedly a valuable source for new potential anti-tuberculosis agents to be explored [2]. According to WHO, almost 65% of the global population uses medicinal plants for primary health care [3]. The scientific evaluation of plants used by traditional healers becomes an urgent need [4]. In this study, the crude extracts from different parts of seven plants from Mozambique, used to treat respiratory disorders in traditional medicine, were screened for their ability to inhibit the growth of *Mycobacterium smegmatis*. From those plants, the methanolic extract and resulting fractions from the bark of *Cassia abbreviata* Oliver (Leguminosae) showed significant inhibitory activity when tested both by disc and wells diffusion methods. Three milligrams of each extract were applied on the discs/wells. Rifampicin was used as a positive control (30 μg). The inhibition zones of the tested extracts oscillate between 10 and 27.5 mm, and the positive control showed an inhibition zone of > 19 mm. The screening of the ability of those extracts to kill intracellular bacteria in macrophages, including pathogenic species such as *Mycobacterium tuberculosis* and *M. avium* is under progress. TLC analysis of the extracts, using the diphenylboric acid 2-aminoethylester reagent, revealed the pre-

sence of phenolic compounds as a major group of constituents. **References:** [1] Pauli, G.F. et al. (2005) Life Sci. 78: 485 – 494. [2] Gautam, R. et al. (2007) J Ethnopharmacol. 110: 200 – 234. [3] Molina-Salinas, G.M. et al. (2006) Arch Med Res. 37: 45 – 49. [4] Eldeen, I.M. et al. (2005) J Ethnopharmacol. 102: 457 – 464.

P 160

Search for antimalarial compounds from *Pycnanthus angolensis*

Ramalhete C[1], Abrantes M[1], Mil-Homens T[1], Duarte N[1], Lopes D[2], Cravo P[2], Madureira MC[3], Ascenso J[4], Ferreira MJU[1]
[1]CECF, Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083 Lisboa, Portugal; [2]CMDT, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, R. da Junqueira 96, 1349 – 008 Lisboa, Portugal; [3]Instituto Superior de Ciências da Saúde Egas Moniz, Quinta da Granja, 2829 – 511 Monte de Caparica, Portugal; [4]CQE, Instituto Superior Técnico, Av. Rovisco Pais, 1049 – 001 Lisboa, Portugal

Malaria is presently one of the most concerning infectious diseases, especially in African countries due to resistance of *Plasmodium falciparum* to the clinical available drugs. In these countries, traditional medicine plays a crucial role, as population has few means of accessing medical treatment. Important antimalarial compounds have been isolated from plants, such as quinine and artemisinin. Resistance to the main antimalarials underscores the need to search for new active compounds from plants used in traditional medicine. *Pycnanthus angolensis* Welw. Ward (Myristicaceae) is a plant used in traditional medicine against several diseases. Its bark has been used to treat fever and malaria in São Tomé and Príncipe islands. The dichloromethane extract of the bark revealed antimalarial activity against 3D7 *P. falciparum* strain (IC_{50}= 1.6 μg/mL) and was submitted to chromatographic bio-guided fractionation yielding the lignans 4,4'-dihydroxy-3-methoxylignan, heliobuphthalmin, (-)-dihydroguaiaretic acid, talaumidin, hinokinin, the labdane diterpene ozic acid and the steroids stigmast-4-en-6β-ol-3-one, stigmasterol and β-sitosterol. Furthermore, other compounds were obtained by derivatization. Structural identification was achieved by physical and spectroscopic methods (IR, EIMS, ^{1}H NMR, ^{13}C NMR and 2D NMR experiments). The *in vitro* antimalarial activity of the compounds was evaluated against 3D7 and Dd2 *P. falciparum* strains. In contrast with the crude extract and fractions, the compounds have not shown significant antimalarial activity in both strains. Unless the active compounds were lost during fractionation, these results might be explained by synergistic effects between the different components of the complex extracts and could suggest that a standardization of the bark extract might be the best solution to a rational use of this traditional antimalarial plant.

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Synergistic effects between macrocyclic diterpenes and doxorubicine on resistant cancer cells

Duarte N[1], Járdánházy A[2], Ramalhete C[1], Molnár J[2], Ferreira MJU[1]
[1]CECF, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600 – 083 Lisbon, Portugal; [2]Department of Medical Microbiology, University of Szeged, H-6720, Szeged, Hungary

The large structural diversity and complexity of plant-derived compounds makes them a very important source of leads in drug discovery and development. Due to the widespread increase of multidrug resistance (MDR), particularly in areas such as oncology and infectious diseases, there is an urgent need to identify new and effective molecules, which can act either as anticancer and antimicrobial drugs, or modulators of multidrug resistance.[1] MDR is often associated with the overexpression of P-glycoprotein (Pgp), which can prevent the accumulation of drugs by expelling them from the cell membrane before they are able to interact with their cellular targets.[2] *Euphorbia* species have been a source of a great variety of macrocyclic diterpenes, with the lathyrane and jatrophane skeleton, which have been reported to be potent modulators of MDR in cancer cell lines.[3], [4] In our search for biologically active compounds from plants, three new macrocyclic diterpenes with the jatrophane skeleton have been isolated from the methanolic extract of *Euphorbia tuckeyana* (whole plant) by chromatographic methods. Moreover, one lathyrane diterpene previously isolated from *Euphorbia lagascae* [5] was derivatized using several reagents, to afford three new ester diterpenes. All the structures were deduced from their physical and spectroscopic data (IR, MS, 1D and 2D NMR). Their multidrug resistance modulating properties were evaluated using the rhodamine-123 assay, in both MDR1-gene transfected and parental mouse lymphoma cell lines. Verapamil was used as a positive control. Furthermore, the antiproliferative effects of the anticancer drug doxorubicine, in combination with these macrocyclic diterpenes, were studied on the same cell lines, showing a synergistic effect. All of the tested compounds were able to reverse MDR on human MDR1 gene transfected mouse lymphoma cells, being much more active than the positive control. Jatrophane diterpenes were found to be potent inhibitors, exhibiting a dose dependent activity. **References:** [1] Butler, M. et al (2006) Biochem. Pharmacol. 71: 919; [2] Hendrich, A. et al (2003) Bioch. Biophys. Res. Com. 304: 260; [3] Duarte, N. et al (2007) Bioorg. Med. Chem. 15: 546; [4] Corea, G. et al. (2005) J. Med. Chem. 48: 7055; [5] Duarte, N. et al (2006) Planta Med. 72: 162.

P 162

Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages

Ebrahimi SN[1], Hadian J[1], Mirjalili MH[1], Yousefzadi M[2]
[1]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran, [2]Department of Biology, Faculty of Science, Tarbiat Modaress University, Tehran, Iran

The genus *Thymus* L., known as "Avishan" in Persian, is a well known aromatic perennial herb. Among 215 species of this genus grown in the world, 14 species are distributed in Iranian flora [1]. Thymus species are well known as medicinal plants because of several biological and pharmacological properties [2]. *Thymus caramanicus* Jalas is an endemic species in Iran. Variations in the quantity and the quality of the essential oil of wild populations of *Thymus caramanicus* at different phenological stages including vegetative, floral budding, flowering and seed set have beem investigated. The essential oils of air dried samples were obtained by hydrodistillation. The yields of oils (w/w %) at different stages were in the order of: flowering (2.5%), floral budding (2.1%), seed set (2%) and vegetative (1.9%). The oils were analyzed by GC and GC-MS [3]. In total 37, 37, 29 and 35 components were identified and quantified in vegetative, floral budding, full flowering and seed set, representing 99.3%, 98.6%, 99.2% and 97.8% of the oil, respectively. Carvacrol was the major compound in all samples. The ranges of major constituents were as follows: carvacrol (58.9 – 68.9%), *p*-cymene (3.0 – 8.9%), γ-terpinene (4.3 – 8.0%), thymol (2.4 – 6.0%) and borneol (2.3 – 4.0%). Antibacterial activity of the oils and their main compounds were tested against seven Gram-positive and Gram-negative bacteria. Their minimum inhibitory concentration (MIC) values were determined. The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil of *T. caramanicus*, were in the range of 15 – 36 mm and 0.5 – 15.0 mg/ml, respectively. The oils showed high activity against all tested bacteria. Thus, they represent an inexpensive source of natural antibacterial substances. **References:** [1] Jalas, J. (1982) Thymus, in Flora Iranica. ed. by Rechinger, K. H., Akademische Druck-u Verlagsanstalt. Graz, Austria. No 150, pp 536 – 538. [2] Zargari, A. (1990) Medicinal Plants. Tehran University Press Tehran, Iran, Vol. 4, pp 28 – 42. [3] Adams, R. (2001) Identification of Essential Oil Components by Gas Chromatography/Quadropole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, USA.

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Phytochemical analysis of *Cotinus coggygria* heartwood. Identification of isolated colorants in historical art objects

Stathopoulou K[1], Magiatis P[1], Karapanagiotis I[2], Valianou L[2], Chryssoulakis Y[3]
[1]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens 15771, Greece; [2]"ORMYLIA" Art Diagnosis Centre, 63071 Chalkidiki, Greece; [3]National Technical University of Athens, Department of Chemical Engineering, 15773, Athens, Greece

The heartwood of *Cotinus coggyria* Scop. (Anacardiaceae) has been used since the antiquity [1] as a source of a yellow dye known as young fustic. Although young fustic had been used in art objects for centuries, its exact chemical consistency is not well studied [2]. Phytochemical investigation of the methanol extract of the heartwood led to the isolation and structure elucidation by NMR and MS of several phenolic compounds, mainly aurones, chalcones, dihydroflavones and dihydroflavonols. Among them, the most abundant were sulfuretin (**1**) as well as 7,3',4'-trihydroxy-dihydroflavone (**2**), 5,7,4'-trihydroxy-dihydroflavone (**3**), 4,2',4'-trihydroxychalcone (**4**), 3,7,3',4'-tetrahydroxy-dihydroflavone (**5**), 3,5,7,3',4'-pentahydroxy-dihydroflavone (**6**), fisetin (**7**) and methyl gallate (**8**). Compounds **2-6** are described for the first time as constituents of young fustic. The isolated compounds were used as standards for the development of an analytic methodology using HPLC-DAD. HPLC analysis of the organic colorants extracted by acid hydrolysis from a series of ecclesiastical garments of the post-Byzantine period has led to the identification of most of the isolated compounds coming from *Cotinus coggyria*, along with other natural dyes. Prior to the analysis of the historical yarns, the ability of the extraction procedure to recover the dye components was tested by using reference dyed fibers. It was concluded that both extraction procedure and HPLC methodology are effective and can be used for the unambiguous identification of young fustic in historical textiles. *Acknowledgement*: The project was funded by the General Secreteriat for Research and Technology of Greece (Program PENED). **References:** [1] Baumann H (1993) Greek Wild Flowers and Plant Lore in Ancient Greece, Herbert Press: London. [2] Westenburg, H. et al. (2000) J. Nat. Prod. 63: 1696 – 1698.

P 164

Antibacterial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L.) essential oils

Bozin B[1], Mimica-Dukić N[2]
[1]Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Republic of Serbia; [2]Faculty of Natural Sciences, Department of Chemistry, Trg D. Obradovica 3, 21000 Novi Sad, Republic of Serbia

Use of aromatic plants in phytotherapy is mostly related to different activities of their essential oils, such as antimicrobial, spasmolytic, carminative, hepatoprotective, anticarcinogenic, etc. Many aromatic plants are today considered as the most important sources for the extraction of compounds with strong antioxidant activity [1]. Rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.), Lamiaceae, are two spices widely used in folk medicine, pharmacy and flavoring agents, but also designed as very powerful aromatic plants. In both spices, several phenolic diterpenes and phenolic acids were identified as major antioxidants [2]. However, for the same activity of essential oils very few data are available. Thus, the present study describes antimicrobial and antioxidant activity of rosemary and sage essential oils. Essential oils were obtained by the hydrodistillation and the chemical composition was evaluated by the means of GC-MS and TLC. Antibacterial activity was tested against 13 bacterial and 6 fungal strains by standard antibiogram assay [3]. Antioxidant activity was evaluated using DPPH- (2,2-diphenyl, 1-picrylhydrazyl) and TBA-test, following the effects of essential oils on Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2 induced lipid peroxidation (LP) [3]. Both examined essential oils expressed strong antibacterial activity, especially on different *Escherichia coli* strains, *Salmonella typhi* and *Shigella sonei*. Rosemary essential oil exhibited stronger antifungal activity on five dermatomycetes and *Candida albicans* than sage oil. The investigated essential oils reduced the DPPH radical formation (IC_{50}= 3.82 µg/ml for rosemary and 1.78 µg/ml for sage) in dose dependent manner. Strong inhibition of LP in both systems of induction was observed for the rosemary essential oil. Obtained results suggest that both essential oils could serve not only as flavour agents, but also as natural supplements in preventing deterioration of foodstuff, cosmetic and pharmaceutical products. **References:** [1] Charalambous G. (Ed.) (1994) Spices, Herbs and Edible Fungi. Developments in Food Science. Elsevier Science, Amsterdam, Netherlands. [2] Loliger, J. (1991) Lipid Technology 3(1): 58 – 61. [3] Bzin et al. (2006) Journal of Agricultural and Food Chemistry 54: 1822 – 1828.

P 165

Antimicrobial and antioxidant activity of propolis from Croatia and Brazil: a comparative study

Kosalec I[1], Sankovic K[1], Zovko M[1], Orsolic N[2], Bakmaz M[3], Kalogjera Z[1], Pepeljnjak S[1]
[1]Faculty of Pharmacy and Biochemistry University of Zagreb, HR-10000 Zagreb, Croatia, [2]Faculty od Science University of Zagreb, HR-10000 Zagreb, Croatia, [3]Zagreb City Pharmacy, HR-10240 Rakov Potok, Croatia

The source of the plant material from which honeybees collect propolis has the influence on its chemical profile, and consequently on biological activity. The aim of present study was to compare antimicrobial and antioxidant activity of ethanolic extracts of propolis collected from hives in Croatia (continental part) as typical poplar-type, and in Brazil (Minas Gerais region) as typical green or alecrim-type of propolis. For the antimicrobial evaluation, hole-plate diffusion and macro-broth serial dilution methods were performed. Antioxidant activity was performed by spectrophotometric methods using β-carotene-linoleic acid. The free radical scavenging activity of ethanolic extracts has been determined by electron paramagnetic resonance (EPR) against stable radical DPPH during 2 h. The content of flavonoids was analysed using spectrophotometric methods with $AlCl_3$ and 2,4-dinitrophenylhydrazine. Higher content of flavanones was found in Croatian propolis (38.6%) than in Brazilian propolis (11.4%). The content of flavones/flavonols was also higher in the Croatian propolis sample (1.6% vs. 0.9%, respectively). More potent antimicrobial activity exhibited Croatian propolis with MIC values below 10.38 mg/mL against *Escherichia coli* ATCC 10535, *Pseudomonas aeruginosa* ATCC 27853, and yeast *Candida albicans* ATCC 10231, while Brazilian propolis had MIC values against the same microbes between 10.38 and 20.75 mg/mL. The antioxidant potential was significant using Croatian propolis sample against DPPH radicals. Comparison of rate of scavenging capacity between propolis extracts showed no radicals after 30 min using Croatian propolis while Brazilian propolis after 60 min left 10% of radicals. In conclusion, origin of propolis has influence on the chemical composition of propolis as well as on its antimicrobial and antioxidant activity. According to the presented study, Croatian propolis rich in flavonoid aglycones exerts stronger antimicrobial and antioxidant activity than Brazilian propolis which is rich in other types of polyphenols such as prenylated phenylpropanoids.

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The antimicrobial activity of *origanum oil, basil oil, chamomile blue oil, tea tree oil* and *thyme oil* against Gram-positive bacterial strains

Papadopoulou C[1], Sakkas H[1], Arvanitidou M[2], Leveidiotou S[3]
[1]Food Microbiology Unit, Microbiology Department, Medical School, University of Ioannina, Ioannina, Greece; [2]Department of Hygiene, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece; [3]Clinical Microbiology Department, University Hospital and Microbiology Department, Medical School, University of Ioannina, Ioannina, Greece

The potential antimicrobial activity of five essential oils was investigated against 21 Gram-positive bacteria. The bacterial strains employed in the study were wild strains isolated from food, water and clinical samples, derived from the strain collection of the Food Microbiology Unit and the Clinical Microbiology Laboratory of the University Hospital (Ioannina, Greece). All essential oils were obtained from Sigma-Aldrich (Germany); origanum oil (W282812), basil oil (W211907), chamomile blue oil (W227307), tea tree oil (W390208) and thyme oil (W306509) were tested against methicillin-resistant *Staphylococcus aureus* (n = 7), methicillin-resistant *Staphylococcus epidermidis* (n = 1), vancomycin-resistant *Enterococcus faecium* (n = 2), *Streptococcus pyogenes* (n = 1) resistant to erythromycin and clindamycin and susceptible strains of *Streptococcus pyogenes* (n = 3), *Streptococcus agalactiae* (n = 1), *Listeria monocytogenes* (n = 5) and *Listeria innocua* (n = 1) using the broth macrodilution method. Susceptibility testing to antibiotics was performed using the Bauer-Kirby method and the VITEK II system (BioMerieux). Carvacrol and thymol (*Origanum oil*), thymol, linalool and p-cymene (*Thyme oil*), terpine-4-ol and p-cymene (*Tea tree oil*), methyl chavicol (*Basil oil*) were the main components of the tested oils. *Origanum oil* and *Thyme oil* produced the strongest antimicrobial effect against all tested strains, while *Tea Tree oil* and *Basil oil,* were active to a smaller extend. Chamomile blue oil exhibited no antimicrobial properties at all. Higher inhibitory capacity was observed in the oils containing phenolic components (carvacrol and thymol). The MIC values for the tested strains ranged from 0.063 to 1% (v/v) for *Origanum* and *Thyme oils*, from 0.25 to 2% (v/v) for *Tea Tree oil* and from 0.25 to > 4%(v/v) for *Basil oil*. *Acknowledgements:* This research was financially supported by the HERAKLEITOS project, funded by the General Secretariat of Research and Technology, Greek Ministry of Development. **References:** [1] Preuss H.G. et al. (2005) Mol. Cel. Biotechnology 272: 29 – 34. [2] Hammer K.A. et al. (1999) JAM 86: 985 – 990.

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Evaluation of the anti-TB potential of bryophytes

Schinkovitz A[1], Scher J[2], Zapp J[2], Becker H[2], Wang Y[1], Franzblau SG[1], Crandall-Stotler B[3], Stotler R[3], Pro S[1], Pauli GF[1]
[1]Department of Medicinal Chemistry and PCRPS, College of Pharmacy, University of Illinois at Chicago, Chilcago IL, 60612 USA; [2]Pharmacognosy and Analytical Phythochemistry, Geb. 32, University of Saarland D-66041, Saarbruecken Germany; [3]Department of Plant Biology, Southern Illinois University Carbondale, Carbondale IL, 62901 – 6509, USA

With more than 1.5 million casualties a year and one third of the world's population latently infected, tuberculosis (TB), together with HIV, represents one of the most menacing diseases of mankind [1, 2]. The contemporary TB treatment regimen requires the combination of at least 4 different antibiotics for 6 – 9 months. This situation and the increasing number of multi-drug resistant strains (MDR) of *Mycobacterium tuberculosis* indicate an urgent need for new antimycobacterial compounds and treatment strategies [2, 3]. Hitherto, many plants were found to exhibit antimycobacterial activities [4, 5]. However, bryophytes have hardly been investigated in this respect. We therefore conducted a bioassay-guided evaluation strategy on 13 North American bryophytes in order to evaluate their potential antimycobacterial activities against virulent *M. tuberculosis* (H37R*v*) in an Alamar Blue assay (MABA). Our preliminary data showed significant inhibition (≥ 90%) of *M. tuberculosis* (H37R*v*) in the crude petrol-ether and dichloromethane extracts of *Thuidum recognitum* Hedw. (Thuidaceae), and *Leucobryum glaucum* (Hedw.) Angst. (Leucobryaceae). Applying fast centrifugal partition chromatography (FCPC), and high-speed current counter chromatography (HSCCC), sub-fractions exhibiting ≥ 90% inhibition at 64 ug/ml could be obtained from both plants and will be analyzed further. With regard to potential lead structures, two active diterpenes, namely *ent*-3β-acetoxy-trachyloban-18-al (MIC: 59.34 μg/ml) and *ent*-trachyloban-17-all (MIC: 24.36 μg/ml) could be isolated from lipophilic extracts of *Jungermannia exsertifolia* Steph. (Jungermanniaceae). Our results suggest that bryophytes are a remarkable group of plants that deserve further attention in the search of lead structures for new anti-tubercular drugs. *Acknowledgements:* Austrian Science Fund (FWF), Vienna, Austria. **References:** [1] http://www.who.int/mediacentre/news/releases/2007/pr08/en/index.html, [2] Chakrabarti, B. et al. (2007) Future Microbiology 2: 51 – 61. [3] American Thoracic Society et.al. (2003) Am J Respir Crit Care Med 167: 603 – 62. [4] Newton, S. et. al. (2002) Phytother Res 17: 303 – 22, Cantrell, L et. al. (2001) Planta Med. 67: 685 – 94.

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Antimicobacterial and antioxidant activitiy of *Helichrysum devium* Johns. from Madeira Archipelago

Gouveia S[1], Camacho E[1], Weinhold T[1], Castilho PC[1], Luna-Herrera J[2]
[1]Centro de Química da Madeira, Dept. Química Universidade da Madeira, Campus da Penteada, 9000 – 390 Funchal, Portugal; [2]Departamento de Inmunología, Laboratorio de Inmunoquimica II. Escuela Nacional de Ciencias Biológicas. Prolongación de Carpio y Plan de Ayala Colonia Casco de Santo Tomás C.P. 11340 Ciudad de México, Mexico

Plants of the *genus Helichrysum* belongs to the Asteraceae family. In Madeira Archipelago (Portugal) *Helichrysum devium* Johns. and *Helichrysum melaleucum* Rchb. ex Holl, endemic sub-species of *Helichrysum*, are used in traditional folk medicine for treating bronchitis and pharyngitis. Preliminary biological tests such as evaluation of *Artemia salina* mortality and antimycobacterial activity were performed with the crude methanol extract of the aerial parts (flowers and leaves) for those two *Helichrysum* species. The extracts showed high activity on *Mycobacterium tuberculosis* (< 50 μg/mL). Subsequently, aerial parts of *H. devium* were extracted with solvents of increasing polarity (hexane, chloroform, ethyl acetate and methanol). In the determination of antimycobacterial activity it was observed that the hexane and chloroform extracts have an significant activity. The same extracts were very toxic (~ 100% of mortality) in the *Artemia salina* test. All extracts showed remarkable radical scavenging activity in the DPPH assay. For the present studies, *H. devium* flowers and leaves were evaluated separately, the extracts being obtained by the same method. The antioxidant activities of the leaves and flowers extracts were evaluated using two methods: α-α-Diphenyl-β-picrylhydrazyl (DPPH) and 2,2'azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assays.[2, 3] The results are in good agreement and show that the hexane extract of flowers exhibits the highest antioxidant activity, followed by the ethyl acetate, chloroform and methanol extracts, respectively. The ethyl acetate extract of the leaves exhibits the highest antioxidant activity, followed by the chloroform, hexane and methanol extracts. *Acknowledgements:* This work was supported in parts by the PhD grant of FCT SFRH/BD/24227/2005.

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Evaluation of the antibacterial and antidiarrheal activities of *Heeria insignis* O. Ktze (Anacardiaceae)

Agunu A[1], Ahmadu AA[2], Zezi AU[3], Yaro AU[4], Ehinmidu JO[5]
[1]Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria; [2]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria; [3]Department of Pharmacology and clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria; [4]Department of Pharmacology, Bayero University, Kano; [5]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria

Heeria insignis O. Ktze (Anacardiaceae) is an indigenous African shrub used in treatment of diarrhea, venereal diseases, tapeworm, hookworm, schistosomiasis, kidney trouble and for increasing lactation in women after childbirth [1,2,3]. The methanol and dichloromethane extracts of the leaves were evaluated for antibacterial activity (using agar-diffusion method) and antidiarrheal activity (using isolated rabbit jejunum and castor-oil induced diarrhea in mice) [4]. The methanol extract gave higher antibacterial activity than the dichloromethane extract. The order of susceptibility of test microorganisms to the methanol extract were *Salmonella typhi* > *Pseudomonas aeruginosa* > *Staphyloccocus aureus* > *Bacillus subtilis* > *Escherichia coli* which were greater than that of dichloromethane and comparable to standard. The minimum inhibitory concentration (MIC) of the methanol extract for these microorganisms was also conducted [5]. The MIC values (in mg/ml) of methanol extract against microorganisms are as follows: *B. subtilis* (3.9), *S. aureus* (1.95), *E. coli* (62.5), *Ps. aeruginosa* (3.9) and *S. typhi* (1.95). On the isolated rabbit jejunum evaluation, both extracts produced concentration-dependent relaxation of isolated rabbit jejunum that was not blocked by phentolamine, suggesting that extracts act via mechanisms other than α-adrenergic receptors. In the castor oil-induced diarrhoeal test, each extract gave 80% protection at 200 mg/kg which is comparable to loperamide 2 mg/kg with 80% protection. This finding may explain the use of the plant in diarrhea and bacterial diseases. **References:** [1] Daziel, J. M. (1955): The useful plants of West Tropical Africa. Crown agents for overseas Government and Administration, Millbank, London. [2] Burkill, H. M. (1985): The useful plants of West Africa, Vol. 1, 2nd edition, Royal Botanical Gardens, Kew. [3] Gelfand, M., Mavi, S. (2002): Pharm. Biol. 40: 74 – 76. [4] Agunu, A. et al. (2005): J. Ethnopharmacol 101: 27 – 30. [5] Ehinmidu, J., Ibrahim, Y. K. E.. (2004): Nig. J. Exp. Appl. Biol. 5: 133 – 139

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Medicinal Plants: the untapped source of bioactive peptides/proteins

Shahid M, Khan MM, Jamil A
Protein Molecular Biology Laboratory, Department of Chemistry (Biochemistry), University of Agriculture, 38040, Faisalabad, Pakistan

Traditionally used indigenous seeds of medicinal plants viz *Hygrophila auriculata, Abrus precatorius, Moringa oleifera, Croton tiglium, Withania somnifera* and *Psoralae corylifolia* were evaluated for antifungal against *Aspergillus niger, Mucor mucedo, Fusarium solani, Ganoderma lucidum* and antibacterial against *Bacillus subtilis, Pasturella multocida, Escherichia coli and Staphylococcus aureus.* The seeds were extracted in potassium phosphate buffer (pH = 7.0). The extracts of *M. oleifera, C. tiglium, W. somnifera* and *P. corylifolia* showed significantly high antimicrobial activity (disk diffusion method) against the selected strains. Further purification was performed by 80% $(NH_4)_2SO_4$, dialysis, gel filtration (sephadex G-100) and DEAE-cellulose chromatography. SDS-PAGE analysis revealed a broad spectrum antimicrobial 50 kDa protein from *Croton tiglium* and 14.4 kDa peptide from seeds of *M. oleifera* which have not been reported in the literature. The protein/peptide showed antifungal activity against *Mucor mucedo*, *Aspergillus niger, Fusarium solani* and *Metarhizium aniscoplae* and antibacterial activity against *P. multocida, S. aureus, E. coli* and *B. subtilis* (fluconazole and rifmpacin as positive control for antifungal and antibacterial respectively). Minimum inhibitory concentration for fungal and bacterial strains ranged (± SD) 13.5 ± 1.5 – 31 ± 3.44 and 10.2 ± 1.15 – 19.5 ± 2.5 μg/mL, respectively. The crude seed proteins showed strong potential in the treatment of waste water, particularly in reduction of microbial load (95%). The seed extracts of *W. somnifera*, *C. tiglium* and *H. auriculata* were found to be arrestant and toxic to assorted workers of *Microtermes obesi*. Seed extracts of these plants also showed effects on behavior and physiology of *Odontotermes obesus* (Isoptera: Termitidae). The antimicrobial and antitermitic of these medicinal plants demonstrated their potent therapeutic ability which might be explored multifariously. *Acknowledgement:* Higher Education Commission, Government of Pakistan for funding the grant for this project.

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Antiprotozoal activity of sequiterpene lactones – modelling of biological target properties and quantitative structure-activity relationships

Schmidt TJ[1], Nour AMM[1], Kaiser M[2], Brun R[2]
[1]Westfälische Wilhelms-Universität Münster, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstraße 56, D-48149 Münster, Germany; [2]Swiss Tropical Insitute (STI), Socinstraße 57, CH-4002, Basel, Switzerland

In continuation of earlier studies where we found a high activity of some sesquiterpene lactones (STL) against *Trypanosoma brucei rhodesiense* (causing East African sleeping sickness) [1, 2], we have now conducted a structure-activity study on a set of 39 STL against *T. brucei rhodesiense*, *T. cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. Furthermore, cytotoxic activity against murine cells was assessed. Some of the compounds possess high activity, especially against *T. brucei* (e.g. helenalin and some of its esters with IC_{50}-values of 0.05 – 0.1 μM which is about 10 times lower than their cytotoxic activity). The accumulated data clearly show that antiprotozoal activity is governed at least in part by different structural features than the cytotoxic effect against mammalian cells. In order to illuminate such factors underlying the observed selectivity, a QSAR study was conducted using the program *Quasar* [3]. This program constructs hypothetical surfaces of the presumed biological target based on the superimposed 3D molecular structures of the compounds. A *Quasar* model correlates the computed binding affinities of the ligands to the receptor surface with their experimental bioactivity data. Since the molecular mechanism underlying the studied activities is presumed to be related to covalent modification of the target by Michael addition, the standard setup of *Quasar* was modified by including the energy of each compound's lowest unoccupied molecular orbital (εLUMO) as a descriptor of electrophilic reactivity. Models of good to excellent descriptive and predictive quality could be obtained for the *T. brucei* and cytotoxicity data. Models for the other mentioned bioactivities are still under development. *Acknowledgements:* Thanks to Dr. A Vedani, Biographics Laboratory 3R, Basel, Switzerland, for valuable discussions concerning *Quasar* and to C. Heitland and J. Mack, Münster, for conducting some of the calculations during an undergraduate research project. **References:** [1] Schmidt TJ et al. (2002) Planta Med. 68: 750 – 751. [2] Nour AMM et al. (2006) Planta Med. 72: 1004 – 1005. [3] Vedani A, Dobler M. (2002) J. Med. Chem. 45: 2139 – 2149.

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Antibacterial, anti-diarrheal activity of *Daniellia oliveri* and *Ficus sycomorus* and their constituents

Ahmadu AA[1], Agunu A[2], Enhimidu JO[3], Magiatis P[4], Skaltsounis AL[4]
[1]Department of Pharmaceutical and medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria; [2]Department of Pharmacognosy and Drug development, Ahmadu Bello University, Zaria-Nigeria; [3]Department of Pharmaceutics and Pharm.Microbiology, Ahmadu Bello University, Zaria-Nigeria; [4]Laboratory of Pharmacognosy and Natural Products Chemistry, Universityof Athens, Greece

In our continuing search for bioactive compounds from Nigerian medicinal plants, the leaves of *Daniellia oliveri* Hutch and Dalz, and *Ficus sycomorus* L. were investigated. Both plants are used in traditional medicine of Northern Nigeria to treat diarrhea, skin infection, inflammation, wounds and diabetes [1, 2]. The ethanol and n-butanol extracts of leaves of both plants were screened for antimicrobial activity by agar diffusion technique [3]).The n-butanol extracts of both plants inhibited the growth of standard bacteria and fungal species which include S.aureus, E.coli, Ps.aeruginosa, B.subtilis T.rubrum and C.albicans. The zones of inhibition for *D. oliveri* were: S.aureus 20.0 mm, E.coli 21.0 mm, Ps.aeruginosa 15.5 mm, T.rubrum 10.5 mm while both C.albicans and B.subtilis showed no activity. Zones of inhibition for *F. sycomorus* were: S.aureus 26.00 mm, E.coli 25.5 mm, Ps. aeruginosa 25.0 mm, B.subtilis 27.5 mm and C.albicans 16.5 mm. Both extracts exhibited significant antidiarrheal activity using castor oil induced diarrhea in mice [4]. The LD 50 of both extracts were found to be > 5000 mg/kg for Daniellia and 1161.4 mg/kg for Ficus sycomorus. Fractionation of the n-butanol extract of *D. oliveri* over silica gel G and Sephadex LH-20 gave flavonoids identified as quercetin-3-O-rutinoside, quercitrin, quercimeritrin and isorhamnetin-3-O-rutinoside by 1 H-NMR and 13C-NMR spectroscopy and by comparison with data reported in the literature [5, 6], while the dichloromethane soluble part of the ethanol leaf extract of *F. sycomorus* gave lupenone, lupenol, β-amyrin and stigmasterol, their structures were elucidated by comparison of their NMR spectra with that reported in the literature. **References:** [1] Hutchinson, J and Dalziel, J.M(1957) Flora of West Tropical Africa. A crown agent for oversea Publication, London. 110 – 114 [2] Onwukeama, DN and Udoh, F(1999). Nig. Journ. of Nat. Products and Medicine. Vol.3 39 – 41 [3] Collins, H and Patricia, NL(1970) Microbiological methods. [4] Agunu, A et al(2005). Journ of Ethnopharm. 61, 209 – 213 [5] Anderson, M.O and Markham, K.R(2005) Flavonoids, chemistry, Biochemistry and application. 54 – 62 [6] Mabry, T.J et al(1970) The Systematic identification of Flavonoids. 296 – 301

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Use of genetically modified bakers yeast in high sensitivity screening of antifungal and multidrug resistance modulatory activities in extracts of plants used in traditional medicine

Kołaczkowski M[1], Kołaczkowska A[1], Środa K[1], Ramalhete C[2], Michalak K[1], Mulhovo S[3], Ferreira MJU[2]
[1]Department of Biophysics, Wrocław Medical University, Chałubińskiego 10, PL-50 – 368 Wrocław, Poland; [2]CECF, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600 – 083 Lisbon, Portugal; [3]Department of Medicinal Plants and Traditional Medicine, Ministry of health, Maputo, Mozambique

The majority of human fungal infections are caused by two yeast species *Candida albicans* and *Candida glabrata*, with the prevalence rate of about 70% and 15% respectively. Although less frequent, *Candida glabrata* infections are difficult to treat and are associated with high mortality rates due to innate increased tolerance towards azole antifungal drugs. Azoles constitute the most important of the limited number of antifungals classes suitable for treatment of systemic infections. The incidence of *Candida albicans* infections resistant to azoles treatment also increases. The underlying mechanism frequently found in azole resistant strains is the overproduction of efflux transporters Cdr1 p and Cdr2 p from the ATP binding cassette superfamily that actively extrude azoles out of cells. In face of the raising resistance there is an urgent need for the identification of novel antifungal compounds not affected by efflux transporters as well as inhibitors of these proteins able to sensitize resistant strains towards currently available antifungals. Bakers yeast *Saccharomyces cerevisiae* due to its well characterized network of genes involved in multidrug resistance, close similarity to pathogenic yeast and ease of genetic manipulation is well suited for antifungal screening purposes. In this study we have screened a panel of 63 crude extracts of plants used in traditional medicine, mainly from South and South-east African regions, for their ability to inhibit growth of strains showing different levels of expression of endogenous multidrug efflux transporters. We have also used a strain heterologously overproducing Cdr1 p to screen for modulators of its efflux activity. Extracts were also assayed against *Candida* species. Our results indicate the advantage of genetic modulation of activity of drug efflux transporters in the process of antifungal activity screening in natural products.

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In vitro antituberculotic activity of several lichen metabolites

Tasdemir D[1], Franzblau SG[2]
[1]Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK; [2]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, USA

Current epidemiological evidence suggests that one-third of the world's population is infected with *Mycobacterium tuberculosis* with 8 million new cases and 2 million deaths per year. Lichens are symbiotic associations between a fungus and an alga, some of which (e.g. Iceland moss) have been known for antibiotic effects throughout the ages [1]. Earlier investigations published in the 1950 s point to antimycobacterial potential of several pure lichen metabolites [2,3], however most of the studies have either involved other *Mycobacterium* species than *M. tuberculosis,* and/or the MIC values are missing. In the continuation of our search to identify natural products with antimycobacterial activity [4], we aimed to reinvestigate four commercially available lichen metabolites, (+)-usnic acid, evernic acid, psoromic acid and vulpic acid, for their in vitro antituberculotic effects. $H_{37}Rv$ strain of *Mycobacterium tuberculosis* and the well-established microplate alamar blue assay (MABA) [5] were used for the determination of the MIC values. Rifampicin served as the reference drug (MIC: 0.07 μg/ml). (+)-Usnic acid proved to be the most potent antituberculotic agent (MIC: 5.2 μg/ml), followed by psoromic acid (MIC: 44 μg/ml) and vulpic acid (MIC: 140 μg/ml). Evernic acid was found to be inactive at highest concentrations tested (MIC: > 200 μg/ml). Ingolfsdottir and his coworkers have recently reported the antibiotic activity of usnic acid against *Mycobacterium aurum*, a non-pathogenic organism with a similar sensitivity profile to *M. tuberculosis* [6]. Hence, this is the first study reporting the growth inhibitory activity of all four compounds against the causative agent of pulmonary tubercle, *M. tuberculosis.* This presentation will deal with the in vitro antimycobacterial effects of these lichens metabolites. Possible biological target(s) of the compounds will also be discussed. **References:** [1] Vartia, K.O. (1973) The Lichens. Academic Press, New York. 547 – 561. [2] Shibata, S. et al. (1948) Jap. Med. J. 1: 152 – 155. [3] Stoll, A. et al. (1950) Schweiz. Z. Path. Bakt. 13: 729 – 751. [4] Tasdemir, D. et al. (2006) Chem. Biodivers. 3: 1230 – 1237. [5] Collins, L., Franzblau, S.G. (1997) Antimicrob. Agents Chemother. 41: 1004 – 1009. [6] Ingolfsdottir, K. et al. (1998) Eur. J. Pharm. Sci. 6: 141 – 144.

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Antifungal activity of *Helianthus annuus* L

Karlickova J, Jahodar L, Buchta V, Rehakova Z, Kubikova K
Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Heyrovkeho 1203, Hradec Kralove, CZ-50005, Czech Republic

On the basis of previous research [1] *Helianthus annuus* L. was selected for testing of *in vitro* antifungal activity. An ethanolic extract from inflorescence (include thalamus and involucrum; INFEE) yielded two concentrated fractions – kaurenoid (IKF) with main compound *ent*-kaur-16-en19-oic acid) and phytosterol (IPF) with main compound stigmasterol. The same extract from leaves was fractionated into three parts -dichlormethane (LCH_2Cl_2), butanolic (LBuOH), ethanolic extract (LEtOH). Microdilution broth test M27A-M1 in a microplate [2] was performed on all types of extracts and fractions (INFEE, IKF, IPF, LCH_2Cl_2, LBuOH, LEtOH) with 13 strains of fungi (*Candida albicans* ATCC 44859, *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. tropicalis* 156, *C. krusei* ATCC 6258, *C. krusei* E28, *C. glabrata* 20/I, *C. lusitaniae* 2446/I, *Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Microsporum gypseum* 27339/01). The activity was evaluated as minimum inhibitory concentrations which were determined as the lowest concentrations that showed 80% growth reduction as compared with the growth of the drug-free control wells. The positive control was ketoconazole. IKF displayed significant *in vitro* antifungal activity against all used fungi (the range of MIC_{80} 0.016 – 0.125%). INFEE and IPF were found to be active only against *Trichophyton mentagrophytes* 445 (MIC_{80} 0.031%) and *Microsporum gypseum* 27339/01 (MIC_{80} 0.025%). Only the ethanolic part (LEtOH) of the crude leaf ethanolic extract showed significant *in vitro* antifungal activity against all used fungi (the range of MIC_{80} 0.032 – 0.128%). LCH_2Cl_2 and LBuOH were found to be active against *Trichophyton mentagrophytes* 445 only (MIC_{80} 0.064% and 0.032% respectively). *Acknowledgements:* This work is supported by the Research project 118/2006/B-BIO/FaF of Charles University Grant Agency. **References:** [1] Jahodar L. et al. (2003) 3 rd International Symposium on Natural Drugs, Proceedings, Naples: 249. [2] National Committee for Clinical Laboratory Standards: NCCLS document M27-P (1992): 771E.

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Antioxidant and antimicrobial activities of *Tamus communis* L. ssp. *cretica* (L.) Kit Tan

Erdemoglu N[1], Turan NN[2], Güvenç A[3], İşcan G[4], Aydın A[5]
[1]Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Ankara, Turkey; [2]Gazi University, Faculty of Pharmacy, Department of Pharmacology, 06330 Ankara, Turkey; [3]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Ankara, Turkey; [4]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskişehir, Turkey; [5]Gulhane Military Medical Academy, Department of Pharmaceutical Sciences, 06500 Ankara, Turkey

Aim: Tamus communis L. ssp. *cretica* (L.) Kit Tan (Dioscoreaceae), is a perennial herbaceous climbing plant with large fleshy tubers [1]. The aim of work was to examine antioxidant and antimicrobial properties of *T. communis*. ssp. *cretica*. **Methods:** The applied methods for the antioxidant activity of aqueous extract from the aerial parts of the plant were DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging, flow injection analysis-luminol chemiluminescence (FIA-CL) and thiobarbituric acid (TBA). The antimicrobial activity of the *n*-hexane, chloroform, ethyl acetate and ethanol extracts of the aerial parts of *T. communis* ssp. *cretica* was assessed towards selected bacteria. In addition, mineral composition of the raw plant was examined by atomic absorption spectroscopy. Main **Results:** DPPH free radical scavenging activity of the plant (IC_{50}= 2.85 ± 1.30 mg/ml) was the weakest antioxidant activity comparing with BHT and quercetin. In FIA-CL system, the aqueous extract has a weak antioxidant activity for H_2O_2 (log IC_{50}= 3.8 ± 0.09 mg/ml) and HOCl (log IC_{50}= $1.3 \times 10^{-3} \pm 4.9 \times 10^{-4}$ mg/ml) compared with ascorbic acid. In the TBA assay, the aqueous extract exerted the weakest antioxidant activity (IC_{50}= 3.82 ± 1.67 µg/mL) compared to propil gallat. The total phenolic content was 56.66 ± 0.21 mg/g. The extracts showed moderate activity with MIC values of 250 – 500 µg/ml against selected microorganisms. According to our results of mineral composition, the raw plant has low amount of toxic elements (Pb, Cd and Al), macro- (Ca and Mg) and microelements (Cu, Fe, Mn and Zn). Conclusion: *T. communis* ssp. *cretica* has a weak antioxidant activity and moderate antimicrobial activities against selected organisms. **References:** [1] Kit Tan (1984) Tamus L. In: "Flora of Turkey and the East Aegean Islands", Vol. 8, ed. by P.H. Davis, University Press, Edinburgh, pp. 552 – 554.

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Antibacterial and antiviral proteins from *Litchi chinensis* seeds

Ferreira CH[1], Alves SBV[1], Ramos LA[1], Oliveira LDR[1], Ribeiro CA[1], Coutinho MS[1], Lazzari AM[1], Melo FR[1]
[1]União Pioneira de Integração Social, SEPS 712/912 Conj. A, Brasília – DF, CEP 70390 – 125, Brazil

Litchi chinensis fruits have been used in Brazilian popular medicine as antibiotic and anti-viral agent on the treatment against several diseases. Some plants possess proteins which presents inhibitory activity against microbe and virus. For this reason, we decide to investigate the presence of these proteins in the plant mentioned above. Crude extract from seeds was prepared using 0.1 M NaCl, 0.01 M HCl solution (1:5, w/v, meal to solution ratio) with continuous stirring during 2 h. The material was centrifuged and the supernatant was precipitated with ammonium sulphate (0 – 90% saturation). This fraction, after dialysis against water, was used to determine the antibacterial activity against *Rhodococcus equi* and *Staphylococcus aureus* in a disk test (disk diffusion, solid culture media, 37 °C for 48 h). Protein quantification was done by Bradford [1] assay and disks containing 1.0 microg/mL of proteins were used. Distilled water was utilized as a negative control. After 48 h, inhibition halos zones of 7 – 10 mm of diameter were observed around the disks. On the antiviral assay was used the *Herpes simplex* virus. The protein fraction was able to inhibit the attack from *H. simplex* to bovine cells. The proteins were visualized in SDS-PAGE [2], indicating proteins with molecular mass between 18 kDa and 60 kDa. Ionic exchange chromatography was done by using CM-cellulose (HPLC-Akta, Superdex 75 column). Some of these proteins could be candidates to the production of antibiotic and antiviral products for applications in the human medicine and veterinary medicine. *Acknowledgements:* EMBRAPA Cerrados, Planaltina -DF, Brazil, Marília Santos Silva. **References:** [1] Bradford M. M., Anal Biochem. 1976;72: 248 – 54. [2] Laemmli U. K., Nature. 1970 Aug 15; 227(5259):680 – 5.

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Antibiotic proteins from *Apeiba tibourbou*, *Bixa orellana* and *Mucuna urens* seeds actives against *Rhodococcus equi*

Oliveira LDR[1], Ramos LA[1], Alves SBV[1], Ferreira CH[1], Zuchelli JM[1], Araújo CS[1], Lazzari AM[1], Melo FR[1]
[1]União Pioneira de Integração Social, SEPS 712/912 Conj. A, Brasília – DF, CEP 70390 – 125, Brazil

A number of animal-associated infections occur in persons infected with the human immunodeficiency virus (HIV), including those due to *Rhodococcus equi*. The principle of pathogenicity is the survival inside macrophages. Cavitary pneumonia is the most common manifestation of *R. equi* disease, but extrapulmonary infections are seen as well. Long term treatment with intracellularly active antimicrobial agents is required [1]. Actually, several antimicrobial proteins and peptides from seeds plants have been described [2]. Here, we describe the antibiotic activity of *A. tibourbou*, *B. orellana* and *M. ureis* proteins against *R. equi*. A crude extract was prepared using

seeds flours and 0.1 M NaCl, 0.01 M HCl solution (1:2, w/v, meal to solution ratio) with continuous stirring during 2 h. The material was centrifuged at 10.000 x g, 4 °C for 30 min and the supernatant was used to prepare an ammonium sulphate protein fraction (0 – 70% saturation). These fractions, after dialysis against water, were used to determine the antibacterial activity against *R.equi* in a disk test (disk diffusion, solid culture media, 37 °C for 48 h). Protein quantification was done by Bradford [3] assay and disks containing 1.0 microg/mL of proteins were used. Distilled water was utilized as a negative control. After 48 h, inhibition halos zones of 9 – 10 mm of diameter were observed around the disks containing the proteins from all fractions. SDS-PAGE [4] was done and protein bands between 10 and 60 kDa were observed in all samples. These proteins represent candidates for antibiotic production for applications in the human and veterinary medicine. *Acknowledgements:* EMBRAPA Cerrados, Planaltina -DF, Brazil, Marília Santos Silva. **References:** [1] – Rozsypal H, Aster V., Cas Lek Cesk. 2007;146(2):163 – 7. [2] – Hancock RE, Chaple DS., Antimicrob Agents Chemother. 1999 Jun;43(6):1317 – 23. [3] – Bradford M. M., Anal Biochem. 1976 May 7;72: 248 – 54. [4] – Laemmli U. K., Nature. 1970 Aug 15; 227(5259):680 – 5.

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Ecological factors, chemical composition and antibacterial activity of the essential oil from *Achillea millefolium* L. in the north of Iran

Mazandarani M[1], Behmanesh B[2], Rezaei MB[3], Ghaemi EO[4]
[1]Islamic Azad University, Gorgan Branch. Po.Box: Gorgan – 49175 – 384, Iran; [2]Faculty Range Management Gorgan University, Iran; [3]Forests and Rangeland Institute, Tehran, Iran; [4]Golestan University of Medical Sciences

This research was designed to investigate the autecology, ethnobotany, chemical composition and antibacterial activity of *Achillea millefolium* L. locally called "Maramboo", which has been used by the rural healers of Golestan province for healing wounds, digestive disorders, dysmenorrheal and fever. Phenology and ecological characteristic were obtained by field observation over an annual period in 2006 in its endogenous localities from 2100 – 2400 meter above the sea level, as the main fragrant medicine herb in Caharbagh village with wide distribution in sunny areas, dry cool climate, clay loam texture soil, annual rain 305 mm, annual temperature 7 pH in 7.6, EC in 5.4, T.N.V rang in 3.5 – 7.5% and organic carbon in 2.7% in west slops. Phenology showed that it is blooming in late June. The composition of essential oil from aerial parts of *Achillea millefolium* L was analyzed by GC- MS. Twenty seven components of the oil were characterized with geranyl acetate (35.9%), geraniol (16.5%), linalool (6.8%), davanone (6.7%),camphor (6.5%)and 1,8cinole (3.5%) being the major constituents. The crude essential oil was evaluated for antibacterial activity by a disc diffusion method against four bacteria *Staphylococcus aureus, Escherchia coli, Pseudomonas aeroginosa* and *Bacillus cereus*. We concluded that the essential oil showed antibacterial activity against tested bacteria, but the Gram-positive bacteria were more sensitive, especially *St. aureus* and *B. cereus*. This study confirmed that the essential oil of *A. millefolium* possesses antibacterial properties in vitro and its ethnopharmacological uses in wound healing. **References:** [1] Agihotri.V.K, Lattoo.S.K., Thappa.R.K.Kaul.P, Oazi.G.N,.2005. Planta Med, 71(3):280 – 3. [2] Baser.K.H, Demirci.B, Kocak.S, Akinci.C, Malyer.H, Gulerus.G. 2002, Planta Med. 10: 941 – 3. [3] Benzic.N, M.Skocibusic, V.Dunkic, A.Radonic. 2003. J.Essential, Phytother Res, 17(9):1037 – 40.

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Composition and antimicrobial activity of essential oil of *Ferulago longistylis* Boiss. fruits

Özkan AMG[1], Demirci B[2], Demirci F[2], Baser KHC[2]
[1]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Tandoğan, Ankara, Turkey; [2]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskişehir, Turkey

The genus *Ferulago* W. Koch (Apiaceae), is represented in Turkey by thirty species, of which sixteen are endemic. It is interesting that only forty-five *Ferulago* species are described in the world, suggesting that the gene center for this genus is Anatolia [1,2]. The species of this genus are known as kuzukemirdi, kuzubaşı, çakşır and resemble *Ferula* and *Prangos* species also widely abundant in Turkey. These three genera are used for many purposes in Turkish folk medicine. But they are mainly used as aphrodisiacs and preferred as fodder to increase animal productivity [3]. *Ferulago longistylis* Boiss. is a rare endemic, perennial species up to 150 cm high[1]. The essential oil from the fruits of *Ferulago longistylis* Boiss. (Apiceae) collected from Erzincan, north eastern Turkey was obtained by hydrodistillation. Simultaneous analyses by GC and GC-MS resulted in the identification of fifty-nine compounds representing 96% of the essential oil. The major constituents found were 2,3,6-trimethylbenzaldehyde (29%), α-pinene (17%), (Z)- β-ocimene (16%), sabinene (6%), myrcene (6%) and bornyl acetate (4%). The essential oil was also screened for its antimicrobial properties against various Gram negative (*Bacillus cereus, Enterobacter aerogenes, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium*) and Gram positive (*Staphylococcus epidermidis, S. aureus,* methicillin resistant *S. aureus*) bacteria including the yeast *Candida tropicalis*. Using a broth microdilution assay, only moderate to weak inhibitory activity (0.5 – 1 mg/mL) was observed against the microorganisms screened in this assay when compared to the standard antimicrobial agents. **References:** [1] Davis P.H. (Ed.) (1972) Flora of Turkey and the East Aegean Islands, Vol. 4. Edinburgh University Press: Edinburgh. [2] Akalın E. (1999) Pharmaceutical Botanical Investigation of Ferulago Species Growing in Western Turkey, Ph.D.Thesis, Istanbul Univ., Istanbul, Turkey. [3] Baytop T. (1999) Therapy with Medicinal Plants in Turkey – Past and Present, Second Edition. Nobel Tıp Basımevi: İstanbul Turkey.

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Screening of Iranian species for antibacterial activities

Amin G[1], Salehi Sourmaghi MH[1], Aynechi Y, Badami N[2], Kamal F, Amin M[2], Asgari T[1]
[1]Faculty of Pharmacy & [2]Faculty of Health, Tehran University of Medical Sciences, P.O.Box: 14155 – 6451, Pursina Ave, Tehran, Iran

The landscape of Iran includes a great range of ecological diversity and a rich flora much still unstudied for phytochemistry or bioactivity. In the present study antibacterial activity of a number of native Iranian plants species are reported, the antifungal effect's has been reported before. Some of this species are used by rural inhabitants as herbal medicine and were collected from different locations of Iran since 1994. In this study 532 species were collected from different regions of Iran. The voucher specimens were authenticated and deposited in the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences. The air-dried plant materials were powdered and extracted in a Soxhlet extractor with methanol 80%. The extracts were tested at a concentration of 100 µg/ml against organisms using Kirby Bauer method [1]. Gentamycin and methanol were used as positive and negative control, respectively. Antibacterial assay organisms were: *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus haemolyticus, Shigella sonei, Salmonella paratyphi-B, Esherichia coli*, *Nocardia astroides, Bacillus subtilis, Bacillus antracis, Proteus vulgaris, Pseudomonas aeroginosa, Proteus mirabilis, Vibrio cholerae, Klebsiella pneumoniae*. Four species, i. e. *Pistacia vera* L., *Cymbopogon parkerii* Stapf., *Myrtus communis* L. and *Echinophora platyloba* DC showed the best antibacterial effects.The most sensitive

organism was *Nocardia astroides* (+4, no growth) and *Pseudomonas aeruginosa* was the least sensitive (-, full growth). Among all tested plants the *Anacardiaceae* were highest ranked with 6 effective species. **Reference**: [1] Baron EJ and Finedgold SM.(1990),Diagnostic microbiology, 8th ed. Mosby Co.Philadelphia, USA

P 182

In vitro culture and biological activity of *Solanum dulcamara*, a medicinal plant

Cansever E, Turker AU
Department of Biology, Faculty of Science and Art, 14280 Bolu, Turkey

Solanum dulcamara L. (bittersweet) is a medicinal plant that has been used to treat skin diseases, warts, tumors, felons, arthritis, rheumatism, bronchial congestion, heart ailments, ulcerative colitis, eye inflammations, jaundice and pneumonia. A reliable *in vitro* culture protocol for bittersweet was established. Explants (leaf and petiole segments) were cultured on Murashige and Skoog minimal organics (MSMO) medium with various hormone combinations. Leaf explants formed more shoots than petiole explants. Best shoot proliferation was obtained from leaf explants with 3 mg/l BA plus 0.5 mg/l IAA and petiole explants with 3 mg/l Kinetin plus 0.1 mg/l 2,4-D. Regenerated shoots were transferred to rooting media containing different levels of IAA, IBA, NAA or 2,4-D. Most shoots developed roots on medium with 0,5 mg/l IBA, 3 mg/l IAA and 1 mg/l IBA. Rooted plants were transferred to Magenta containers including vermiculate for acclimatization. After 3 weeks, they were transferred to soil. Biological activities of bittersweet extracts were assessed using selected bench-top bioassays (antibacterial and antitumor). Four different kinds of plant extracts were analyzed (IM, FM, IW and FW); methanolic extracts of *in vitro*-grown (IM) and field-grown (FM) plant materials and aqueous extract of *in vitro*-grown (IW) and field-grown (FW) plant materials. In general, methanolic extracts of field-grown leaves and stems were the most effective and showed antibacterial activity against *Staphylococcus epidermidis*, *S. aureus*, *Klebsiella pneumonia*, *Salmonella typhimurium* and *Serratia marcescens*. *In vitro* grown plant material showed less antibacterial activity than field grown plant material. All four extracts showed antitumor activity and the percentage inhibition was more than 40% in experiments comparing with control (water). Methanolic extracts showed better antitumor activity than water extracts and field-grown leaves and stems were better than *in vitro*-grown leaves and stems. *Acknowledgements:* The Scientific and Technological Research Council of Turkey (TUBITAK) [TBAG-2278(103T024) and TBAG-HD/27(105T040)], Assist.Prof.Dr. Hakan Turker.

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Phytochemical investigation on leaves of *Cordia salicifolia* Cham. (Fam. Boraginaceae)

Menghini L[1], Epifano F[1], Tirillini B[2], Angelini P[3], Pagiotti R[3], Curini M[4]
[1]Dipartimento di Scienze del Farmaco, Via dei Vestini, 31, 66013, Chieti Scalo (CH), Italy; [2]Istituto di Botanica ed Orto Botanico, via Bramante, 61023 Urbino, Italy; [3]Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Borgo XX Giugno 74, 06126 Perugia, Italy; [4]Dipartimento di Chimica e Tecnologia del Farmaco, Sezione di Chimica Organica, Via del Liceo, 06123 Perugia, Italy

Cordia salicifolia Cham. (Fam. Boraginaceae) (sin. *C. ecalyculata* Vell.), also known with several common names such "porangaba" "chá de bugre" or "café do mato" is a small tree growing 8 – 12 meters in height with a trunk 30 – 40 cm in diameter, having as peculiar feature the production of red fruits resembling a coffee bean, which are roasted and brewed into tea as a coffee substitute. It is indigenous in Brazil but can be found also in tropical forest areas of Argentina and Paraguay. *C. salicifolia* is largely used in the ethnomedical traditions of Brazilian people, in particular in Minas Gerais, Bahia, Acre and Goias regions. Extracts of this plant are commercialized in Brazil as diuretic, appetite suppressant and weight loss products [1]. in the present communication we wish to report results obtained on the qualitative and quantitative analysis of some secondary metabolites extracted from leaves of *C. salicifolia*. Finely powdered dried leaves (100 g) were exhaustively extracted by maceration with dichloromethane for 24 h. After evaporation of the solvent under vacuum, the brown syrup obtained was purified by SiO_2 gel column chromatography using dichloromethane as eluent. The major components found in the leaves extract were (+)-spathulenol (0.53%) and β-sitosterol (0.24%), that were identified by comparison with commercially available samples. (+)-Spathulenol exhibited a very weak activity as inhibitor of growth of *Helicobacter pylory* (strain DSMZ 4867, originated from human gastric samples in vitro (MIC = 200 μg/mL). *Acknowledgements:* Regione Abruzzo (L.R. 35/97) Project 'Tutela della Biodiversita', University "G. d'Annunzio", Ufficio Relazioni Internazionali". **References:** [1] Cruz, G.L. (1994) Dicionário das plantas úteis do Brasil, 5 ed. Bertrdand. Rio de Janeiro.

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Enhanced bioactivity of *Citrus limon* (Lemon Greek cultivar) extracts, essential oil and isolated compounds before and after encapsulation in liposomes

Gortzi O[1], Lalas S[1], Tsaknis J[2], Chinou I[3]
[1]Dept. of Food Technology, Technological Educational Institution (T.E.I.) of Larissa (Karditsa Annex), GR-43100, Karditsa, Greece; [2]Dept. of Food Technology, Technological Educational Institution (T.E.I.) of Athens, GR-12210, Egaleo, Athens, Greece; [3]Dept. of Pharmacognosy-Chemistry of Natural products, School of Pharmacy, University of Athens, Athens GR-15771, Greece

The antibacterial property of essential oils is the most widely studied area in human nutrition, food preservation and animal production [1,2]. The chemical composition of the essential oil from *Citrus limon* (Lemon Greek cultivar) was analysed by GC-MS. Limonene, β-pinene, γ-terpinene, neral and α-terpineol were identified as the main compounds. The antimicrobial properties of the essential oil was tested [using the diffusion technique of Bauer-Kirby (disc method) [3] against four Gram positive, four Gram negative bacteria, three human pathogenic fungi and the food-pathogen bacteria *Listeria monocytogenes*. In order to investigate any possible synergistic or antagonistic effect limonene, β-pinene, and γ-terpinene were isolated from the essential oil and their antimicrobial activity was determined before and after encapsulation in MLV liposomes (with ratios originally founded in the essential oil). The antioxidant activity was determined using two different methods (Rancimat and DSC) and compared with common commercial antioxidants BHT and α-tocopherol. Essential oil and its main compounds were encapsulated in liposomes and their antioxidant action was again estimated. Thermal-oxidative decomposition of the samples (liposomes pure and encapsulating extracts) was studied by DSC method. In all cases, the encapsulated material (essential oil or compound) proved to posses much stronger activity, than itself in pure form. *Acknowledgements:* The study has been co-funded by 75% from E.E. and 25% from the Greek Government under the framework of the Education and Initial Vocational Training Program – Archimedes II. **References:** [1] Keller, B.C. (2001) Trends Food Sci. Tech.: 12, 25. [2] Litwinienko, G. et al. (1999). Thermochim. Acta: 331, 79 – 86. [3] Bauer, A.W. et al. (1966) Am. J. Clin. Pathol.: 45, 493.

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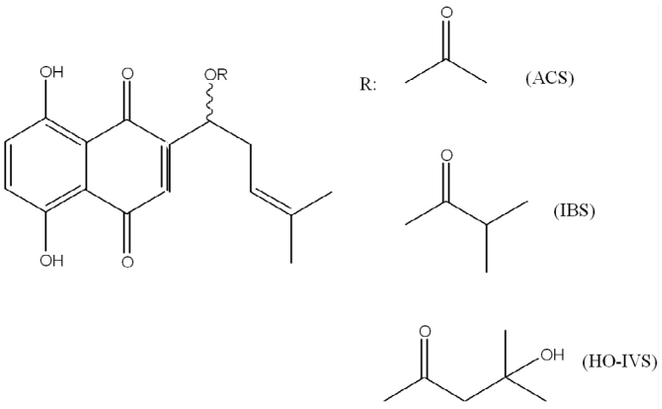
Shikonin pigments from hairy root culture of *Lithospermum canescens*

Damianakos H[1], Graikou K[1], Pietrosiuk A[2], Sykłowska-Baranek K[2], Chinou IB[1]
[1]Dept. of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Zografou, 15771, Athens, Greece; [2]Dept. of Biology and Pharmaceutical Botany, Warsaw Medical University, Banacha 1, 02 – 097 Warsaw, Poland

Pigments of shikonin type, beyond cosmetic applications, show inflammatory, antibacterial, antifungal, immunosti- mulating and anticancer activities [1]. *Lithospermum canescens* (Michx.) Lehm. (Boraginaceae) is a common plant in northern America also known as Indian paint. Recently, the natural roots of *L. canescens* have been found to contain the red pigments acetylshikonin, isobutyl shikonin as well as isovalerylshikonin and α-methyl butyrylshikonin [2]. In the pesent study, hairy root cultures of *L. canescens* were established using three strains of *Agrobacterium rhizogenes*: ATCC 15834, LBA 9402 and NCIB 8196. In the n-hexane extract of the hairy root culture of *L. canescens* [3]) the presence of acetylshikonin (ACS), isobutylshikonin (IBS) and β-hydroxyva- lerylshikonin (HO-IVS) has been confirmed so far, by means of 1 H-NMR, 13C-NMR and COSY techniques. Antioxidative, antimicrobial and cytotoxic activities assays are under research



Acknowledgements: This study has been financially supported by the project 70/3/7596 and the PENED 2005 (70/3/8586). **References:** [1] Couladouros, E. et al.1997 Tetrahedron Lett. 38: 7263; [2] Pietrosiuk, A. and Wiedenfeld, H. 2005 Pharm. Biol.43: 189. [3] Pietrusiuk, A. et al. 2006 Plant Cell Rep. 25: 1052

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Bovine tuberculosis: a zoonotic target for medicinal plants

McGaw LJ[1], Lall N[2], Hlokwe T[3], Michel A[3], Meyer JJM[2], Eloff JN[1]
[1]Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04 Onderstepoort 0110, South Africa; [2]Department of Botany, University of Pretoria, Hatfield Pretoria 0002, South Africa; [2]Tuberculosis Laboratory, ARC-Onderstepoort Veterinary Institute, Private Bag X5 Onderstepoort 0110, South Africa

Bovine tuberculosis (TB), caused by *Mycobacterium bovis*, is an important zoonotic disease that can spread to humans, especially immunocompromised individuals, through infected livestock products including unpasteurised milk. In southern Africa, medicinal plants are employed to relieve TB-related symptoms such as chest complaints and coughing, for example root preparations of *Euclea undulata* and *E. natalensis*. These plants, and compounds isolated from them, are known to have activity against *M. tuberculosis*. The objective of this study was to determine firstly the activity of *E. undulata* and *E. natalensis* species against infective *M. bovis*, and secondly the extent to which the non-pathogenic vaccine strain, *M. bovis* BCG, can be used as a model for detection of activity against slow-growing pathogenic *Mycobacterium* species. Activity against two fast-growing saprophytic species, *M. smegmatis* and *M. fortuitum*, was also evaluated. Extracts of *E. undulata* and *E. natalensis* roots were tested for antimycobacterial activity against *M. bovis* ATCC 19210, *M. bovis* BCG, *M. smegmatis* and *M. fortuitum* using a twofold serial dilution assay in microtitre plates. A compound with known antimycobacterial activity present in both *Euclea* species, diospyrin, was included in the assays. The anti-TB drug isoniazid was used as a positive control. After incubation, MIC values were detected using a tetrazolium salt indicator. MICs of the plant extracts and diospyrin were similar against the *M. bovis* slow-growing strains, with MICs ranging between 5.70 and 16.28 μg/ml. Against the fast-growers, the MIC for the extracts was approximately 300 μg/ml and for diospyrin 15.63 – 62.50 μg/ml. In conclusion, it was shown in this instance that *M. bovis* BCG is a better model for antimycobacterial activity against *M. bovis* than *M. smegmatis* or *M. fortuitum* although it has the disadvantage of a slower growth rate. *Acknowledgements:* Claude Leon Foundation, National Research Foundation (South Africa).

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A new antifungal dihydroisocoumarin from *Xyris pterygoplephara* Kunth (Xyridaceae)

Braga CF[1], Guimarães KG[1], Mares-Guia TR[2], Souza Filho JD[3]
[1]Faculty of Pharmacy, UFMG, Av. Antônio Carlos 6627, Belo Horizonte, CEP 31270 – 010, Brazil; [2]Department of Biochemistry, Insitute of Chemistry, USP, Av. Prof. Lineu Prestes 748, São Paulo, CEP 00508 – 900, Brazil, [3]Department of Chemistry, ICEX, UFMG, Av. Antônio Carlos 6627, Belo Horizonte, CEP 31270 – 010, Brazil

Xyris plants are small shrubs, popularly known as "sempre-vivas" (everlasting plants). Some are collected for ornamental purposes and for medicinal uses, to treat eczemas and dermatitis [1]. Around 90% of the 152 *Xyris* species found in Brazil are endemic and over harvesting has put some into extinction risk [2]. Both the chemistry and the biological activities of *Xyris* spp. are poorly studied. We have previously described the isolation of a new anthraquinone from *X. pilosa* L. with antifungal activity [3]. In the present study, we report the phytochemical study of *X. peterygoblephara*. The ethanol extract from its aerial parts was evaluated against 9 microorganism strains (*Bacillus subtillis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Epidermophyton floccosum*, *Mycrosporum canis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*) by the microdilution and agar diffusion assays. Fractionation of the extract by silica gel column chromatography and partition between immiscible solvents, following purification by semi preparative NP-HPLC and Sephadex LH20 column chromatography, resulted in the isolation of three compounds. The structures were assigned by spectroscopic data as moronic acid, quercetin and (3R,4R)-(-)-6-methoxy-3,4-dihydro-4-acethoxy-5-*n*-pentil-1 H-2-benzopyran-1-one (**1**), a new compound. The absolute configuration of **1** was defined using circular dichroism spectroscopy. Assay of **1** (100 μg/disc) by the agar diffusion method against isolates of the dermatophytous fungi *E. floccossum* (inbibition zone, mm ± s.d.: 4.5 ± 0.8), *T. mentagrophytes* (4.8 ± 0.4) and *T. rubrum* (10.2 ± 0.8) revealed similar activity to the positive control amphotericin B (32 μg/disc; 5.0 ± 0.2, 5.0 ± 0.6 and 8.8 ± 1.2, respectively). The obtained data corroborate the ethomedical use of *Xyris* species to treat dermatitis. *Acknowledgements:* CNPq for a research fellowship (FCB), UFMG/PRPq (Projetos Estruturantes) for the financial support, Fundação Zoo-Botânica for collecting the plant material. **References:** [1] Pio Corrêa M., Penna L. (1969) Dicionário das plantas úteis do Brasil e das exóticas cultivadas. Ministério da Agricultura. Rio de Janeiro. [2] Sajo, M.G. et al. (1997) Bol. Bot. USP 16: 15 – 19. [3] Cota et al. (2004) Biochem. Sys. Ecol. 32: 391 – 397.

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Antibacterial and anticandidal activity studies on *Sonchus erzincanicus* Matthews

Yiğit D[1], Yiğit N[2], Özgen U[3], Sevindik HG[3], Aktaş AE[4]
[1]Erzincan University, Faculty of Education, Erzincan, 24030, Turkey; [2]Atatürk University, Health Services Vocational Training School, Medical Laboratory Program, Erzurum, 25070, Turkey; [3]Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, 25240, Turkey; [4]Atatürk University, Faculty of Medicine, Microbiology and Clinical Microbiology Department, Erzurum, 25240, Turkey

Sonchus erzincanicus (Compositae) is an endemic species for Turkey [1]. Six *Sonchus* species grow in Turkey and are known as "sütlük, kuzu gevreği, eşek marulu" in Turkey [2]. It has been found that some *Sonchus* species contain sesquiterpene lactone glucosides, flavonoids, triterpenes and steroids [3,4]. In this study, the antibacterial and anticandidal activity of aerial parts of *Sonchus erzincanicus* Matthews methanol extract and fractions ($CHCl_3$, EtOAc, aqueous) prepared from methanol extract were evaluated against clinical bacterial and *Candida* strains.

Tested organism	Zone of inhibition (mm)			
	Water	MeOH	EtOAc	$CHCl_3$
Escherichia coli	10	15	13	14
Staphylococcus aureus	-	10	-	5
Enterobacter aerogenes	-	10	-	-
Candida albicans	-	13	12	13
Candida glabrata	-	10	10	-
Candida parapsilosis	-	-	10	10

Ampicillin (10 µg) > 19 – 10 mm sensitive Amphotericin B (10 µg) > 15 – 10 mm sensitive **References:** [1] Matthews, V.A. (1975) Sonchus L. Flora of Turkey and the East Aegean Islands, University Pres. Edinburgh, Volume: 5, pp. 690 – 696 (edited by Davis, P. H.). [2] Akartürk, R. (2001) Şifalı Bitkiler Flora ve Sağlığımız, Orman Genel Müdürlüğü Mensupları Yardımlaşma Vakfı (in Turkish). [3] Helal A.M. et al. (2000) Phytochemistry 53(4), 473 – 477. [4] Devkota, K.P. (2005). Ph. D. Thesis, Research Institute Of Chemistry/University of Karachi.

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Antimicrobial studies on *Origanum acutidens* (Hand.-Mazz.) Ietswaart

Yiğit N[1], Yiğit D[2], Özgen U[3], Sezen E[3], Sevindik HG[3], Aktaş AE[4]
[1]Atatürk University, Health Services Vocational Training School, Medical Laboratory Program, Erzurum, 25070, Turkey; [2]Erzincan University, Faculty of Education, Erzincan, 24030, Turkey; [3]Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, 25240, Turkey; [4]Atatürk University, Faculty of Medicine, Microbiology and Clinical Microbiology Department, Erzurum, 25240, Turkey

Many *Origanum* species (Lamiaceae) known as "Kekik, kekik otu, keklik otu, mercanköşk" are used by the public especially as a spice and for its several medicinal effects in Turkey [1]. *O. acutidens* is an endemic species and grows mainly in Central and East Anatolia [2]. Ursolic and oleanolic acid; rosmarinic and lithospermic acid; vicenin-2, betulalbuside A, 8-OH-linaloyl glucoside were isolated from aerial parts of *O. acutidens* [3]. In this study, the antibacterial and anticandidal activity of *O. acutidens* methanol extract and fractions (n-hexane, $CHCl_3$, EtOAc, aqueous) prepared from methanol extract were evaluated against clinical bacterial and candida strains. Antimicrobial test was then carried out by disc-diffusion method using suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast spread on Mueller-Hinton Agar (MHA) and Saboraud Dextrose Agar (SDA) respectively. The disc (6 mm in diameter) were impregnated with test fractions at concentrations 100 µg/disc. The methanol extract and the other fractions did not show any anticandidal activity.

Tested organism	Zone of inhibition (mm)				
	Water	MeOH	EtOAc	$CHCl_3$	n-hexane
Escherichia coli	7	18	15	8	18
Staphylococcus aureus	5	10	10	5	15

Positive control: Ampicillin (10 µg) > 19 – 10 mm sensitive (It was measured all bacterial strains) **References:** [1] Baytop, T. (1999) "Therapy with Medicinal Plants in Turkey (Past and Present)", 2nd ed. pp. 325 – 326, Nobel Tıp Kitabevleri, İstanbul. [2] Ietswaart, J.H. (1982) Origanum L., "Flora of Turkey and the East Aegean Islands", Vol. 7, pp. 297 – 313, University Press, Edinburgh (edited by P.H. Davis) [3] Özgen, U., et. al. (2005) Phytochemical Studies on Origanum acutidens (Hand.-Mazz.) Ietswaart, 53rd Annual Congress of GA, Florence, Italy, August 21st-25th.

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Quantitative HPLC Analysis of Deoxyshikonin, Acetyl shikonin and 3-Hydroxy-isovaleryl shikonin in *Onosma armeniacum* root

Özgen U[1], Öztürk M[2], Atila A[2], Sevindik HG[1], Kadıoğlu Y[2], Coşkun M[3]
[1]Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, 25240, Turkey; [2]Atatürk University, Faculty of Pharmacy, Deparment of Analytical Chemistry, Erzurum, 25240, Turkey; [3]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, 06100, Turkey

Onosma armeniacum Klokov (Boraginaceae) has been used for wounds, burns and hemorrhoids and to treat stomach ulcer and tonsillitis in Turkey [1]. It is known as "havacıva" in Erzurum Province (Turkey). The roots of some *Onosma* species contain naphthoquinone compounds [2]. In this study, the quantitative analysis of deoxyshikonin (**1**), acetyl shikonin (**2**) and 3-hydroxy-isovaleryl shikonin (**3**) isolated from n-hexane-dichloromethane (1:1) extract of *O. armeniacum* roots was carried out. HPLC analysis was performed on a Thermoquest HPLC system, equipped with a DAD detector (Thermo UV 6000). For all analysis, a RP-C 18 column (250 x 4.6 mm, 5 µm particle size, ACE®) was used. The mobile phase consisted of acetonitril-methanol-water (2% acetic acid) (60:20:20), which were applied in the isocratic elution. The analysis temperature was kept constant at ambient temperature, flow rate and sample volume were set to 1.0 ml/min and 20 µl, respectively. All analysis were monitored at 525 nm. Peaks were assigned by spiking the samples with authentic samples of **1**, **2** and **3**, and comparison of the UV-spectra and retention times. Relative contents of the acetyl shikonin, deoxyshikonin and 3-hydroxy-isovaleryl shikonin in the extracts were 23.82%, 3.63% and 2.53%, respectively. **References:** [1] Özgen U., Coşkun M. (2000) Ilıca (Erzurum) İlçesine Bağlı Köylerde Halk İlacı Olarak Kullanılan Bitkiler, XIII. Bitkisel İlaç Hammaddeleri Toplantısı, 20 – 22 September, İstanbul (in Turkish). [2] Khajuria, R.K., Jain, S.M. (1993). Indian J. Chem. B 32, 390 – 391.

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Essential oil in fruits of *Peucedanum alsaticum* L. and *Peucedanum cervaria* (L.) Lap. Composition and antibacterial activity

Skalicka-Woźniak K[1], Los R[2], Głowniak K[1], Malm A[2]
[1]Medical University of Lublin, Department of Pharmacognosy with Medicinal Plant Garden, 1 Chodzki Str., 20 – 093 Lublin, Poland, [2]Medical University of Lublin, Department of Pharmaceutical Microbiology, 1 Chodzki Str., 20 – 093 Lublin, Poland

Peucedanum alsaticum L. and *Peucedanum cervaria* (L.) Lap are plants belonging to Apiaceae family. The composition of essential oils in fruits was investigated. Oils were obtained by used of hydrodistillation and head-space solid-phase microextraction and then analyzed by GC-MS method. Identification of the individual components was based on comparison of obtained mass spectra with those of standards and NIST libraries. 42 kind of compounds were identi-

fied among which α-pinene and sabinene were dominant in both species. HS-SPME method seems to be better for analysis since it gave higher or similar results, had very good sensitivity and needs less time and amount of sample. The *in vitro* antibacterial activity of essential oils obtained from fruits of *P. cervaria and P. alsaticum* was evaluated against 10 reference microorganisms, including 6 Gram-positive (*Staphylococcus epidermidis* ATCC 122228, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240) and 4 Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453). The minimal inhibitory concentrations (MICs) were determined by agar dilution method using Mueller-Hinton agar. Both essential oils obtained from fruits of *P. cervaria* and *P. alsaticum* had no influence on the growth of Gram-negative bacteria even at the highest concentration tested (2000 mg/l). Among Gram-positive bacteria only *B. subtilis* and *M. luteus* were sensitive to these oils (MIC = 2000 mg/l).

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Screening of some Sudanese plants against *Mycobacterium tuberculosis*

Hassouna RA[1], Khalid AS[2], Khalid HS[3]
[1]Dept. of Microbiology University of Bakht El Roda Universty; [2]Dept. of Microbiology – University of Nelein; [3]Medicinal & Aromatic Plants Research Institute – National Centre for Research – P.O Box 11496

The paper evaluates the activity of Sudanese plants used in folk-medicine against *Mycobacterium tuberculosis*. Preliminary screening was carried out using 20 plant extracts. The screening revealed that the petroleum ether extracts were devoid of any activity while the methanolic extracts showed a weak activity. An experiment was designed to compare the in-vitro activity of two drugs of choice for treatment of *tuberculosis* (streptomycin & rifampcin) with the activity of the plant extracts [1,2]. A series of sensitivity tests were conducted to evaluate the effect of combined therapy, consisting of the methanolic extract of each plant plus streptomycin on *M. tuberculosis*. The results revealed that thirteen methanolic extracts have a positive effect on *Mycobacterium tuberculosis* when mixed with Streptomycin. The minimum inhibitory concentration of each extract was determined. The results indicated that the most effective plant extracts are *Asphodelus tenuifolius*, *Nigella sativa* and *Kigelia africana*. **References:** [1] Cruickshank, R; Duguid, P.J; Marmion, P.B and Swain, H.A.R (1975) Medicinal Microbiology, Voulme 2, The Practice of Medical Microbiology 12th Edition. Churchill Livingstone. [2] Khalid, E. Amna, (2003) Personal communication.

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Antioxidant and antimicrobial potential of *Garcinia hombroniana* Pierre bark

Hofstätter H[1], Kaiser M[1], Wungsintaweekul J[2], Wattanapiromsakul C[2], Tewtrakul S[2], Feierl G[3], Brantner AH[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4/I, A-8010 Graz, Austria; [2]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90112, Thailand, [3]Institute of Hygiene, Medical University Graz, Universitaetsplatz 4, A-8010 Graz, Austria

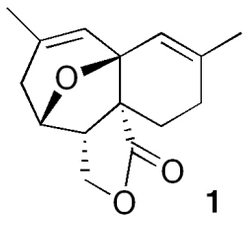
The crude methanolic extract from the bark of *Garcinia hombroniana* (Clusiaceae), a tree which is native to tropical areas in Southeast Asia, showed a promising antioxidant activity tested by the DPPH assay (EC_{50} 2.11 μg/ml) [1]. After partitioning between chloroform/water and subsequently bioassay-guided fractionation by chromatographic methods the antioxidant effect could be further increased. The red brown aqueous phase which was further separated by a Sephadex LH-20 column resulted in an antioxidant activity of EC_{50} 0.61 μg/ml in fraction 6. The activity could be referred to the high content of tannins. Additional phytochemical investigations elucidated the occurrence of flavonoids, saponins, terpenoids, essential oils and coumarins [2,3]. The methanolic extract (1 mg/disc; disc diameter 9 mm) as well as the fractions were tested [4] against 8 gram-positive and 6 gram-negative bacterial strains including the causative peptic-ulcer bacteria *Helicobacter pylori* and against two *Candida* strains. The plant extract demonstrated an inhibiting effect on the growth of *Bac. cereus* (inhibition zone diameter (IHD) 15 mm), *Bac. subtilis* (IHD 12 mm), *E. faecalis* (IHD 12 mm), *Helicobacter pylori* (IHD 13 mm), *Staph. aureus* (IHD 13 mm) and *Staph. epidermidis* (IHD 12 mm). The strongest effect was found against *Bac. cereus*. An interesting activity against *Helicobacter pylori* was detected. *Acknowledgements:* ASEA-UNINET grants to H.H. and M.K. and the Pharmacy PSU grants are acknowledged. **References:** [1] Hatano, T et al. (1988) Chem. Pharm. Bull. 36(6): 2090 – 2097 [2] Rukachaisirikul, V. et al. (2000) 55: 183 – 188. [3] Rukachaisirikul, V. et al. (2005) 68: 1222 – 1225. [4] Pharmacopoeia Europaea, ed. 5 (2005).

P 194

Anthecularin, a sesquiterpene lactone with a novel ring system from *Anthemis auriculata* exert dual inhibitory activity against plasmodial FabG and FabI enzymes

Karioti A[1], Skaltsa H[1], Linden A[2], Perozzo R[3], Brun R[4], Tasdemir D[5]
[1]Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, GR-15771 Athens, Greece; [2]University of Zurich, Institute of Organic Chemistry, CH-8057 Zürich, Switzerland; [3]School of Pharmaceutical Sciences, University of Geneva, 1211 Geneva 4, Switzerland; [4]Department of Medical Parasitology, Swiss Tropical Institute, CH-4002 Basel, Switzerland; [5]Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK

One of the most promising targets emerged from *Plasmodium falciparum* genome project is the *de novo* fatty acid biosynthesis. *Plasmodium falciparum* fatty acid synthase (*Pf*FAS) is a type II multienzyme complex, as found in plants and bacteria, and as such, differs markedly from human type I FAS. FabG and FabI are two key enzymes of the *Pf*FAS-II system and are ideal targets for malaria drug discovery. We have previously reported three linear sesquiterpene lactones from Greek *Anthemis auriculata* [1]. In continuation of our studies into this plant, we now describe a new sesquiterpene lactone, anthecularin (**1**), which was isolated in minor amounts by repeated column chromatography. The structure of **1** was established by high field NMR, HREIMS and X-ray crystallography. A literature survey indicates that **1** possesses a novel sesquiterpene skeleton. Anthecularin showed dual inhibitory activity against the FabG and FabI enzymes (IC_{50}s 14 and 28.3 μg/ml), which was well-correlated with its antimalarial potential (IC_{50} 23.3 μg/ml). It also exhibited trypanocidal activity (*T. brucei rhodesiense*, IC_{50} 10.1 μg/ml) without any cytotoxicity on mammalian cells (IC_{50} > 90 μg/ml). Considering the structural novelty, biological activity and safety, anthecularin (**1**) appears as a new drug lead for the design of novel antimalarial agents.



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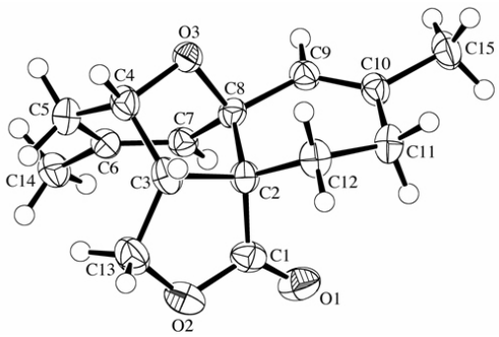

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Novel bioactive bacterial metabolites from a marine derived bacterium *Nocardia Sp.* Merv 21695

Hawas UW[1], El-Gendy MM[2], Jaspars M[1]
[1]University of Aberdeen, Aberdeen, Scotland, UK; [2]Department of Chemistry of Natural Compounds, National Research Centre, Dokki, Cairo, Egypt

The marine environment may contain more than 80% of world's species. Marine microorganisms constitute the largest and yet most poorly explored source of new biologically active secondary metabolites. Marine microbiology is still under utilized as a starting point field for academic drug discovery and biological programs. We have recently started a program aimed to investigate the bioactive secondary metabolites of marine derived microbial strains. We report here the isolation, structure elucidation and antimicrobial activities of the secondary metabolites isolated from the Egyptian actinobacterial strain as well as the taxonomy of the producing strain. Egyptian marine strain Merv 21695, which was found to be the most promising bioactive strain, was isolated from the marine algae *Laurencia spectablilis* of Ras – Gharib beach of the Red Sea, Egypt. Merv 21695 strain was identified according to detailed identification studies: morphological; culture-based; physiological; biochemical and 16 S rDNA sequencing, as a new species of *Nocardia*. The cultivation and chemical analysis of this species yielded four structurally related compounds namely, asphodelin [1], justicidin B [2], chrysophanol 8-methyl ether [3] and 1,1-dicloro-4-ethyl-5-(4-nitro-phenyl)-hexan-2-one by a series of steps: solvent extraction; silica gel column chromatography; sephadex LH-20 column chromatography and semipreparative HPLC. The structures were secured by detailed spectroscopic analysis of the MS and NMR data and single crystal x-ray diffraction studies. By using the serial dilution technique [4], these compounds displayed antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as antifungal activity with minimum inhibitory concentration (MIC) ranged from 0.5 ~ 10 µg/ml. **References:** [1] Ulubelen A. and Tuzlaci E. (1985) Phytochemistry 24: 2923. [2] Yang M. et al. (2006) Magn. Reson. Chem. 44: 727. [3] Hongzhu G. et al. (1998) Phytochemistry 49: 1623. [4] Egorov, N. S. (1985) Antibiotics. A scientific approach. Mirpublishers. Moscow. (translated from the russian by Alexander Poshinkin).

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Antibacterial effects of alcoholic extracts of different parts of *Mentha longifolia* and *Ziziphora tenuior*

Tajadod G, Majd A
Biology Department, Islamic Azad university, North Tehran branch, passdaran, dowllat, No481,1945873613, Tehran, Iran

Mentha species are grown in most parts of Iran and have a wide range of pharmacotherapeutic uses such as treatment for bronchitis, colds and cough. In this survey ethanolic extracts of different parts (stem, flower and root) of two species (*Mentha longifolia and Ziziphora tenuior*) with the concentration of 100 mg/ml were used against two species of Bacillaceae bacteria (*B.subtillis and B.cereus*) and one fungus *Alternaria alternaata*, with the use of three methods, 1) well method 2) dilution in solid media 3) dilution in broth media. These tests showed that all the extracts of different parts had antibacterial effects. The well method showed that the root extract of *Ziziphora* with the inhibitory zone of 17 mm was most effective on both bacteria and stem extracts of *Mentha* with an inhibitory zone of 15 mm on *B. cereus* had the least antibacterial effect. The other two methods showed the same results. As no growth of fungi on the media with extracts of different parts of the two plant species was observed fungicide effects of the extracts were proved too. Minimum inhibitory concentration (MIC) of 80% ethanolic extracts against *B. subtilis* was 50 mg/ml. *Acknowledgment*: Islamic Azad University, North Tehran Branbch. **Reference:** 1. Avilia- Acevado (1998) Int. J. Pharmacol. 31:1, 61 – 64. 2. Diaz, R. (2001) Fitoterapia 59: 329 – 322; 3. Gergis, V. (2003) Flavour Fragr. J. 6:93 – 95; 4. Paniz, L. (2005) J. Ethnopharmacol. 39: 3, 167 – 170; 5. Tantaway, E (2006) Biochem 2: 256 – 261

P 197

Antibacterial activity of Ecuadorian plant extracts

Gachet MS, Fabian C, Schühly W, Bucar F, Bauer R
Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Universitaetsplaz 4/I, 8010 Graz, Austria

The hexane, dichloromethane and methanol extracts of 13 plant taxa from Ecuador (200 µg/mL) were tested against the human pathogenous bacteria *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (Laboratory strain) and *Candida albicans* (ATCC 10028). Antibacterial activity of the samples was assayed using the agar diffusion method.[1] The plant extracts that showed activity are indicated (in brackets: the inhibition zone in millimeters) for *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*. In case activity was not found in the plant extract mentioned the abbreviation nao (no activity observed) will occupy the space. *Minquartia guianensis* Aubl. (Olacaceae) showed activity in the hexane (2.5, 1.0, 1.5), dichloromethane (5.0, 6.0, nao) and methanol (1.5, 2.5, nao) extracts. *Bocconia integrifolia* Bonpl. (Papaveraceae) presented activity in the dichloromethane (2.5, 1.7, 1.6) and methanol (4.5, 4.5, 4.5) extracts. *Protium subserratum* (Engl.) Engl. (Burseraceae) was active in the dichloromethane (2.5, 0.5, nao) and methanol (1.7, nao, nao) extracts. The dichloromethane (0.5, nao, nao) and methanol (1.5, nao, nao) extracts from *Piptadenia pteroclada* Benth. (Fabaceae) also showed activity. *Croton menthodorus* Benth. (Euphorbiaceae) (3.0, 3.5, nao) and *Pentagonia macrophylla* Benth. (Rubiaceae) (0.7, nao, nao) only present activity in the methanol extract while *Syngonium podophyllum* Schott (Araceae) (1.0, nao, nao) only did it in the hexane extract. *Acknowledgements:* This research is part of a dissertation funded by the Austrian Exchange Service (ÖAD). **References:** [1] Frei, B.; et al., (1998) Phytomed. 5: 177 – 86.

P 198

In vitro antibacterial and antiadherence properties of flavonoid-rich extract of *Pistacia atlantica* hull against microorganisms involved in dental plaque

Kamrani YY[1], Amanlou M[1], Esmaeelian B[1], Rahimi M[2]
[1]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Medical Sciences/University of Tehran, P.O.Box: 14155 – 6451, Tehran, Iran; [2]Department of microbiology, Veterinary center of Tehran, Tehran, Iran

Natural products have recently undergone more thorough investigation for their potential in preventing oral disease, particularly plaque-related diseases such as dental caries. It is well known that *Streptococcus mutans* and other cariogenic bacteria are the major etiological agents in dental caries. We investigated the antibacterial

effects of *Pistacia atlantica* subsp. *kurdica,* an Iranian species of the family of *Anacardiaceae, to* inhibit the growth and acid production of microorganisms involved in dental plaque. *P. atlantica* fruit-hull extract prepared by percolation method using 70% ethanol. A flavonoid-rich extract of *Pistacia atlantica* (FEPA) was prepared by adsorption on macroporous resin and desorption by ethanol. Total flavonoid content was determined according as the aluminum chloride colorimetric method. The growth inhibitory activity of FEPA was tested against *Streptococcus mutans*, *S. salivarious*, *S. sobrinus, S. sanguis and Actinomyces viscosus.* Total flavonoid content of FEHP was 789.2 ± 2.9 mg/g. The MIC value of the FEPA was 0.71 – 0.86 mg mL^{-1}. In-vitro studies had shown that FEPA at concentrations of 10%, could inhibit strongly acid-producing ability of *S. mutans* and salivary glycolysis up to 5 h post rinsing when compared to Listerine (a commercial mouthwash) control (*P*< 0.05*).* Meanwhile 10% FEPA was 55% effective in inhibiting bacterial adherence, as shown by the low weight of accumulated *S. mutans* plaque to nichrome wire mesh, while Listerine tested was no better than the water control. In conclusion, the antiacidogenic effect of *P. atlantica* suggests that this material could be a useful source for the development of promising anticariogenic agents and led to the acceptance of traditional use of this medicinal plant. *Acknowledgements:* 1. Tehran University of Medical Sciences and Pharmaceutical Sciences Research Center. 2. Mr. AS. Nasreldin Heidari

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Comparative antitrypanosomal activity of *Terminalia chebula* dried fruits against *Trypanosoma evansi*

Shaba P[1], Pandey NN[1], Sharma OP[2], Rao JR[3], Singh RK[4]
[1]Division of Medicine, Indian Veterinary Research Institute, Izatnagar - 243122, India, [2]Regional Station, Indian Veterinary Research Institute, Palampur 176061, [3]Division of Parasitology, Indian Veterinary Research Institute, Izatnagar -243122, India, [4]Regional Station, Indian Veterinary Research Institute, Palampur 176061, India

Trypanosoma evansi, one of the causative agents of trypanosomosis in animals was used to evaluate the trypanocidal potential of *Terminalia chebula* dried fruits. Powdered *T. chebula* dried fruits was comparatively extracted with solvents of different polarities (hexane, chloroform, methanol and water) and obtained dried extracts were solubilized in 1% dimethylsulphoxide. Alsever medium supplemented with inactivate bovine serum at 56 °C in ratio of 1:1 was incubated at 37 °C and under 5% CO_2 for 5 h. 20 µl of each extract was added to 180 µl of Alsever medium with trypanosomes (1×10^6/ml) at different concentrations of extracts (250 – 1000 µg/ml) and incubated for 5 h. *In vitro* trypanocidal activity was in order of methanol, aqueous, chloroform and hexane. At 250 µg/ml, methanolic plant extract (MPE) exhibited strong immobilization and killing of trypanosomes. At same concentration, there was complete killing of trypanosomes at 5 h of incubation, which was same as diminazine aceturate (50 µg/ml) standard drug at 4 h. Trypanocidal activity observed in all extracts was in concentration-time dependent faction. The cytotoxicity test was carried out on Vero cell line maintained in Dubecco's Modified Eagle Medium (DMEM) and seeded in 96-wells flat bottom ELISA plates. Each well contained 500,000 cells/ml. Different concentrations of methanolic plant extract (MPE) (1.56 – 100 µg/ml) were dissolved in DMEM, solubilized in 1% dimethylsulphoxide and incubated at same conditions for 72 h. MPE of *T. chebula* and diminazine aceturate were cytotoxic to Vero cells except at 6.25 and 1.56 µg/ml, respectively. *In vivo*, mice inoculated with 1×10^4/ml trypanosomes became parasitemic after 48 h and were subsequently treated with methanolic extract using concentrations (12.5, 25, 50 100 and 200 mg/kg body weight) at dose rate of 100 µl per mouse via intraperitoneal route consecutively for 3 days. However, mice treated with MPE of *T. chebula* at a dose of 200 mg/kg body weight showed a survival time of maximum 8 days.

P 200

In vitro trypanocidal activity of methanolic extract of *Picrorrhiza kurroa* rhizomes against *Trypanosoma evansi*

Shaba P[1], Pandey NN[1], Sharma OP[2], Rao JR[3], Singh RK[4]
[1]Division of Medicine, Indian Veterinary Research Institute, Izatnagar - 243122, India, [2]Regional Station, Indian Veterinary Research Institute, Palampur 176061, [3]Division of Parasitology, Indian Veterinary Research Institute, Izatnagar -243122, India, [4]Regional Station, Indian Veterinary Research Institute, Palampur 176061, India

In the course of screening of medicinal plants for possible antitrypanosomal drug against important flagellate blood protozoan parasite of the genus Trypanosoma, *Picrorrhiza kurroa* rhizomes were screened for their antitrypanosomal activity and cytotoxicity. Reports of the resistance to currently used trypanocides are on the increase in different parts of the world. No new drug has been introduced in the market for more than half a century and prospect for a vaccine remains elusive due to high genetic variability of trypanosomes. Vero cell line maintained in Dubecco's Modified Eagle Medium (DMEM) in ELISA plates was incubated with *Trypanosoma evansi* for more than 12 h. Methanolic plant extract (MPE) of *Picrorrhiza kurroa* was solubilized in 1% dimethylsulphoxide. MPE was added to the Vero cell culture medium at different concentrations (250 – 1000 µg/ml) and incubated at appropriate conditions. Cytotoxicity test of MPE was carried out on Vero cell line at different concentrations (1.56 – 100 µg/ml) dissolved in the same medium and incubated at same conditions for 72 h. For acute toxicity testing MPE at concentration of 2000 mg/kg body weight was administered to mice which were observed for more than two weeks. In vitro, *Picrorrhiza kurroa* induced immobilization and killing of the parasites in concentration-time dependent manner. At 250 µg/ml of the test MPE, concentration of trypanosomes was reduced from 40.00 ±.00 to 4.667 ± 0.67. But at 500 µg/ml, trypanosomes were completely killed at 4 h of incubation which was same as diminazine aceturate (50 µg/ml), standard reference drug with significant difference ($P \leq 0.01$). Both immobilized (clumped trypanosomes) and apparently killed trypanosomes were injected into two groups of mice which were observed for a period of 30 days. Mice injected with immobilized trypanosomes developed parasitemia while, the other group did not. MPE of *Picrorrhiza kurroa* and diminazine aceturate appeared to be toxic to mammalian cell line at same concentration (6.25 µg/ml). No mortality was recorded during acute toxicity test

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Antimicrobial activity of some edible flowers in Thailand

Wessapan C, Charoenteeraboon J, Wetwitayaklung P, Limmatvapirat C, Phaechamud T
Faculty of Pharmacy, Silpakorn University, Nakhon-Pathom, Thailand, 73000

In Thailand, many flowers were used for long time as vegetable, tea, dye and also Thai traditional medicine. However, biological activity of some flowers have never been reported. In this study, some of Thai edible flowers were investigated in their anti-microbial activities. The methanolic extracts were screened using agar disc diffusion method at 1 mg/disc and only extracts that generated the clear zone were selected to determine the minimum inhibitory concentration (MIC) using microdilution plate method. Antimicrobial activities against *Staphylococcus aureus* with MIC at 50 – 800 µg/ml were found in five of 19 flowers, *Mesua ferrea* L., *Sonneratia caseolaris* Gaerth, *Quisqualis indica* L., *Saraca indica L.* and *Antigonon leptopus* Hook. *Candida albicans* were inhibited by flower extracts from *Sonneratia caseolaris* Gaerth *and Quisqualis indica* L., with MIC at 50 and 800 µg/ml, respectively. However all extracts had no inhibitory effect on *E. coli.* Total phenolic content, using Folin-Ciocalteu colorimetric method, in all extracts was 1.27 – 31.49%. All flower extracts with antimicrobial activity in this study, except *Seraca indica* L., also generated antioxidant activity in ABTS assay with the trolox equivalent antioxidant capacity (TEAC) of 0.15 – 0.70. These results im-

plied that some flowers have potential to be further developed as medicinal food or pharmaceutical products, however it needs more investigation. *Acknowledgements:* Faculty of Pharmacy, Silpakorn University **References:** [1] Hutapatt, K. (2004) Edible flowers. Greenmedia and Product. Bangkok. Thailand. (Thai). [2] Casey, JT. (2004) J Microb Methods 58: 327 – 334.

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Antioxidative and antimicrobial activity of ancient Egyptian medicinal plants

Abdalla AEM
Food Science Department, Faculty of Agriculture Saba Basha, Alexandria University, 22 Tag El-Roassa Street, Saba Basha, P.O. Box 2153 Bolkly, Alexandria, Egypt

Egyptian medicinal plants are being used as pharmaceutical ingredients, and in other innovative ways as in food or feed supplementation [1, 2, 3]. Therefore, the present study was carried out to screen the bioactive compounds in selected Egyptian medicinal plants and to evaluate the antioxidant and antimicrobial activities of plant extracts in model system and in Egyptian food. Phenolic compounds of methanol extracts were separated and identified using HPLC. The antioxidant activity of plant extracts was tested in β-carotene-methyl linoleate system and also evaluated in sunflower oil at ambient temperature and during frying of potato chips. The antimicrobial activities of extracts were determined by disc diffusion with Gram-positive and Gram-negative species and also evaluated in salted fish [4]. The results showed that rosemary, sage, thyme and garlic extracts (600 ppm each) were the most active natural antioxidants in model system and in sunflower oil during storage in the dark at ambient temperature [3]. Combination of these four extracts (100 ppm each) showed the strongest antioxidant activity in sunflower oil during frying. Garlic, thyme and ginger extracts (1200 ppm, 30 mg extract/disc) were found to be active against all tested microorganisms. Combination of extracts (300 ppm each) prevents the growth of *Staphylococcus aureus* in salted sardine. It could be concluded that the combination of tested plants showed promising antioxidant and antimicrobial activities, thus justifying their traditional and innovative uses. **References:** [1] Abdalla, A.(1999) Adv. Food Sci.(CMTL) 21: 100. [2] Abdalla, A., Roozen, J.(2001) Eur. Food Res. Technol., 212: 551. [3] Abdalla, A. (2004). 3 rd Euro Lipid Congress, Edinburgh Univ., Scotland (5 – 8 Sept.). [4] Abdalla, A., Daud, S. (1994) J. Agric. Res. Tanta Univ. 20: 252

P 203

Phytoncidal effects of rare and endemic species of the *Rosaceae* family from Dinaric Alps

Redžić S[1], Redzic A[2], Tuka M[3]
[1]*Dep. of Botany, Faculty of Science & Faculty of Pharmacy University of Sarajevo, 33 – 35 Zmaja od Bosne St., 71 000 Sarajevo, Bosnia and Herzegovina;* [2]*Medical Faculty University of Sarajevo, 71 000 Sarajevo, Bosnia and Herzegovina;* [3]*Private pharmaceutical company "Apoteka", Kiseljak, Bosnia and Herzegovina*

Many species of *Rosaceae* family contain tannins [1] which have phytoncidal and antimicrobial effects and are regarded as effective antioxidants and antimicrobials [2]. In this study special attention has been given to the endemic and rare plants of the Rosaceae as a source of new natural antibiotics [3] and to evaluate the phytoncidal activity plants growing in the Dinaric wilderness. During 2006, the herbal material has been gathered at the Central Dinaric area. Vouchers of the analyzed species are located at the Herbarium of University of Sarajevo. The antimicrobial activity of *Potentilla, Alchemilla*, *Dryas* and *Geum* species was analyzed. Aqueous extracts (decocts) were made from the dried rhizomes and roots, followed by maceration and ethanolic extraction at different concentrations. Agar diffusion method was used at 37 °C, the incubation lasted for 48 hours. Following test microorganisms were used: *Bacillus subtilis* (ATCC 441), *Staphylococcus aureus* – Sa (ATCC 25923), *Escherichia coli* – Ec (ATCC 25922), *Pseudomonas aeruginosa*- Pa (ATCC 27853), *Proteus vulgaris*- Pv (MTCC 1771) and *Candida albicans*- Ca (MTCC 277). The microorganisms and official reference antibiotics were obtained from the Institute of Public Health of Canton Sarajevo. The results are showing different phytoncidal and antimicrobial activity of the extracts depending on type of the extract as well as the concentration. The most sensitive was *Sa* with the zones of inhibitions of 10.3 mm -12 mm (*P. palustris*, *P. reptans*, *P. montenegrina* and *P. speciosa*). Similar sensitivity has as well been noted for Bs with zones of inhibitions of 7 mm -15 mm (*P. speciosa*, *P. apennina*, *P. clusiana*, *P. caulescens* and *G. montanum*), as well as for Ec – 4,3 mm – 7,2 mm (*P. reptans*, *P. anserina*, *P. montenegrina*, *A. velebitica* and *D. octopetala*). As a control the following antibiotics were used (inhibition zone): Penicillin (25 mm), Erythromycine (22 mm) and Oxatetracycline (21 mm). **References:** [1] Tomczyk, M. (2006) Bioch Syst & Ecol 34: 770 – 773. [2] Oszmianski, J. et al. (2007) Food Chemistry 100: 579 – 583. [3] Redzic, S. (2006) Proc.1st IFOAM Intern. Conf. Organic Wild Production, 117 – 141.

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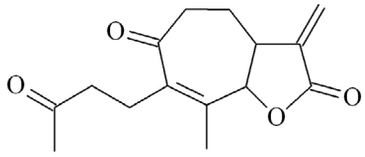
Chemical constituents from three *Inula* species. Biological activities

Panoutsopoulos G, Aligiannis N, Chinou IB
Dept. of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens,-Zografou, 15771, Athens, Greece

The genus *Inula* consists of variable perennial herbs distributed in Asia, Europe, Africa and predominantly in the Mediterranean region (Asteraceae family, tribe Inuleae). Plants of the genus are used as traditional herbal medicines throughout the world [1]. *Inula viscosa* (L.) Aiton. (syn. *Dittrichia viscosa* (L.) Greuter) is a widespread Mediterranean plant proven to be a rich source of sesquiterpenes, lactones and flavonoids. *Inula pseudolimonella* (Rech. F.) and *I. candida subsp. candida* are both Greek endemic plants and have never been studied phytochemically before [2]. The non-polar (dichloromethane) and the polar (methanolic) extracts of all species were investigated thoroughly and afforded a new sesquiterpenoid lactone



1

(**1**) together with the known inusoniolide and tomentosine. Among the constituents that have been isolated from the three species are the triterpenoids dammara-20,24-dien-3-ol and dammara-20,24-dien-3β-yl-acetate, stigmasterol and α and β-amyrin as well as the monoterpenoid thymol. The polar extracts also yielded the well known oligosaccharide inulin. Their structural elucidation was performed by modern spectral means such as1D-, 2D-NMR and MS and literature data. All extracts and the isolated compounds were tested for their antimicrobial activity against nine human pathogenic bacteria and fungi by both disc diffusion and dilution methods as well as against two cancer cell lines by MTT assay. Through antimicrobial screening, the extracts proved to be active against several of the human pathogenic bacteria – fungi, while the pure isolated compounds showed a very interesting profile. The new natural compound **1** exhibited a strong cytotoxic activity (IC50 6.0 and 6.9 μg/ml respectively). *Acknowledgements:* This study has been financially supported by PENED 2001 (70/3/6352) **References:** [1] Ceccherelli, P. et al, Phytochemistry, 24, 453 (1985). [2] Ball, P. W. and Tutin, T. G. (1976) "Flora Europaea", Vol. 4, Cambridge University Press, Cambridge, pp. 133.

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A study of the seasonal variation in the antimicrobial constituents of the leaves of *Loranthus micranthus* sourced from *Percia americana*

Osadebe PO, Dieke CA, Okoye FBC
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, 41001, Enugu State, Nigeria

A comparative study of the antimicrobial constituents of the leaves of *Loranthus micranthus* (parasitic on *Percia americana*) harvested at different seasons of the year, namely, January, April, July and November, was carried out. The air-dried and pulverized leaves harvested at the stated periods were extracted with petroleum ether and the extracts subjected to antimicrobial screening and phytochemical investigation. Using various solvent treatments the powdered leaves harvested in April were fractionated into four fractions, A, B, C and D; each fraction was screened for antimicrobial activity and phytochemical constituents. Phytochemical analysis of the extracts showed the presence of tannins, flavonoids, alkaloids, terpenoids and saponins with some of these constituents showing variations across the seasons. Broad spectrum antibacterial activity was observed for all the extracts. However, the activity against *Bacillus subtilis* and *Salmonella typhi* was significantly ($p < 0.001$) lower for the extracts of the leaves harvested in January when compared with the extracts of the leaves harvested in the other months. Only the extracts of the leaves harvested in April showed antifungal activity. Fractions A, B and D showed antimicrobial activity comparable ($p < 0.05$) to standard antibiotic, chloramphenicol. These fractions are rich in tannins (A and D), alkaloids (A) and terpenoids (B). The presence of alkaloids only in April and July may explain the higher antimicrobial activity observed in these months. Earlier reports showed that alkaloids may be responsible for the antimicrobial activity [1]. In conclusion, mistletoe used as a herbal remedy for nonspecific infections may be preferentially harvested in April and July. *Acknowledgments*: The authors are grateful to Mr. Ozioko of Bio-resource Development and Conservation Centre Nsukka, Enugu State, Nigeria for authenticating the plant material. **References:** [1] Osadebe PO and Ukwueze SE. (2004). J of Bio. Res. and Biotechn. 2: 18 – 23.

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Comparison of antimicrobial activity of traditionally used herbal uroantiseptics against clinical isolates of uro-genital pathogens

Kosalec I[1], Zovko M[2], Kalogjera Z[2], Pepeljnjak S[1]
[1]*Department of Microbiology and* [2]*Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry University of Zagreb, HR-10000 Zagreb, Croatia*

In present study, we compared the antimicrobial activity of ethanolic extracts of four traditionally used drugs in the treatment of urogenital infections (Ericaceae flos, Myrtilli folium, Uvae ursi folium, Vitis ideae folium) [1] with arbutin, hydrochinone, and the commonly used uroantiseptic norfloxacin. The activity against clinical isolates of the most common urinary pathogens was evaluated against gram-negative (*Escherichia coli* p-fimbriae positive-strains, *Proteus mirabilis*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*) and gram-positive bacterial species (*Enterococcus faecalis*, *Staphylococcus aureus*), as well as against the yeast *Candida albicans*, using hole plate diffusion and broth dilution methods. In general, the activity of the extracts was species-dependent. In hole plate diffusion method, the extracts of Uvae ursi folium and Myrtilli folium showed stronger bactericidal activity than the rest of the extracts with average inhibition zones of 28.47 and 25.47 mm, respectively. In the same method none of the extracts showed noticeable antifungal activity against *Candida albicans*. Using broth dilution method minimal inhibitory concentrations (MICs) were determined. All the investigated bacterial species were sensitive to the extracts with MICs below or equal to 10.42 mg/mL while the MIC values for the isolates of *C. albicans* yeast were between 8.33 and 25 mg/mL. The highest activity was demonstrated by Uvae ursi folium extract against *E. faecalis* (MIC 2.60 mg/mL) and *P. aeruginosa* strains (3.13 mg/mL). The antimicrobial activity of the extracts was lower than the activity of hydrochinone and norfloxacin, but higher than arbutin. Statistical analysis did not reveal clear correlation between content of arbutin, methylarbutin, polyphenols or tannin in extracts and *in vitro* MIC values. However, the extracts with higher amount of investigated substances tended to be more active in both methods. The results of the study confirm the justifiability of use of these drugs in treatment of urinary infections caused by investigated bacterial species. **Reference:** 1. Kustrak, D. (2005) Farmakognozija-Fitofarmacija. Golden Marketing-Tehnicka knjiga. Zagreb

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Screening for natural antimicrobials from Thai Medicinal plants

Vadhanasin S[1], Singkhonrat J[2], Pojjanapimol S[3]
[1]*Dept. of Food Science & Technology, Faculty of Science & Technology. Thammasat University, Klong Luang District, Pathumthani 12121. Thailand;* [2]*Dept. of Chemistry, Faculty of Science & Technology. Thammasat University, Klong Luang District, Pathumthani 12121. Thailand;* [3]*Dept. of Food Science & Technology, Faculty of Science & Technology. Thammasat University, Klong Luang District, Pathumthani 12121. Thailand*

Twenty six Thai edible plants that are traditionally used for prevention or treatment some infective problems were screened for antimicrobial activity against 4 pathogens. Sixteen were sensitive against at least one strain studied, whereas five gave strongest inhibition zones against all four strains and two gave medium positive results against all strains. Six plants were selected for further study, including *Sandoricum nervosum* Car. (Zingiberaceae), *Psidium guajava* L. (Myrtaceae), *Piper betle* L. (Piperaceae), *Garcinia atroviridis* Griff. (Guttiferae), *Garcinia mangostana* L. (Guttiferae) and *Punica granatum* L. (Punicaceae). After confirmation by disk susceptibility test with 8 pathogens (4 resistant strains of Gram-negative bacteria and fungi were also added), all extracts were studied and compared according to extraction techniques and solvents applied. The minimum inhibitory concentration (MIC) of individual extracts was also determined. It was concluded that in most cases, extraction with water and ethyl acetate (5 times) was the best, but the worst in term of % yield of the extract. A comparison between methanol and ethylacetate reflux extraction was varying, depending on individual plant, but ethylacetate was more preferable for *P. betle*, even though all extracts from *P. betle* were active against all 8 strains studied, of which, its sensitivities were as follows: *L. monocytogenes* ≥ *S. aureus*≥ *C. albicans*> *B. cereus* ≥ *S.* typhimurium ≥ *A. niger* > *E. coli* > *P. aeruginosa* at MICs < 0.10, < 0.10, < 0.10, 0.10, 0.10, 0.10, 0.68 and 1.82 mg/ml respectively. However, extracts from methanol reflux in all cases gave highest % yields. The *P. betle* from water and ethylacetate extract as separated by thin layer chromotography (TLC) yielded two substances of Rf 0.57 and 0.35 which could be chavibetol and allylpyrocatechol (APC).

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Plants used traditionally in Ecuador with potential anti-leishmanial activity

Gachet MS[1], Schühly W[1], Bauer R[1], Kaiser M[2], Brun R[2], Muñoz R[3]
[1]*Institute of Pharmaceutical Sciences, Pharmacognosy, Karl-Franzens-University Graz, Universitaetsplaz 4/I, 8010 Graz, Austria;* [2]*Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland;* [3]*Laboratorio de Química Orgánica e Investigaciones Aplicadas, Escuela Politécnica Nacional, Ladron de Guevara E11 – 253, POBOX: 17 – 01 – 2759, Quito, Ecuador*

Leishmaniasis is a disease caused by an intracellular parasite of the genus Leishmania, which is transmitted to humans by sand-flies. This illness is common in 88 tropical countries around the world

with an annual incidence of 1 – 1.5 million cases and one of its clinical forms, visceral leishmaniasis, is the second largest parasitic killer in the world [1]. As part of a project based on ethnobotanical research in Ecuador [2], 13 plant taxa have been found to have potential in the treatment of Leishmaniasis. The plants were collected from the coast, highlands and jungles of Ecuador during August-September 2006. The plant material (bark, leaves, etc.) was milled and extracted successively with hexane, dichloromethane and methanol. The crude extracts were tested against *Leishmania donovani* at two concentrations, 9.7 μg/mL (high) and 1.6 μg/mL (low). The biological screening shows that the bark of *Bocconia integrifolia* Bonpl. (Papaveraceae), at the two concentration levels assayed high/low, presents a percentage of growth inhibition of 84.1/49.8 for the hexane extract, of 96.2/73.1 for the dichloromethane extract and of 95.9/72.2 for the methanol extract. The leaves of *Minquartia guianensis* Aubl. (Olacaceae) show growth inhibition of 96.5% and 83.5% for the high and low concentration levels tested but only for the dichloromethane extract. *Acknowledgements:* This research is part of a dissertation funded by the Austrian Exchange Service (ÖAD). **References:** [1] World Health Organization WHO, Special Programme for Research an Training in Tropical Diseases TDR. Electronic publication: "Strategic Direction for Research: Leishmaniasis". Published online: February 2002. Access date: March 15, 2007. www.who.int/tdr. [2] De la Torre, L., et al., Catálogo de Plantas Útiles del Ecuador (in preparation).

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Antimicrobial effect of *Helichrysum plicatum* DC. subsp. *plicatum* extracts on *Escherichia coli* O157:H7

Aslan M[1], Demir A[2], Mercanoglu B[2], Aykac A[2], Yesilada E[3]
[1]Gazi University, Faculty of Pharmacy, Department of Pharmacognosy 06330 Etiler-Ankara, Turkey; [2]Hacettepe University, Faculty of Engineering, Department of Food Engineering Beytepe-Ankara, Turkey; [3]Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy İstanbul, Turkey

Members of the genus *Helichrysum* have been used in folk medicine mainly as anti-microbial, anti-inflammatory, digestive, choleretic and diuretic [1 – 3]. *Escherichia coli* O157:H7 was first recognized as a pathogen in 1982 and is considered as the main cause of hemorrhagic colitis. Despite many investigations on the antimicrobial activity of different *Helichrysum* species, the effect on *E. coli* O157:H7 has not yet been evaluated in detail. The present study investigates with the antibacterial activity of *H. plicatum* DC. subsp. *plicatum* (HPP) against *E. coli* O157:H7. The extracts used in this study, that were obtained from the ethanol and water extraction of flower and leaf parts of HPP were Flower EtOH (HFE), Leaf EtOH (HLE), Flower Water (HFW) and Leaf Water (HLW) extracts; and the sub-extracts were obtained by successive solvent extractions of HFE by n-hexane (HFEH), chloroform (HFEC), ethyl acetate (HFEEA), n-butanol (HFEB), the remaining aqueous part was designated as HFER. We investigated the effect of plant extracts at different concentrations on the survival of overnight culture of *E. coli* O157:H7 at 37 °C for a week. All extracts (except for HFE) did not show any significant activity in this screening test even at a concentration of 2.5%. Although antibacterial activity of 0.5% concentration of HFE against *E. coli* O157:H7 was less than the activity of the other concentrations, all concentrations of HFE extract had a bacteriostatic or bactericidal effect against *E. coli* O157:H7 (MIC value 62.5 μg/ml). Since HFE was found to be the most effective extract of the plant, the study has been carried out on the sub-extracts of it. Next, we tested the effect of sub-extracts of HFE at different concentrations on the survival of *E. coli* O157:H7 at 37 °C for a week. Of the 5 sub-extracts, HFEC, HFEB and HFER were observed to be inactive on *E. coli* O157:H7 at all concentrations. However, both of at 2.0 and 2.5% concentrations HFEEA extract showed a partial inhibition on growth of the pathogen on the fifth and the seventh day, respectively. In the same way, HFEH extract started its bacteriostatic or bactericidal effect on the seventh day at a concentration of 2.5%. **References:** [1] Sezik, E. et al. (1991). Journal of Ethnopharmacology 35: 191 – 196. [2] Fujita, T. et al. (1995). Economy Botany 49: 406 – 422. [3] Sezik, E. et al. (2001). Journal of Ethnopharmacology. 75: 95 – 115.

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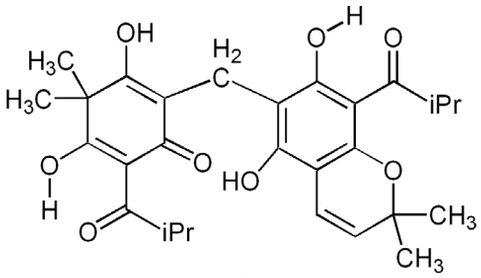
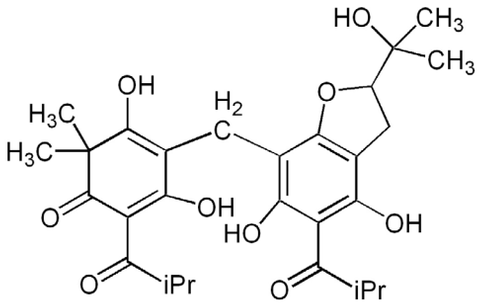
A new phloroglucinol derivative and anti-MRSA active phloroglucinols from *Hypericum mutilum* L. (Clusiaceae)

Schühly W[1], Hammer E[1], Fabian C[1], Kunert O[2], Bauer R[1], Bucar F[1]
Institute of Pharmaceutical Sciences, [1]Department of Pharmacognosy and [2]Department of Pharmaceutical Chemistry, Karl-Franzens-University Graz, 8010 Graz, Austria

In the present investigation the secondary metabolites of *Hypericum mutilum* L. (Clusiaceae, sect. Trigynobrathys), a native plant of Eastern North America, were studied. In an antibacterial screening against gram-positive and gram-negative bacteria, the dichloromethane extract of *H. mutilum* was found to exhibit a strong inhibitory activity against *Staphylococcus aureus* and *St. epidermidis* in the disk diffusion assay. During the phytochemical analysis, several phloroglucinol derivatives carrying these strong antibacterial activities were isolated and structurally elucidated. Among these, the known uliginosins A and B (**1**) together with a hitherto unknown phloroglucinol of isouliginosin type (**2**) were found. Uliginosin A and B showed at a concentration of 10 μg considerable activity against the methicillin-resistant *St. aureus* MRSA NCTC 10442 PVL (-) with inhibition zones of 1.5 and 5 mm, respectively.



(**1**)



(**2**)

Uliginosin A and B were first described from *H. uliginosum* H.B.K. [1], later also found in other members of the section Trigynobrathys. This is the first report on their activity against methicillin-resistant *St. aureus*. **References:** [1] Taylor, HL et al. (1969) Lloydia 32: 217 – 19

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Antifungal and antibacterial activities of six different extracts of *Harungana madagascariensis* stem bark

Iwalewa EO, Suleiman MM, Mdee LK, Eloff JN
Department of Paraclinical Sciences, (Phytomedicine Programme), Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, 0110, Pretoria, RSA

Ethnoveterinary usefulness of *Harungana madagascariensis* Choisy Poir (Clusiaceae) has been documented in Africa and in Europe for the treatment of animals with anaemia and various infections [1]. Most of these studies were on the aqueous extract of the leaves. We have found out in our laboratory that aqueous extract of plant material does not show prominent antimicrobial activity. Therefore in this study, hexane (H), dichloromethane (D), chloroform (C), ethyl acetate (E), acetone (A) and methanol (M) extracts were subjected to antimicrobial activity in-vitro testing against fungi (*M. canis, C. albicans, C. neoformans, S. schenckii,* and *A. fumigatus*) and bacteria *(E. faecalis, E. coli, P. aeruginosa,* and *S. aureus)* using the TLC fingerprint and bioautography [2]. The % yield of extractants indicated for M (11.1%), A (10.5%), C (5.8%), D (5.2%), E (5.1%) and H (2.4%). TLC fingerprint and bioautography were best eluted in benzene: ethanol: ammonia (BEA) (18: 2: 0.2) and showed at least 8 different compounds that are active against the different strains of fungi and bacteria used. These compounds are common to D, C, E, A and M, but were not present in H extract. The MIC values of the six extracts showed inhibition of the fungi and bacteria organisms in various degrees. D and H extracts exhibited the lowest (6.3 – 104.1 μg/ml) and the highest (27.9 – 500.0 μg/ml) MIC on fungi pathogens respectively as compared to Amphotericin B which gave (0.62 μg/ml). Likewise, the same D and H extracts also gave the lowest (3.6 – 7.8 μg/ml) and the highest (13.0 – 26.0 μg/ml) MIC values respectively on bacteria pathogens as compared to Gentamycin which gave 0.36 μg/ml. The study therefore showed that antimicrobial compounds also reside in the stem bark like in the leaves [3, 4] with the highest activities found in the D extract. **References:** [1] Committee for Veterinary Medical Product (1999): Harungana madagascariensis. The European Agency for the Evaluation of Medicinal Products. http://www.eudra.org/emea.html. [2] Eloff. J.N. (1998) J. Ethnopharmacol. 60 1 – 8. [3] Moulari, B. et al. (2006) J. Ethnopharmacol. 106 272 – 278. [4] Okoli A.S. et al. (2002) Phytother Res/16:/174 – 179.

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Antioxidant and antibacterial properties of *Lecaniodiscus cupanioides*

Sofidiya MO[1,2], Jimoh FO, Aliero AA[2], Afolayan AJ[2], Odukoya OA[1], Familoni OB[3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, university of Lagos, Nigeria; [2]Department of Botany, University of Fort Hare, Alice 5700, South Africa; [3]Department of Chemistry, Faculty of Science, University of Lagos, Nigeria

Lecaniodiscus cupanioides Planch. Ex Bth (Sapindaceae) is widely used in Nigerian folk medicine for the treatment of inflammatory conditions, hepatomegaly and bacterial infections. This study investigated the antioxidant and antibacterial activity of the methanolic extract of the leaves to justify its use in traditional medicine. DPPH, Reducing power and ABTS assays were used as antioxidant models. Total phenolic content was calculated as tannic acid equivalents (TAE mg). Total flavonoid content was estimated as quercetin and proanthocyanidin expressed as catechin equivalent. Antimicrobial activity was determined by agar radial diffusion method against selected strains of gram +ve (*Bacillus cereus, Staphylococcus epidermidus, Staphylococcus aureus, Micrococcus kristinae and Streptococcus pyrogens*) and gram -ve (*Escherichia coli, Salmonella pooni, Serratia marcescen, Pseudomonas aeruginosa and Klebsiella pneumonae*) bacteria. The extract exhibited strong DPPH and ABTS radical scavenging activity greater than BHT and was comparable to ascorbic acid. At a dose of 0.1 mg/ml the extract inhibited DPPH and ABTS radicals up to 99.4% and 98.5% respectively. The multiple antioxidant activity of the extract was evident, as it also possessed moderate reducing power. TAE was 37.67 ± 1.66 mg/g. This is higher than that reported in many other plant extracts, based on dry extract. Flavonoid and proanthocyanidin contents were 4.14 ± 0.06 and 2.54 ± 0.32 mg/g respectively. Strong correlation was recorded between ABTS/TAE (R^2= 0.89) DPPH/TAE (R^2= 0.90). Antimicrobial activity was highest on gram +ve organisms *B. cereus, S. aureus, M. kristine and S. pyrogens* (MIC value < 1.0 mg/ml). Gram-ve *S. pooni* and *P. aeruginosa* (MIC value ≤ 2.0 mg/ml). The results of this study attributed the antioxidant potential of *L. cupanioides* leaf extract to its strong proton donating ability and justified its use for the treatment of bacterial infections in ethnomedicine. *Acknowledgements:* NRF, South Africa, and University of Lagos, Nigeria.

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Development and validation of ultra-violet spectrophotometric assay method for the powdered leaves, extracts and formulations of *Loranthus micranthus*

Uzochukwu IC[1], Osadebe PO[2]
[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria; [2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

A simple, precise and reproducible ultraviolet spectrophotometric method for the assay of powdered leaves, extracts and formulations of *Loranthus micranthus* was developed and validated. The complexation of the flavonoids of *L. micranthus* with methanolic aluminum nitrate solution was employed for the assay [1]. The absorbance of the formed complex was determined spectrophotometrically at λ_{max} of 300 nm. The precision, repeatability, linearity, optical characteristics and accuracy of the method were studied [2]. Beer's law was obeyed in the concentration range of 0.4 to 3.6 mg%. The calibration curve had a regression coefficient of 0.9913. The limit of detection and the limit of quantitation of the proposed method were found to be 0.04 and 0.12 mg% respectively. Percentage recoveries between 92 and 112% were obtained. The proposed method proved to be robust and therefore recommended for the assay of powdered leaves, extracts and formulations of *Loranthus micranthus*. **References:** [1] Crozier, A., Jennifer B., et. al., (2000). Biological Research 33, 3. [2] European Medicine Evaluation Agency (2006). Validation of analytical procedures: definitions and methodology, ICH Harmonised Tripartite Guideline, EMEA, London, UK. p1 – 15.

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Biocide effects of *Chrysactinia mexicana* Gray

Juárez-Flores BI, Jasso Pineda Y, Cárdenas Ortega NC, del Valle Coulon ML, García-Chávez E, Aguirre Rivera JR
Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí, Altair 200 Col. Del Llano, CP 78377, San Luis Potosí, México

Chrysactinia mexicana Gray (Asteraceae), commonly known as St. Nicholas's herb or "false Damiana", is a small aromatic shrub widely distributed throughout central and northern Mexico, where it is extensively used in folk medicine treating several diseases [1]. In order to corroborate and to explain the qualities attributed to this species, diverse experimental works have been undertaken of which a review will be presented: (a) The antifungal activity of the alcoholic extract of this species has been evaluated on *Aspergillus flavus*. Antifungal screening was performed using Czapek agar. *A. flavus* SRRC 1273 from the NCAU (Peoria, IL) was used for the determination of antifungal activity; the diffusion method was from wells on agar plates. The alcoholic extract was placed in a well of 6 mm in diameter made on the test plate and cultured for 72 h at 28 ± 1 °C. Inhibition diameters were determined after incubation. The antifungal screening showed that the extract caused significant inhibition

of *A. flavus* growth. The extract was fungistatic and fungicidal against *A. flavus* [2]. (b) Also the antibacterial effect of the ethanolic and aqueous extracts (200 mg/ml of dry matter) of *C. mexicana* against *Shigella flexneri, S. boydii, S. sonnei, S. dysenteriae*, *Salmonella enteridis, S. typhi* and *Escherichia coli* was shown by the method of Kirby-Bauer by diffusion in Muller Hinton agar, modified as well diffusion technique. The aqueous extract significantly reduced the development of *S. boydii* and *S. flexneri,* whereas the alcoholic extract displayed antibacterial activity against all the studied bacteria except *E. coli* [3]. **Conclusions:** The daily drinking of St. Nicholas' herb infusion seems to be widely justified by its antibacterial and fungicide actions, since the problems of digestive tract are frequent and the causal agents can be multiple and from diverse nature. **References:** [1] Rzedowski J. y G. Rzedowski (1985). Flora Fanerogámica del Valle de México, Vol II, ENCB, IE, México. [2] Hernández del A. F. A., et al. (2000) Acta Cient. Potos. 15 (1): 40 – 53. [3] Pérez G. J. M. (2002) Actividad amebicida y antibacterial de cinco especies de la familia Asteraceae. Tesis de Licenciatura. Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, S. L. P. México, 39 p

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Antimicrobial activity and preliminary phytochemical screening of *Turraea heterophylla* and *Terminalia glaucescens* used in Togo ethnomedecine to treat common infections

Ingabire G[1], Koumaglo HK[2], De Souza C[3], Dotse CK[2], Anani K[3], Kabera J[1], Mukazayire MJ[1]

[1]Institute of Research Science and Technology (I.R.S.T.), Center of Research in Phytomedecine and life Science, B.P. 227 Butare, Rwanda; [2]University of Lomé (Department of Chemistry): Laboratoire d'Extraits Végétaux et Arômes Naturels (LEVAN); [3]University of Lomé- Laboratoire de microbiologie: Ecole Supérieure Des Techniques Biologiques et Alimentaires (ESTBA)

Three crude extracts, including hexane, dichloromethane and methanolic extracts from *Turraea heterophylla* and *Terminalia glaucescens* used in Togo ethnomedicine to treat common infections, were screened *in vitro* for antimicrobial activity. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli, Salmonella typhimurium, Mucor sp., Rhisopus stolonifer, Trichoderma viride, Microsporum ferrugineum* and *Candida albicans* have been used as test organisms. The dichloromethane extract of *Turraea heterophylla* roots was the most active with a minimum inhibitory concentration (MIC) between 12.5 mg/ml and 50 mg/ml. The inhibition percentage is above 98% for bacterial strains and 70% for dermatophytes. The methanolic extract of stem bark, stem and roots of *Terminalia glaucescens* showed antimicrobial activity with a minimum inhibitory concentration (MIC) between 6,25 mg/ml and 12,5 mg/ml and the inhibition percentage above 99,95% for bacterial strains. The preliminary phytochemical screening revealed the presence of tannins, alkaloids, flavonoids and saponosides. *Acknowledgement*: The Rwandese Government and the Ministry of Education are gratefully acknowledged for the grant to Ingabire Goretti

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Antibacterial activity of some Serbian aromatic plants in relation to selected phytopathogenic bacteria

Stanojevic D, Stefanovic O, Comic L, Cekovic J, Stanojkovic A
Faculty of Science, 34000 Kragujevac, Serbia

Water, ethanol and ethyl acetate extracts of 24 Serbian aromatic plants were screened for antibacterial activities against selected phytopathogenic bacteria. The following plants were tested: *Achillea millefolium*, *Artemisia absinthium, Calendula officinalis, Centaurea cyanus, Cichorium intybus, Eupatorium cannabinum, Helichrysum arenarium, Inula helenium, Lavandula officinalis, Matricaria chamomilla, Melissa officinalis*, *Mentha piperita*, *Mentha pulegium*, *Ocimum basilicum*, *Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis*, *Satureia montana*, *Solidago virga-aurea, Taraxacum officinale, Teucrium montanum*, *Tussilago farfara, Thymus serpyllum*, *Thymus vulgaris.* Overground parts of plants were tested (*Cichorium intybus* overground part and root). The antibacterial activities were tested by filter disc diffusion method in relation to the following bacterial species*: Agrobacterium radiobacter pv. tumefaciens, Bacillus subtilis, Erwinia carotovora, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens, Sarcina lutea* and *Staphylococcus aureus.* All the aromatic plants investigated showed antimicrobial activities against selected phytopathogenic bacteria tested. 22 of the 24 species examined showed antibacterial activities. The most active were water and ethanol extracts of *Achillea millefolium* especially in relation to *Pseudomonas aeruginosa.* Ethanol extracts of *Helichrysym arenarium*, *Melissa officinalis* and ethyl acetate extracts of *Salvia officinalis* and *Rosmarinus officinalis* also showed significant antibacterial properties in relation to almost all tested bacterial species. The most sensitive bacteria were *Agrobacterium radiobacter* pv. *tumefaciens* and *Erwinia carotovora.* The most resistant bacterial species was *Escherichia coli* which was significantly inhibited by ethanol extracts of *Inula helenium, Calendula officinalis, Cichorium intybus(*root) and ethyl acetate extracts of *Inula helenium, Cichorium intybus(*root) and *Centaurea cyanus.* These results are encouraging and indicate potential use of plant extracts in the control of selected phytopathogenic bacteria. **References:** [1] Petrovic, J. et al. (2004) Fitoterapia 75: 737 – 739. [2] Boatto, G. et al. (1994) Fitoterapija 6: 279 – 280. [3] Hammer, K. A. et al. (1999) Journal of Applied Microbiology 86: 985 – 990

P 217

Antibacterial activity of some plants from the family Apiaceae growing wild in Serbia

Stefanovic O, Stanojevic D, Comic L, Brkovic D
Faculty of Science, University of Kragujevac, Serbia

Twelve plants from the family Apiaceae were selected for preliminary screening for their antibacterial activities *in vitro*. The following plants were tested: *Aegopodium podagraria, Angelica silvestris, Chaerophyllum bulbosum, Daucus carota* subsp. *carota, Foeniculum vulgare, Heracleum sphondylium, Pastinaca sativa, Peucedanum cerevaria, Peucedanum oreoselinum, Pimpinella saxifraga, Sanicula europea, Torilis anthriscus*. Aerial parts of plants were used. The antibacterial activities of water, ethanol and ethyl acetate extracts were tested by disk diffusion method against 11 bacterial species*: Agrobacterium radiobacter* var. *tumefaciens*, *Azotobacter chroococcum, Bacillus mycoides, Bacillus subtilis, Erwinia carotovora*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas fluorescens*, *Pseudomonas glycinea* and *Staphylococcus aureus*. The water extracts of *Torilis anthriscus* and *Daucus carota subsp. carota* showed significant inhibitory activity. Ethanol extracts of *Chaerophyllum bulbosum, Heracleum sphondylium, Peucedanum oreoselinum* and *Pimpinella saxifraga* were active in relation to 10 tested bacteria. The most active extract of *Pastinaca sativa, Foeniculum vulgare* and *Peucedanum cerevaria* was the ethyl acetate extract. The extracts of *Sanicula europea* did not show inhibitory activity. The comparative analysis of the antibacterial activities of the tested plants showed that the intensity of their antibacterial effect depends on taxonomic characteristics of bacteria**.** The least resistant bacteria were *Enterobacter cloacae*, *Pseudomonas glycinea, Agrobacterium radiobacter* var. *tumefaciens* and *Bacillus subtilis*.The greatest resistance showed *Escherichia coli* which was significantly inhibited only by the ethanol extract of *Peucedanum oreoselinum*. **References:** [1] Brković, D. et al. (2006) Kragujevac J Sci. 28: 65 – 72. [2] Ozcelik, B.,et al. (2004) Pharmaceutical Biol. 42: 526 – 528

3. Analysis and biopharmaceutics of herbal medicinal products and herbal drugs

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Antimalarial properties of galloylated epicatechin derivatives: a first report

Sannella AR[1], Anke J[2], Hensel A[2], Vincieri FF[3], Severini C[1], Maiori G[1], Bilia AR[3]

[1]Department of Infectious, Parasitic and Immunomediated Diseases, Vector-Borne Diseases and International Health Section, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; [2]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany; [3]Department of Pharmaceutical Sciences, University of Florence, Via U. Schiff 6, I-50019 Sesto Fiorentino, Florence, Italy

Malaria is one of the most important tropical diseases. According to the World Health Organization (WHO), between 300 and 660 million clinical cases of malaria occur every year and more than 1 million persons are killed by malaria annually [1]. Due to the increasing development of resistant strains of *Plasmodium falciparum*, the development of a new antimalarial substance with a new chemical skeleton, and probably having a novel mode of action, is a global health priority. Plant natural products have always played a key role in the discovery of antimalarial agents and recently we reported on the antimalarial activity of a standardised extract of green tea as well as two of its main constituents. Epigallocatechin-3-O-gallate (IC_{50} ~30 μM for 3D7 strain) and epicatechin-3-O-gallate (IC_{50} ~7 μM for 3D7 strain), strongly inhibit *Plasmodium falciparum* growth *in vitro* [2]. Thus, in continuing the study on this class of constituents, five galloylated proanthocyanidin dimers and trimers isolated from *Rumex acetosa* L. [3] were tested. All the investigated substances showed antimalarial activity whereas galloylation generated an increased effect. Pronounced plasmodicidal effects against 3D7 parasite strains were measured for a di-galloylated (4β→6)-dimer (IC_{50} ~25 μM) and a mono-galloylated trimer with A-type linkage (IC_{50} ~15 μM). *Acknowledgements:* The Ente Cassa di Risparmio di Firenze is gratefully acknowledged for generous financial support. **References:** [1] Snow, R.W. et al. (2005) Nature 434: 214. [2] Sannella, A.R. et al. (2007) Biochem Biophys Res Commun 353: 177 – 181. [3] Anke, J. et al. (2006) 54rd Annual Congress of the Society for Medicinal Plant Research, Helsinki, P 039.

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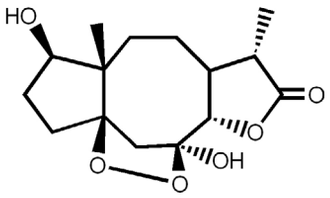
A new antimalarial agent; effects of extracts of *Artemisia diffusa* against *Plasmodium berghei*

Rustaiyan A[1], Nahrevanian H[2], Kazemi M[3], Larijani K[1]

[1]Department of Chemistry, Science and Research Campus, Islamic Azad University, P.O.BOX 14515 – 775, Tehran, Iran; [2]The Malaria Research Group, Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran; [3]Faculty of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran

Malaria is one of the most serious health problems in many parts of the world, particularly in Africa, Asia and Latin America. There are at least 300 million acute cases of malaria each year globally, resulting in more than a million deaths. Around 90% of those deaths occur in Africa; mostly in young children. Drug-resistant malaria is a major world wide public health problem. In Southeast Asia, for example, *Plasmodium falciparum* strains have become resistant to all of the classical antimalarias. Fortunately, these strains are still susceptible to the artemisinin derivatives. All of the artemisinin compounds contain stable endoperoxide bridges. The genus *Artemisia* including some Iranian species has been studied chemically and the presence of monoterpenes, sesquiterpenes, especially sesquiterpene lactones and essential oils was reported. The extract of the aerial parts of *A. diffusa* collected in the Province of Khorassan (Iran) afforded, in addition to several eudesmanolides, a new type of sesquiterpene lactone (Tehranolide) with an endoperoxide group that probably has the same effect as the antimalarial agent Artemisinin. We report here the antimalaria properties of a crude extract of the same species (*Artemisia diffusa* Krasch. ex Poljakov). The study especially examined the inhibitory effects of the extracts on developmental stages of *in vivo* of *Plasmodium berghei* in mice. Since the endoperoxide group is an essential requirement for the antimalarial activity of artemisinin, we presume the antimalarial properties of the crude extract of *A. diffusa* are attributed to Tehranolide. In the preliminary experiments, the toxicity of the ethanolic extract was tested, and judging from the high doses that were tolerated without significant overt mortality or signs of toxicity, it was estimated that the plant ethanolic extract is of relating low toxicity.



Tehranolide

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Comparison between the chemical composition and radical scavenging activities of essential oils of leaves, flowers and fruits of *Callistemon lanceolatus* DC grown in Egypt

El-Sawi SA

Pharmacognosy Department, National Research Centre, Dokki, 12622, Cairo, Egypt

The essential oils obtained by hydrodistillation from leaves, flowers and fruits of *Callistemon lanceolatus* were analyzed by GC/MS. The oil yields were 0.68%, 0.52% and 0.25%, respectively. Thirty six compounds were identified in leaf oil, while thirty four compounds were identified in both the flower and fruit oils. 1,8-Cineole was the main component in the three oils in percentage 87.8% in leaf oil, 73.8% in flower oil and 40. 9% in fruit oil, followed by p-menth-1-en-8-ol which present in higher percentage in fruit oil (4.3%) than in leaf oil (2.9%). The flower oil was characterized by its high content of alpha -pinene (27.2%) and *cis*-asarone (9.7%). *In vitro* radical scavenging activities of these oils obtained by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, were also evaluated between 5 and 20 mg/ml. At 20 mg/ml, leaf, fruit and flower oils showed good capacities to act as a non specific donor of hydrogen atoms or electrons, quenching 91.6, 71.5 and 52% of the radicals, respectively. The IC_{50} values of the oils were 7.6, 11.8 and 19.1 mg/ml, respectively. **References:** [1] Guenther, E. (1961). The Essential Oils, Vol VI, Van Nostrand, NewYork. [2] Adams, R. P. (1995). Identification of Essential Oils Components by Gas Chromatography/Mass Spectrometry. Allured Publication, Carol Stream, Illinois. [3] Calliste, C. A. et al. (2001). J. Agr. Food Chem. 49: 3321 – 3327.

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Quantification of bioactive constituents and determination of free radical scavenging activity in mangosteen fruit rind extracts

Pothitirat W, Gritsanapan W

Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Rd., Ratchatewi, Bangkok 10400, Thailand

The pericarp of mangosteen (*Garcinia mangostana* L., Guttiferae) has been used as dyeing agent and traditional medicine, e.g. as antidiarrhoea and antidysentery agent and for the treatment of wounds [1]. In this study, the content of bioactive constituents and free radical scavenging activity of unripe and ripe fruit rinds of *G. mangostana* were determined and compared. Ethanolic extracts of the unripe and ripe fruit rinds were examined for the content of total phenolic compounds, total flavonoids, and total tannins using Folin-

Ciocalteu procedure [2], aluminium chloride colorimetric method [3], and protein precipitation method [4], respectively. The results showed that the content of total phenolic compounds (42.57 ± 0.11 g GAE/100 g extract) and total tannins (51.25 ± 0.20 g TAE/100 g extract) in unripe fruit rind extract were higher than in the ripe fruit rind extract, while the highest content of total flavonoids was found in the extract of ripe fruit rind (4.08 ± 0.07 g QE/100 g extract). Validated analytical methods, i.e., TLC-densitometry and HPLC, were developed for determination of α-mangostin contents in unripe and ripe fruit rinds of *G. mangostana*. The highest content of α-mangostin was found in the ripe fruit rind extract (16.65 ± 0.38% w/w by TLC-densitometry, and 13.63 ± 0.06% w/w by HPLC). Free radical scavenging activity was evaluated from EC_{50} value using DPPH scavenging method. The unripe fruit rind extract gave higher free radical scavenging activity (EC_{50}, 5.56 ± 0.12 μg/ml). This study demonstrates differences in the content of chemical components and free radical scavenging activity between unripe and ripe fruit rinds of *G. mangostana*. This work also compared the content of α-mangostin in the fruit rind extracts using different analytical methods; TLC-densitometry, and HPLC. These methods are useful for quantitative analysis of α-mangostin in raw materials, extracts, pharmaceuticals, and cosmetic products from this plant. *Acknowledgement:*: This study was supported by a research grant from Mahidol University, Bangkok, Thailand. **References:** [1] Saralamp, P., Chuakul W., et al. (1996) Medicinal Plants in Thailand. Amarin printing and publishing Public Co., Ltd. Bangkok. [2] Kim, KH. et al. (2006) Food Chem 95: 466 – 473. [3] Meda, A. et al. (2005) Food Chem 91: 571 – 577. [4] Hagerman, AE. and Butler, LG. (1978) J Agric Food Chem 26(4): 809 – 812.

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Gene expression of enzymes related to biosynthesis of antioxidative flavonoids in Siamese neem tree leaves

Sithisarn P[1], Suksangpanomrung M[2], Gritsanapan W[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand; [2]National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand

Siamese neem tree (*Azadirachta indica* A. Juss. var. *siamensis* Valeton) is a medicinal plant of which the young leaves and flowers are popularly consumed as a bitter tonic vegetable in Thailand [1]. The study of flavonoid biosynthesis gene expression by reverse transcription-polymerase chain reaction (RT-PCR) and electrophoresis showed that Siamese neem tree leaves accumulate mRNAs corresponding to plant F3′H, FLS, DFR and F3′5′H genes. The nucleotide and deduced amino acid sequences share higher similarities to the sequences identified in fruits and leaves than those identified in flowers, which confirmed the identification of the enzyme isoforms specifically accumulated in the leaves of Siamese neem. The leaf extract of Siamese neem tree exhibited higher antioxidant activity than extracts from the fruits and seeds. Antioxidant activity and total flavonoid content determination of the leaf extract of Siamese neem tree from different developmental stages showed that all leaf extracts exhibit high antioxidant activity and total flavonoid content. The expression of FLS, DFR and F3′5′H genes in the leaves is also higher than in other parts of Siamese neem tree. This could imply that FLS, DFR and F3′5′H gene expression are related to total flavonoid content and antioxidant activity of Siamese neem tree leaves. The expression of flavonoid biosynthesis genes in Siamese neem tree, especially for FLS, DFR and F3′5′H is strong in the early stages of leaf development such as in top shoot, first young leaves, top part and basal part of young leaves while the expression is lower in mature leaves. Comparing among different Siamese neem tree tissues, the expression levels of FLS, DFR and F3′5′H are clearly higher in leaves than other tissues of Siamese neem tree while F3′H expression level is equally detected in all tested tissues. The results may implicate roles of FLS, and F3′5′H contributing to the production of flavonoids related to antioxidant activity. Flavonols/dihydroflavonols may contribute to the activity. There has been no report about nucleotide or amino acid sequences of flavonoid biosynthesis related enzymes and also no report about F3′H, FLS, DFR and F3′5′H gene expression in Siamese neem tree and other *Azadirachta* plants before. Therefore, this study could be beneficial for further biotechnological work about flavonoid biosynthesis and antioxidant activity of Siamese neem tree. This also confirms that the early developmental stages or young leaves of Siamese neem tree exhibit higher antioxidant activity, total flavonoid content and flavonoid biosynthesis related enzyme expression than the mature leaves or other plant parts supporting the ethnomedical uses of the young leaves of Siamese neem tree as bitter tonic vegetables. *Acknowledgements:* The Royal Golden Jubilee PhD. Program, Thailand Research Fund. **References:** [1] Clayton T. et al. (1996) Medicinal plants in Thailand volume 1. Amarin Printing. Bangkok.

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Variation of curcuminoids and free radical scavenging activity of rhizome extracts of *Curcuma longa* in Thailand

Pothitirat W[1], Gritsanapan W[1], Brantner AH[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Rd., Ratchatewi, Bangkok 10400, Thailand; [2]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University, A-8010 Graz, Austria

The rhizome of *Curcuma longa* L. or turmeric is a popular herbal drug of Thailand and is used as a coloring agent, household medicine and spice. Medicinal uses of the rhizome are based on the volatile oil which has carminative and antifungal activity [1] and yellow curcuminoids which have anti-oxidative and anti-inflammatory properties [2]. At present, the extract of turmeric is used in herbal cosmetics as natural colorant and additive, and also in drug preparations. So, it is necessary to develop analytical methods for quality assessment of *C. longa* extracts. TLC-densitometric and HPLC methods were used for quantitative analysis of major antioxidant components; curcumin, demethoxycurcumin, and bisdemethoxycurcumin, in 10 ethanolic extracts of *C. longa* cultivated in several parts of Thailand. The contents of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in the extracts were in the range of 12.57 – 18.41, 6.29 – 9.99, and 9.55 – 15.68% (w/w), respectively when determined by TLC-densitometry. By HPLC, the contents of these compounds were 8.55 – 15.88, 1.50 – 4.16, and 5.54 – 9.33% (w/w), respectively. Free radical scavenging activity of the ethanolic extracts of *C. longa* was evaluated using DPPH scavenging method. EC_{50} values of all extracts ranged from 18.24 – 26.36 μg/ml. The content of these bioactive compounds and the free radical scavenging activity of the extracts will be useful as a guidance for further standardization of turmeric extracts used in pharmaceutical and cosmetic products. *Acknowledgements:* This study was supported by research grants from Drug program of National Research Council of Thailand (NRCT), Mahidol University, Bangkok, Thailand and ASEA UNINET. **References:** [1] Apisariyakul A, Vanittanakorn N, et al. (1995) J Ethnopharmocol 49: 163 – 69. [2] Masuda T, Jitoe A, et al. (1993) Phytochemistry 32(6): 1557 – 60.

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Lichen metabolites inhibit UV light and nitric oxide-induced plasmid DNA damage and cell growth in human melanoma cells

Russo A[1], Piovano M[2], Lombardo L[3], Vanella A[1], Cardile V[3], Garbarino J[2]
[1]Dept. of Biological Chemistry, Medical Chemistry and Molecular Biology, University of Catania, V.le A. Doria 6, 95125, Catania, Italy; [2]Dept. of Chemistry, University T.F. Santa Maria, Casilla 110-V, Valparaiso, Chile; [3]Dept. of Physiological Sciences, University of Catania, V.le A. Doria 6, 95125, Catania, Italy

In humans both UVA and UVB cause gene mutations and suppress immunity, and these two biological events caused by UV lead to skin cancer. Inhibition of reactive oxygen species (ROS) and reactive

nitrogen species (RNS) appears particularly promising as ROS and RNS production by both UVA and UVB contributes to inflammation, immunosuppression, gene mutation and carcinogenesis [1]. Chilean lichens live in regions where the UVR is particularly intensive, and in these conditions, they are stimulated to synthesize metabolites with a strong absorption in the UV region, that exhibit a large variety of biological activity. In a previous study we isolated sphaerophorin, pannarin and epiphorellic acid-1 from *Sphaerophorus globosus*, different species of the genus *Psoroma* (*Psoroma reticulatum*, *P. pulchrum*, *P. palladium*) and *Cornicularia epiphorella,* respectively [2,3]. In this work, we evaluated, in cell free systems, the effect of lichen compounds on pBR322 DNA cleavage induced by nitric oxide and by hydroxyl radicals, generated from UV-photolysis of hydrogen peroxide, and their superoxide anion scavenging capacity. In addition, we investigated the growth inhibitory activity of these natural compounds against a human melanoma cell line (M14). Under our experimental conditions, sphaerophorin, pannarin and epiphorellic acid-1 showed a protective effect on DNA damage and exhibited a superoxide dismutase like effect, but only sphaerophorin and pannarin were able to reduce significantly ($p < 0.001$) the vitality of M14 cells, inducing apoptotic cell death, as demonstrated by the high fragmentation of genomic DNA and by a significant increase ($p < 0.001$) of caspase-3 activity. **References:** [1] Russo, P.A.J. and Halliday, G.M. (2006) Photobiol. 155: 408 – 415. [2] Qhilhot, W. et al. (1989) Ser. Cient. INACH 39: 75 – 89. [3] Piovano, M. et al. (1989) J. Nat. Prod. 52: 191 – 192.

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The fungal metabolite militarinone A induces cells death and differentiation

Küenzi P[1], Kiefer S[1], Koryakina A[1], Hamburger M[1]
[1]Institute of Pharmaceutical Biology, Klingelberstr. 50, 4056 Basel, Switzerland

As neurodegenerative diseases are a major challenge for aging societies, the development of small, non-peptidic molecules with neurotrophic activity is of vital importance as such compounds easily cross the blood-brain barrier. We recently reported that the fungal metabolite militarinone A (MiliA), from the entomopathogenic fungus *Paecilomyces militaris* [1], stimulates neuronal outgrowth in PC12 cells within 24 hours [2]. Application of MiliA to other cell lines such as the neuroblastoma cell line N2a, however, resulted in immediate onset of apoptosis. Differentiation in PC12 cells is induced by persistent activation of the same pathways that are involved in the NGF-mediated differentiation. This is namely the PI3-K/PKB and the MEK/ERK pathways as well as the activation of the transcription factor CREB. The continuous activation of these pathways finally led to up-regulation of p53, release of AIF from mitochondria and activation of the c-Jun/AP-1 transcription factor. Consequently, PC12 cells succumbed to cellular death as well after 48 to 72 hours of treatment. Application of MiliA to N2a cells resulted in immediate onset of apoptosis by nuclear translocation of AIF, activation of caspases and c-Jun/AP-1 transcription factors. The main difference between the two cell types was found to be the basal level of p53 expression, which was very high in N2a but low in PC12. Pre-treatment of N2a cells with the PI3-K inhibitor LY294.002 delayed onset of apoptosis as it lowered basal expression levels of p53 but could not prevent its activation. **References:** [1] Schmidt, K. et al. (2002) Org Lett. 4: 197 – 199, [2] Riese, U. et al. (2004) FEBS Lett. 577: 455.

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Comparative phytochemical study on rhizome and tissue culture of *Ruscus aculeatus* L

Balica G[1], Vlase L[2], Deliu C[3], Tămaş M[1], Crişan G[1]
[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu", Emil Isac 13, 400023, Cluj-Napoca, Romania; [2]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu", Emil Isac 13, 400023, Cluj-Napoca, Romania; [3]Institute of Biological Research, Ghe Bilaşcu 48, 400015, Cluj-Napoca, Romania

Ruscus aculeatus L. (*Ruscaceae*), butcher's broom, is a known medicinal plant used in the treatment of venous diseases such as varicose veins and hemorrhoids [1]. It contains steroidal saponins having as aglycons ruscogenin and neoruscogenin [1,2]. Our study was focused on quantitative determination of steroidal sapogenins resulted by acid hydrolysis [3] of 7 samples of *R. aculeatus* from wild flora of South-Western Romania (Dealurile Lipovei). These were: rhizomes with roots, rhizome, roots, alcoholic extract (1:1) from rhizome with roots and shoots, callus and callus with roots obtained by *in vitro* tissue culture. The quantitative analysis has been carried out by HPLC-MS/MS, using ruscogenin and neoruscogenin as external standards. Chromatographic separation was performed on reverse phase SB-C18 column (Agilent). The mass spectrometer was operated using an ESI source in the positive mode and was set for isolation and fragmentation of sodium-dehydrated adduct ions of ruscogenin and neoruscogenin respectively, with $m/z = 431.3$ and 429.3. The HPLC-MS/MS assay showed that the highest sapogenin concentration is present in the rhizome samples (0.11% ruscogenin and 0.17% neoruscogenin) and among the *in vitro* tissue culture samples the highest sapogenin concentration is present in shoots (0.017% ruscogenin and 0.075% neoruscogenin). On the other hand, all samples contain more neoruscogenin than ruscogenin. *Acknowledgements:* This work was supported by the grant ET 3263/2005 CNCSIS. **References:** [1] Bruneton J. (1993), Pharmacognosie, Phytochimie Plantes Medicinales, 2ed, Ed. Tec&Doc, Paris: 556 – 558. [2] Hostettman J., Marston A., (1995), Saponins, Ed. Cambridge University Press, Great Britain: 84, 298, 302. [3] Drapeau D., et. al. (1986), Planta Med. 6: 474 – 478.

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Variation of curcuminoids in ethanolic extract of *Curcuma zedoaria* Rhizomes in Thailand by HPLC

Paramapojn S, Gritsanapan W
Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Road, Ratchathewi, Bangkok 10400, Thailand

Curcuminoids, major components in the rhizomes of *Curcuma zedoaria* Roscoe were determined using a high performance liquid chromatographic (HPLC) method. The analysis was carried out at 425 nm using a BDS Hypersil C_{18} column as a stationary phase, 0.1% acetic acid aqueous solution and acetonitrile as mobile phase [1, 2]. Ethanolic extracts of *C. zedoaria* rhizomes collected from various parts of Thailand contained curcumin, demethoxycurcumin and bisdemethoxycurcumin in the range of 1.46 ± 0.45 to 5.73 ± 0.11%w/w (average 2.73 ± 1.24%w/w), 3.15 ± 0.15 to 10.98 ± 0.28%w/w (average 7.37 ± 2.71%w/w) and 0.49 ± 0.02 to 2.99 ± 0.20%w/w (average 1.40 ± 0.82%w/w), respectively. The highest average total curcuminoids content in the extracts was found to be 16.83 ± 0.62%w/w while the lowest content was 6.09 ± 1.79%w/w. This information will be useful as guidance for further standardization of *C. zedoaria* extracts of which the content has not been reported elsewhere.

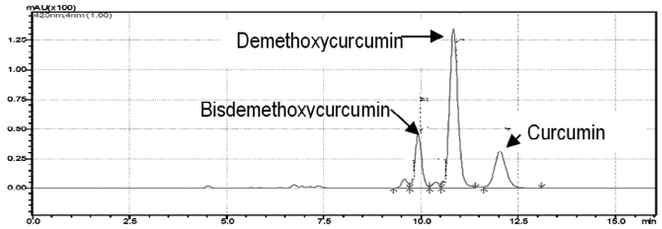

Fig. 1: Chromatogram of 70% ethanolic extract of *C. zedoaria* rhizomes.

Acknowledgement: This study was supported by the Royal Golden Jubilee Ph.D. Program of the Thailand Research Fund. **References:** [1] Pothitirat, W (2006) Standardization and antioxidant activity of Curcuma longa rhizome extract (M.Sc. Thesis in Pharmaceutical Chemistry and Phytochemistry). Faculty of Graduate studies, Mahidol University: 117. [2] Guddadarangavvanahally, K.J. et al. (2002) J Agric Food Chem. 50: 3668 – 72.

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Development and validation of an HPLC – method for quality control of *Pueraria lobata* Willd. flower plant material

Bebrevska L, Bravo L, Apers S, Pieters L, Vlietinck A
Laboratory of Pharmacognosy, University of Antwerp, Universiteitsplein 1, Wilrijk 2610, Belgium

Pueraria lobata (Willd.) Ohwi (Fabaceae) is a medicinal plant widely used in the traditional Chinese medicine. *Pueraria* isoflavones are currently a significant target in the pharmaceutical and nutritional research owing to their numerous health promoting properties. Many commercial preparations of isoflavone extracts from the root of the plant are sold as dietary supplements. Nowadays research is more and more focused on the flower of *P. lobata* and its isoflavone constituents. The efficacy and the safety of such phytopreparations depend on the actual content of active compounds, which is directly linked to the quality of the raw material used for the production. This fact indicates the need for improved quality control. In this study the development, optimisation and validation of an HPLC – method suitable for Quality Control of *Pueraria* flower plant material is presented. This analytical method for quantification of the isoflavones: (1) Tectorigenin-7-O-[β-D-xylopyranosyl-(1 – 6)-β-D-glucopyranoside]; (2) Tectorigenin-7-O-β-D- glucopyranoside and (3) Tectorigenin, was fully validated according to the ICH guidelines in terms of linearity, precision and accuracy. The extraction procedure, the extraction solvent, the extraction yields and the HPLC-conditions were evaluated and optimised. The samples were analysed on a RP C 18 column gradiently eluted with a two-phase system consisting of formic acid, water and methanol; detection was at 262 nm. The isoflavons identified in the samples are not commercially available and therefore, genistin and genistein were chosen as external standards. Tectorigenin used in the recovery experiments was isolated and purified in our laboratory.

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Free Radical Scavenging Activity of some Anticancer Herbs

Odukoya OA, Sofidiya MO
Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria

Dietary and endogenous antioxidants prevent cellular damage by reacting with and eliminating oxidizing free radicals. However, in cancer treatment, a mode of action of certain chemotherapeutic agents involves the generation of free radicals to cause cellular damage and necrosis of malignant cells. This study determined the 1 – 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, total phenolics, flavonoids and anthocyanins of 4 methanolic extracts from 2 indigenous wild plant species [*Dialium guineense* Willd. (Leguminosae) and *Spondias mombin* L. (Anacardiaceae)], indicated as anticancer plants in Nigerian traditional medicine. Assays were determined spectrophotometrically. Free radical scavenging activity was measured as maximum fading power of DPPH at 517 nm in presence of known concentration of extracts. Vitamin E, a natural antioxidant, was used as a control. The content of total phenolics was according to the Folin-Ciocalteu assay calculated as Gallic acid equivalents (GAE), total flavonoids and anthocyanins were calculated as rutin and catechin respectively. Table1. Scavenging activity and the extract content of phenolics, flavonids and anthocyanins

Samples	% Scavenging activity (SA)	Phenolics (GAE) (mg100 g^{-1})	Flavonoids (TF) (mg100 g^{-1})	Anthocyanins (TA) (mg100 g^{-1})
Dialium leaves	51.40 ± 0.04	1.42 ± 0.01	15.63 ± 1.22	0.54 ± 0.06
Dialium seeds	88.99 ± 0.50	4.46 ± 0.12	49.18 ± 0.75	3.72 ± 0.00
Spondias leaves	57.87 ± 0.55	14.68 ± 0.03	13.63 ± 0.11	5.31 ± 0.19
Spondias fruits	0.67 ± 0.05	2.75 ± 0.00	0.65 ± 0.01	0.80 ± 0.04

The correlation between scavenging capacities and the content of total flavonoids was high SA/TF (R^2= 0.8959). But a low R^2 with total phenolics and anthocyanins respectively. SA/GAE (R^2 = 0.2448); SA/TA (R^2= 0.5936). These medicinal plants associated with anticancer might be a potential source of potent natural antioxidants and therapeutic chemopreventive agents.

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Lipid peroxidation as index of activity in aphrodisiac herbs

Muanya CA, Odukoya OA
Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria

Reactive Oxygen Species (ROS) are important mediators of sperm function. Production of malondialdehyde (MDA), an end product of LPO, has been reported in spermatozoa [1]. [*Anthocleista djalonensis* A. Chev (Loganiaceae), *Carpolobia lutea* G. Don (Polygalaceae), *Cassia sieberiana* DC (Leguminosae), *Chasmanthera dependens* Hochst (Menispermaceae), *Cissus populnea* Guill & Perr (Vitaceae), *Cnestis ferruginea* DC (Connaraceae), *Dioscorea cayenensis* Lam. (Dioscoreaceae), *Lecaniodiscus cupanioides* Planch (Sapindaceae) and *Microdermis keayana* J. Leonard (Pandaceae)] are used locally in Nigeria to boost libido, induce erection, increase sperm count and consequently male fertility. Inhibition of lipid peroxidation by these plant extracts as index of male fertility was assessed. MDA was assayed by thiobarbituric acid (TBA) reaction on lipid peroxidation in raw/cooked fish homogenates and measured as the amount of Thiobarbituric Acid Reactive Sample (TBARS) in nmol mg^{-1} protein. Extracts significantly inhibited the extent of lipid peroxidation. *A. djalonensis* was most active while *D. Cayenensis* was least active in both raw and cooked fish homogenates. TBARS ±(SEM) values at 10% concentration of extracts are: *A. djalonensis* (5.18 ± 0.00) > *C. lutea* (4.02 ± 0.04) > *C. ferruginea* (2.97 ± 0.19) > *L. cupanioides* (2.85 ± 0.52) > *M. keayana* (2.43 ± 0.58) > *C. dependens* (2.35 ± 0.02) > *C. populnea* (2.27 ± 0.04) > *C. sieberiana* (2.10 ± 0.68) > *D. cayenensis* (1.54 ± 0.01) for raw fish homogenates and *A. djalonensis* (7.52 ± 0.00) > *C. populnea* (6.52 ± 0.01) > *C. dependens* (3.96 ± 0.00) > *C. lutea* (3.76 ± 0.01) > *C. ferruginea* (2.86 ± 0.01) > *M. keayana* (2.17 ± 0.01) > *C. sieberiana* (2.07 ± 0.00) > *L. cupanioides* (1.81 ± 0.01) > *D. cayenensis* (1.82 ± 0.00) for cooked fish homogenates. The correlation between TBARS activity and concentration was R^2 = 0.9533 for raw and R^2= 0.9739 for cooked fish homogenates. Thus these plants may be considered as cheap and readily available sources of treating sexual dysfunction in men. **References:** [1] Darley-Usmar, V. et al. (1995) FEBS Letters 369: 131 – 135.

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Astringent herbs as vasoconstrictors in haemorrhoid therapy

Odukoya OA, Ilori OO, Sofidiya MO
Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria

Haemorrhoids are cushions of tissue and varicose veins located in and around the rectal area. Treatment includes over-the-counter topical medications, surgery and herbal therapy. Astringency is not a taste but a feeling of dryness or roughness that is sensed by the tongue as a result of increased friction between the tongue and the surfaces inside the mouth. This is thought to be caused by tannin, which reduces lubrication by precipitating salivary proteins. As part of our ongoing investigation on astringent herbs, some medicinal plant extracts used locally in the treatment of haemorrhoids [*Achyranthes aspera* Linn. (Amaranthaceae), *Adansonia digitata* L. (Bombacaceae), *Dialium guineense* Willd (Leguminosae), *Harungana madagascariensis*, *Newbouldia leavis* Seem. (Bignoniaceae), *Spondias mombin* L. (Anacardiaceae)], were subjected to an astringency assay. This was based on the characteristic of astringents to tannins. Astringency was measured as the amount of tannin precipitated by a standard protein bovine serum albumin (BSA). Extracts were added to a solution of BSA in a buffer that allowed maximum precipitation of the tannin-BSA complexes. The precipitate was collected by centrifugation, dissolved in a basic buffer containing detergent that disrupted the hydrogen bonds holding the protein-tannin complexes together in the precipitate. The soluble tannin present in the precipitate was measured using ferric chloride. Ferric ion complexes with the tannins and forms a blue chromophore with an absorbance maximum at 510 nm. The blue product was determined photometrically and compared to a standard curve prepared with tannic acid. Astringency was reported as mg/L tannic acid equivalents. Astringency of extracts was in the order of Spondias (746.86 ± 0.65) > *Dialium* (471.17 ± 0.65) > *Harungana* (132.09 ± 0.10) > *Newbouldia* (96.48 ± 0.08) > *Adansonia* (82.98 ± 0.43) > *Achyranthes* (36.03 ± 1.05). It is proposed that these astringent herbs accomplish haemorrhoid activity by plugging up minute leaks and holes in the veins and capillaries thereby promoting vein elasticity and acting as vasoconstrictors in the perianal area.

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Lippia dulcis Trevis. an Aztecan sweet herb of potential interest

Nayal R, Melzig MF
Free University of Berlin, Institute of Pharmacy, Königin-Luise-Str. 2+4, 14195 Berlin, Germany

The demand for new alternative sweeteners for diabetic purposes has increased worldwide [1]. Recent research for the discovery and evaluation of potentially non-cariogenic sweeteners from plants has focused on plant derived compounds of terpenoid types like in our herb [2]. *Lippia dulcis* is a Verbenaceae native to tropical America and contains sesquiterpenoid sweeteners in the essential oil like (+)-hernandulcin and (+)-4β-hydroxyhernandulcin [3,4]. We have isolated the essential oils from two populations of this species, originated from Mexico and Panama, by steam distillation and analyzed them with the gas chromatography. The oil of the Mexican plants contains about 30% camphor and about 10% hernandulcin, whereas the oil of the Panamic plants contains about 17% hernandulcin and only traces of camphor < 0,015%. Sweetness estimation of hernandulcin which was purified by column chromatography showed that it may be app. 500 times sweeter than sucrose. The cytotoxicity of ethanolic extract of the Mexican plants, camphor, hernandulcin, and the two types of essential oils were assessed in HepG2 cells by measuring the reduction of the MTT [5] and IC_{50} values were determined. The essential oils and hernanadulcin showed mild toxicity on HepG2- cells [IC_{50} app. 150 μg/ml (oil of Mexico), 70 μg/ml (oil of Panama), and 68 μg/ml, respectively]. However, the ethanolic extract and the component camphor showed no effect on these cells at concentrations up to 250 μg/ml. **References:** [1] Kim, NC. et al. (2002) Arch Pharm Res. 25: 725 – 746. [2] Kinghorn, AD. et al. (2002) Pure Appl. Chem. 74: 1169 – 1179. [3] Kaneda, N. et al. (1992) J. Nat. Prod. 55: 1136 – 1141. [4] Compadre, CM. et al. (1985) Science 227: 417 – 419. [5] Mosmann, T. (1983) J. Immunol. Methods 65: 55 – 63.

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Headspace volatiles from unifloral honeys of *Satureja montana* L. and *Salvia officinalis* L. of Croatian origin isolated by solid phase microextraction (SPME)

Jerković I[1], Marijanović Z[2], Jelić M[2]
[1]Faculty of Chemistry & Technology, N. Tesle 10/V, 21 000 Split, Croatia;
[2]Marko Marulić Polytechnics of Knin, P. Krežimira IV 30, 22 300 Knin, Croatia

Unifloral honeys of *Satureja montana* L. and *Savila officinalis* L. are one of the major honey harvests in Dalmatian region of Croatia with remedy properties (especially antiseptic). The aim of this work is to determine, for the first time, their headspace "fingerprint" due to the general lack of their physicochemical data for unifloral origin determination. Screening of unifloral honey origin was based on pollen analysis (pollen percentage at least 20%). A simple solvent-free technique, solid phase microextraction (SPME), has recently been developed for isolation of honey headspace volatiles [1]. Different types of SPME fibres have been evaluated and we found better results with PDMS/DVB fibre. The main headspace volatiles of *Salvia* honey were: phenylacetaldehyde, α-isophorone, 4-ketoisophorone, *p*-anisaldehyde, hotrienol followed by minor quantity of 2-phenylethanol, *cis*- and *trans*-linalool oxides and others. *Salvia* honey headspace volatiles was shown to be distinct by high content of norisoprenoides (3,5,5-trimethylcyclohex-2-ene derivatives) with attractive sensory properties and low odour thresholds [2]: 3,5,5-trimethylcyclohex-2-en-1-one (α-isophorone) and 3,5,5-trimethylcyclohex-2-ene-1,4-dione (4-ketoisophorone). The main headspace volatiles of *Satureja* honey were: ethyl benzoate, hotrienol, *cis*- and *trans*-linalool oxides followed by minor quantity of acetoin, 2-phenylethanol, phenylacetaldehyde, β-phenylacetate, linalool and others. Hotrienol is probably formed during ripening [3]. Phenylacetaldehyde, 2-phenylethanol and benzaldehyde are ubiquitous in many types of honeys [4]. *Acknowledgements:* Ministry of Science, Education and Sports of the Republic of Croatia (grant 011 – 0982929 – 1329). **References:** [1] Cuevas-Glory L. F. et al. (in press) Food Chem. [2] Bianchi, F. et al. (2005) Food Chem. 89: 527 – 532. [3] Alissandrakis, E. et al. (2007) 100: 396 – 404. [4] Piasenzotto, L. et al. (2003) 83: 1037 – 1044.

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Screening of statin production by fungal strains

Colombo ML[1], Bianco MA[2]
[1]Dept. Science and Drug Technology, Via P. Giuria 9, 10125 Torino, Italy;
[2]Dept. Plant Biology, Via Mattioli 25, 10125 Torino, Italy

Statins were first isolated from *Aspergillus terreus* (lovastatin) and from *Penicillium citrinum* (mevastatin). Statins inhibit the hydroxyl methylglutaryl coenzyme A reductase (HMGR) involved in the synthesis of cholesterol and they have become very important for control of cholesterol levels in patients. Many clinical studies demonstrated that HMGR inhibitors (statins) have antioxidant power. This work aimed at screening of statin production by fungi via the total antioxidant activity of the fermentation broth extracts, following the reaction with the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical. The cultivated fungi were *Polyporus mori* (Pollini) Fr., *Collybia marasmioides* (Britz.) Bresinsky & Stangl, *Trametes ochracea* (Pers.) Gilb. & Ryv., *Perenniporia fraxinea* (Fr.) Ryv., *Schizopora paradoxa* (Fr.) Donk, *Phanerochaete chrysosporium* Burdsall, *Lactarius subdulcis* (Fr.) S.F.Gray, *Ganoderma pfeifferi* Bres, *Collybia acervata* (Fr.) Karst., *Stropharia semiglobata* (Fr.) Quèl., *Crucibulum laeve* (Bull.) Kambly, *Cyathus stercoreus* (Schw.) deToni, *Hebeloma trunca-*

tum (Schaeff.) Kumm., *Ustilago maydis* (D.C.) Corda, *Calvatia utriformis* (Bull.) Jaap, *Bovista plumbea* Pers., *Antrodia sinuosa* (Fr.) Karst., *Phlebia radiata* Fr., *Hymenochaete rubiginosa* (Dicks) Lév., *Entoloma lividum* (Bull.) Quél., *Gloeophyllum odoratum* (Wulf.) Imaz., *Gloeophyllum sepiarium* (Wulf.) Karst. The color reaction of the fungal extracts with 0.1 mM DPPH was measured spectrophotometrically (517 nm) monitoring the loss of DPPH radical as a function of its reduction. The results, as IC_{50} (mg/mL) values of antioxidant activity, are from 0.66 [*P.mori*] to 12.8 [*C.marasmoides*] mg/mL. Other species have intermediate values. *U.maydis*, *C.utriformis*, *B.plumbea, A.sinuosa, P.radiata, H.rubiginosus, E.lividum, G.odoratum* and *G.sepiarium* do not show a significant antioxidant activity. The statin contents (HPLC analysis) correlate well with the results in radical scavenging activity.

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Chemical composition of extracts from green, brown and black leaves of *Bergenia crassifolia* L

Shikov AN[1], Pozharitskaya ON[2], Dorman HJD[3], Makarov VG[1], Tikhonov VP[2], Hiltunen R[3]

[1]Interregional Center "Adaptogen", 47/5, Piskarevsky pr., 195067, St-Petersburg, Russia, [2]"Diod" Ltd, 11[a]Derbenevskaya Ave., 113114, Moscow, Russia, [3]Faculty of Pharmacy, Division of Pharmaceutical Biology, University of Helsinki, P.O. Box 56 (Viikinkaari 5E), FIN-00014, Finland

Siberian tea *Bergenia crassifolia* L. is widely used in Russian ethnomedicine. Black leaves which have passed 2 winters are used as adoptogen; furthermore, they are used to treat struma. Green leaves are used as an anti-inflammatory preparation for the treatment of burns, as a diuretic and an antimicrobial remedy. *B. crassifolia* rhizomes have been included in the Russian State Pharmacopoeia. The chemical composition of bergenia rhizomes has been studied quite extensively. However, the comparative composition of secondary metabolites from green, brown and black leaves has not been studied to date. The purpose of the present work was to study the chemical composition of different leaves of *B.crassifolia* by the technique of reverse phase-high performance liquid chromatography (RP-HPLC) coupled with photodiode array detection (PDA). Green, brown (after 1 winter) and black (after 2 winters) leaves of *B.crassifolia* were obtained from the MTT Agrifood Research Finland. The air-dried botanical material was extracted by MeOH. The results of the qualitative-quantitative analysis of the *B.crassifolia* methanol extracts are shown in Table 1. Table 1. Extract yield, total phenolics, and HPLC-PDA qualitative-quantitative data for methanol extracts of *B. crassifolia*.

Sample	Extracts	Total	Identified components [mg/g]					
	yield	phenolics	Arbutin	Hydroquinon	Gallic acid	Protocat. acid	Bergenin	Ellagic acid
Green	30.4	49.5	70.87 ± 8.48	0.08 ± 0.03	1.02 ± 0.09	ND	5.58 ± 0.89	0.47 ± 0.09
Brown	16.8	49.2	15.06 ± 0.24	15.57 ± 0.43	8.57 ± 0.27	0.37 ± 0.03	6.90 ± 0.66	2.00 ± 0.09
Black	8.4	32.5	2.79 ± 0.28	5.62 ± 0.32	6.51 ± 0.36	0.06 ± 0.02	5.05 ± 0.23	1.95 ± 0.13

The amount of extractable components after 2 winters natural fermentation decreased from 30.4 mg/g (green) to 8.4 mg/g (black). Maximal changes in composition of leaves were observed for arbutin (decreased 25 times), hydroquinone (increased 194 times after first year and decreased 3 times after 2 years), protocatechuic acid (increased 37 times after first year and decreased 6 times after 2 years) and ellagic acid (increased 4 times). However, the concentration of total phenolics decreased only 35%. *Acknowledgements:* Authors are grateful to Mr. B. Galambosi from MTT Agrifood Research Finland for providing of *B. crassifolia* samples

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The Microbiological Quality of Herbal Teas and Herbal Medicinal Products

Bosnić T[1], Softic DZ[1], Jerg-Simanovic D[1], Pilipovic S[1]

Institute for Quality Control of Medicines, Sarajevo, Titova 9, 71000, Bosnia and Herzegovina

Plant material are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market. Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil, while a large range of bacteria and fungi form the naturally occurring micro flora of herbs, aerobic spore-forming bacteria frequently predominate [1]. Eighty five herbal teas and sixteen herbal medicinal products obtained from different suppliers were examined for microbial contaminants. The maximum acceptable limits were determined according to European pharmacopoeia [2]. The microbiological examination was carried out in accordance with the Ph.Eur. The total aerobic plate counts for herbal teas were about 10^5 CFU/g, so the limits were met. The same applied to the moulds and yeasts whose aggregate values reached 10^4 CFU/g. While none of the herbal tea samples contained *Salmonella spp*., six samples exceeded the limit of 10^2 CFU/g for *E. coli*. The total aerobic plate counts for herbal medicinal products were about 10^2 CFU/g. **References:** [1] Beckmann, K. et al. (2003) Pharmeuropa 15: 291 – 298. [2] European Pharmacopoeia. (2005) Council of Europe. Strasbourg.

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Pharmaceutical quality of yarrow (*Achillea millefolium* L. s. l.) – Investigation of 40 commercial drug samples by means of the bioactive compounds

Benedek B, Rothwangl-Wiltschnigg K, Rozema E, Gjoncaj N, Reznicek G, Jurenitsch J, Glasl S, Kopp B

Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria

Yarrow (*Achillea millefolium* L. s. l.) is traditionally used in the treatment of inflammatory and spasmodic gastro-intestinal complaints, hepato-biliary disorders, as an appetite enhancing drug, against skin inflammations and for wound healing due to its antiphlogistic, choleretic and spasmolytic properties. The main pharmacologically active principles were shown to be the essential oil (antimicrobial), proazulenes and other sesquiterpene lactones (antiphlogistic), dicaffeoylquinic acids (choleretic) and flavonoids (antispasmodic) [1 – 4]. In order to assess the pharmaceutical quality of the drug we evaluated the content of these bioactive compounds in 40 commercial drug samples. The essential oil and the proazulenes were analysed according to the European Pharmacopoeia, whereas the content of dicaffeoylquinic acids and flavonoids was determined by SPE-HPLC/UV. In this comprehensive survey the amount of the main bioactive constituents in a broad range of commercial *Achillea* samples was assessed for the first time. It revealed that the quality of the drug material was very heterogenous, and only 50% of the samples met the standards of the European Pharmacopoeia. Moreover, this study gives information about the content of phenolic compounds in the drug and allowed us to establish tentative reference values which may be used as additional parameters in the quality control of the drug. **References:** [1] Karamenderes, C. et al. (2002) Acta Pharm Turc 44: 221 – 5. [2] Kastner, U. et al. (1993) Planta Med. 59: A 669. [3] Benedek, B. et al. (2006) Phytomedicine 13: 702 – 6. [4] Lemmens-Gruber, R. et al. (2006) Arzneimittel-Forsch 56, 582 – 8.

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Parthenolide and essential oil content in the aerial parts of *Tanacetum larvatum*

Tadić VM[1], Aljanèić IS[2], Vajs VE[2], Milosavljević SM[3], Todorović N[3], Menković NR[1], Đorđević I[3], Gođevac D[3]
[1]Institute for Medicinal Plant Research "Dr. Josif Panèić", 11000 Belgrade, Serbia; [2]Institute for Chemistry, Technology and Metallurgy, 11000 Belgrade, Serbia; [3]Faculty of Chemistry, University of Belgrade, 11000 Belgrade, Serbia

^{1}H NMR spectroscopy has been used to measure parthenolide content in *Tanacetum larvatum* (Gris.) Kanitz, Asteraceae, an endemic plant of Montenegro [1]. Parthenolide, found in significant amount in *T. parthenium* (L.) Schultz-Bip., Asteraceae (feverfew), has been linked to the anti-migraine action. This examination provides evidence that *T. larvatum* contains a high level of parthenolide, comparable to that in feverfew. Investigation of the essential oil of the aerial parts put insight into its chemical composition, with the aim to give a comment on the differences in comparison to feverfew essental oil. Aerial parts of *T. larvatum*, were collected in July and August during five years period, starting 2001, in Montenegro on several locations: mountain Komovi (Sample *a*), mountain Prokletije (Sample *b*) and mountain Sinjajevina (Sample *c*). The Parthenolide level, monitored during 2001 – 2005 ranged from 1.93 to 0.41% (Sample *a*). Although the variations in the percentage were registered, the parthenolide content in all samples was comparable to that found in feverfew [2]. Comparing to feverfew essential oil which contains mainly camphor and *trans*-chrysanthenyl acetate [3], *trans*-chrysanthenyl acetate has not been identified in the essential oils of the investigated samples. On the other hand, sabinyl acetate (37.5 and 55.6% in Samples *a* and *b*, respectively), responsible for potential teratogenicity in mice [4], has not been detected in the feverfew essential oil. Sample *c* contains santolina triene and β-pinene as principal components (13.0 and 30.1%, respectively), with no detected compounds known for their toxic effects. **References:** [1] Dostal, J. (1942) Preslia, vìstník èeské botanické spoleènosti v Praze XX-XX1, Praha. [2] Awang, DV. et al. (1991) J Nat Prod 54: 1516 – 21. [3] Lawrence B. (1999) Perfumer & Flavorist 24: 45 – 63. [4] Pages, N. et al. (1992) Phytother Res 6: 80 – 3.

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Simultaneous determination of monomeric and oligomeric alkannin and shikonin by HPLC-DAD-APCI-MS

Assimopoulou AN[1], Ganzera M[2], Stuppner H[2], Papageorgiou VP[1]
[1]Aristotle University of Thessaloniki, Department of Chemical Engineering, 541 24 Thessaloniki, Greece; [2]Institut für Pharmazie, Abteilung Pharmakognosie, Leopold-Franzens-Universität Innsbruck, Innrain 52, Josef-Moeller-Haus, A-6020 Innsbruck, Austria

Alkannin and shikonin (A/S) derivatives possessing a wide spectrum of biological activities have been found in roots of several Boraginaceous species [1,2]. As reviewed in [3], determination of monomeric A/S derivatives in commercial samples, root extracts and tissue cultures by HPLC has been described previously. Although the presence of oligomeric A/S derivatives has been confirmed by size exclusion chromatography, their analysis by HPLC has not been reported. Therefore, in the present study a rapid and simple HPLC-DAD-APCI-MS method was developed to detect and determine monomeric and oligomeric alkannins/shikonins simultaneously for the first time. Commercial samples of alkannin and shikonin were analyzed for the respective compounds and the structures of the oligomeric derivatives were identified based on their MS-spectra. Under the experimental conditions applied monomeric A/S was detected at a retention time (r.t.) of 12.7 min in the LC-MS chromatogram (with a molecular ion of m/z 288), while two other major peaks were observed at r.t. 20.3 and 20.6 min, each of them showing a molecular ion at m/z 558 representing A/S dimers. In smaller proportions, three additional A/S dimers with m/z 574 (r.t. 17.8, 18.0, 18.1 min) and four trimers (m/z 828; r.t 24.9 – 25.4 min) were detected. The molecular ion at m/z 558 is in absolute accordance with the dimeric structure proposed recently by our group [4], representing the main dimeric component in the alkannin commercial sample. The proposed structures of the other oligomers detected will be discussed. **References:** [1] Papageorgiou, V.P., Assimopoulou, A.N. et al. (1999) Angewandte Chemie, Int. Edition, 38(3): 270 – 301. [2] Papageorgiou V.P., Assimopoulou, A.N. et al. (2006) Current Organic Chemistry, 10(16): 2123 – 2142. [3] Papageorgiou V.P., Assimopoulou, A.N. et al. (2006) Current Organic Chemistry, 10(5): 583 – 622. [4] Spyros A., Assimopoulou, A.N. et al. (2005) Biomedical Chromatography, 19: 498 – 505.

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An extract of *Ammi visnaga* L. prevents cell damage caused by oxalate

Vanachayangkul P[1], Byer K[2], Khan S[2], Butterweck V[1]
[1]Department of Pharmaceutics, College of Pharmacy, University of Florida PO Box 100494 1600 SW Archer Rd. Gainesville, FL 32610 USA; [2]Department of Pathology, College of Medicine, University of Florida PO Box 100275 1600 SW Archer Rd. Gainesville, FL 32610 USA

Urinary stones affect 10 – 12% of the population in industrialized countries. Epidemiological data collected during several decades showed that the majority of stones, up to 80%, are composed mainly of calcium oxalate (CaOx) [1]. Egyptians have been traditionally using extracts prepared from the seeds of *Ammi visnaga* L. (syn. Khella, Apiaceae) as a remedy to treat kidney stones [2]. It was the aim of our study to evaluate the effect of a Khella extract on CaOx crystallization *in vitro* using cell culture experiments. The extract was phytochemically characterized by means of HPLC. The quantitative amount of the marker compounds khellin and visnagin was 2.88 and 1.72 mg/100 mg Khella extract, respectively. LLC-PK1 and MDCK cell lines were exposed to 300 μM oxalate (Ox) or 133 μg/cm^2 CaOx in presence or absence of 10, 50, 100 or 200 μg/ml Khella extract. To evaluate the cell damage, cell viability was assessed by determining the release of lactate dehydrogenase (LDH). Khella extract (100 μg/ml) significantly decreased LDH release from LLC-PK1 (Ox control: 8.46 ± 0.76%, Ox + 100 μg/ml Khella: 5.41 ± 0.94%, $P < 0.001$) as well as MDCK cells (Ox control: 30.9 ± 6.58%, Ox + 100 μg/ml Khella: 17.5 ± 2.50%, $P < 0.001$), which indicates a prevention of cell damage. Results presented here demonstrate that exposure of renal epithelial cells to Ox is associated with a significant release of LDH. Our research provides an experimental basis for examining the role of Khella extract in kidney stone disease. *Acknowledgement:* Department of Pharmaceutics, University of Florida, USA **Reference**: 1. Sierakowski, R. et al. (1978) Invest Urol 15:438 – 441. 2. Ahsan, S.K. et al. (1989) J Ethnopharmacol 26:249 – 254.

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Computer-aided discovery of Smac-mimetics within the plant kingdom

Pfisterer PH[1], Schuster D[2], Rollinger JM[1], Langer T[2], Stuppner H[1]
[1]Institute of Pharmacy/Pharmacognosy, Leopold-Franzens University, 6020 Innsbruck, Austria; [2]Institute of Pharmacy/Pharmaceutical Chemistry, Leopold-Franzens University, 6020 Innsbruck, Austria

Pharmacophore-based virtual screening of natural product databases is an efficient and trendsetting strategy to exploit nature's multitude of bioactivities [1]. Endogenous Smac (second mitochondrial derived activator of caspase) binds at the Bir3 domain of XIAP (X-linked inhibitor of apoptosis protein) and induces apoptosis in this way. Using a computational approach we intend to isolate new potent Smac-mimetics for the discovery of chemosensitizers upon treatment with conventional chemotherapeutics [2]. In a previous study, a pharmacophore model of the Bir3 domain was generated and validated [3]. Commercial databases were virtually screened and synthetic hits were tested on their XIAP inhibition properties. Based on these experimental data, the validated model was refined

in this work. Virtual screening filtering experiments were conducted using two in-house made 3D multi-conformational databases of natural molecules designated INP (> 110 000 compounds) and DIOS (> 9 000 natural products from medicinal plants) [4]. The databases produced hit rates of 0.65% (713 hits) and 0.24% (22 hits), respectively. A first evaluation of the hit lists disclosed some well-known phytochemical classes of higher plants such as acetogenins, terpenoids, alkaloids and lignans. Among the obtained virtual hits 18.6% of the constituents of higher plants are already described in literature to act as cytotoxic, apoptotic, anti-neoplastic or anti-tumor natural agents. In order to verify this computer-aided strategy, compounds of interest will be analyzed from the plant matrix by LC-MS and phytochemically enriched and/or isolated by different chromatographic methods. In further studies, the isolated compounds will be tested in wild type and XIAP overexpressing human leukemia S-Jurkat cells for their potential to induce apoptosis either alone or when combined with low doses of etoposide in order to identify etoposide specific chemosensitizers. **References:** [1] Rollinger, J.M. et al. (2006) Planta Med. 72: 671 – 678. [2] Fulda, S. et al. (2002) Nat. Med. 8: 808 – 815. [3] Bliem, C.B. et al. (2006) Planta Med. 72, P084: 1008. [4] Rollinger, J.M. et al. (2004) J. Chem. Inf. Comput. Sci. 44: 480 – 488.

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Quantitative determination of alpha lipoic acid in dietary supplements using HPLC with different detection modes

Durrani AI[1], Schwartz H[1], Schmid W[2], Sontag G[1]
[1]Department of Analytical and Food Chemistry, University of Vienna, Währinger Straße 38, A-1090 Vienna, Austria [2]Institute of Organic Chemistry, University of Vienna, Währinger Straße 38, A-1090 Vienna, Austria

Alpha lipoic acid (α-LA) is a natural antioxidant used both in the prevention and treatment of various oxidative stress related diseases [1]. The present paper deals with the development of a rapid and selective method for the quantitative determination of the α-LA content in dietary supplements. The method is based on HPLC with coulometric electrode array detection (CEAD) and ESI-MS detection modes. First, α-LA was extracted with methanol by sonication. The chromatographic separation for CEAD was then achieved isocratically [acetonitrile/methanol/50 mM potassium dihydrogen phosphate 305:65:630, v/v/v, pH 3] using an ACE 3-C-18 column (150 x 3 mm, 3 μm particle size) at a flow rate of 0.45 ml/min. For ESI-MS (negative mode), an ACE 3-C-18 column with 2.1 mm I.D. was used. The mobile phase consisted of 0.1% acetic acid in water/acetonitrile 55:45, v/v and was used at a flow rate of 0.2 ml/min. Both chromatographic methods were validated and the results were in good correlation [Table 1]. High sensitivity and selectivity make these methods useful in pharmacological studies. Table 1: Content of α-lipoic acid in dietary supplements

Supplement	Claimed (mg/g)	Found (mg/g)	
		HPLC-CEAD	HPLC-MS
a	34.9	33.6	33.9
b	0.7	0.4	0.5
c	1.3	0.9	0.9
d	20.6	12.1	13.2
e	13.1	9.9	10.5
f	1.0	0.4	0.4

Acknowledgements: 1) Higher Education Commission, Pakistan 2) Austrian Exchange Service, Austria. **References:** [1] Navari-Izzo, F. et al. (2002) Plant Physiol Biochem 40: 463 – 470.

P 243

Changes in content of benzophenanthridine alkaloids in three species of the family *Papaveraceae* during the vegetation period

Suchomelova J[1], Taborska E[1]
[1]Department of Biochemistry, Faculty of Medicine, Masaryk University, Komenského nám. 2, 662 43, Brno, Czech Republic

Some species of the family *Papaveraceae*, especially *Dicranostigma lactucoides* (HOOK.f.et THOMS), *Macleaya microcarpa* (Willd.), and *Stylophorum lasiocarpum* (Oliv.), are known by their high content of quarternary benzo[c]phenanthridine alkaloids (QBA) in roots. Many papers deal with biological activity of the main QBA sanguinarine and chelerythrine [1]. On the other hand, knowledge about the effects of minor QBA is very poor [2]. We monitored the content of five QBA sanguinarine (SA), chelerythrine (CHE), chelirubine (CHR), chelilutine (CHL), and macarpine (MAC) in roots of the named species during the vegetation period using RP-HPLC. The aim was to determine the best period for the collection of plants and subsequent isolation of QBA. Methanol extracts were prepared for analysis. The Phenomenex reverse phase C-12 column Synergy™ MAX-RP was used and mobile phase consisted of heptanesulfonic acid (0.01 M) and triethanolamine (0.1 M) in redestilled water, pH 2.5 (H_3PO_4) acetonitrile gradient 25 – 60% during 30 minutes. Detection was perfomed at 280 nm. The amount of QBA had sinusoidal character for all tested species. For *D. lactucoides,* which is a biannual plant, the highest content of QBA was determined in May of the second year of cultivation (1.99% for SA, 3.43% for CHE and 0.31% for CHR). In *M. microcarpa* (perennial plant), the QBA content culminated in May (0.73% CHE and 0.46% SA) and June (0.23% CHR, 0.14% CHL, and 0.20% MAC). In *S. lasiocarpum* (perennial plant), the highest amount of CHE, CHR, CHL, and MAC (0.08%; 0.07%; 0.01% and 0.17%) was found in August and of SA (0.13%) in September. According to these results, *M. microcarpa* is a suitable source of minor QBA, while the content of SA and CHE is highest in *D. lactucoides*. **References:** [1] Dvorak, Z. et. al. (2006) Heterocycles 68 (11): 2403 – 2422. [2] Slaninova, I. et al. (2001) Cell biology and Toxicology 17: 51 – 63.

P 244

Essential oil content and composition of German Chamomile affected by age of seedling and date of sowing

Rafieiolhossaini M[1,2], Adams A[3], De Kimpe N[3], Van Damme P[1]
[1]Laboratory of Tropical and Subtropical Agriculture and Ethnobotany, Coupure Links 653, B-9000 Gent, Belgium; [2]Department of Agronomy, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran; [3]Department of Organic Chemistry, Faculty of Bioscience engineering, Coupure Links 653, B-9000 Gent, Belgium

German chamomile (*Matricaria chamomilla*) is an annual plant belonging to Asteraceae family. Since chamomile is cultivated commercially, the optimum time for sowing has been the subject of a number of studies [1, 2, 3]. The aim of the present investigation was to determine the influence of sowing time and age of seedling on the essential oil content and composition of German chamomile grown in Belgium. The experiment was carried out on a loamy sand soil at Ghent University, Belgium, during 2005. The treatments consisted of 4 different times of planting (15 April, 1, 15 and 30 May) in combination with 5 seedling ages (30, 45, 60, 75 and 90 days) replicated thrice in a randomized complete block design. Seedlings were transplanted at a spacing of 10 cm x 10 cm. Plot size under the trial was 70 x 50 cm^2. After drying the flowers, the essential oil was isolated by Likens-Nickerson extraction followed by GC-MS analysis.The results indicate that for the first date of sowing, the measured traits were not significantly influenced by the age of seedling at transplanting except for the spathulenol content whereby the highest amount was obtained for 90 days-old seedlings. The planting time for 30 days seedlings had significant effects on the content of (E)-β-farnesene, bisabolol oxide (A), bisabolon oxide and

spathulenol. Results show that the planting time has a more pronounced effect on essential oil content and composition of German chamomile as compared to the age of seedling. **References:** [1] Gasic, O. et al. (1991) J. Ess. Oil Res. 3: 295 – 302. [2] Johri, A.K. et al. (1992) Indian J. Agron. 37: 302 – 304. [3] Hadj Seyed Hadi, M. et al. (2002) Iranian J. Crop Sci. 4: 208 – 217.

P 245

Influence of polysaccharides from *Viscum album* L. on human dermal fibroblasts *in vitro*

Craciunescu O, Balan M, Gherghina E, Moldovan L
National Institute R&D for Biological Sciences, 296, Splaiul Independentei, Bucharest, 060031, Romania

Polysaccharides (Pz) play an important role in different biological activities, such as inflammation, fertilization, cell-cell adhesion, signal transduction, etc [1]. The purpose of this study was to isolate and characterize Pz from European mistletoe (*Viscum album L.*) and to evaluate their *in vitro* influence on human dermal fibroblast (HDF), in order to use them in dermatological therapy. Water-soluble Pz (PZE) was fractionated by ethanol precipitation (A1-A4 fractions) and A2 fraction was purified by ion-exchange chromatography on DEAE-Spherodex LS (B1-B6 fractions). For all these fractions, carbohydrate, uronic acid and the protein content were determined. The influence of carbohydrate-rich Pz fractions (A2 and B3) and of PZE on fibroblast cell behaviour has been evaluated under *in vitro* conditions by MTT assay [2], light microscopy and gelatin-zymography [3]. Our results indicated that B3 fraction concentrations ranging from 0.2 to 100 μg/ml stimulated the fibroblast mitochondrial activity. The highest registered value, corresponding to a concentration of 100 μg/ml B3, was 126.5 ± 3.4% compared to the control (100 ± 2.9%). Moreover, light micrographs showed that cells maintained the typical fibroblast morphology and a proliferation rate similar to that of the control. A2 fraction and PZE did not induce cytotoxic effects on HDF in the range 0.2 – 100 μg/ml. On the other hand, the majority of cells cultivated in the presence of A2 fraction and PZE, in concentrations above 100 μg/ml, presented an alteration of their morphology and a lower proliferation rate (65%). Similarly, the synthesis of matrix metalloproteinases in culture medium was influenced by the concentrations of Viscum album Pz. In conclusion, Viscum album Pz stimulated HDF proliferation in a dose-dependent manner and in terms of their purification degree. *Acknowledgements:* This work was supported by Romanian Project BIOSTAR PN-06 – 400107. **References:** [1] Deters, A.M. et al. (2005) Planta Med. 71: 33 – 39. [2] Mossman, T. (1983) J Immunol Methods 65: 55 – 61. [3] Cimpean, A. et al. (1998) J Med Biochem 2: 313 – 322.

P 246

Simultaneous determination of oenothein B and quercetin glucuronide in aqueous extract of *Epilobii angustifolii* herba

Bazylko A, Kiss AK, Kowalski J
Department of Pharmacognosy and Molecular Basis of Phytotherapy, Faculty of Pharmacy, Medical University of Warsaw, 1 Banach St., 02 – 097 Warsaw, Poland

Epilobium angustifolium L.(Oenotheraceae), a representant of willow-herbs species found throughout northern temperate regions, is used in folk medicine for benign prostate hyperplasia (PBH) and associated with problems of micturition. Its use to treat prostatic adenoma has also been mentioned. The dimeric ellagitannin oenothein B (OeB) appeared to be responsible for some observed biological activity of extracts [1,2,3]. Flavonoids possibly act synergistically with oenothein B [3,4,5]. The method of separation and quantitative determination of OeB and quercetin glucuronide (GQ) in an aqueous extract of Epilobii angustifolii herba by HPTLC-densitometry was developed. Powdered plant material was extracted with water (1:10) in an ultrasonic water bath. The extract was filtrated and lyophilised. Samples were applied by an automatic applicator. Chromatography was performed in a vertical chamber. HPTLC RP-18 WF_{254} plates were used. As a first mobile phase 25% of MeCN in water (+ 50mM H_3PO_4) was used (distance of 8 cm), then acetonitrile was used as a second mobile phase (distance of 4 cm). Plates were dried and scanned. Densitometry was carried out by using of Shimadzu CS-9301PC densitometer. The absorption spectra were recorded at 270nm (OeB) after first developing and at 350nm (GQ) after second. The amount of OeB and GQ in the aqueous extract of Epilobii angustifolii herb using the developed method were 152.46 ± 4.92 mg g^{-1} and 22.07 ± 1.38 mg g^{-1}respectively. **References:** [1] Hiermann A., Radl B. (1998) J Chromatogr A 803: 311. [2] Vitalone A. et al. (2003) Pharmacol 69: 79. [3] Kiss A. et al. (2006) Pharmazie 61: 66. [4] Shikov A.N. et al. (2006) J Agric Food Chem 54: 3617. [5] Kiss A. et al. (2004) Planta Med. 70: 919.

P 247

Synthesis of a putative substrate for malonyl-coenzyme A: 21-hydroxypregnane 21-*O*-malonyltransferase, an enzyme involved in cardenolide formation, and development of an HPLC method for the quantification of its malonylated derivative

Kuate SP[1], Pádua RM[1], Poumale HMP[2], Kreis W[1]
[1]*Chair of Pharmaceutical Biology, Friedrich-Alexander University of Erlangen-Nürnberg, Staudstr. 5, D-91058 Erlangen, Germany;* [2]*Department of Organic and Biomolecular Chemistry, Georg-August-University of Göttingen, Tammannstr. 2, D-37077 Göttingen, Germany*

The butenolide ring is the main common characteristic of all cardenolides. Its formation is supposed to be initiated by the transfer of a malonyl moiety from malonyl-coenzyme A to the 21-hydroxypregnane [1, 2]. Since all methods (HPLC, TLC, GC) tried so far for the determination of malonyl-coenzyme A: 21-hydroxypregnane 21-O-malonyltransferase activity had their weak points, a reliable, fast and sensitive method had to be developed. A surrogate substrate was synthesized (Fig. 1) containing a side chain resembling the sugar side chain attached to C-3 of putative cardenolide precursors and a chromophor allowing UV/HPLC detection. We synthesized 3β-benzoyloxy-5β-pregnane-14β, 21-dihydroxy-20-one and its 21-O-malonylated derivative, the latter being the expected product of the enzyme reaction. The new substrate was well accepted by the enzyme. An UV/HPLC method has been established for the detection and quantification of 3β-benzoyloxy-5β-pregnane-14β, 21-dihydroxy-20-one and its 21-O-malonylated derivative, 3β-benzoyloxy-5β-pregnane-14β-hydroxy-20-one 21-O-malonyl hemiester, the product of enzyme action. The method was validated. *Acknowledgments:* German Academic Exchange Service (DAAD) for the PhD Grant awarded to SPK. **References:** [1] Stuhlemmer, U. and Kreis, W. (1996) Tetrahedron Lett., 37: 2221 – 2224. [2] Kreis, W. et al. (1998) Planta Med. 64: 491 – 499.

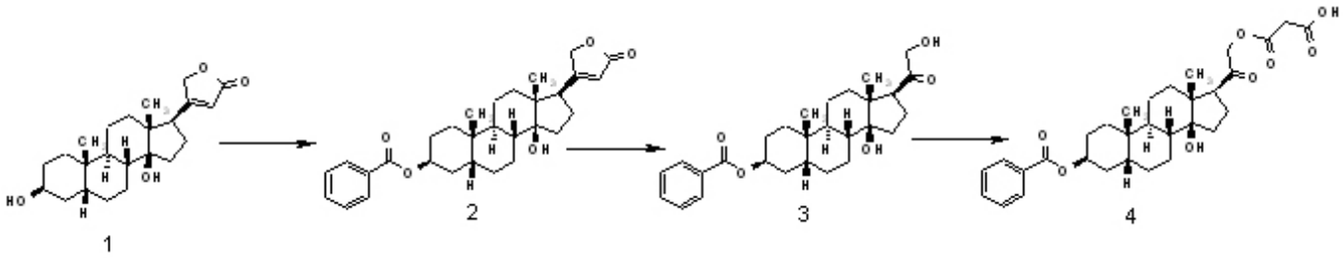

Fig. 1: Main compounds involved in the preparation of 3β-benzoyloxy-5β-pregnan-14β-hydroxy-20-on-21-malonylhemiester (4) from digitoxigenin (1). Benzoyldigitoxigenin (2), 3β-benzoyloxy-5β-pregnan-14β, 21-dihydroxy-20-one (3).

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Antioxidant activities and DNA fingerprint of 4 varieties *Lotus* stamens

Phonkot N[1], Wangsomnuk PP[2], Aromdee C[3]
[1]Graduate student, Faculty of Pharmaceutical Sciences, Khon Kaen University, 40002, Thailand; [2]Department of Biology, Faculty of Science, Khon Kaen University, 40002, Thailand; [3]Corresponding author, Faculty of Pharmaceutical Sciences, Khon Kaen University, 40002, Thailand

Stamens of sacred lotus (*Nelumbo nucifera* Gaerth), or Royal Lotus in Thai, are used as a heart stimulant and as an active ingredient in many Thai traditional recipes. This work is part of the standard and specification establishment of lotus stamens used in Thai traditional medicine [1]. Stamens from four varieties of *Nelumbo nucifera* (Gaerth) were investigated for their DNA fingerprints, and their antioxidant activities, DPPH and TBARS [2 – 6]. The PCR amplification was used for identification of Lotus DNA by using OPS 3, OPS 11, OPS 13 and OPE3 random-decamer primers. The result showed variety-specific markers of Pathum, Sattabongkot, Boontharik and Sattabutre varieties. The antioxidant activities (IC_{50}) of these four Lotus varieties by 2,2-diphenyl-1-picrylhydrazyl (DPPH) model were 69.0 ± 6.3, 62.5 ± 4.0, 32.3 ± 3.4 and 40.5 ± 1.5 $\mu g.mL^{-1}$, respectively. The thiobarbituric acid reactive substance (TBARS) model were 47.3 ± 6.3, 45.3 ± 2.3, 23.0 ± 5.6 and 29.1 ± 2.3 $\mu g.mL^{-1}$, respectively. The results showed that IC_{50} in both methods of Sattabongkot were significantly low ($p < 0.05$, at the confidence level of 95%). **References:** [1] Aromdee, C.; Phonkot, N. (2005) The Thai Journal of Pharmaceutical Sciences. Supplement 29(-): 30. [2] Belitz HD, Grosch W. (1999). Food Chemistry, 2nd ed., Germany: Springer. p208. [3] Heim, K.E. et al. (2002) Journal of Nutritional Biochemistry. 13(10): 572 – 584. [4] Horwitz W. (2000) Official Methods of Analysis of AOAC International, 17th ed., Vol. 2. Maryland: AOAC International, chapter 45: p5. [5] Jung H.A. et al. (2003) Arch Pharm Res; 26(4):279 – 85. [6] Pourmorad, F. et al. (2006) African Journal of Biotechnology. 5(11): 1142 – 1145.

P 249

Chemical constituents of the rhizomes of *Curcuma spp.* (Wahn Mahamek) and DNA fingerprints

Aromdee C[1], Polrat S[2], Wangsomnuek P[3], Kittiwongsunthorn W[4]
[1]Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand; [2]Graduate School, Khon Kaen University, Khon Kaen, 40002, Thailand; [3]Department of Biology, Faculty of Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand; [4]Regional Medical Science Center Ubonrachathani, Ubonrachthani, 34000, Thailand

Wahn Mahamek's rhizome (rhizome of *Curcuma aeruginosa* Roxb.) is used as carminative, analgesic and against uterus inflammation [1]. Two types of Wahn Mahamek specimen were investigated; one is *Curcuma aeruginosa* Roxb., the other is still being identified. However, both of them are easily distinguished when fresh, but when they were dried and sold in herbal drug stores they were sliced, dried or powdered and therefore hard to identify. In this study 3 techniques were used to differentiate the two Wahn Mahameks. Therefore their identity and standards of Wahn Mahamek can be established. Both species were grown and collected at 10 months age. DNA fingerprint, chemical constituents of their volatile oils by using gas chromatography, linear retention index and some authentic standards to identify the constituents were performed. Besides that methanol extracts were compared by thin layer chromatography. The PCR amplification was used for identification the two plants DNA by using OPA02, OPA03, OPA13, OPA20, OPE4 and OPE7 random-decamer primers. The polymorphic bands between the two specimen studied were found which can be used for identification. The contents of camphor, curzerenone and epicurzerenone of *C. aeruginosa* were 16.85, 16.81 and 3.5% whereas the *C. sp.* contained 6.04, 0 and 62.84% respectively. The thin layer chromatogram revealed that *C. spp.* contains curcumine, whereas none was detected in *C. aeruginosa*. **References:** [1] Faculty of Pharmacy, Mahidol University (2000) Herb...Local Plants (4). Prachachon Ltd. Bangkok 10500

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Determination of haematoxylin and brazilin in *Caesalpinia sappan* extract from various locations in Thailand by high performance liquid chromatography

Temsiririrkkul R, Punsrirat J, Ruangwises N, Wongkrajang Y, Nakornchai S
Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhya, Rajthewi, Bangkok, 10400, Thailand

Caesalpinia sappan L. (sappan wood) has been used in oriental medicine for a long time. The important part of this plant is the heartwood that contains brazilin and haematoxylin, which have several scientific reports in antimicrobial, antihypertension, antioxidant, anticonvulsant and anti-inflammatory activities. This study was undertaken to evaluate haematoxylin and brazilin content by high performance liquid chromatography (HPLC) in the water and methanolic extract of *C. sappan* heartwood collected from 10 locations from the Central, Northern, Northeastern and Western parts of Thailand. Samples were continuously extracted using a soxhlet apparatus with water-methanol (1:1, v/v). The brazilin and haematoxylin content were analyzed by HPLC with methanol and 1% acetic acid as mobile phase. The content of brazilin in 10 samples was 8.7 – 22.2% w/w while no content of haematoxylin was found. **References:** [1] Y.N. Zhao. et al. (2006) Pharmacol. 76: 76 – 83. [2] B.S. Guleria et al. (1997) Asian J Chem. 9: 816 – 818. [3] L. Du. et al. (2004) J Chrom. A (2005) 1077: 44 – 48

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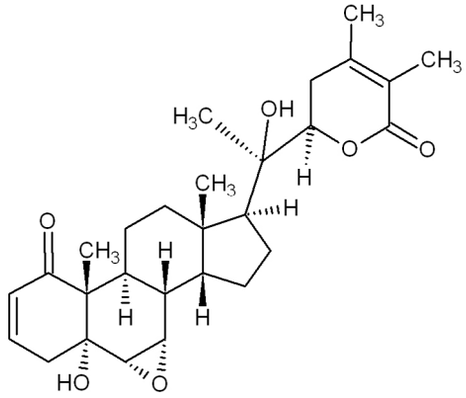
Withania somnifera HPLC-PDA method validation

Penman K[1], Briski T[1], Bone KM[2], Agarwal A[3], Murali B[3], Mundkinajeddu D[3], Mayachari A[3], Shenoy R[3], Lehmann RP[1]
[1]Mediherb Research Laboratories 3/85 Brandl St Eight Mile Plains Brisbane, 4113, Australia; [2]School of Health Science, University of New England, Armidale, 2031 Australia;[3]Natural Remedies Pty Ltd 5B Veerasandra Indl. Area 19thKM Stone, Hosur Road Bangalore 560100 India

Withania somnifera (L.) Dunal or Ashwagandha root has a long traditional history of usage in ayurvedic medicine and remains widely used. Phytochemical characterization studies have highlighted the complexity of this plant with the identification of numerous alkaloids, withanolides and sitoindosides. The phytochemical profile of the withanolides in different chemotypes of *Withania somnifera* varies considerably [1]. Reports of HPLC analysis of *Withania somnifera* typically detect and quantify only 2 – 4 withanolides [2,3]. A routine HPLC-PDA method was developed and validated (using linearity, accuracy, precision, limit of detection, limit of quantification, robustness, stability of standards and test solutions criteria) for the analysis of withanolide content in *Withania somnifera* raw materials and product samples. This assay uses routine methodologies and permits identification of several key withanolides – Withaferin A, 12-Deoxywithastramonolide, Withanolide A, Withanolide B, Withanoside IV and Withanoside V. The HPLC-PDA method developed enables chemotype differentiation and this together with the ability to quantitatively assess these key withanolides ensures a consistent therapeutic product using *Withania somnifera*. **References:** [1] Bassalie, R. et al. (1987) J. Chrom. 389: 195 – 210. [2] Ganzara, M. et al. (2003) Fitoterapia 74: 68 – 76. [3] Khajuria, R. et al. (2004) J. Sep. Sci. 27: 541 – 546.



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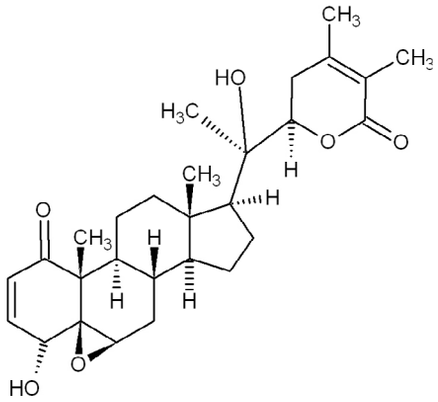

2

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NMR studies found that a commercially supplied standard reference purported to be Withanolide D was instead Withanolide A

Penman K[1], Bone KM[2], Hayes P[3], De Voss J[3], Agarwal A[4], Murali B[4], Mundkinajeddu D[4], Mayachari A[4], Shenoy R[4], Lehmann RP[1]
[1]Mediherb Research Laboratories 3/85 Brandl St Eight Mile Plains Brisbane, 4113, Australia; [2]School of Health Science, University of New England, Armidale, 2031 Australia; [3]School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, 4072, Australia; [4]Natural Remedies Pty Ltd 5B Veerasandra Indl. Area 19thKM Stone, Hosur Road Bangalore 560100 India

Formal and accurate identification of a number of withanolides in the HPLC-PDA traces of Withania somnifera samples was required as part of the development of a routine assay for samples of this herb. A large number of withanolides have been isolated from Withania somnifera, many with only minor structural differences between them. Sophisicated NMR techniques provide the best approach for the discrimination of these similar compounds [1,2,3] and this approach was used to elucidate the structural differences as well as confirm the identities of commercially available reference compounds. Detection of an incorrectly assigned reference material was made. NMR data proved that the Withanolide D (structure 1) standard was in fact Withanolide A (structure 2). This work reinforces the importance of applying rigorous checks to reference materials. **References:** [1] Gottlieb, H. et al. (1981) Org. Mag. Res. 16(1):20 – 25. [2] Gamoh, K. et al. (1984) J.Chem.Soc. Perkin Trans.1: 449 – 454. [3] Pelletier, S. et al. (1979) J. Nat. Prod. 42(5): 512 – 521.

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LC-MS analysis of alkamides in *Echinacea purpurea* and *Echinacea pallida* cultivated in Turkey

Kartal M[1], Kan Y[2], Gülpinar AR[1]
[1]Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Tandogan-Ankara, Turkey; [2]Selcuk University, Agricultural Faculty, Department of Field Crops, 42070 Kampus-Konya, Turkey

Root extracts of the *Echinacea purpurea* (L.) Moench and *E. pallida* (Nutt.) Nutt. are widely used for medicinal purposes. Effective quality control of these extracts requires rapid methods to determine their chemical composition [1]. *E purpurea and E. pallida* were cultivated in the experimental farm of Selcuk University, Faculty of Agriculture in ecological conditions of Konya. *Echinacea purpurea* and *E. pallida* contain caffeic acid derivatives and alkamides. They are known as immunstimulant plants. Their specifications and some analysis methods have been described in the monographs of the European Pharmacopea [2]. Liquid chromatography is a fundamental separation technique in the life sciences and related fields of chemistry. Mass spectrometers also generate three dimensional data. In addition to signal strength, they generate mass spectral data that can provide valuable information about the molecular weight, structure, identity, quantity, and purity of a sample. We used an HPLC/electrospray ionization mass spectrometry method for simultaneous analysis of alkamides in the root extracts of *Echinacea purpurea* and *Echinacea pallida* cultivated in Turkey. The analysis was carried out with reversed phase HPLC coupled to electrospray ionization mass spectrometry (ESI-MS). The mass spectra were taken on a Waters ZQ micromass LC-MS spectrometer (Waters Corporation, Milford, MA, USA) by using ESI(+) method. The mass spectrometer was operated with a scan range of 90 – 900 *m/z*, a capillary temperature of 200 °C, a maximum column pressure of 300 bar. Voltages of capillary, cone, extractor and RF lens are 3.6 kV, 24 V, 2 V, 0.1 V. İt was tuned in the positive ion mode. Total analysis time was

20 minutes. Alkamides (undeca-2Z,4E-diene-8,10-dynoic acid isobutylamide, undeca-2E,4Z-diene-8,10-dynoic acid 2-methylbutylamide, dodeca-2E,4E,8Z,10Z-tetraenoic acidisobutylamide) were separated and determined using a xterra MS C18 (4.6 x 250 mm; 5 µm) column by isocratic elution with flow rate 0.5 ml/min. The mobile phase composition was methanol- Acetonitrile- 0.1% TFA in water (55:35:10) (v/v/v). **References:** [1] Cech, N. B. et al. (2006) J. Chromatogr. 1103 (2): 219 – 228 [2] European Pharmacopoeia (2005), 5th edition, Council of Europe, Strasbourg.

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Analysis of galanthamine and related alkaloids in plant extracts and during the purification process by GC-MS

Berkov S, Codina C, Viladomat F, Bastida J
Departament de Productes Naturals, Facultat de Farmàcia, Universitat de Barcelona, 08028 Barcelona, Spain

Recently, GC-MS has been applied for the search and quantification of galanthamine in plant samples [1, 2] but not for monitoring of the extraction process and the active substance which is the aim of the present work. Ten alkaloids were detected in the samples (A and B – plant extracts of *Leucojum aestivum* L., C and D – products of galanthamine purification). Eight of them were identified by comparing their MS and Kovats retention indexes (*RI,* HP-5 MS column) with those of authentic compounds isolated in our laboratory. Compound **6** was identified by NIST 05 database and **2** (galanthamine type) was left unidentified. GC-MS provided rapid information on the ratio of the alkaloids in the plant extracts, reliable identification of the impurities which are at trace amounts (< 0.1% of TIC) in the active substance (sample D) and structural information on the unidentified compounds. The presence of **2** (0.1%) which co-eluted in the peak tail of **1** was unequivocally confirmed after deconvolution of its MS by AMDIS® 2.64 (NIST). The combination of GC-MS, *RI* and mass spectral deconvolution is a powerful tool, providing a high specificity for the analysis of impurities at trace amounts and identification of co-eluted compounds with chemical structures similar to galanthamine.

Compound	*RI*	A	B	C	D
Galanthamine (**1**)	2405	65.71	74.94	94.41	99.78
A (**2**)	2422		1.8	0.05	0.1
N-Demethylgalanthamine (**3**)	2440	0.15	11.16	4.6	0.02
Epigalanthamine (**4**)	2450		0.13	0.02	0.02
Narwedine (**5**)	2479	1.47	3.76	0.88	0.04
Anhydrolycorine (**6**)	2501	0.53	0.34		
Demethylmaritidine (**7**)	2507		1.25		
N-Allylnorgalanthamine (**8**)	2540		0.06	0.02	
Lycorine (**9**)	2746	31.91	5.09		
N-Formylnorgalanthamine (**10**)	2812	0.24	1.49		
Nonalkaloid compound	2548			0.02	0.02
Nonalkaloid compound	2827				0.02

Acknowledgements This work was partially financed by the Generalitat de Catalunya (2005SGR-00020). S. Berkov thanks the Spanish Ministerio de Educación y Ciencia for a research fellowship (SB2004 – 0062). The authors thank Galen-N Ltd (Bulgaria) for the provided samples. **References:** [1] Gotti, R. et al. (2006) J Pharm Biomed Anal 42: 17 – 24. [2] Berkov, S. et al. (2007) Phytochem Anal (in press).

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Direct metabolic fingerprinting by NMR spectroscopy as a new method for the analysis of herbal tinctures

Politi M, Prieto JM, Heinrich M
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London (UK), WC1N 1AX

In natural product chemistry, NMR spectroscopy has traditionally been used for identification of pure compounds, while recent efforts have focused on the use of NMR for fingerprinting in combination with multivariate data analysis [1]. Here we describe the application of an NMR-based approach for direct fingerprinting herbal tinctures. This method does not need the evaporation of the solvent; only the addition of 10% of deuterated water to the tinctures was necessary for the acquisition of the spectra. Compared to the HPLC approach [2], this method does not require the optimization of the conditions for each specific herbal tincture. In fact, the same NMR experiments were here successfully applied for fingerprinting tinctures prepared from different medicinal plants. Simple proton spectra of the herbal tinctures show practically exclusively the intense signals of the solvents (ethanol and water). However, using different NMR experiments, such as 1D-DOSY and the 1D-NOESY pulse sequence with suppression of water and ethanol signals [3], it was possible to reduce the intensity of the solvent signals in the spectra and a better resolution of the metabolic profiles was then achieved. Tinctures of Cannabis (prepared in our lab), Ginkgo and Echinacea (commercial products) were successfully analyzed with this rapid and versatile method. The major limitation for this NMR approach is due to variation of the chemical shifts that occur when a component is dissolved in different solvents, and tinctures containing different amount of alcohol must be compared carefully. *Acknowledgment:* We thank the European Commission for financial support under the FP6 (COOP-CT-2004 – 512696). **References:** [1] Sumner, L.W., et al. (2003) Phytochemistry, 62: 817 – 836. [2] Bilia, A.R., et al. (2001) Chromatographia, 53: 210 – 215. [3] Duarte I., et al. (2002) J. Agric. Food Chem. 50: 2475 – 2481.

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The potential of PCR-related methods to identify medicinal plants in herbal medicinal products

Kersten T[1,2], Daniel C[2], König GM[2], Knöß W[1]
[1]Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger-Allee 3, 53175 Bonn, Germany; [2]Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, 53115 Bonn, Germany

Methods established and described in pharmacopoeias to test the identity of medicinal plants are generally based on a morphological anatomical analysis or on the analytics of natural products. Nevertheless, in some cases a reliable confirmation of the raw material of medicinal plants and its identity or purity is difficult. Various molecular biological techniques, including PCR-based methods, have been used for authentication of medicinal plants and herbal drugs during the last years [1,2,3]. However, there is a need not only for reliable authentication of raw plant material but also for suitable methods to verify the medicinal plants used in later steps of production. Our approach is to establish a validated method based on Polymerase Chain Reaction which should be applicable for a broad range of medicinal plant species. As major model for analytical development and validation, we have chosen the genus Matricaria because of the availability of different preparations in the market. In addition to RAPD techniques we investigated the potential of amplification of specified parts of the nuclear ribosomal DNA-cluster. We were able to demonstrate, that amplifiable DNA could be successfully extracted from herbal preparations such as fluid or dry extracts and finished herbal medicinal products like tablets. However, RAPD-fingerprints were not suitable to identify the raw material: due to the degradation of DNA during processing of the medicinal products data were hardly reproducible. Several fragments of the ETS- and ITS-regions were amplified and sequenced. Thus, comparisons of sequences with data bases may be useful to identify medicinal plants by this method. **References:** [1] Joshi, K. et al. (2004). Curr. Sci. India 87(2): 159 – 165. [2] Slanc, P. et al. (2006). Pharmazie 61(11): 912 – 915. [3] Techen, N. et al. (2004). Curr. Med. Chem. 11(11): 1391 – 1401.

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Accumulation of phenolic compounds by *in vitro* cultures of Spanish Sage (*Salvia lavandulifolia* L.)

Braga PSC[1], Andrade PB[2], Gonçalves RFG[2], Valentão P[2], Seabra RM[2], Fernandes-Ferreira M[1]
[1]Departamento de Biologia, Escola de Ciências, Universidade do Minho, Campus de Gualtar, 4710 – 057 Braga, Portugal; [2]CEQUP/Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 4050 Porto, Portugal

Spanish sage (*S. lavandulifolia* L.) grows wild in Spain and Southwest France and is used in perfumes and food flavouring [1]. Several reports indicate that extracts of this species have anticholinesterase, antioxidant and anti-inflammatory effects [2]. Our group has been studying the production of secondary metabolites by *in vitro* cultures of *S. lavandulifolia*, in order to evaluate its potential use for the production of phytomedicines. To our knowledge, this type of study, including the establishment of *in vitro* cultures, was not yet performed for this species. In this presentation the accumulation of phenolic compounds by *in vitro* cultures of *S. lavandulifolia* is reported. Aseptic shoot cultures were established from surface sterilized seeds and maintained by culturing nodal segments excised from the *in vitro* growing seedlings. Calli cultures were induced from leaves of these shoots. Suspension cultures were established by transference of calli to liquid medium. Dried biomass of *in vitro* shoots, *calli* and suspended cells was extracted with acetone and analyzed by HPLC-DAD. An extract from the aerial parts of in Nature growing *S. lavandulifolia* (*in vivo* plants) was also analyzed for comparison. Total phenolics in the different samples, some of them unidentified, decreased according to the following order: *in vivo* plants > *in vitro* shoots > suspension cells > calli. Luteolin, apigenin, cirsimaritin and genkwanin were identified in both shoots and *in vivo* plants. Salvigenin, the main flavonoid from shoots, was not detected in Nature growing plants. This one, as well as other flavonoids, was not detected in calli neither in suspended cells. Phenolic diterpenes accumulated in higher amounts in Nature growing plants and in shoots and were not detected in suspended cells. The accumulation of phenolic acids, namely rosmarinic acid, on the contrary, was higher in suspended cells and shoots than in *in vivo* plants. *Acknowledgements:* This work was sponsored by EU (FSE/FEDER) and Portuguese Republic Government (FCT) through the Grant SFRH/BD/18908/2004 and the Project SageBiotech (POCTI/AGR/62040/2004). **References:** [1] Kintzios, SE (2000), Sage, The genus Salvia, Harwood academic publishers, Amsterdam, Netherlands. [2] Perry, NSL (2003) Biochem Pharmacol Behav 75: 651 – 659.

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A simple HPLC-method for the quality assurance of Noni (*Morinda citrifolia* L.) juice

Basar S, Westendorf J
Institute of Experimental and Clinical Pharmacology and Toxicology, University Clinic Hamburg Eppendorf, Vogt-Kölln-Straße 30, D-22527 Hamburg, Germany

In 2003 pasteurized Noni (*Morinda citrifolia)* juice was approved as novel food by the EU commission. In the meantime the juice became very popular as a remedy against a broad variety of physical disorders. In order to investigate the quality of Noni juice products on the market, we developed an easy HPLC method based on an extraction of 1 ml juice samples with ethyl acetate. Two pharmacologically active marker compounds, scopoletin and 2(*E*)-4(*Z*)-7(*Z*)-decatrienoic acid (CPX), were used for the characterization of the juices. It was shown that both compounds are present in fresh Noni fruits independent of the ripeness. The location of harvest was also of minor effect on the concentration, however an increase of the scopoletin content was observed after fermentation of the Noni juice. Considerable differences in the concentration of the marker compounds were observed after investigation of a variety of about 17 commercial Noni juices. The content of original Noni juice in these brands varied between almost 100% to less than 1%, even if these juices were labelled as "prepared from 100% pure Noni juice". Our investigations demonstrate the importance of a quality control for Noni juices and provide an easy and quick analytical method for this purpose.

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A rapid TLC screening method for the quality control of *Sedum acre* L. homeopathic tinctures

Gehrmann B[1], Melzig MF[2]
[1]Einhorn-Rats-Apotheke, Markt 10 – 12, 25813 Husum, Germany; [2]Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin, Germany

Sedum acre L., wall pepper or stone crop is a perennial member of the Crassulaceae family native to Europe, Western Siberia, the Caucasian region and to North America. Ethanolic tinctures of Sedi acris herba are used in homeopathy in the treatment of haemorrhoids and anal pain [1, 2]. *Sedum acre* L. is monographed in the homoeopathic pharmacopoeia (HAB) 2005 [2]; however, the described TLC procedure is a general comparative method. Thus, we propose a rapid and simple TLC investigation applying the H-separating chamber and using different solvent systems containing e.g. ethyl acetate, methanol, water at different proportions as mobile phase (optimum 75+20+10) and diverse silica gel plates (e.g. Si 60, HPTLC-, RP-material) as stationary phase. The optimised TLC conditions are performed on different samples of homeopathic wall pepper tinctures and provide chromatograms showing satisfying distributions of characteristic zones, e.g of arbutin, quercitrin, piperidin derivatives, in the range of R_f values from about 0.2 to 0.8 (detection: UV 254, UV 366, natural product reagent). The described TLC methods require short running times of about 4 min, minute sorbens plates (5 x 5 cm), and small amounts of solvents (mobile phase approx. 2 ml/performance) as well as of homeopathic tinctures (application of 10 – 20 µl/performance). These rapid TLC-analytical investigations may be proposed for an improved HAB monograph of *Sedum acre* L. **References:** [1] Brendler Th., Grünwald, J., Jänicke, Chr., Editors. (2003) Herbal Remedies, CD-ROM, medpharm, Scientific Publishers, Stuttgart. [2] Deutsches Homöopathisches Arzneibuch (2005), Monograph Sedum acre (Edition 2001).

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Flavonoid and polyphenol content of *Aloe vera* (*Aloe barbadensis* Mill.) flowers and their *in vitro* antioxidative capacity

Keyhanian S, Stahl-Biskup E
University of Hamburg, Institute of Pharmacy, Dept. of Pharmaceutical Biology and Microbiology, Bundesstrasse 45, D-20146 Hamburg, Germany

Aloe vera has become very popular in cosmetics and nutraceutical formulations due to the ascribed beneficial properties of the inner gel from the fleshy leaves (Aloe vera gel). The big yellow flowers of *A. barbadensis* are not of commercial interest yet although the *Aloe* flowers were shown to contain various biologically active substances like e.g. phenolcarboxylic acids and flavonoids [1,2,3] and a few products, e.g. "Aloe flower herbal tea", have appeared on the market. The aim of this study was to investigate the correlation of the polyphenol content and the antioxidative capacity. Therefore the polyphenols and their flavonoid portions of three batches of the years 2003, 2004, and 2005 were determined and the *in vitro* antioxidative capacity was measured. The polyphenol content was analysed with the Folin-Ciocalteu reagent (λ= 760 nm, water extracts) and the flavonoid content with oxalic/boric acid (λ= 410 nm, 60% ethanolic extracts). The antioxidative capacity was determined by the TEAC method [4] and the ORAC method [5]. The polyphenol content of the dried Aloe flowers ranged between 0.73% – 1.01% (± 0.05%) and the flavonoid content between 0.24% – 0.34%

(± 0.01%) correlating from batch to batch. The hydrophilic antioxidative capacity amounted to 85.7 – 94.9 (± 0.50) µmol Trolox equivalent (TE)/g dried *Aloe vera* flowers (TEAC) and 79.8 – 134.2 (± 5.6) µmol TE/g dried Aloe vera flowers (ORAC). The individual data show a direct correlation between the antioxidative capacity and the polyphenol content on the one side and the flavonoid content on the other side. **References:** [1] Keyhanian, S., Stahl-Biskup, E. (2007) Planta Med.: in press. [2] Hou, D.Y. et al. (2004) J Zhejiang University 31: 438 – 41. [3] Sigler, A., Rauwald, H.W. (1994) Z. Naturforsch. 49 c: 286 – 92. [4] Re, R. et al. (1999) Free Radic. Biol. Med. 26: 1231 – 37. [5] Prior, R. L. et al. (2003) J. Agric. Food Chem. 51: 3273 – 79.

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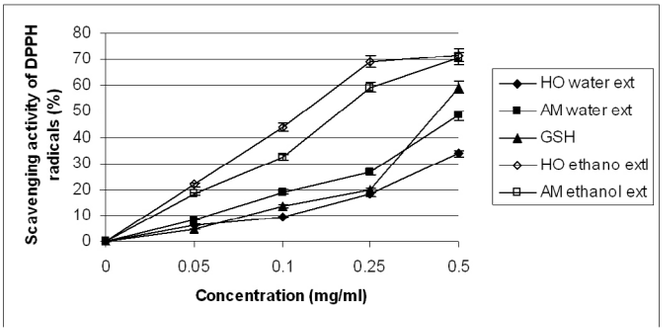
Antioxidant activity, phenolics and flavonoid content of some selected Romanian medicinal plants

Alexandru V, Balan M, Gaspar A, Coroiu V
National Institute R&D for Biological Sciences Bucharest, 296 Spl. Independentei, 060031, Romania

Extracts of *Achillea millefolium* L. (AM) and *Hyssopus officinalis* L. (HO) are widely used as wound – healing agents in Romanian traditional medicine. In the present study, we tested the antioxidant activity of ethanol and water extracts prepared from selected plants. Total phenolics [1], total flavonoids [2] and 1,1-Diphenil-2-Picrazyl Hidrazil Radical (DPPH) scavenging activity [3], were determined spectrophotometrically. Ethanol extract of AM had the highest phenolic content (118 ± 1.9 mg caffeic acid/g D.W), followed by ethanol extract of HO (86.7 ± 2.6 mg caffeic acid/g D.W.) and water extracts. The flavonoid content of the ethanol extracts of AM (36.3 ± 1.6 mg quercetin/g D.W.) was higher then that of HO (31.4 ± 1.3 mg quercetin/g D.W.) and water extracts. We found that ethanol extracts had higher radical scavenging activity then standard control (59.28 ± 3.8%), reduced gluthatione (GSH) and water extracts (see the figure). The highest scavenging effect was observed for ethanol extract of HO (71.59 ± 3.5%), followed by AM (70.36 ± 3.2%) ethanol extract, at 0.5 mg dry matter/ml concentration. In conclusion, AM and HO possess antioxidant activity. The therapeutic benefit of these plants might be due to antioxidant effects exerted by their phenolic compounds.



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Nanosystems with taxifolin for solid dosage form and its bioavailability *in vitro*

Urakova I[1], Pozharitskaya O[1], Shikov A[1], Makarov V[1], Tikhonov V[2]
[1]St-Petersburg Institute of Pharmacy, 27 – 424, Partizanskaya str., 195248, St.-Petersburg, Russia, [2]"Diod" Ltd, 11[a]Derbenevskaya ave., 113114, Moscow, Russia

Flavonoids are naturally-occurring substances possessing some positive effects on human health. Taxifolin is widely distributed in the rind of Siberian and Dahurian larchs. In recent years taxifolin has shown capillaryprotective, anti-inflammatory, antioxidant, and hepatoprotective activities [1 – 3]. Flavonoids are slightly soluble in water and show a slow dissolution rate from solid oral forms, restricting their use in therapy. It is well known that the drug dissolution rate can be the critical limiting step in the bioavailability after oral administration and the therapeutic effect of the drug. The aim of this work was to increase the dissolution rate of taxifolin. Nanosystems with taxifolin were formed by solid dispersion polymers: polyethylene glycol 6000 (PEG) and Kollidone 17PF (PVP). Solid dispersions of taxifolin (SDT) were prepared in different ratios of drug:carrier (1:5; 1:7; 1:10; 1:13, w/w) by using melting (PEG) and solvent (PVP) methods. The differential scanning calorimetry (DSC) and microscopy data were applied to estimate the physical state and interactions of taxifolin with polymers. The dissolution tests of SDT were performed according to the basket method. Water, simulated gastric (pH 1.2) and intestinal (pH 6.8) liquids were dissolution medium. The DSC and microscopy data have shown that in solid dispersion systems a crystal structure of pure taxifolin is not determined. In SDT there was a considerable enhancement in the dissolution rates of taxifolin in comparison with the pure compound. The best bioavailability in vitro of taxifolin were obtained from SDT with 1:10 drug:carrier ratio. The values of dissolution constants determined by first order model for SDT dissolution were markedly higher than for pure drug dissolution in all dissolution media. The constants of rate release of SDT (PVP), SDT (PEG) and taxifolin in water were $0.0819 \pm 0.004\ min^{-1}$, $0.0398 \pm 0.002\ min^{-1}$ and $0.0097 \pm 0.001\ min^{-1}$, respectively. It was established that polymer nature and its ratio with taxifolin allow to develop nanosystems with controlled release. **References:** [1] Habtemariam et al. (1997) J. Nat. Prod. 60: 775 – 778. [2] Nielsen et al. (1998) Xenobiot. 28: 389 – 401. [3] Teselkin et al. (2000) Phytother. Res. 14: 160 – 162.

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Self-microemulsifying drug delivery systems as nanosystems for bioavailability enhancement of taxifolin *in vitro*

Karlina M, Pozharitskaya O, Shikov A
St-Petersburg Institute of Pharmacy, 27 – 424 Partizanskaya str, 195248, St.-Petersburg, Russia

Taxifolin, allocated from wood of larch, has a wide spectrum of biological activity, showing antioxidative, capillaroprotective, anti-inflammatory and antithrombotic effects [1,2]. Taxifolin is a poorly water soluble compound and has low bioavailability. There is an actual development of new drug forms on the basis of taxifolin. Self-microemulsifying drug delivery systems (SMEDDS) are perspective drug delivery systems for oral, nasal and transdermal applications. They represent thermodynamically stable systems with high bioavailability of the compounds. The purpose of the present work was to prepare SMEDDS of taxifolin and to study the release rate of taxifolin *in vitro*. A water-in-oil nanoemulsion containing 2% taxifolin, surfactant (Tween 80) and cosurfactant (propylene glycol), oil phase (Labrafil® M 1944 CS) and water was prepared. *In vitro* release was investigated by the USP 28 paddle over disc method at 100 rpm (temperature 32 ± 1 °C, dissolution medium water) at modeling of transdermal application and by using the USP 28 paddle method at 100 rpm (temperature 37 ± 1 °C, dissolution medium 1-octanol and water [3]) at modeling of oral application. Concentrations of taxifolin were analyzed by RP-HPLC. It was shown, that

release of taxifolin from nanoemulsion is described by the equation of first order kinetics, constants of rate release for modeling of transdermal and oral application were 0,9306 and 0,8479 h^{-1}, accordingly. Taxifolin release from SMEDDS was about 95% in 2 and 3 h in models of transdermal and oral application, accordingly. Particle size in the nanoemulsion was evaluated upon dilution with aqueous media as described by [4]. It was concluded that bioavailability of taxifolin was enhanced greatly by SMEDDS. Alternative mechanisms, such as improved lymphatic transport pathway, other than improved release may contribute to enhancement of bioavailability of taxifolin. **References:** [1] Middleton E. et al. (2000) Pharmacol. Rev. 52: 673 – 751. [2] Silva M.M. et al. (2002) Free Radical Res. 36: 1219 – 1227. [3] Karlina M. et al. (2006) Voprosy Biol. Med. Pharm. Chem. 9: 42 – 46. [4] Ghosh P.K. et al. (2006) AAPS Pharm Sci Tech, 7: art. 77

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Comparison of ginkgolide, bilobalide and flavonoid content in tea from *Ginkgo biloba* leaf tea-bags with standardized dry extract drugs

Kosman V, Pozharitskaya O, Urakova I
Interregional Center "Adaptogen", 47/5, Piskarevskiy pr, 195067, St-Petersburg, Russia

Ginkgo biloba L. (Ginkgoaceae) is a famous medicinal plant. The biological activity of Ginkgo extracts is associated with the sesquiterpenoid bilobalide, diterpenoid lactones – ginkgolides A, B, C and J, and flavonoids – quercetin and kaempferol glycosides [1]. Extracts of *G. biloba* leaves are widely used for the treatment of cerebrovascular diseases [2,3]. It was shown that ginkgolides A, B, C can inhibit the platelet-activating factor (PAF) and *G.biloba* drugs are natural PAF antagonists [4]. The aim of the present work was to compare concentrations of major biologically active compounds in *G. biloba* leaf tea from tea-bags and drugs with standardized dry extract. Terpenes were analyzed by HPTLC with densitometry, flavonoids – by RP-HPLC with UV-detection. Ginkgolide A (Ga), ginkgolide B (Gb), ginkgolide C (Gc), bilobalide (B), rutin, quercetin (QU), and kaempferol (KF) were used as reference substances (Sigma, Germany). Analytical methods were developed and validated. Infusions of tea-bags of *G. biloba* leaves (St-Medipharm, Russia) and capsules Bilobil (KRKA, Slovenia) as reference drugs were investigated. The contents of the main components were compared. The main data are presented in the table. **Table:** The results of qualitative-quantitative analysis of major Ginkgo compounds

Sample	Ga	Gb	Gc	B	Total terpens	QU	KF	Total flavonoids
Tea from tea-bag (mg/100 ml)	0.55 ± 0.10	0.40 ± 0.08	0.80 ± 0.20	0.25 ± 0.05	2.0 ± 0.4	2.5 ± 0.2	2.2 ± 0.2	12.0 ± 0.9
Bilobil (mg/1capsule)	0.69 ± 0.14	0.46 ± 0.09	0.58 ± 0.12	0.91 ± 0.18	2.6 ± 0.5	2.0 ± 0.1	1.8 ± 0.1	9.8 ± 0.7

It was shown that 100 ml of tea obtained from one tea-bag of *G. biloba* and one Bilobil capsule have similar concentrations of ginkgolides, bilobalide and flavonoids. Tea making procedure during 5 – 7 min is enough for the extraction of terpenoids and flavonoids. Thus, application of freshly prepared tea from tea-bags is similar to administration of drugs with a dry extract of *G. biloba* leaves. **References:** [1] Van Beek T.A. (2002) J. Chromat. 967: 21 – 55. [2] Kressmann S. et al. (2002) J. Pharm. Pharmacol. 54: 661 – 669. [3] Sierpina V. et al. (2003) American Family Physician. 68: 923 – 926. [4] Smith P.F. et al. (1996) J. Ethnopharmacol. 50: 131 – 139.

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Cascade of bioactive compounds from *Plantago lanceolata* L. cultivated in Romania

Pintilie G, Paraschiv I, Manaila N, Ocnaru D, Armatu A, Colceru S, Pirvu L, Rughinis D, Nita S
National Institute for Chemical- Pharmaceutical R&D, 112 Vitan st., code 031299, Bucharest, Romania

The aim of this work: a cascade method to isolate triterpene acids, flavonoids, phenylpropanoids, iridoid glycosides, polysaccharides from *Plantago lanceolata* L. cultivated in Romania, in order to further study their possible antagonistic or synergistic actions concerning antiinflammatory and antimicrobial effects. The dried powdered vegetable material was succesively extracted with dichloromethane, methanol and water in an ultrasound field. The components were isolated as follows: -triterpene acids: from dichloromethane extract through extraction with acetone, ethanol, discolouration and crystallization; -flavonoids: from methanolic extract through extraction with ethyl acetate and CC on a polymeric phase with methanol/water as eluent; -phenylpropanoids and iridoid glycosides: from methanolic and aqueous extracts through extraction with n-butanol and CC on a polymeric phase with a gradient of methanol/water; -polysaccharides: from aqueous extract through precipitation in ethanol, discolouration and repeated precipitations. Quantification of constituents was carried out through HPLC and TLC using ursolic and oleanolic acid, apigenin-7-glycoside, luteolin-7-glycoside, acteoside, aucubin and catalpol as reference substances. HPLC -column type: Eurospher 100 – 5C 18, -mobile phase: -methanol/acetonitril/ortho-phosphoric acid pH 2.5 (40/10/50 v/v/v) (flavonoids); -methanol/ortho-phosphoric acid pH 3 (40/60 v/v) (phenylpropanoids); -acetonitril/methanol/water (3/5/92 v/v/v) (iridoids); TLC -stationary phase: Silica gel 60F-254, -mobile phase: -ethyl acetate/formic acid/water (20/2/3 v/v/v) (flavonoids); -ethyl acetate/methanol/10% acetic acid (75/20/5 v/v/v) (phenylpropanoids); -chloroform/methanol/2N acetic acid (70/30/6 v/v/v) (iridoids); -petroleum ether/benzene/ethyl acetate/ethanol (20/10/7/0.5 v/v/v/v) (triterpene acids). The obtained products are appropriate to be pharmacologically tested.

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Antioxidant activity of compounds in *Euphrasia officinalis* L. – revaluation of a traditional medicinal plant

Blazics B, Kéry Á
Semmelweis University, Department of Pharmacognosy, Budapest, Üllői u. 26., H-1085, Hungary

Euphrasia officinalis L. (eyebright) is a valuable plant in traditional medicine as a remarkable remedy for inflamed eye disorders since ancient times. Our knowledge is still lacking exact details in phytochemical composition, and way of mechanism of active compounds of eyebright. Our aim was to contribute to the phytotherapeutical revaluation of *Euphrasia officinalis* L. by investigating the main chemical groups of the herb and to understand their role in the inflammatory process, in which free radicals are considered to play a significant role via different biochemical pathways. After a successive extraction of *Euphrasiae herba* with n-hexane, chloroform, ethylacetate and methanol, as follows, the main compounds were identified as iridoids and phenolics/flavonoids. The content of Flavonoids, polyphenols, tannins and hydroxycinnamic derivatives were determined, results are: 0.38 g/100 g, 1.47 g/100 g, 0.56 g/100 g, 1.97 g/100 g, respectively. Then preliminary assays were performed to study the free radical scavenging activity (DPPH, ABTS and TBA assay). The lyophilised decoctum of *Euphrasiae herba* showed a considerable scavenging activity, so it was reasonable to elucidate the main chemical groups, and find correlation between the active compounds and the free radical scavenging activity. Methanolic extract of eyebright herb was fractionated by polyamid column chromatography using water (7 fractions: iridoids) and methanol (8 fractions: phenolics/flavonoids). For the qualitative analyses of the fractions

TLC, HPLC and LC-MS/MS methods were developed and used. We have found that the fraction containing simple caffeoyl-derivatives provided the highest scavenging activity in both DPPH and ABTS assays. Those fractions which were rich in flavonoids were also notable antioxidants. The scavenging effect of *Euphrasia officinalis* L. is a combination of the iridoids and phenolics/flavonoids, however the phenolic components basically determine the effect.

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Anti-inflammatory and anti-leukemic activities of the flower extracts from Thai medicinal plants

Noysang C[1], Schmidt K[2], Efferth T[3], Luanratana O[4], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University, Universitaetsplatz 4, 8010 Graz, Austria;[2]Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, Karl-Franzens-University, Universitaetsplatz 4, 8010 Graz, Austria; [3]German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany;[4]Faculty of Pharmacy, Department of Pharmacognosy, Mahidol University, 10400 Bangkok, Thailand

Based on the traditional medical use in Thailand, the flowers of six medicinal plants were investigated for anti-inflammatory and anti-leukemic activities. In the screening, the *n*-hexane, dichloromethane, methanol and water extracts were tested for anti-inflammatory activity by the inducible nitric oxide synthase assay (iNOS) [1] and for anti-leukemic activity by a growth inhibition assay against human CCRF-CEM leukemia cell line [2]. The results from the iNOS assay showed that the tested extracts were only weak inhibitors with inhibition rates of 0 – 53% at 10 µg/ml. The *n*-hexane extracts of *Mammea siamensis* (Miq.) T. Anderson, *Mesua ferrea* Linn. and the dichloromethane extracts of *Mesua ferrea* Linn. and *Michelia alba* DC. exhibited very strong cytotoxic effect against human CCRF-CEM leukemia cells at 10 µg/ml with inhibition values of 85 – 99%. Bioassay-guided fractionation of the active extracts is in progress. *Acknowledgements:* C.N. is thankful to Rajamangala University of Technology Thanyaburi for a Ph.D. scholarship. **References:** [1] Baer, H.P. et al. (1995) Life Sci. 57: 1973 – 80. [2] Efferth, T. et al. (2002) Blood Cells Mol. Dis. 28: 160 – 8.

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The Mercury Concentration in Particular Parts of *Taraxacum officinale* (Dandelion) in different Areas of Slovakia

Kimáková T, Bernasovská K
Institute of Hygiene, Faculty of Medicine University of P. J. Safarik, Šrobárova 2, Kožice, 041 80, Slovakia

The main aim of the presented work was determination of the mercury concentration in particular parts of the plant *Taraxacum officinale* (Dandelion) in the chosen areas of Slovakia. We monitored mercury (Hg) presence in plant *Taraxacum officinale* (n = 53) and we also analyzed the soil. For finding the concentration of mercury in some commodities we used the analytic method for stating mercury by fireless atomic absorption spectrometry AAS AMA 254 [1]. Findings were put into 3 groups from zero level up to permissible limit. 2 samples were put within the span of 0.01 mg/kg, within 0.01 mg/kg up to 0.02 mg/kg Hg, there were 5 samples and in the last one from 0.02 mg/kg up to 0.03 mg/kg there were 11 samples. Samples over the limit 0.03 mg/kg contained to 35. The measured values of samples were in the scale from 0.01 mg/kg up to 7.05 mg/kg Hg. Analysing the plants we measured the mercury concentration in the roots, stalks and leaves. We found nearly linear dependence between the amounts of mercury in particular parts of the plant. The lowest concentration was in roots – 0.00037 mg/kg, the higher one in stalks within 4.83 mg/kg and 6.65 mg/kg and the highest concentration was measured in leaves – 11.56 mg/kg. In all plants from soil contaminated by Hg amounts over the limited concentration of Hg were measured [2]. We have to take into account that the soil is important and the amount of mercury is important as well as the distribution of mercury in the parts of a plant. That is the reason why it is important to continue monitoring the mercury concentration in medicinal plants. **References:** [1] Handbook – AAS AMA 254. (1998) Praha. [2] Kimáková T., Bernasovská K. (2005): Slovak Veterinary Journal. XXX:6. 369 – 371 p.

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Active constituents profiling of *Rhodiola rosea* L.

Egger P[1,2], D'Ambrosio M[1], Aiello N[2], Contrini C[1], Fusani P[2], Scartezzini F[2], Vender C[2]
[1]University of Trento, Laboratory of Bioorganic Chemistry, via Sommarive 14, 38050 Trento-Povo (TN), Italy; [2]CRA-ISAFA (Forest and Range Management Research Institute), piazza Nicolini 6, I-38050 Trento-Villazzano, Italy

A rapidly upward demand for medicinal and aromatic plants entails the revive and increasing interest in natural products. The plant constituents are greatly influenced by climatic factors as well as by variations among populations coming from different geographical regions. Five species typical of Trentino Alpine region are evaluated within a project named PARMA (**P**iante **A**limentari, a**R**omatiche e **M**edicinali **A**lpine = edible, aromatic and medicinal plants of the Alps). The third analysed species *Rhodiola rosea* L. (golden root or rose root) is a perennial plant widely distributed at high altitudes in arctic and mountainous regions. Its long use in traditional medicine is connected to biological activities such as antiallergic effects, effect to memory, antidepressant, anti-aging and anti-inflammatory properties, and effects in cancer therapy. The authors perform cultivation trials in the Trentino region on 1250 m, starting in 2004 and working out the proper agronomic technology. The phytochemical characterisation of dried roots was studied by LC-ESI/MS^n, quantifying the six most important constituents salidroside, tyrosol, rosarin, rosavin, rosin and rosiridin, which are isolated from 3 year old plants and derive from four different accessions. In comparison to cited data [1], higher concentrations of salidroside (5.58 ± 0.95 mg/g), rosavin (9.56 ± 0.21 mg/g) and rosiridin (6.91 ± 1.03 mg/g) are detected. An additional quality-parameter is introduced by the separated harvest of male and female plant material. In general, higher amounts of salidroside and tyrosol are found in male plants, whereas rosarin, rosavin, rosin and rosiridin are more abundant in female plants. *Acknowledgement*: This work has been supported by the grant of the Autonomous Province of Trento, 9th July 2004 no. 1587. **References:** [1] Ganzera, M. et al. (2001) Chem Pharm Bull 49: 465 – 467.

P 270

Spectrophotometric determination of acteoside in the herbal drugs Plantaginis lanceolatae folium and Plantaginis majoris folium

Gažpar Randić Z[1], Malež [2]
[1]JADRAN Galenic Laboratory Ltd., Pulac b.b., 51000 Rijeka, Croatia; [2]Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39, 10000 Zagreb, Croatia

Plantaginis lanceolatae folium (*Plantago lanceolata* L., *Plantaginaceae*) and Plantaginis majoris folium (*P. major* L.) are well known herbal drugs that have been used for treating inflammation of the mouth and throat. Chemical investigations have detected various constituents. Acteoside as phenylethanoid is a major phenolic constituent and reportedly has antiphlogistic, antihepatotoxic, antioxidant, spasmolytic, antimetastatic and antiviral activity. According to the requirement of Ph Eur Plantaginis lanceolatae folium should contain not less than 1.5% of total ortho-dihydroxycinnamic acid derivatives expressed as acteoside calculated with reference to the dried drug. Leaves of the species *P. lanceolata* and *P. major* were collected at the different locations in Croatia and investigated in comparison with Plantaginis lanceolatae folium from commercial origin. The content of acteoside was determined spectrophotometrically according to Ph Eur and ranged from 2.28 to 7.61%. Leaves of

P. lanceolata contained a larger quantity of acteoside compared to the leaves of *P. major*. The results of this investigation indicated that all samples meet the standards of Ph Eur. The quantity of acteoside depended on the various factors, such as plant species investigated, the type of soil, microclimatic conditions and geographic position.

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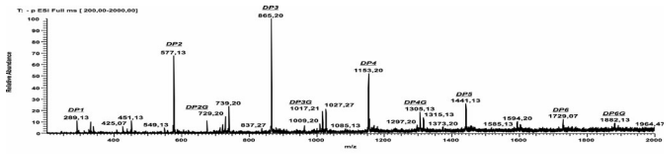
The LC-ESI/MS/MS determination of procyanidins in grape seed extracts

Janda B, Stochmal A, Oleszek W
Institute of Soil Science and Plant Cultivation, State Research Institute, ul. Czartoryskich 8, 24 – 100 Pulawy, Poland

The extracts of grape seeds are widely used as dietary supplements. They show a number of health beneficial effects including antitumor, chemopreventive, cardioprotective, antioxidant, apoptosis inducing and gene modulating activities [1]. The main chemical constituents responsible for these activities are procyanidins. Their identification and determination in plant material is rather complex. In the present work the liquid chromatography-mass spectrometry technique was applied for identification and determination of procyanidins in alcohol and water extracts from grape seeds. The number of monomer, dimer, trimer, tetramer procyanidins, as well as their gallate derivatives were monitored by direct injection mode ESI-MS (Figure 1)



m/z **289**	**DP1**: catechin, epicatechin		
m/z **577**	**DP2**: catechin-catechin	*m/z* **729**	**DP2G**: catechin-catechin-gallate
m/z **865**	**DP3**: catechin-catechin-catechin	*m/z* **1017**	**DP3G**: cat-cat-cat-gallate
m/z **1153**	**DP4**: cat-cat-cat-cat	*m/z* **1305**	**DP4G**: cat-cat-cat-cat-gallate
m/z **1441**	**DP5**: cat-cat-cat-cat-cat	*m/z* **1593**	**DP5G**: cat-cat-cat-cat-cat-gallate
m/z **1729**	**DP6**: cat-cat-cat-cat-cat-cat	*m/z* **1881**	**DP6G**: cat-cat-cat-cat-cat-cat-gallate

The amount of procyanidin monomers, dimers and trimers was calculated after LC separation, from the selective ion chromatograms (SIC) using catechin as an external standard. The concentration of procyanidins in grape seed water extract was about 25% of dry matter, while in ethanol extract this was on the level of 10% of dry matter. **References:** [1] Bagchi D, Sen Ck, et al. (2003) Mutat. Res. 523 – 524: 87 – 97.

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Evaluation of phenolic compounds in *Sempervivum tectorum* L.

Alberti Á, Szőke É, Kéry Á
Semmelweis University, Department of Pharmacognosy, Budapest Üllői út 26., 1085 Hungary

Houseleek (*Sempervivum tectorum* L., Crassulaceae) has been used in folk medicine as an anti-inflammatory remedy. Some studies have described its antinociceptive, liver- protecting and membrane stabilizing effects [1, 2, 3]. These may correlate with diverse phenolic compounds such as polyphenols, proanthocyanidines, flavonols and flavonol- glycosides, which act as potent antioxidant agents. The aim of our study was to evaluate phenolic composition and content of *Sempervivum tectorum* L. Further purpose was to reveal the contribution of these compounds in radical scavenger action. Polyphenol, tannin, proanthocyanidine and flavonoid contents of lyophilized houseleek leaves were determined by spectrophotometry according to the instructions of the European Pharmacopoeia 5.08 (2007). Contents are, respectively: 4,9 mg^*g^{-1}, 2,0 mg^*g^{-1}, 2,7 mg^*g^{-1} and 9,4 mg^*g^{-1}. Flavonoid fingerprints of extracts before and after acidic hydrolysis have been evaluated by TLC and HPLC methods. Also numerous catechin and proanthocyanidine compounds were detected by TLC. HPLC separations were performed on a Supelcosil C 18 (250*4,6 mm; 5 μm) column. For elution, acetonitrile and 2,5 v/v% acetic acid were used. The main flavonoid aglycone was kaempferol, its presence has been proven by standard addition and UV spectra. The antioxidant activity of different extracts was determined by neutralization of the free radicals ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) [4]. We have found that the scavenger activity of extracts containing flavonoid aglycones was the highest ($EC50_{DPPH}$= 1,29*10^{-5} g^*ml^{-1}, $EC50_{ABTS}$= 0,75*10^{-5} g^*ml^{-1}), similar to that of kaempferol standard ($EC50_{DPPH}$= 1,91*10^{-5} g^*ml^{-1}, $EC50_{ABTS}$= 0,85*10^{-5} g^*ml^{-1}). Extracts with different chromatographic profiles of flavonoid glycosides were less effective scavengers of DPPH and ABTS free radicals ($EC50_{DPPH}$= 7,87*10^{-5} g^*ml^{-1}, $EC50_{ABTS}$= 3,73*10^{-5} g^*ml^{-1}). **References:** [1] Kekesi, I. et al. (2003) Phytother Res 17: 1032 – 1036. [2] Blázovics, A. et al. (1993) Phytother Res 7: 98 – 100. [3] Blázovics, A. et al. (2000) J Ethnopharmacol 73: 479 – 485. [4] Re, R. et al. (1999) Free Rad Biol Med 26: 1231 – 1237.

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Phenolics as radical scavengers in *Plantago* species

Beara I[1], *Jovin E*[1], *Bozin B*[2], *Mimica-Dukić N*[1]
[1]*Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg d. Obradovica 3, 21000 Novi Sad, Serbia;* [2]*Department of Pharmacy, Faculty of Medicine, Hajduk Veljkova 1 – 3, 21 000 Novi Sad, Serbia*

The genus *Plantago* L. (Plantaginaceae) comprises about 275 species found all over the world. Ancient use of plantains as herbal remedies is a consequence of their adstringent, anti-toxic, antimicrobial, anti-inflammatory, expectorant and diuretic properties. Although studies concerning the antioxidant activity of some plantains are available [1, 2], there are no reports on the antioxidant activity of some other *Plantago* species. The aim of this study was to examine antioxidative properties of methanolic extracts from four *Plantago* species (*P. maior* L., *P. media* L., *P. lanceolata* L. and *P. holosteum* Scop.) collected from three different locations in Serbia. The extracts were investigated regarding their composition by different colorimetric techniques, such as the content of total phenolic compounds by the Folin-Ciocalteu assay [3] and flavonoids by $AlCl_3$ reagent [4]. Their radical scavenger capacity (RSC) was evaluated measuring spectrophotometrically the decrease of DPPH* (diphenylpicrylhydrazyl radical) [5]. The content of total phenolic compounds (expressed as g chlorogenic acid equivalents/g of dry extract) varied from 0.1281 (*P. maior* location N. Sad) to 0.2774 (*P. media*-Mt. Kopaonik). Flavonoid content was the highest in *P. holosteum*, followed by *P. media*, *P. lanceolata* and *P. maior* (from 19.76 to 7.77 mg quercetine equiv/g of dry extract, respectively). The most potent extracts of *P. media* (Mt. Kopaonik) and *P. holosteum* (Mt. Stara Planina) inhibited the DPPH* by 50% (IC_{50}) at 4.25 and 4.28 μg/ml respectively indicating higher activity than the synthetic antioxidant BHT (IC_{50} 4.62 μg/ml). The extracts of *P. maior* and *P. lanceolata* also showed significant scavenging capacity. Beside estimated correlation between the scavenging potency and the total phenolic content of the extracts, this study presented *P. media* and *P. holosteum* as a possible new source of natural antioxidants. **References:** [1] Galvez, M. et al. (2005) J. Agric. Food. Chem. 53(6):1927 – 33. [2] Pourmorad, F. et al. (2006) Afric. J. Biotech. 5: 1142 – 1145. [3] Singleton, V.L. et al. (1999) Methods in enzymology, Acad. Press, New York. [4] Chang, C.C et al. (2002) J. Food Drug Anal. 10: 178 – 182. [5] Soler-Rivas, C. et al. (2000) Phitochem. Anal. 11: 330 – 338.

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Comparison of biological activity and composition of extracts from *Rhaponticum carhamoides* grown on Polish and Siberian plantations

Biskup E[1], Szynklarz B[1], Kumirska J[2], Golebiowski M[2], Stepnowski P[2], Lojkowska E[1]
[1]Department of Plant Protection and Biotechnology, Intercollegiae Faculty of Biotechnology UG & MUG, Kladki 24, 80 – 822 Gdansk, Poland [2]Department of Environmental Analytics, Faculty of Chemistry, UG, Sobieskiego 18/19, 80 – 952 Gdańsk

A wide range of plant compounds gained attention as pharmaceuticals, cosmetics or food supplements. Therefore obtaining high-quality plant material for research and industrial purposes is of great importance. The biochemical profile of plant material may be influenced e.g. by soil quality or climate. *Rhaponticum carthamoides* (Willd) Iljin, used in Asian folk medicine, grows naturally in Siberia, Sayan and Altai Mountains. This plant synthesizes a wide range of secondary metabolites, famous for their anabolic (ecdysteroids, [1]) and antioxidant (flavonoids, [2]) properties. In 2003 the first Polish plantation was established in Lubiewice by FITOSTAR™. Since the geographic and climatic conditions differ from the natural ones, we checked whether the extracts' composition and biological activities are similar. Polish plant material was collected every five days during summer 2006 from two fields differing in soil class. Siberian material was supplied by FITOSTAR™. Three leaf extracts (aqueous, methanolic and chloroform) were analysed for their composition and biological activities. Chemical composition was compared using Matrix-assisted Laser Desorption/Ionization mass spectrometry (MALDI-MS). Two matrices (2,5-dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid) were employed and the range of compounds of ca. 100 – 1000 MW was screened, showing that Polish extracts are more abundant. We also examined the extracts' capacity to prevent lipid peroxidation, to scavenge DPPH free radical and to reduce molybdenum. Statistically significant differences were observed only in the DPPH assay for methanolic extracts (IC_{50}= 16,9 and 34,5 μg/ml for Polish and Siberian plants respectively). Cytotoxicity of Polish and Siberian plants (MTT assay) was also similar and rather low (IC_{50} > 100 μg/ml for aqueous extracts). Therefore we assume, that introducing *R. carthamoides* to Polish growing (climatic and soil) conditions is possible and may result in obtaining adequate plant material. **References:** [1] Slama K. et al. (1996) Experientia 52: 702 – 706; [2] Miliauskas G. et al. (2005) J. Nat. Prod. 68: 168 – 172

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Phenolic Content of a Commercial *Sambucus nigra* Preparation

Chrubasik C[1], Maier T[2], Schieber A[2]
[1]Institute of Forensic Medicine, University of Freiburg, Albertstr. 9, 79104 Freiburg, Germany, [2]Institute of Food Science and Biotechnology, University of Hohenheim, August-von Hartmann-Str. 3, 70599 Stuttgart, Germany

Elderberries contain various flavonoids such as hyperoside, isoquercitrin and rutinoside and up to 1% of anthocyanins [1]. **Aim** of this investigation was to evaluate the flavonoid content in a commercially available elder product (elderberry juice concentrate prepared from 120 g fresh berries supplemented with elderflower juice and extract based on 3.9 g dried flowers per 200 mL). **Methods:** We used the method described by Kammerer et al. [2]. The liquid product was extracted three times with acidic methanol (approx. 60 min per extraction). After evaporation, the residue was dissolved in 1 mL of acidified bidistilled water (pH 3). Polyphenols separated by HPLC were quantified using commercially available standards (calibration curves) or, if standards were not available, corrected by a molecular weight factor according to Chandra et al. [3]. Phenolic compounds were characterized and quantified using HPLC with diode array and mass spectrometric detection. **Results:** Five major compounds were detected at 320 nm (hydroxycinnamates) and 370 nm (flavonols) and unambiguously identified as neochlorogenic acid (125 mg/L), chlorogenic acid (623 mg/L), rutin (1441 mg/L), kaempferol-3-rutinoside (44 mg/L), and isorhametin-3-rutinoside (324 mg/L). Only very small amounts of anthocyanins were found in the product (4 mg/L). **Conclusion:** Our results show that rutin and chlorogenic acid were the predominant phenolic compounds present in the product. It seems likely that most of the antioxidative compounds have been destroyed during preparation of the proprietary juice concentrate. **References:** [1] Anonymous. (1994) Hagers Handbuch der Pharmazeutischen Praxis 6: 579 – 86, Springer Press, Heidelberg. [2] Kammerer, D. et al. (2004) J. Agric. Food Chem. 52: 4360 – 4367. [3] Chandra, A. et al. (2001) J. Agricult. Food Chem. 49: 3515 – 3521.

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Prantschimgin content of a methanolic extract of the roots of *Ferulago platycarpa* Boiss. & Bal. (Umbelliferae)

Şatır E[1], Coşkun M[1]
[1]Ankara University Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Ankara, Turkey

Umbelliferae (Apiaceae) is a large family mainly containing coumarins and volatile oils. *Ferulago platycarpa* Boiss.& Bal. is a perennial endemic species, growing in Nevşehir, Turkey [1]. The species and also the other species of this genus are called "Çakşır" or "Çağşır". In addition, species of the genera *Ferula* and *Prangos* are also known by these names and though they are reported to have other usages, mainly used as aphrodisiacs in Turkey [2]. In this study, we determined prantschimgin content of the methanolic extracts obtained from the roots of *Ferulago platycarpa* by HPLC. Prantschimgin was identified for the first time in the roots of *F. meioides* (L.) Boiss. [3]. It is reported to be present in some other *Ferulago* species as well [4 – 6]. External standard method was used to determine prantschimgin content of the methanolic extract of the roots of *F. platycarpa* and was found to be 1.90%. This value is higher than the prantschimgin content of *F. isaurica* Peşmen and *F. syriaca* Boiss which had been determined under similar HPLC conditions (1.17% and 0.91% respectively). We think that this furanocoumarin is one of the major compounds present in the roots and can both serve as a chemotaxonomic marker for the genus *Ferulago* and also may be responsible for the biological effect. **References:** [1] Davis, P. H. (1972) Flora of Turkey and the East Aegean Islands Volume 4, Edinburgh University Press, Edinburgh, UK. [2] Baytop, T. (1999) Therapy with Medicinal Plants in Turkey – Past and Present, 2nd Edn. Nobel Tıp Basımevi, İstanbul, Turkey. [3] Ongyanov, I., Botcheva, D. al (1969) Planta Med. 17(1): 65 – 70. [4] Doğanca, S. et al. (1997) Fitoterapia 58(1): 80. [5] Jimenez, B. et al. (2000) Phytochem. 53: 1025–1031. [6] Erdurak Kılıç, C.S., Coşkun, M., (2006) Chem. Nat. Compd. 42(3): 351 – 352.

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Isoflavone characterization in different genotypes of soybean

Æeran V[1], Miladinović J[2], Cvejić J[1]
[1]Laboratory of Analysis of Natural and Pharmaceutical Products, Department of Pharmacy, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia; [2]Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia

Soy isoflavones are phytochemicals of high interest since consumption of soy products has been linked to a variety of health protective effects including risk reduction of cardiovascular disease, lowering rates of prostate and mammary cancers and improvement of bone health. The aim of this work was to select soybean genotypes which

contain a high isoflavone concentration and therefore may enhance the health beneficial effects of soy products. Twenty soybean samples (from USA, Serbia, Ukraine and China) of different genotype origin were screened for their isoflavone content. All samples were cultivated and harvested under the same conditions on experimental fields at the Institute of Field and Vegetable Crops, Novi Sad. After extraction with methanol-water (8:2, v/v), the isoflavone content was determined by C_{18} reversed phase HPLC, coupled with a photodiode array detector [1]. The highest and lowest isoflavone contents were 8.52 and 2.64 mg/g soy, respectively, while the average was 6.01 mg/g soy. Analysis of variance showed that there were significant differences between cultivars (*P* value < 0.05). On the other hand, *t*-test showed that there is no significant difference in content of isoflavones between different countries of origin (*P* value < 0.05). This demonstrates that each of investigated countries has a cultivar that is significantly rich in isoflavones, as well as one that has low isoflavone levels. Considering significant differences among different soy genotypes on the basis of the results of this study, some cultivars with the highest content of isoflavones could be recommended as potential food and supplement ingredients. **References:** [1] Lee JH. et al. (2004) J. Agric. Food Chem. 52: 2647 – 2651.

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Exploring chemical differences in five subpopulations of *Hieracium pilosella L.* by HPLC-ESI-MS/MS and comparison with genetic variability

D'Ambrosio M[1], Egger P[1,2], Guerriero A[1], Komjanc M[3], Zini E[2,3]
[1]Università degli Studi di Trento, Laboratorio di Chimica Bioorganica, via Sommarive 14, 38050 Trento-Povo (TN), Italy; [2]CRA-ISAFA (Consiglio per la Ricerca e la sperimentazione in Agricoltura), Piazza Nicolini 6, I-38050 Trento-Villazzano, Italy; [2]Istituto Agrario di San Michele all'Adige, Via E. Mach 1, 38010 San Michele all'Adige (Trento), Italy

The project PARMA (ARomatic and Medicinal Plants of the Alps) is intended to stimulate the cultivation of five officinal plants from the Trentino region (Italy). However, the first step is their characterization in terms of genetic variability as well as concerning their content of active principles. In European traditional medicine [1], the infusion from the whole fresh plants of *Hieracium pilosella* L. is used as diuretic or in the treatment of rheumatic forms of renal and vesical affections. A preliminary investigation on 10 different accessions of *H. pilosella* was performed by the technique of microsatellite markers. The Valpiana location (Val di Sole) was divided into five plots, each having approximately the same area and corresponding to a plant community: A, B, C, D and E. The microsatellite analysis revealed high genetic variation among and between these subpopulations. Then, a quantitative evaluation of three classes of natural products (phenolic acids, coumarins, flavonoids) was undertaken in order to assess chemical diversity within those subpopulations at Valpiana. The content in metabolites among the five samples of fresh aerial parts was obtained by using LC-ESI-MS/MS. To give a couple of results, luteolin ranged between 1.3 and 0.50 mg/g fresh plant material and umbelliferone between 0.52 and 0.32 mg/g whereas values between 0.54 and 0.26 mg/g were found for chlorogenic acid. In addition, we observed steady low or high amounts of all the examined compounds for the same subpopulation sample. Our data are in accordance with those reported in the literature [2]. *Acknowledgement*: This work has been supported by the grant of Provincia Autonoma di Trento, 9th July 2004 no. 1587 **References:** [1] Fournier P. (1948) Le livre des plantes médicinales et vénéneuses de France. P. Lechevalier Ed. Paris. [2] Zidorn, C. et al. (2002) Plant Systematics and Evolution 231: 39 – 58

P 280

Metabolomic fingerprinting of medicinal plants using ^{1}H NMR and HPLC/ELSD in combination with PCA

Daniel C[1], Kersten T[1,2], Kehraus S[1], König GM[1], Knöß W[2]
[1]Institute for Pharmaceutical Biology, Nußallee 6, 53115 Bonn, Germany; [2]Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger-Allee 3, 53175 Bonn, Germany

Quality control is a basic requirement in the manufacturing of herbal medicinal products. In the past, metabolomic fingerprinting with NMR and HPLC, respectively, in combination with PCA (Principal Component Analysis) proved to be an alternative method for control of quality [1] and origin [2] and for the discrimination between species [3]. However, most projects in this context are only investigating small groups of plants, mostly one species or one genus. The intention of this research project is to develop a method which is applicable to a broader range of medicinal plants, and to evaluate the possibilities of applications to analyse also finished herbal medicinal products. In order to obtain consistent data the experimental procedure was standardised after a series of preliminary experiments. The dried and ground starting material is extracted first with dichloromethane and subsequently with methanol. The ^{1}H NMR spectra and the HPLC/ELSD chromatograms of the plant extracts are recorded and statistically analysed by PCA. Different samples of *Matricaria recutita* L. and *Chamaemelum nobile* L. are analysed as well as *Anthemis cotula* L., an adulteration of chamomile. First results showed that dichloromethane extracts from *M. recutita, C. nobile* and *A. cotula* samples could be clearly classified into three groups corresponding to the species by PCA of ^{1}H NMR spectra. So far, there was no definite discrimination into groups possible when comparing the ^{1}H NMR spectra of methanol extracts by means of PCA using default settings. Moreover, the development of a method with HPLC/ELSD and PCA turned out to be challenging as even slight shifts in retention times led to high variability in PCA. *Acknowledgement*: We thank the Federal Institute for Drugs and Medical Devices for financial support. **References:** [1] Wang Y et al. (2004) Planta Med. 70: 250 – 255 [2] Zhao LH et al. (2005) ChemPharmBull 53: 1054 – 1057 [3] Choi YH et al. (2005) J. Agric. Food Chem. 53: 1237 – 1245

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Seasonal changes and water stress effects on ambrosin and damsin of *Ambrosia maritima* plant

El-Mergawi RA[1], Mahassen Sidky MA[2]
[1].Botany Department, National Research Centre, Dokki, Giza, 12311, Egypt; [2].Medicinal and Aromatic Plant Research Department, Horticulture Research Institute, Dokki, Giza, 12311, Egypt

Ambrosia maritima is used for treatment of renal colic and calculi and for control of bilharzia [1]. A field experiment was conducted to determine ambrosin and damsin in different plant organs throughout the first two years of plant age. Seedlings were transplanted in the field in November, eight cuts were collected in a two month-interval from june to october of the first and second year of plant age. Another field experiment was conducted to study the effect of water stress. Plants were subjected to two water treatments, after 60 days from transplantation, normal plants irrigated every two weeks and water stressed plants irrigated every six weeks. Three cuts were taken after 4, 6, and 8 months from the beginning of water treatment. Five plant samples were taken from each cut and subjected to analysis by HPLC [2]. Amount of sesquiterpene lactone was calculated and analyzed using least significant differences (LSD). Ambrosin and damsin were present at high concentrations in leaves and flowers in all evaluated cuts, while stems and seeds contained only traces. These two organs accumulated more than 90% of sesquiterpene lactones produced by plant. Seasonal changes in ambrosin and damsin content in different plant organs nearly had the similar trend throughout two years of plant growth. It is increased reaching maximum values in August in the first year of plant

age and in June in the second year and decreased by October. The highest yield of ambrosin (5.29 mg/g dry matter and 2086 mg/plant) and damsin (6.87 mg/g dry matter and 2707 mg/plant) occurred in June of the second year of plant age. Ambrosin concentration was slightly affected by water stress, but damsin decreased in leaves and increased in flowers. Water stress had a depressive effect on the amount of ambrosin and damsin accumulated per plant. **References:** [1] El-Sawy, M. et al. (1984) Tropenmed. Parasitol.35: 100. [2] Geissman, T. et al. (1996) Phytochem. 8: 145.

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Identification of undeclared synthetic drugs in herbal products commercialized in Brazil

Moritz MIG[1], Lang KL[1], Baratto L[1], Caro MSB[2], Falkenberg M[1], Schenkel EP[1]
[1]Department of Pharmaceutical Sciences, Federal University of Santa Catarina, CIF/CCS/UFSC, Campus Trindade, 88.040 – 900 Florianópolis, Brazil; [2]Department of Chemistry, Federal University of Santa Catarina, QMC/CFM/UFSC, Campus Trindade, 88.040 – 900 Florianópolis, Brazil

The adulteration of herbal products with undeclared synthetic drugs was recently described in many countries [1]. A product called "Indiano Talún", with the complementary descriptions: "C. DA ÍNDIA TALÚN", "is a plant used to treat back pain, arthritis, osteoarthritis ..." besides "Registration free Product. Decrete 79994/77 Art. 28 and 29 Law. 7.370" was involved in several intoxications in Southern Brazil [2]. The product does not meet the obligatory declarations according to Brazilian law and was therefore declared as illegal, since it has no registration. The analysis of two "Indiano Talún" samples was performed. The content of 15 capsules from each sample was extracted three times with methanol. The methanolic extracts were concentrated, affording 920 mg residue for sample A and 1.0 g for sample B. Both were analysed by TLC in different systems. Sample A presented two main compounds and sample B, four. Both extracts were fractionated by column chromatography and from each sample one compound was isolated. UV, IR and NMR spectra were obtained and the structures elucidated as piroxicam (A) and ketorolac (B). These nonsteroidal anti-inflammatory agents possess several adverse effects and drug interactions, with increased risks for elderly [3], what could explain the severe intoxications registered. The production and commercialization of "Indiano Talún" was forbidden by the National Agency of Health Vigilance (ANVISA) in June 2006 [4], but after some time besides "Indiano Talún" another product called "Fator P", with similar package and labeling, could be found in the market. TLC comparative analysis allowed the identification of "Fator P" as being similar to sample (A). Both "Indiano Talún" and "Fator P" are still being commercialized, mainly through internet and also in other regions of the country. These results confirm the urgent need for more intensive surveillance by health authorities and for a specific regulation concerning commerce of health products in the internet. **References:** [1] Ernst, E. (2002) J. of Intern. Med. 252: 107 – 13. [2] Centro de Informações Toxicológicas de Santa Catarina. CIT/SC (unpublished data). [3] Lacy, C.F. et al. (2000) Drug Information Handbook. Lexi-Comp/American Pharmaceutical Association. Hudson (Cleveland). [4] http://www.anvisa.gov.br/inspecao/medicamentos/apreendido_2006.htm

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The efficiency comparison of ramentaceone isolation from *Drosera aliciae* plants using normal and reversed- phase preparative liquid chromatography techniques

Gilgenast E[1], Romanik G[1], Maciejewska A[1], Królicka A[2], Kamiński M[1]
[1]Technical University of Gdansk, Chemical Faculty, Department of Analytical Chemistry, 11/12 Narutowicza St., Pl-80 – 952 Gdańsk; [2]Department of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Kładki 24 St, Pl-80 – 822 Gdańsk

Preparative HPLC is a profitable technique for receiving usable amounts of substances from various mixtures. The work presents two ramentaceone isolation procedures from a chloroform extract obtained from *Drosera aliciae* Raym.-Hamet (Droseraceae) cultured *in vitro* using normal- and reversed-phase preparative liquid chromatography (NP-, RP-PLC). A LiChroprep RP-18, 12 μm, 250 x 16.8 mm column and mixture of methanol: water 8:2 v/v as solvent were used in the reversed phase mode. Samples of plant extracts were also separated using a LiChrosorb silica gel column (200 x 16.8 mm, 10 μm) and a mixture of n- hexane/methyl t-butyl ether 9:1 v/v was used as mobile phase (NP-PLC). The mobile phase flow rate was 7 mL/min (in the reversed phase mode) and 5mL/min (in the normal phase mode). The HPLC column was backflushed after elution of ramentaceone in each analysis to remove strongly adsorbed compounds. Step elution is an alternative to backflush, however, it requires activation of the PLC column. For a specified purity of products the maximum level of column overloading, efficiency of process and productiveness of PLC- columns were compared. The process efficiency was defined as an amount of received substance in a unit of time. Under the NP-PLC conditions we received about 10 mg ramentaceone of 99,80% purity in one experiment and under the RP-PLC conditions 2 mg of 92,50% purity. Run time was 60 min and 40 min for NP-PLC and RP-PLC, respectively. Productiveness of the PLC- column was defined as an amount of substance of maximum purity received in unit of time in relation to column cross section area. Productiveness of NP-PLC column was found to be 4,5 $\mu g/cm^2 min$, concerning the the RP-PLC column it was only 1,3 $\mu g/cm^2 min$. *Acknowledgements:* State Committee for Scientific Research, Grant No KBN 0430/P04/2004/26

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Carotenoid content of the flower of tansy (*Tanacetum vulgare* L.)

Horváth G[1], Turcsi E[2], Molnár P[1], Szabó LG[1], Deli J[2]
[1]University of Pécs, Institute of Pharmacognosy, Pécs, Rókus u. 2, 7624, Hungary; [2]University of Pécs, Department of Biochemistry and Medical Chemistry, Pécs, Szigeti út. 12, 7624, Hungary

Tansy (*Tanacetum vulgare* L.) is a medicinal plant, also listed by the Council of Europe as a natural source of food flavouring (Category N3). Main constituents are volatile oils, sesquiterpene lactones including arbusculin-A and tanacetin, steroids and tannins. Tansy is stated to possess anthelmintic, carminative and antispasmodic properties [1]. In medicinal plants carotenoids may give protection against some life-threatening conditions, for example, cancer and heart disease [2 – 3]. In this study our research focuses primarily on the separation and indentification of carotenoids in the flower of tansy. The fresh inflorescence flowers were separately extracted with MeOH/Et_2O. The combined extract was saponified in heterogeneous phase (30% KOH/MeOH) and was distributed between MeOH: H_2O (9:1) and hexane. The distribution resulted in hypophasic and epiphasic fraction. The HPLC (column: 250 x 4,6 mm, Merck LiChrospher 100 RP-18 endcapped; solvent system: (A) 12% H_2O/MeOH, (B) MeOH, (C) 50% acetone/MeOH, gradient program; detector: Dionex PDA-100, λ= 450 nm) and CC ($CaCO_3$ adsorbent, eluent: mixture of toluene and hexane) analysis of the hypophasic fraction resulted in the identification of following carotenoids: violaxanthin, lutein-5,6-epoxide, (9*Z*)-violaxanthin, lutein, (9*Z*)-lutein-5,6-epoxide, (9*Z*)+(9'*Z*)-lutein, (13*Z*)+(13'*Z*)-lutein. In the epiphasic fraction lutein, (9*Z*, 9'*Z*)-lutein, (9*Z*)+(9'*Z*)-lutein, (13*Z*)+(13'*Z*)-lutein, α-cryptoxanthin-5,6-epoxide, α-cryptoxanthin, (9*Z*)+(9'*Z*)-α-cryptoxanthin, β-carotene-5,6,5',6'-diepoxide, β,β-carotene, (*Z*)-isomers of β,β-carotene were identified. *Acknowledgements:* This study was supported by the grant OTKA K 60121 (Hungarian National Research Foundation). **References:** [1] J. Barnes, L. A. Anderson, J. D. Phillipson (2002) Herbal Medicines. Pharmaceutical Press, London, pp. 460 – 461. [2] G. Britton, S. Liaaen-Jensen, H. Pfander (1995) Carotenoids, Vol. 1A, Isolation and Analysis, Birkhäuser Verlag, Basel-Boston-Berlin [3] N. I. Krinsky, E. J. Johnson (2005) Mol. Asp. Med. 26: 459 – 516.

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Qualitative and quantitative analysis of components of some Senegalese essential oils by GC-MS and Nuclear Magnetic Resonance spectroscopy

Dall'Acqua S, Campesato L, Innocenti G
Dipartimento di Scienze Farmaceutiche, Università di Padova, Via Marzolo 5, 35131 Padova, Italy

NMR spectroscopy is a powerful tool for the fingerprint analysis of natural extracts as well as of essential oils and could be very useful to assess quality of herbal drugs [1 – 5]. In this communication, we report the preliminary results of our study on the chemical composition of some essential oils determined by means of GC, GC-MS and NMR spectroscopy. The essential oils were obtained by steam distillation of the following Senegalese plants: *Eucalyptus camaldulensis, Fagara leprieurii, Hyptis suaveolens, Lippia chevalieri, Melaleuca leucadendron, Mezoneuron benthamianum, Ocimum gratissimum, Ocimum canum, Xylopia aethiopica,* and *Citrus aurantium* (Petitgrain de bigarade). Oils were preliminarily analysed by GC-MS and quantitative determinations were performed by GC-FID. ^{1}H, ^{13}C, and bidimensional NMR experiments (HMQC HMBC COSY, TOCSY and NOESY) were used to determine the chemical structures of compounds in the crude oil. Quantitative determinations of the selected constituents were obtained using caffeine as internal standard. For example thymol (39.6%), γ-terpinene (19.2%) and p-cymene(11.2%) were determined as main constituents of *Ocimum gratissimum*, while major components of *Xylopia aethiopica* oil were identified as β-pinene (35.4%), β-terpinene (16.5%) and α-pinene (13.4%). The obtained results show that NMR spectroscopy is a good analytical tool for both qualitative and quantitative analysis of some of the investigated essential oils, offering advantages over the classical chromatographic methods. **References:** [1] Bilia A.R. et al. (2002) J. Agric. Food. Chem. 50: 5016 – 25. [2] Rivero-Cruz B. et al. (2006) J. Nat. Prod. 69: 1172 – 76. [3] Guerrini A. et al. (2006) J. Agric. Food Chem. 54: 7778 – 88. [4] Al-Burtamani S.K.S. et al. (2005) J. Ethnopharmacol. 96: 102 – 117. [5] Kubeczka K.H., Formàcek V., (2002) Essential oil analysis by capillary GC and Carbon-13 NMR spectroscopy, 2nd Ed., Wiley: New York.

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Isolation and identification of carotenoids in the fruit of cornelian cherry (*Cornus mas* L.)

Horváth G[1], Turcsi E[2], Molnár P[1], Szabó LG[1], Deli J[2]
[1]University of Pécs, Institute of Pharmacognosy, Pécs, Rókus u. 2, 7624, Hungary; [2]University of Pécs, Department of Biochemistry and Medical Chemistry, Pécs, Szigeti út. 12, 7624, Hungary

The edible fruits of Cornelian cherry are most frequently used to produce drinks, sweets, gels and jams as well as in cookery. They can be utilized in medicine, as well, against diarrhoea and enteritis due to their tannin content. The fresh fruits of this medicinal plant have an antioxidant effect that can be attributed to their flavonoids and anthocyanidins [1]. Most current research focuses on the proposed role of carotenoids as lipid antioxidants. Consumption of fruits has been shown to be effective in the prevention of chronic and degenerative diseases, including cancer [2 – 3]. In this study our research focuses primarily on the separation and identification of carotenoids in the fruits of Cornelian cherry. The fresh berries were separately extracted with MeOH/Et_2O. The combined extract was saponified in heterogeneous phase (30% KOH/MeOH) and was distributed between MeOH: H_2O (9:1) and hexane. The distribution resulted in a hypophasic and an epiphasic fraction. The HPLC (column: 250 x 4,6 mm, Merck LiChrospher 100 RP-18 endcapped; solvent system: (A) 12% H_2O/MeOH, (B) MeOH, (C) 50% acetone/MeOH, gradient program; detector: Dionex PDA-100, λ= 450 nm) and CC ($CaCO_3$ adsorbent, eluent: mixture of toluene and hexane) analysis of the hypophasic fraction resulted in the identification of the following carotenoids: (all-*E*)-neoxanthin, (9'*Z*)-neoxanthin, epimers of neochrome or luteoxanthin, lutein-5,6-epoxide, lutein, (9*Z*)+(9'*Z*)-lutein, (13*Z*)+(13'*Z*)-lutein. In the epiphasic fraction lutein, β-cryptoxanthin, β-carotene-5,6-monoepoxide, β,β-carotene were identified. *Acknowledgements:* This study was supported by the grant OTKA K 60121 (Hungarian National Research Foundation). **References:** [1] I. Schönfelder, P. Schönfelder (2001) Der neue Kosmos Heilpflanzenführer. Franckh-Kosmos Verlags- GmbH and Co., Stuttgart [2] G. Britton, S. Liaaen-Jensen, H. Pfander (1995) Carotenoids, Vol. 1A, Isolation and Analysis, Birkhäuser Verlag, Basel-Boston-Berlin [3] P. Molnár, M. Kawase, N. Motohashi (ed. N. Motohashi) (2005) In Functional Polyphenols and Carotenoids with Antioxidative Action, a review book series of Chem. Pharm. Sci. (RSFLASH, Trivandrum-695 023, Kerala, India), pp. 111 – 131.

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Characterization and determination of compounds in willow bark using HPLC-MS and mf-MELDI-MS

Kasemsook S[1], Stecher G[1], Sultana T[1], Hashir MA[1], Abel G[2], Popp M[2], Bonn GK[1]
[1]Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, Innsbruck 6020, Austria; [2]Bionorica AG, Kerschensteinerstr. 11 – 15, Neumarkt 92318, Germany

Willow bark (*Salix* sp.) is officinal in German Pharmacopeia and approved in the Commission E monographs. It is used as a main component of analgesic and anti-rheumatic drugs. Constituents of willow bark include 1.5 – 11% of phenolic glycosides, mainly salicylates (1.0 – 10.2%), including salicin and its derivatives (salicortin and tremulacin), 8 – 20% of tannins, mainly catechin tannins, some gallotannins and condensed tannins (procyanidins) and 1 – 4% of flavonoids, including isoquercitrin and naringin and its glycoside. The aim of this study was the qualitative and quantitative analysis of target compounds relevant for the pharmacological activity of *Salix* extracts using high performance liquid chromatography-mass spectrometry (HPLC-MS) and matrix free-material enhanced laser desorption ionization-mass spectrometry (mf-MELDI-MS). A special purification protocol was used to purify the extracts for identification of compounds in *Salix* extracts. Employing polyamide column, Sephadex LH-20 column and Sep-Pak C18 SPE cartridges fractions were obtained, containing phenolic acids, flavonoid glycosides, monomeric catechins, oligomeric procyanidins and polymeric procyanidins. Qualitative analysis was performed by HPLC-MS and mf-MELDI-MS. Especially mf-MELDI-MS was suitable for qualitative investigation, as it delivered data in few minutes with accurate mass identification. For quantification several SPE cartridges were evaluated to gain highly purified fractions prior to HPLC analysis. Strata-X SPE cartridge delivered highest amounts of interesting compounds and yielded purified samples. *Acknowledgement*: A scholarship BMWF (Technology Grant) for S.K. is gratefully acknowledged

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Pressurized liquid extraction of tectoridin and tectorigenin in *Belamcanda chinensis* and pressurized water extraction of phyllodulcin in *Hydrangea macrophylla*

Lee HJ[1], Um BH[1], Yoon KD[2], Kim J[2], Kim CY[1]
[1]KIST Gangneung Institute, Gangneung Techno Valley, Gangneung, Gangwon-do 210 – 340, Korea; [2]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151 – 742, Korea

Pressurized liquid extraction (PLE) was applied to extract tectoridin and tectorigenin from *Belamcanda chinensis* (Iridaceae) with aqueous ethanol, an environment-friendly solvent. Tectoridin and tectorigenin were proven to be the main biologically active constituents of *B. chinensis* with anti-inflammatory, anti-angiogenic, and anti-tumor activities [1]. Several compositions of water and ethanol, temperatures, and static extraction times were studied to optimize extraction protocol. The optimized conditions are as follows: (i) tectoridin; solvent, 60% ethanol/water (v/v); temperature, 150 °C;

static extraction time, 10 min; (ii) tectorigenin; solvent, 60% ethanol/water (v/v); temperature, 180 °C; static extraction time, 15 min. In addition, extraction efficiency of PLE for tectoridin and tectorigenin was compared to conventional methods such as soxhlet and sonication extraction. The highest extraction efficiency of tectoridin and tectorigenin was achieved by PLE. Pressurized hot water extraction (PHWE) was developed for phyllodulcin, a well known sweetener, in *Hydrangea macrophylla* var. *thunbergii* Makino (Saxifragaceae) [2]. PHWE is a non-flammable, harmless, readily available and eco-friendly extraction method. The extraction yield was highest at 150 °C and 20 min. In addition, extraction efficiency of PHWE for phyllodulcin was compared to sonication extraction with methanol. The extraction yields of phyllodulcin were 10.4 mg/g for PHWE, and 17.4 mg/g for sonication with methanol. From these results, it is possible to state that PLE and PHWE could be promising alternative extraction methods for natural products. *Acknowledgements:* No. RT105 – 01 – 02 from the Regional Technology Innovation Program of the Ministry of Commerce, Industry and Energy (Republic of Korea), Gangneung Marine Bio Foundation (Korea). **References:** [1] Jung, S. H. et al. (2003) Planta Med. 69: 617 – 622. [2] Yasuda, T. et al. (2004) J. Nat. Prod. 67: 1604 – 1607.

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Determination of allantoin in Dioscoreae Rhizoma by HPLC using CN Column

Yoon KD[1], Chin YW[1], Yang H[1], Yang MH[1], Ryu MY[1], Nam SI[2], Park JH[2], Kim J[1]

[1]College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151 – 742, Korea; [2]R&D Institute, Tong Yang Moolsan Co., Ltd., Young-In, Kyunggi-Do 449 – 825, Korea

Dioscoreae Rhizoma has been widely used as food and traditional medicine in the world. It contains purine derivatives, saponins, starch and mucilage as main constituents. Among these components, allantoin is one of the biologically active compounds in Dioscoreae Rhizoma [1, 2]. There are a number of methods to determine allantoin in this medicinal plant. However, these methods are somewhat complex because of time-consuming sample preparation and adjusting of buffer conditions. In this study, we developed an easy and reliable HPLC method using CN columns, and applied it to analyze the allantoin content of four Dioscoreae Rhizoma samples. It is possible to separate and quantify allantoin from other polar constituents using CN columns such as YMC-Pack CN, Zorbax SB-CN, and DiscoveryP® Cyano columns. The intraday precision was 0.58 – 3.33% for YMC-Pack CN, 0.41 – 2.20% for Zorbax SB-CN and 0.45 – 1.93% for DiscoveryP® Cyano columns, whereas interday variations were 0.09 – 1.84%, 0.04 – 2.59% and 0.87 – 5.18% for YMC-Pack CN, Zorbax SB-CN and DiscoveryP® Cyano columns, respectively. The recoveries of allantoin were in the range of 98.8 – 102.6% (RSD 1.1 – 1.6%) for YMC-Pack CN, 99.7 – 110.5% (RSD 1.3 – 4.9%) for Zorbax SB-CN, and 97.2 – 110.1% (RSD 1.8 – 5.7%) for DiscoveryP® Cyano columns. The content of allantoin in four Dioscoreae Rhizoma samples was in the range of 4.1 – 7.1 mg/g dry weight. This study showed that analysis of allantoin using CN column is a simple and reliable method, which could be adopted to verify allantoin content in various Dioscorea preparations. *Acknowledgements:* This study was financially supported by a grant (No. PF06102 – 00) from Plant Diversity Research Center of the 21C Frontier R&D Programs. **References:** [1] Fu, Y.C. et al. (2006) Food Chem. 94: 541 – 549. [2] Fu, Y.C. et al. (2005) LWT-Food Sci. Technol. 38: 735 – 744.

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New Solid Phase Materials for Phytoanalytical Purposes

Krieg C[1], Stecher G[1], Popp M[2], Abel G[2], Bonn GK[1]

[1]Institute of Analytical Chemistry and Radiochemistry, Leopold Franzens University, Innrain 52a, 6020 Innsbruck, Austria; [2]Bionorica AG, Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Solid phase extraction (SPE) is a powerful tool for analytical chemists in the fields of extraction, purification and enrichment. The aim of this work is focused on the development of new solid phase extraction materials for the preconcentration and purification of pharmacologically active components out of different plant materials. Special interest is put on molecules with phenolic and polar functions like oleuropeine due to their positive effects on human health. By now, almost all commercially available SPE-Materials are either hydrogencarbon derivatised spherical silica particles or polymers which are in general adequate for most analytical purposes but not sufficient in all cases of complex analytes or difficult matrices. In this approach, the surfaces of different substrate materials, diamond powder, titan oxide, porous and non porous silica gels, are coated by radical polymerisation with different mixture ratios of functionalised phenols and divinylbenzene to achieve strong π -π and OH interactions between analyte molecules and stationary phases during concentration and cleaning progress [1]. Therefore not only the influence of different kinds of bulk material and their morphology but also the different ratios of the coating monomers on extraction efficiency are investigated. Scanning electron microscopy (SEM) investigations are proving the successful surface coatings and are showing different morphologies depending on the bulk materials. The materials are tested with standard mixtures, with real samples of olive oil and olive leave extracts. The recovery results are compared to commercially available SPE materials with special focus on the extraction of phenolic targets like oleuropein. **References:** [1] M. Sultan, et al., (2005) Curr. Med. Chem. 12: 573 – 588

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Chemical investigation of the Azorean *Lepidium sativum* seeds as a phytonutrient alternative for rheumatic diseases

Lima E, Baptista J

Departamento de Ciências Tecnológicas e Desenvolvimento (DCTD)/Centro de Investigação de Recursos Naturais (CIRN), Universidade dos Açores, 9501 – 855 Ponta Delgada, S. Miguel, Açores, Portugal

Lepidium sativum Lin. (LS) seeds have been used as a traditional anti-rheumatic formula in Azores Islands for treatment of arthritic diseases. Osteoarthritis, the most common form of the disease, results from progressive catabolic loss of cartilage proteoglycans and standard therapy is only of palliative benefit and may exacerbate loss of cartilage [1]. To evaluate the anti-rheumatic properties of LS seeds, the long-chain fatty acids and plant sterols were determined by GC/MS. LS is very rich in α-linolenic (37.4%) and eicosenoic (12.4%) acids and presents a ω_3/ω_6 ratio of 2.71. The plant sterols by descending order were β-sitosterol (224.8 mg), campesterol (129.3 mg), stigmasterol (53.1 mg) and brassicasterol (50.9 mg), per 100 g of dry seeds weight. Neutral sugars (arabinose, galactose, xylose, fucose and ramnose) and amino sugars were determined by HPLC. LS revealed a content of neutral sugars (13.5 μg), glucosamine (456 mg) and glucosamine-2-sulphate (1301 mg), per 100 g of dry seeds. Fat- and water-soluble vitamins were also determined by HPLC. According to the chemical composition, the phytoneutraceutical LS seeds may have potential properties for rheumatic disorders. **References:** [1] Steinberg, J. et al. (1983) Bioch. Bioph. Acta 757: 47 – 58.

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The metabolism of *Lonicera japonica* by artificial gastric juice

Lin L[1], Wang Z[2], Li C[3], Wang W[4]
Institute of Materia Medica, China Academy of Chinese Medical Sciences, No.16 Nanxiaojie Street, Dongzhimennei, Beijing, 100700 China

Using high-performance liquid chromatographic method, the metabolism of multi-constituents in the decotion of *Lonicera japonica* by artificial gastric juice was simultaneously studied. The original decotion and the incubated samples were separated on Agilent-C_{18} column using methanol-water as mobile phase. The chromatographic peaks of chlorogenic acid, caffeic acid, galuteolin and rutin were identified in all samples. At different intervals (0.5, 1.0, 1.5, 2.0, 4.0, 6.0 h) after incubating the decotion of *Lonicera japonica* by artificial gastric juice, the concentrations of the constituents chlorogenic acid, galuteolin and rutin, were detected and compared with the ones in the original decotion and the metabolic tendencies were described. The experimental results showed: The major chromatographic peaks of the incubated samples were relegated to the original decotion, no ingredients emerged nor disappeared. The content of chlorogenic acid and galuteolin in incubated samples was lower than in the original decotion. The metabolic curves of chlorogenic acid and rutin presented an adscendent tendency, but the one of galuteolin showed little change. It was concluded, in brief, that the study established the bases for searching for *L. japonica*'s pharmacodynamic components. *Acknowledgements:* This project was supported by the National Natural Science Foundation of China (Grant No. 30672601). **References:** [1] The pharmacopeia committee of China, China pharmacopeia of 2005 edition, publishing company of chemical industry:117 – 118. [2] L. Lin, et al. (2007) China J. CMM. (Accepted).

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Variation of the morphological characteristics and active principles in wild and cultivated *Gentiana lutea* L. from Romania

Tanase M[1], Sand C[1], Bobit D[2], Lazurca D[2], Boncut M[1], Barbu CH[1], Pop MR[1]
[1]"Lucian Blaga" University of Sibiu, 10 Victoriei Bv., 550024 Sibiu, Romania; [2]ICDCSZ – Braşov, Medicinal Plant Laboratory, 2 Fundaturii St., 500470 Brasov, Romania

Gentiana lutea L., whose use as Radix Gentianae is well-known [1], is an endangered plant because of its large scale cropping and now is protected in Romania [2]. In order to start its large scale cultivation in Sibiu area, by in vitro multiplication, we have analyzed several plants from various locations, considering both certain morphological characteristics, as well as the amount of volatile oil and active principles. The results are briefly shown in the table below.

Origin	Material	Bitterness degree	Volatile oil (%)
Experimental field Braşov – 1	radix	11400	0,025
	folia	4270	
Experimental field Braşov – 2	**radix**	**9230**	**0,022**
	folia	**4090**	
Piatra Craiului wild flora	radix	10450	0,028
	folia	4600	
Postăvarul (wild flora)	**radix**	**11150**	**0,031**
	folia	**5600**	

Due to these characteristics and morpholigical data (radix length and weight, crown diameter, number of fruits, shoot height) we decided to focus our attention and start the in vitro multiplication from plants originating from the Experimental field Brasov – 2, and Postavaru Mountain. The gas chromatography-mass spectrometry analysis of the hydro-ethanolic (50:50, v/v) radix extracts [3] gave the following results (%, DW): Gentian from Postavaru Mountain – amarogentin: 0.05 – 0.5, gentiopicrin: 3.5 – 2.0, gentianin: 0.03, sugars: 27.0 – 35.0; experimental field Brasov (2) – amarogentin: 0.02 – 0.2, gentiopicrin: 3.0 – 1.9, gentianin: 0.01, sugars: 25.3 – 29.0. *Acknowledgements:* The research was performed within the Romanian CEEX grant no.2619/2006. **References:** [1] Lange, D. (1998) Europe's medicinal and aromatic plants: their use, trade and conservation. TRAFFIC International, Cambridge, Great Britain. [2] Parvu, C. (2005) Enciclopedia Plantelor, Ed. Stiintifica, Bucuresti. [3] Arino A. et al. (1997) Z. Lebensm.-Unters. -Forsch. A, Eur. Food Res. Technol., 205(4): 295 – 299

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Free radicals scavenging and hepatoprotective activities of the rwandese medicinal herb, *Crassocephalum vitellinum* and composition of the essential oil

Mukazayire MJ[1,2], Faes M[1], Allaeys V[3], Tomani JC[2], Buc Calderon P[3], Chalchat JC[4], Duez P[1]
[1]Free University of Brussels (ULB), Laboratory of Pharmacognosy, Bromatology and Human Nutrition, Institute of Pharmacy, CP 205 – 9, B-1050 Brussels, Belgium. (32 – 2 – 650.5283); [2]Institute of Research Science and Technology, (I.R.S.T.), Center of Research in Phytomedecine and life Science, B.P. 227 Butare, Rwanda; [3]Louvain Catholic University, Unit of Pharmacokinetics, Metabolism, Nutrition and Toxicology, School of Pharmacy, 1200 Brussels, Belgium; [4]Laboratory of Photochemistry Molecular and Macromolecular, Chemistry of Essential Oils, Blaise Pascal Clermont University, 63177 Aubière Cédex, France

The treatment of liver diseases occupies an important place in the Rwandese traditional medicine and several plants or combinations of plants, among which are *Crassocephalum vitellinum* leaves, are used by the traditional healers. The present work investigates the claimed antihepatotoxic and antioxidant activities of leaves polar extracts, comparing to N-acetyl cysteine (reference antihepatotoxicant) and both quercetin and Trolox (reference antioxidants). In order to evaluate the direct and protective effects of *C. vitellinum* methanolic extract on liver, rats precision cut liver slices (PCLS) were prepared and incubated in a Williams medium E with 1, 2, 5, 10 mg/ml of the extract either alone or in the presence of acetaminophen (hepatotoxicant, 10mM). The measurement of ATP level and CYP2E1 activity were used as endpoints to assess liver toxicity and metabolic activity. Chlorzoxazone was used as a probe to assess CYP2E1 enzymatic activity and the metabolite 6-OH-chlorzoxazone formation was quantified by HPLC [1]. In addition to a significant hepatoprotective activity (1 mg/ml), the methanolic extract of *C. vitellinum* leaves strongly scavenged the stable radical 1, 1-diphenyl-2-picryhydrazyl (DPPH) and reduced the peroxidation of linoleic acid. The essential oil was isolated by hydrodistillation (0,046% v/w yield) and analyzed with GC and GC/MS [2]. Identified predominating constituents that represent 95.8% of the total oil profile are limonene (34.8%), (E)-β-ocimene (21.8%), β-pinene (8.5%), α-pinene (6.6%), myrcene (6.3%), β-phellandrene (5.5%), germacrene-D (4.04%), α-phellandrene (3.6%),terpinolene (1.8%), Sabinene (1.7%), and β-caryophyllene (1.14%). **References:** [1] Evdokinova E. et al. (2001) Toxicology in vitro 15: 683 – 690. [2] Lameck S. et al. (2004) Flavour and Fragrance Journal 20: 316 – 317

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Anti-inflammatory activity of a new triterpenoidal saponin from *Bauhinia variegata* Linn. (Fabaceae)

Mohamed MA, Rabee M
Department of Medicinal Chemistry, [2]Department of Pharmacology, Theodor Bilharz Research Institute, Giza, Egypt

Many *Bauhinia* species are used extensively for traditional medicinal uses especially as anti-inflammatory agents [1,2,3]. The present study aimed to the identification of triterpenoidal saponins and polyphenols from the leaves of *B. variegata*. The *n*-butanol and ethyl acetate extracts of *B. variegata* leaves were separately fractionated on silica gel columns, followed by successive chromatography on cellulose and sephadex LH-20 for final purification of the new 23-hydroxy-3α-[(*O*-α-L-$^1C_4$-rhamnopyranosyl-(1"→4')-*O*-α-L-$^4C_1$-arabinopyranosyl-oxy]olean12-en-28-oic acid *O*-α-L-$^1C_4$-rhamnopyranosyl-(1""→4"")-*O*-β-D-$^4C_1$-glucopyranosyl-

(1''''→6''')-*O*-β-D-$^4C_1$-glucopyranosyl ester (**1**) and eight known compounds, i.e. quercetin, 3,3'-dimethoxy-quercetin, 3,3',6-trimethoxy-quercetin, isoquercitrin, quercetin 3-*O*-β-D-galacturonopyranoside, quercetin 3-*O*-β-D-neohespridoside [4], caffeic acid and ferulic acid, based on UV, HRESI-MS, 1D and 2D NMR. Moreover, as a conclusion for the biological study on male Swiss Albino mice (18 – 20 g), it was found that the new saponin (**1**) is non-toxic (LD_{50} 1000 mg/kg b. wt.) and has a significant anti-inflammatory activity in comparison with indomethacine. A significant anti-inflammatory effect was concluded from the reduction recorded in granuloma diameter on treatment with **1** (19.2% and 20.9%) in comparison with indomethacin (32.1 and 35.5%) at 32 and 48 days after ova injection, respectively, relative to control group. PGE2 level in granuloma was also reduced after treatment with **1** (36.1 and 22.1%) with respect to that of indomethacine (44.4 and 32.4%) at 32 and 48 days after ova injection, respectively. **References:** [1] Reddy, V. M. S., Yadava, R N. (2003). Nat Prod Res 17: 165. [2] El-Khatib, A. S, Kaleel, A. E. (1995). Bull Fac Pharm (Cairo Univ) 64: 892. [3] Sosa, S. et al. (2002). Phytomedicine 9: 646 – 53. [4] Agrawal, P. K. (1989). Studies in organic chemistry 39,^{13}C NMR of flavonoids. Elsevier science, New York.

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Determination of carbohydrates in medicinal plants – comparison of different techniques

Nasimullah Qureshi M[1], Stecher G[1], Abel G[2], Popp M[2], Bonn GK[1]
[1]Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, 6020 Innsbruck; [2]Bionorica AG, Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Determination of carbohydrates is a challenging and therefore a complex analysis field in phytochemistry. Owing to the high degree of isomerization and epimerization, to the low volatility and to the lack of UV absorbing moieties several analytical techniques cannot be employed without prior modification of target molecules. Additionally within medicinal plants beside carbohydrates several other substances and analytes are present in the produced extract. Aim of this study was the evaluation of different analytical approaches in order to recognize the most suitable technique for qualitative and quantitative analysis of carbohydrates. Focus within the study was placed on thin layer chromatography (TLC), gas chromatography (GC) and a newly developed mass spectrometric method, i.e. matrix free material enhanced laser desorption ionization time of flight mass spectrometry (mf-MELDI-MS) [1]. Samples employed within the study were standards and microwave assisted water extracts from *Quercus cortex*. TLC was developed using a solvent mixture of butanol:acetic acid:H_2O (8:3:2). The visualization of different carbohydrates was performed after reaction with a mixture of acetone – aniline -diphenylamine – phosphoric acid. Mono-, di- and trisaccharides were detected in reference to the carbohydrate standards. Gas chromatography with MS detection has been employed after derivatisation of targets. For di- and tri-saccharides single peaks were obtained, which was not the case for monosaccharides: Glucose for example delivered two signals, but at a constant ratio. Within mf-MELDI-MS from mono- to deca-saccharides clear potassium and sodium adduct signals could be obtained. Evaluation of all three employed techniques clearly proved the performance of mf-MELDI-MS for the qualitative analysis of complex mixtures, as target don't need modification and analysis needs only a few minutes. Beside that GC-MS is suitable for quantitative analysis. **Reference**s: [1] G. Bonn, A. Hashir, G. Stecher, R. Bakry; Matrix free MELDI mass spectrometry, 2006, patent pending.

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Quantitative Analysis of capsaicin in *Capsicum frutescens L.* by ^{1}HNMR

Nazari F[1], Nejad Ebrahimi S[2], Talebi M[1], Kazemizadeh Z[1], Hamzehloei A[1], Shabani S[3]
[1]Department of Phytochemistry, Academic Centre for Education Culture & Research, Shahid Beheshti Branch, Evin, Tehran, P.O. Box 19835 – 371, Iran; [2]Department of Phytochemistry, Medicinal Plants & Drug Research Institute, Shahid Beheshti University, Evin, Tehran, P.O. Box 19835 – 371, Iran; [3]Department of Chemistry, Faculty of Science, Shahid Beheshti University, Evin, Tehran, P.O. Box 19839 – 4716, Iran

Capsicum frutescens L. also known as red chilli, is a member of Solanaceae family and used in folk medicine for the treatment of rheumatoid arthritis, osteoarthritis and neuropathies [1]. In order to develop an efficient laboratory scale extraction of capsaicin from red chilli different methods have been compared. Ultrasonication, microwave assisted extraction and Soxhlet extraction methods were evaluated for their efficiencies and ^{1}H-NMR spectrometry was applied to the analysis of the capsaicin in extracts without chromatographic purification. In the ^{1}H-NMR spectrum integration range of peak 4.349 – 4.360 ppm was chosen for quantitative analysis. These integrals were compared to integrated value of dimethyl formamide as an internal standard at the range of 8.006 – 8.030 ppm. Among the tested methods microwave showed the highest yield of capsaicin (0.67%) compared to ultrasonic extraction (0.53%) and conventional Soxhlet method (0.4%). *Acknowledgements:* We are sincerely grateful for financial support by Research and Technology Deputy of ACECR under the contract No. 982 – 11. **References:** [1] Al-Qarawi A., Adam, E. (2003) Phytother. Res. 17: 92 – 95.

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Flavonoids of horse chestnut seeds

Kapusta I, Janda B, Stochmal A, Oleszek W
[1]Institute of Soil Science and Plant Cultivation, State Research Institute, ul. Czartoryskich 8, 24 – 100 Pulawy, Poland

Horse chestnut extracts show beneficial effects on venous insufficiency and have many positive pharmacological effects on the skin [1]. The principial extract and medicinal constituent of *Aesculus hippocastanum* seeds is aescin, a mixture of triterpenoid saponin glycosides. Its components include protoaescigenin and barringtogenol C. It can be fractionated into β-aescin, an easily crystallizable mixture and α-aescin, which is water-soluble. The aescin is a potent anti-inflammatory principle, which also reduces capillary fragility and prevents leakage of fluids into surrounding tissues. These saponins have also been used in shampoos, shower foams, creams, lotions and toothpastes. One other group of secondary metabolites in the seeds of horse chestnut is flavonoids. The work performed on flavonoids from the seeds of *Aesculus chinensis* demonstrated their significant antiviral activity [2]. *A. hippocastanum* flavonoids were also identified but their profile and concentration were not researched in detail [3]. Thus, the aim of our present work was to develop a fast UPLC procedure for profiling and determination of *A. hippocastanum* flavonoids. The method allowed good separation during 4.5 min. The particular compounds were identified by spiking the extract with authenticated standards or by LC-ESI/MS/MS analysis. Thirteen compounds could be identified in the profile out of which di- and triglycosides of quercetin and kaempferol were the dominant and their acylated forms occurred just in trace amounts. The total concentration in the seed was 0.88% of dry matter, which was more than two times higher than in a previous evaluation (method not reported) [3]. Considering these findings, it can be concluded that flavonoids of horse chestnut seeds may play a significant role in overall activity of the extracts, and should not be neglected as previously suggested [3]. **References:** [1] Bagchi D, Sen Ck, et al. (2003) Mutat. Res. 523 – 524, 87 – 97. [2] Wie F, Ma S-C, et al. (2004) J. Nat. Prod. 67: 650 – 653. [3] Hubner G, Wray V et al. (1999) Planta Med. 65: 636 – 642.

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Development and densitometric standardization of *Convolvulus pluricaulis* containing herbal medicinal products by quantification of marker compound

Patil UK[1], Dixit VK[2]
[1]Department of Pharmacognosy, VNS Institute of Pharmacy, Bhopal (M.P.) 462044 India; [2]Department of Pharmaceutical Sciences, Dr. H.S.Gour University, Sagar (M.P.) 470003 India

Convolvulus pluricaulis (Fam. Convolvulaceae) is a perennial herb with a small woody-branched rootstock and occurs in tropical and subtropical countries. It is available in Asian market as Shankhpushpi. A thin layer chromatographic method with densitometric UV detection at λ= 226 nm has been developed for standardization of *Convolvulus pluricaulis* containing herbal medicinal products by quantification of one isolated marker compound. The isolated marker compound was characterized as 3β, 23, 24-trihydroxyolean-12-en-28-oic acid. TLC analysis was performed on aluminium backed silica gel 60 F_{254} plates with n-hexane-ethyl acetate, 7:4 (v/v), as a mobile phase. Under these experimental conditions the method was highly sensitive (the limit of detection was 14.2 ng) and recovery was satisfactory (from 94.46% to 97.24%). The results obtained during validation of the method were confirmed in standardization of herbal syrups and tablets by quantification of isolated marker, because high precision and accuracy were achieved. *Acknowledgements:* One of the authors, UKP is thankful to University Grants Commission of India for providing financial assistance for the present work. **References:** [1] Podolak, I. et al. (2003) J. Planar Chromatography 16: 48 – 51. [2] Taranalii, AD et al. (2000) Pharm Biology 38: 51 – 56.

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The amount of secondary metabolites in cultivated *Gentiana lutea* L

Radanović D, Nastovski T, Janković T, Šavikin K, Menković N, Zdunić G
Institute for Medicinal Plants Research, T. Kožćužka 1, 11000 Belgrade, Serbia

The roots of *Gentiana lutea* are the officinal drug in European pharmacopoeia. However, recent investigation suggested that also the aerial parts of this plant could be useful as medicinal material [1,2]. Samples of leaves and roots of *G.lutea* were collected in June 2006 from three experimental plantations (two, three and five years old) on mountain Tara (1000 m). Second sampling was conducted by the end of vegetation, in October 2006. Plantations were established via nursery plants, produced from the seeds collected from nature (mountain Suvobor, Serbia). In all plantations the same agrotechnique measures were performed. Type of soil was Acid Brown, pH 5.5, clay content 7%, humus content 5%. Air-dried powdered plant material was extracted with methanol in ultrasonic bath for 30 min. Mangiferin (MG), isogentisin (IG) and gentiopicrin (GP) were isolated according to a previously published procedure [3]. Quantification of MG, IG and GP was performed using HPLC and calculated from calibration curves. The amounts of secondary metabolites in leaves and roots varied during the age of plantation, as well as during the vegetation period. The production of GP and MG in leaves and GP in roots is higher in younger plants (two-years old) than in five-years-old plants. Moreover, the seasonal variation in the content of these metabolites is recorded, i.e. their amounts decreased from June to October. IG showed different dynamic of accumulation. With the exception of two-years-old plants, in all samples collected in June this compound was found in traces, while its content increased by the end of vegetation period. Also, the amount of IG increased with cultivation period, being 0.21 and 2.9 mg/g of dry weight in roots of two-years-old and five-years-old plants, respectively, and 1.95 and 4.84 mg/g dry weight in leaves of two-years-old and five-years-old plants, respectively. **References:** [1] Nikolaev, S.M. et al. (1987) Izv. Sib. Otd. Akad. Nauk URSS Ser. Biol. Nauk 0: 3. [2] Menković, N. et al. (2000) Planta Med. 66: 178. [3] Menković, N (1997) Ph D. Thesis, Faculty of Pharmacy, Belgrade.

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An improved HPLC method for the analysis of diterpenoid glycosides in *Stevia rebaudiana*

Hoekstra B[1], Schaneberg B[1]
[1]ChromaDex, Inc., 2830 Wilderness Place, Boulder, CO, 80301, USA

Stevia rebaudiana, also known as sweetleaf, has been used as a sweetner for centuries in South America. Extracts are up to 300 times sweeter than table sugar, which is attributed to the diterpenoid glyscosides, stevioside and rebaudioside A. Although widely used in Japan and Brazil, most other countries do not allow the use of *Stevia* as a food additive. In the US, it can only be labelled as a dietary supplement. Many companies are now focusing on isolation of each of the individual diterpenoid glycosides, not just stevioside and rebaudioside A, for use in products as a low-calorie sugar alternative. Current HPLC methods for the determination of diterpenoid glycosides have been on NH_2-columns under isocratic conditions [1]. An improved HPLC method utilizing a Phenomenex Synergi Hydro-RP column has been developed on a gradient reverse-phase system of 0.1% trifluoroacetic acid in water and acetonitrile at 202 nm which overcomes the limitations of the reported NH_2 method with regards to separation of the minor components of *Stevia*. The method successfully separates rebaudiosides A, B, C, D and F, dulcoside A, isoteviol, steviol, steviol glucuronide, stevioside, steviolbioside, and several unidentified compounds. **References:** [1] Kolb, N. et al. (2001) J Ag Food Chem 49: 4538.

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HPLC evaluation of water-soluble extracts of *Chamaenerion angustifolium* L. and *Pentaphylloides fruticosa* L

Shikov A[1], Poltanov E[2], Pozharitskaya O[2], Dorman HJD[3], Makarov V[1], Tikhonov V[2], Hiltunen R[3]
[1]Interregional Center "Adaptogen", 47/5, Piskarevsky pr., 195067, St-Petersburg, Russia, [2]"Diod" Ltd, 11[a]Derbenevskaya ave., 113114, Moscow, Russia, [3]Faculty of Pharmacy, Div. of Pharm. Biology, University of Helsinki, P.O. Box 56 (Viikinkaari 5E), FIN-00014, Finland

Infusions of aerial parts of Ivan-tea (*Chamaenerion angustifolium,* Onagraceae) and Kurilian tea (*Pentaphylloides fruticosa,* Rosaceae) have been used in Russia for a long time as home made teas. The data about the chemical composition of these plants are limited. Dry water-soluble extracts of *C. angustifolium* (5 series) and *P. fruticosa* were investigated. The botanical material was dried at 40 °C for 5 – 6 h. After comminution, the tea was placed in heaps on outside platforms, wetted and bruised to initiate "fermentation", and fermented for 6 h at 40 – 41 °C (except the unfermented sample). Fermented teas were sampled from the heaps and dried at 40 °C for 4 – 6 h. The material obtained was suspended in water and extracted for 2 h. The resulting aqueous extracts were filtered, reduced in volume in vacuo, dried, and stored at 4 °C. The extracts were analyzed by RP HPLC coupled with PDA detection. Results of the analysis of the extracts are presented in table below. Table 1. Qualitative-quantitative data for a water soluble extracts of C. angustifolium and P. fruticosa

	Identified components, mg/g						
Extract	Total phenols	L-asc. acid	Gallic acid	Protocatechuic acid	Hyperoside	Ellagic acid	Octyl gallate
C. angustifolium							
Express	194.4 ± 7.9	ND	2.08 ± 0.10	2.88 ± 0.14	2.93 ± 0.14	0.96 ± 0.05	0.12 ± 0.01
Lotos	145.5 ± 14.2	ND	1.07 ± 0.05	3.22 ± 0.15	3.00 ± 0.14	0.59 ± 0.02	0.15 ± 0.01
Tver	206.9 ± 19.4	15.9 ± 0.8	4.70 ± 0.18	ND	2.82 ± 0.10	1.07 ± 0.06	0.08 ± 0.01
Diod	156.8 ± 11.4	17.7 ± 0.9	4.46 ± 0.16	ND	3.53 ± 0.15	1.18 ± 0.06	0.08 ± 0.01
Nonfermented	209.4 ± 4.2	6.1 ± 0.1	4.47 ± 0.24	ND	2.92 ± 0.14	1.06 ± 0.05	0.09 ± 0.01
P. fruticosa	160.6 ± 14.5	9.4 ± 0.4	4.04 ± 0.20	ND	3.08 ± 0.14	1.01 ± 0.03	0.08 ± 0.01

All the extracts of *C. angustifolium* contained gallic acid, hyperoside, ellagic acid, and octyl gallate. L-ascorbic acid was identified only in Tver, Diod and nonfermented extracts. Protocatechuic acid was identified only in Express and Lotos extracts. Extract of *P. fruticosa* contained L-ascorbic acid, gallic acid, hyperoside, ellagic acid and octyl gallate. Protocatechuic acid was not detected in this extract. It

can be seen that the water-soluble extracts of *C. angustifolium* and P. *fruticosa* in general have similar chromatographic profiles and active components in equivalent concentration.

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Bioactive Constituents of the edible wild mushrooms *Gomphus clavatus* and *Lactarius salmonicolor*

Makropoulou M[1], Athanasakis G[1], Aligiannis N[1], Fokialakis N[1], Gonou Z[2], Pratsinis H[3], Skaltsounis AL[1]
[1]Division of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Athens, Greece, [2]Division of Mycology, Faculty of Biology, University of Athens, Athens Greece [3]Laboratory of Cell Proliferation & Ageing, Institute of Biology, NCSR "Demokritos", Athens, Greece

Mushrooms are recognized as an excellent nutritious food as well as an important source of biologically active compounds of medicinal value. In a continuation of our research for new bioactive secondary metabolites we have focused on the investigation of two widely consumed mushrooms widespread in North America, Europe and Asia. The wild mushrooms *Gomphus clavatus* and *Lactarius salmonicolor* were collected, lyophilized and extracted with DCM, MeOH and EtOH/H_2O sequentially. The screening for the antioxidant activity of the crude extracts, performed by a modification of DPPH method, revealed that the MeOH extracts were most active. Subsequently the fractionation and investigation of *Gomphus clavatus* MeOH extract lead to the isolation of: ergosterol, 5,8-hyperoxide of ergosterol, serevisterol, 3,5,6-trihydroxyergostan-7,9(11)22-triene, 3,5,6-trihydroxyergostan-7,22-diene, 3β,5α,9α-trihydroxy-(22E)-ergostan-7,22dien-6-one, nicotinic acid and indole-3-carboxylic acid. The investigation of *Lactarius salmonicolor* MeOH extract lead to the isolation of serevisterol, 3β,5α,9α-trihydroxy-(22E)-ergostan-7,22dien-6-one, nicotinic acid, *p*-hydroxybenzoic acid and a new natural azulene type product. The ergosterol derinatives were evaluated for their antioxidant activity and their estrogenic activity in MCF-7 (Breast cancer) and PC-3 (Prostate cancer) cell lines. Concerning estrogenic activity, the samples derived from saponification of DCM extract of *Gomphus clavatus,* rich in ergosterol derinatives and the pure compound of the peroxide of ergosterol, were the most active samples with IC_{50} 15.1 and 15.3 μg/ml in MCF-7 and 13.6 and 13.1 μg/ml in PC-3 cell lines, respectively. Overall the isolated compounds had interesting activities and further research is needed in order to reveal the crucial role that mushrooms could play in human health when collected from the wild and consumed.

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Medicago sativa and *Medicago truncatula* as plant sources of the chemopreventive flavone tricin

Stochmal A, Kowalska I, Oleszek W
[1]Institute of Soil Science and Plant Cultivation, State Research Institute, ul. Czartoryskich 8, 24 – 100 Pulawy, Poland

In recent years many data have been published on health benefits of flavonoids present in human diet. Many studies were performed to characterize potential cancer chemopreventive properties of various plant phytochemicals, including flavonoids. Among this group of secondary metabolites tricin (4',5,7-trihydroxy-3',5'-dimethoxyflavone) has shown cancer preventive activity. The growth inhibitory and cell cycle-arresting properties of tricin in human MDA-MB-468 breast cancer and in nude mice were documented. The ability of tricin to inhibit cyclooxygenase enzymes and to modulate the cyclooxygenase-mediated prostaglandin production may contribute to its chemopreventive efficacy on intestinal carcinogenesis and prostate cancer. Few studies have focused on other pharmacological activities of tricin, and those performed used tricin monomer, which derives from chemical synthesis. Plant derived tricin products were not explored so far. The tricin glycosides are commonly distributed in the plant kingdom. However, there are few plant species with a concentration of tricin which may be of commercial value. Our recent work on flavonoids from *Medicago sativa* (common name alfalfa) and *Medicago truncatula* showed that tricin glucuronides were the main components of the flavonoid fraction of these two species [1, 2]. The total concentration of tricin glycosides in *M. sativa* was 1.4 – 1.7% of dry matter (whole shoots) depending on variety. *M. truncatula* var *truncatula*, *M. truncatula* var *longispina* and *M. truncatula* var Jemalong A17 (leaves) contained 3.23, 2.37 and 3.34% dry matter, respectively. Thus, we suggest that these two species (other *Medicago* species should be studied) can be considered as natural, commercial herbs with high tricin concentration. **References:** [1] Stochmal A. et al. (2001) J. Agric. Food Chem. 49: 5310 – 5314. [2] Kowalska I. et al. (2007) J. Agric Food Chem. 55: 2645 – 2652.

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Indigo naturalis: Qingdai, a TCM monograph proposed for the European Pharmacopoeia (Ph. Eur.)

van der Sluis W[1], Toren J[2], van Alphen C[2], de Kaste D[2]
[1]Universiteit Utrecht, Faculty of Pharmaceutical Sciences. Medicinal Chemistry, PO box 80082, NL-3508 TB Utrecht, Nederland; [2]RIVM/KCF, PO box 1, NL-3720 BA Bilthoven, Nederland

Indigo naturalis is one of the TCM monographs from the Chinese Pharmacopoeia proposed by the European Pharmacopoeia Commission to include in the Ph. Eur. [1]. It is prepared from the leaves or stems of *Baphicacanthus cusia* (Nees) Bremek., *Persicaria tinctoria* (Aiton) Spach., *Isatis tinctoria* L. or *Indigofera tinctoria* L. after fermenting. *Content*: minimum 2.0 per cent of indigo ($C_{16}H_{10}N_2O_2$; M_r 262.3) and minimum 0.13 per cent of indirubin ($C_{16}H_{10}N_2O_2$; M_r 262.3) (dried drug). Several indigo samples, including very old samples from Java (Indonesia) have been analysed by means of TLC and HPTLC and most proved to fulfil the requirements. Proposed **TLC**: *Test solution*: To 0,5 g of the powdered drug (355) add 25 ml chloroform R. Sonicate during 30 min and filter. *Reference solution*: Dissolve 1 mg of indigo R and 1 mg of indirubin R in 5 ml of chloroform R. *Plate*: TLC silica gel plate R (5 – 40 μm [or TLC silica gel plate R 2 – 10 μm]. *Mobile phase*: acetone R, chloroform R, light petroleum R (5:15: 30 V/V/V). *Application*: 20 μl as bands of 10 mm [or 10 μl as bands of 7 mm]. *Development*: over a path of 6 cm [or 3 cm]. *Detection*: immediately after development in daylight because of the instability of blue indigo colour. **HPLC**: *Test solution*. To 25.00 mg of the powdered drug add 25 ml chloroform R and sonicate for about 10 min and add methanol R to 50.00 ml and filter. *Reference solution*: Dissolve 1.000 mg indigo CRS in 50.00 ml chloroform R and sonicate for about 10 min and add methanol R to 100.00 ml and filter. The same for indirubin CRS. Prepare the solutions immediately before use. *Column*: – *size*: *l* = 0.125 m, Ø= 4 mm, – *stationary phase*: octadecylsilyl silica gel for chromatography R (5 μm*). Mobile phase*. water R, methanol R (34:66 *V/V*). Flow rate: 1.0 ml/min. *Detection*: spectrophotometer at 290 nm. Injection*: 10 μl. Acknowledgements:* Dr Riepl, TU München for the indirubin sample. **References:** [1] Pharmeuropa 19.3 (2007)

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Qualitative and quantitative analysis of multiple galloyl derivatives of quinic acid from the medicinal plant *Galphimia glauca* Cav. using HPLC-ESI-MS

Sultana T[1], Stecher G[1], Abel G[2], Popp M[2], Bonn GK[1]
[1]Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, 6020 Innsbruck, Austria, [2]Bionorica AG, Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Galphimia glauca (Malpighiaceae) is used in Mexican traditional medicine for the treatment of nervous excitement. Pharmacological investigations have reported sedative, anticonvulsant, antiasthmatic, antiallergic properties, hypothermic activity, barbiturate potentiation, protection against strychnine- and leptazol-induced convulsions. The present investigations deal with the identification of

Galphimia ingredients with special focus on quinic acid derivatives. Beside evaluation of different extraction techniques, i.e. Aquasolv® extraction [1], microwave assisted extraction (MAE) and traditional extraction, HPLC and mass spectrometric techniques were of central interest for identification and quantification of quinic acid galloyl derivatives. Evaluation of extraction techniques clearly demonstrated that MAE in 50% ethanolic solution afforded highest recovery (concerning dry weight) within shortest time. Mass spectral investigations proved that in addition to the most abundant galloyl derivative of quinic acid, i.e. the tetra-galloyl-quinic acid, several other multiple derivatives were detected differing from each other by 152 mass units. Elution order of these derivatives in reversed phase HPLC system was from lowest mass number to highest one, depending upon increasing hydrophobicity of analytes. Pure quinic acid was also present. Moreover, several different isomers for these multiple galloyl derivatives were identified, e.g. four different isomers both for di- and tri-galloyl-quinic acid, respectively, depending upon the difference in the point of attachment of galloyl moiety to the quinic acid ring structure. Owing to the changing polarity of different target compounds, clear tendencies concerning extraction solvent were observed. Using pure water for MAE delivered highest extraction yields for lower molecular weight derivatives, i.e. mono- to tri-galloyl-quinic acid, while for higher molecular weight galloyl derivatives of quinic acid, 50% EtOH was the right option. Finally the most abundant galloyl derivative found in *Galphimia glauca* (tetra-galloyl-quinic acid) was quantified at 280 nm, delivering approximately 6.9% (w/w). **References:** Bonn, G. et al. (1987) Wood Science and Technology, 21: 179.

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Quality relevant compounds in homeopathic mother tinctures – Isolation and characterisation of 3,5- and 4,5-dicaffeoylquinic acid

Weiß C[1,2], Schnädelbach D[1], Manns D[1,2]
[1]Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger Allee 3, D-53175 Bonn, Germany; [2]Pharmaceutical Institute, Pharmaceutical Chemistry, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

The quality of homeopathic mother tinctures is determined by the quality of the starting material and the method of preparation. In Germany their production and quality parameters are regulated by the official German Homeopathic Pharmacopoeia and the European Pharmacopoeia. According to these Pharmacopoeias, the majority of homeopathic mother tinctures should be prepared from fresh plant material. However, samples of certain mother tinctures from the European market showed an unexpected variability in their thin layer chromatography patterns, which might be caused by mother tinctures prepared from dried plant material instead of fresh plants. The present study contributes to the identification of quality relevant compounds able to distinguish properly manufactured mother tinctures from non-properly manufactured ones. In preliminary tests mother tinctures prepared both from fresh and dried plant material were tested using TLC. In a number of cases there were spots which occurred exclusively in tinctures from dried material. The isolation of the substances responsible for these spots was carried out using *Sambucus nigra* L. For isolation dried flowers were extracted with methanol. The dry residue was dissolved in water and successively re-extracted with solvents of increasing polarity. The EtOAc-soluble fraction was separated by preparative RP-HPLC/PDA yielding two substances which can be used as quality relevant compounds: 3',5'-*O*-dicaffeoylquinic acid and 4',5'-*O*-dicaffeoylquinic acid. It was the first time that the two acids were isolated from *Sambucus nigra* and characterized on the basis of UV-, MS- and NMR spectroscopic data. 3',5'-*O*-dicaffeoylquinic acid had already been identified earlier by LC/MS (dissertation G. Rieger, Graz, in preparation).

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Swertisin chemotype of passionflower (*Passiflora incarnata* L.)

Wohlmuth H[1], Penman KG[2], Pearson T[1], Lehmann RP[2]
[1]Department of Natural and Complementary Medicine, Southern Cross University, PO Box 157, Lismore NSW 2480, Australia; [2]MediHerb Research Laboratories, Brisbane Technology Park, Eight Mile Plains QLD 4064, Australia

Passionflower *(Passiflora incarnata)* is a medicinal plant widely used for its sedative and anxiolytic properties. The botanical drug is included in the current European and British Pharmacopoeias and is characterised phytochemically by a number of flavonoids, including the C-glycosyl flavonoids vitexin, isovitexin, schaftoside and isoschaftoside [1]. Following the identification of *P. incarnata* raw material grown in Australia with an aberrant flavonoid profile, we undertook a survey of dried material originating in Italy (1 sample), India (1 sample) and Australia (8 samples). A commercial extract (Indena SpA) was also included. Five other *Passiflora* taxa, which are potential adulterants of the drug, were also analysed. Ethanolic (70%) extracts were analysed by HPLC and LC-MS. Methanolic extracts were used for TLC analysis. Samples were also examined macro- and microscopically. Other taxa were readily distinguishable from *P. incarnata* by both TLC and HPLC. The *P. incarnata* samples fell into two groups, each with a distinct flavonoid profile. Although both groups contained vitexin and isovitexin (albeit in different proportions), the samples from Italy and India, the commercial extract and two of the Australian samples all contained schaftoside/isoschaftoside, but no swertisin. In contrast, 6 other samples grown in Australia displayed a large swertisin peak and the absence of schaftoside/isoschaftoside. Although the flower colour was not known for all samples, the results suggest that the swertisin chemotype corresponds with the white-flowered phenotype *P. incarnata* 'Alba', while the normal chemotype corresponds with the typical purple-flowered phenotype. The existence of chemotypes may have implications for raw materials selection. **References:** [1] Dhawan, K. et al. (2004) J Ethnopharmacol 94: 1 – 23.

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Rapid determination of nonsteroidal anti-inflammatory properties of alpine medicinal plants (NAIP)

Marcon N, Berthouzoz S, Zonnevijlle F, Grogg AF
HES-SO Valais/Wallis, Institute of Life Technologies, Route du Rawyl 47, 1950 Sion, Switzerland

The determination in liquid medium of the *in vitro* nonsteroidal anti-inflammatory activity of all kinds of matrices has been the subject of quite a number of studies. Such measurements avoid but do not replace, *in vivo* experiments on animals. Nevertheless they are very useful in systematic studies. They enable the quantification of the inhibition of certain enzymes that are responsible for the biochemical signal of inflammations, *e.g.* phospholipase A_2, 5- and 12-lipoxygenase and cyclooxygenase. Commercially available standard microcuvette colorimetric tests work well with pure dissolved substances. However, the usefulness of these tests is limited when the active substance is present in a complex matrix, such as raw and refined plant extracts. Recently it has been shown for acetylcholinesterase [1], that it is possible to apply an enzymatic reaction to a thin layer, after separation of the matrix, in order to characterize by colorimetry the inhibitory effect of each individual fraction. As the characterization of the nonsteroidal anti-inflammatory activity without previous separation of a plant extract is questionable, transferring commercial microcuvette tests to thin layers allows a more reliable direct comparison of raw plant extracts when carrying out a series of comparative measurements. Applying such enzymatic tests requires partial separation of the constituents by TLC, and colorimetric detection *in situ* by spraying well-defined quantities per surface unit of a solution of the enzyme, the substrate (lipoxygenase inhibition) or the developer (phospholipase A2). A

second treatment is sometimes needed after fixation of the reagents (cyclooxygenase). The global *in vitro* nonsteroidal anti-inflammatory activity is presented, based on the IC_{50} values measured for each specific enzymatic reaction. The results obtained for microcuvettes and for thin layers will be compared and presented for screening tests of raw extracts of a dozen alpine plants. The results obtained confirm the advantages of the newly developed method. **Reference**: [1] A. Marston et al. (2002) Phytochem. Anal. 13: 51

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Evaluation of phytochemical markers characterising cultivated and wild mullein flowers (*Verbascum phlomoides* L.)

Bodor Z[1], Alberti Á[2], Zsarnóczai J[2], Kéry Á[2], Németh É[1]
[1]Corvinus University of Budapest, Department of Medicinal and Aromatic Plants, Budapest Villányi út 29. 1118, Hungary; [2]Semmelweis University, Department of Pharmacognosy, Budapest Üllői út 26. 1085 Hungary

Verbasci flos, the drug of mullein is officinal in numerous national pharmacopoeias. Most important compounds of the herb are flavonoids, iridoids, mucilaginous polysaccharides and saponins [1,2,3,4]. Due to its expectorant and diaphoretic activity *Verbasci flos* has been used in folk medicine in cases of cold [1]. The aim of our study was to reveal the chemical composition of flower drugs from the annual cultivated variety 'Napfény' and from a wild biennial *Verbascum phlomoides* population. Further purpose was to compare the two drugs. Active substance assays were performed from representative drug samples, with triple repeats according to the instructions of the European Pharmacopoeia as well as with TLC, TLC-densitometry, HPLC, HPLC/MS, GC and GC/MS methods. Identification of representative compounds was carried out with standard additon and comparison with mass spectra from spectral libraries. We have found that in the accumulation of polar compounds characteristic of flower drugs there could not be detected any significant distinction between wild and cultivated stands. Contents of wild and cultivated stands are as follows: swelling index was 8,2 – 10,2 ml and 8,0 – 11,0 ml; total flavonoid content was (expressed as hyperoside) 0,97 g/100 g and 1,05 g/100 g; total tannin content was (expressed as pyrogallol) 4,12 g/100 g and 3,80 g/100 g, respectively. At valuation of flavonoid/phenolic composition tamarixetin, rutoside, rutin, isoquercitrin, quercitrin and chlorogenic acid contents have been studied and proportional differences have been found. We have observed significant differences in occurence of iridoid and apolar compounds of inflorescence drugs, accordingly in essential oil composition (occurence and proportion of α and β isophorone, minor compounds) and in accumulation of phytosterols and triterpenoids. **References:** [1] Wichtl M. (1997) Teedrogen und Phytopharmaka. Wissenschaftliche Verlagsgesellschaft. Stuttgart. [2] Kraus, J. et al. (1987) Deutsche Apotheker Zeitung. 127: 665 – 669. [3] Klimek B. et al. (1996) Phytochemistry. 43: 1281 – 1284. [4] Papay, V. et al. (1980) Pharmazie 35: 334 – 335.

4. Medicinal plants in animal healthcare

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Detection of essential oil compounds in different animal tissues after feeding a phytogenic feed additive to pigs

Zitterl-Eglseer K[1], Wetscherek W[2], Stoni A[2], Kroismayr A[2], Windisch W[2]
[1]Institute for Applied Botany and Pharmacognosy, Department of Public Health, University of Veterinary Medicine, Veterinaerplatz 1, A-1210 Vienna, Austria; [2]Department of Food Sciences and Technology, Division of Animal Food and Nutrition, University of Natural Resources and Applied Life Sciences, Gregor Mendel Straße 33, A-1180 Vienna, Austria

In the scope of a feeding trial on weaners with the approved phytobiotic zootechnical additive Biomin® P.E.P 1000, on the one hand, and the antibiotic growth promoter avilamycin, on the other in the diet, in comparison to a negative control group, the focus was placed on the bioavailability of the main components of the phytogenic feed additive's essential oil. A recently published analytical method [1] for measuring thymol in human blood plasma after administration of a cough suppressant was adapted for the analysis of animal tissues and faeces. The main compound of the phytobiotic zootechnical additive, carvacrol, was recovered in the blood plasma at amounts of 105.8 – 171.1 ng/ml (n = 12), in faeces (121.2 – 366.6 ng/g; n = 12) and in kidney tissue (50.0 – 240.4 ng/g; n = 12) by means of solid phase micro-extraction interfaced with GC/MS analysis, whereas approximately 10 times lower amounts of thymol were found. The two compounds could not be detected in liver, spleen, muscle or abdominal fat tissue. Metabolites of thymol and carvacrol and others derived from essential oil compounds were also not detectable. Conclusion: The administration of Biomin® P.E.P 1000 as feed additive led to no compilation of essential oil compounds or derived metabolites in tissues of pigs between 8 and 13 kg live weight. The relatively high amount of carvacrol in the kidneys shows the essential role of this tissue in the elimination of essential oil compounds. **References:** [1] Kohlert, C. et al. (2002) J Chromatogr. B 767: 11 – 18.

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Effect of fresh garlic supplements on serum cholesterol levels of broiler chickens

Lijuan W[1], Kupittayanant S[1], Yoohat K[2], Kupittayanant P[2], Chunlun S[3], Papirom P[3]
[1]Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand; [2]Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand; [3]Faculty of Veterinary Medicine, Khon Kaen University, Khon Kean, 40002, Thailand

Garlic (*Allium sativum*) has been used as both food and medicine in many cultures for thousands of years. In broiler chickens, it was found that a commercial garlic powder reduces elevated total cholesterol levels effectively [1]. However, the effect of fresh garlic on lowering cholesterol levels has not been studied. We examined the effect of fresh garlic on serum cholesterol levels in broiler chickens. Fresh garlic was added to the diet of Arbor Acres broiler chickens, and the cholesterol levels, as well as the concentration of the low-density lipoprotein cholesterol (LDL-C) in the serum were evaluated. Three-day-old broilers are studied and divided into four groups, each group containing 40 subjects. A 3-week experimental feeding trial was conducted in which three groups of broilers were fed 6.0, 8.0, and 10.0% of fresh garlic (FG groups) within their diet, respectively, and the result was compared to the control group. The results indicated that cholesterol concentration in serum was found to be considerably lower in FG groups when compared to control group. In addition, in all FG groups, LDL-C was lower than in the control group. In short, the results demonstrated that the cholesterol levels could be lowered by adding fresh garlic to the diet of boiler chickens. **References:** [1] Konjufca, V.H. et al. (1997) Poult Sci 76: 1264 – 71.

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Search for potential plant extracts in the prevention/ treatment of dental caries in animals: GTF inhibition is a poor predictor of biofilm inhibition by plant extracts

Honraet K[1], Thas O[2], Apers S[1], Cimanga K[1], Pieters L[1], Vlietinck AJ[1], Nelis HJ[3]

[1]Laboratory for Pharmaceutical Microbiology, University of Ghent, Harelbekestraat 72, B-9000, Ghent, Belgium; [2]Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure links 653, B-9000, Ghent, Belgium; [3]Laboratory of Pharmacognosy, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium

Streptococcus mutans plays an important role in the formation of dental plaque. [1]. Colonisation of tooth surfaces by this commensal bacterium and the subsequent formation of a biofilm are considered as the first important steps in the induction of dental caries [2]. The key enzymes in the biosynthesis of the extracellular matrix (glucan) of this biofilm are the glucosyltransferases (GTF). Some natural products, e.g. green tea inhibit GTF, the *in vitro* adhesion of *S. mutans* and biofilm formation by the latter *in vivo* [3]. However, so far there are no reports on a possible relationship between the GTF inhibition by plant extracts (PE) and an effect on *S. mutans* biofilms *in vitro*. In the present study, 44 PE (including a green tea extract and an epigallocatechingallate (EGCG) concentrate of green tea) were selected on the basis of their established GTF-inhibiting activity or a phylogenetic relationship to a plant with known GTF-inhibiting properties. All of these PE were tested for their ability to reduce the biomass of *S. mutans* biofilms on hydroxyapatite disks in a Modified Robbins Device (MRD). In addition, the inhibition of the *Leuconostoc mesenteroides* GTF mediated formation of dextran was evaluated in a turbidimetric microtiter plate assay. Although for some PE the results suggest a causal relationship between GTF inhibition and prevention of biofilm formation, most extracts exerted either only one or none of these effects. This finding implies that GTF inhibition is a poor predictive indicator of a possible antibiofilm activity by PE. This conclusion was corroborated by the results obtained in both test systems of fractions of a PE showing GTF as well as biofilm-inhibiting properties. The antibiofilm activity was predominantly present in a non-GTF-inhibiting fraction, whereas the tannin-containing fraction that did inhibit GTF had no effect on biofilm formation. *Acknowledgements:* Oystershell NV, Drongen, Belgium, IWT (KMO Innovation Project). **References:** [1] Spratt D (2003) Medical biofilms: detection, prevention and control, John Wiley & Sons Ltd., Chichester, UK,. [2] Hao, Y. et al. (1997) Infect. Immun. 65: 2292 – 2298. [3] Otake, S. et al. (1991) Caries Res. 25: 438 – 443.

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Antimicrobial activity of the essential oil from *Salvia officinalis* L. against selected pathogenic microorganisms in piglets

Taylorova B[1], Poracova J[1], Salamon I[1], Blascakova M[1]

[1]Presov University in Presov, Faculty of Humanities and Natural Sciences, 1, November 17th St., 080 01 Presov, Slovak Republic

Plant extracts and their active components have shown a large scale of activities: as antimicrobials, anti-oxidants, digestive stimulators, immunomodulators, anti-inflammatory agents, appetisers and performance enhancers [1,2]. The antimicrobial properties of the essential oil from sage (*Salvia officinalis* L., Lamiaceae) were evaluated against anaerobic bacteria, aerobic bacteria, *Escherichia coli*, enterobacteria and enterococci in a model experiment in crossbred piglets (Slovak White x Pietrain) weaned at 28 days of age. The essential oil content of dry leaves, consisting of thujone (24%), borneole (18%) and cineole (15%), was applied daily in a dose of 0.05% into the commercial feed mixture ÈOS 1 and ÈOS 2 starting at the age of 21 days to 7 piglets in an experimental group and pathogen concentrations were compared with a control group of 3 piglets. Faecal samples of piglets were analysed on the 21st, 35th and 42nd days of age and counts of anaerobes, aerobes, *Escherichia coli*, enterobacteria and enterococci were performed. The differences in counts of selected bacteria within the control group during the experiment were not statistically significant. The counts within the experimental group showed statistically significant differences ($p < 0.05$) in number of all selected bacteria between 1st and 2nd samplings. The anaerobic bacteria count was also significantly different on 21st and 42nd day of age. When comparing the control and the experimental group counts of *Escherichia coli* at the age of 35 days, they were significantly lower in the experimental group. All statistically significant differences observed show decrease in selected bacteria counts. Because the essential oil is inhibitory to selected pathogenic microorganisms it may provide an alternative and supplement to conventional antimicrobial additives in feeds. **References:** [1] Burt, S. A., Reinders, R., D. (2003) Lett. Appl. Microbiol. 36: 162 – 167. [2] Dorman, H. J. D., Deans, S. G. (2002) J. App. Microbiol 88: 308 – 316.

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Serum biochemical parameters in piglets after the application of essential oils

Poracova J[1], Salamon I[1], Taylorova B[1], Zahatnanska M[1], Sutiakova I[1]

[1]Presov University in Presov, Faculty of Humanities and Natural Sciences, 1, November 17th St., 080 01 Presov, Slovak Republic

Extracts and essential oils from medicinal plants are used as feed additives in order to increase the quality of food production with animal origin [1, 2]. Today's trend of focusing on protection of the environment and increased production of organic products can be supported using natural ways and resources of protein production with animal origin and application of phytoadditives [3, 4]. Sage (*Salvia officinalis* L., Lamiaceae) and oregano (*Origanum vulgare* L., Lamiaceae) essential oils were applied to 11 piglets Slovak White x Pietrain crossbreed. The main compounds of essential oil from leaves of *Salvia officinalis* L. were thujone (24%), borneole (18%) and cineole (15%). The major component of the essential oil was carvacrol (55%) in *O. vulgare*. In this model experiment, piglets aged 3 weeks were divided into 2 groups containing 5 piglets in a control group (CG) and 6 piglets in an experimental group (EG). They were housed in stall system having water administered *ad libitum.* Piglets in EG were fed with feed mixture ÈOS 1 and ÈOS 2 enriched with sage and oregano essential oils (0.05%) daily for a period of 4 weeks. Biochemical parameters – AST (EC 2.6.1.1, Aspartate transaminase), ALT (EC 2.6.1.2, Alanine transaminase), LDH (EC 1.1.1.27, Lactate dehydrogenase), glucose, total proteins, albumins, and triacylglycerides in blood serum were measured weekly. Significant difference ($p < 0.01$) in activity of ALT comparing CG and EG was found in weeks 1 and 2 of the experiment. Concentration of glucose, total proteins, albumins and triacylglycerides was found to be significantly different ($p < 0.05$) in the 1st and 4th weeks of the experiment. Although only a small number of animals was involved in this model experiment, application of sage and oregano essential oils indicated the probability of having an influence on piglets. **References:** [1] Stashenko, E.E. et al. (2002) Anal.Bioanal. Chem 373: 70 – 74. [2] Vichi, S. et al. (2001) Nahrung 45: 101 – 104. [3] Marcin, A. et al. (2003) Kvalitatívne aspekty pestovania a spracovania lieèivých, aromatických a koreninových rastlín. Agroinžtitút. Nitra. [4] Šutiak, V. et al. (2002) A handbook of veterinary phytoterapy. UVM. Kožice.

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Anthelmintic effects of certain Czech medicinal plants on *Ascaris suum* and *Trichostrongylus colubriformis*

Urban J[1], Kokoska L[2], Langrova I[3]
[1]National Reference Laboratory for Disinfection and Sterilization, The National Institute of Public Health in Prague, Srobarova 48, 100 42 Prague 10-Vinohrady, Czech Republic; [2]Department of Crop Science and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic; [3]Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic

Increasing problems of resistance development in helminths [1] against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. This resistance against synthetic anthelmintics for gastrointestinal trichostrongylids and ascarids is a worldwide problem in sheep, goat and pig breeding [2,3]. Thus we decided to evaluate selected medicinal plants traditionally used in Czech Republic for treatment of nematode infections for their *in vitro* anthelmintic effects. The ethanol extracts of 16 Czech medicinal plants, namely *Allium sativum*, *Artemisia absinthium*, *Artemisia vulgaris, Carum carvi, Consolida segetum, Cucurbita pepo, Daucus carota, Dryopteris filix-mas, Erigeron canadensis, Hedera helix, Inula helenium, Juglans regia, Satureja hortensis, Tanacetum vulgare, Thymus vulgaris* and *Valeriana officinalis* have been tested for their potential *in vitro* anthelmintic effect against eggs of *Ascaris suum* and infectious larvae of *Trichostrongylus colubriformis* using ovocid effects and larval migration inhibition methods [4,5]. The extracts of *A. sativum, A. absinthium*, *C. carvi*, *D. carota,* and *J. regia* possessed the strongest anthelmintic effect on the egg hatching in all concentrations tested (62.5, 125, 250, 500, 1000, 2000 µg/ml). The best results, showing a higher effect against the third-stage larva infects in comparison with the synthetic anthelmintic Zentel (Albendazole), have been obtained for *A. sativum, A. absinthium*, *C. carvi*, *C. segetum*, *I. helenium*, *J. regia*, *S. hortensis,* and *V. officinalis*. Preliminary results of chemical analysis for the three most active extracts showed presence of sulphur components, sesquiterpene lactones and terpenoids for *A. sativum, A. absinthium* and *C. carvi*, respectively. *Acknowledgements:* This research was supported by projects research project MSM 6046070901. **References:** [1] Coles, G.C., et al. (1992) Vet Parasitol 44: 35 – 43. [2] Serrano, F.J., et al. (2001) Parasitology 122: 699 – 707. [3] Waller, P.J. (1994) Acta Trop 56: 233 – 243. [4] Hounzangbe-Adote, M.S., et al. (2005) Res Vet Sci 78, 155 – 160. [5] Rabel, B., et al. (1994) Int J Parasitol 24, 671 – 676.

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Veratrum album L. - Total alkaloid extract with use in veterinary medicine

Ionescu E, Mihailescu R, Chiriac M, Tebrencu C
The S.C. Centre for Research and Medicinal Plant Processing "PLANTAVOREL" S.A. Piatra Neamt, 46, Cuza Voda street, 610019, Romania

Our investigations aimed to obtain, by means of an original method, a total alkaloid extract from *Veratrum album* rhizome standardized as veratrin in a formula of a veterinary pharmaceutical product to be used in digestive affections of ruminants [1,2]. We performed experimental studies to obtain a total alkaloid extract from Veratri rhizoma in several extraction steps, using extraction solvent conditions with controlled pH, and obtained a standardized product with 0.03 – 0.08% total alkaloids expressed as veratrin. Veratrin ($C_{36}H_{51}O_{11}N$) has a ruminant effect and acts synergistically with the other compounds of the extract obtained in the form of a hydroalcoholic solution (ethyl alcohol, hydrochloric acid, total alkaloid extract). The pharmaceutical product may be used in the treatment of acute and chronic affections of the cattle first stomach, acting as an excitant of the first stomach mucosa and of the cattle glandular stomach, reactivating their movements and favouring digestion by stimulating the secretion of gastric juice. *Acknowledgements:* This work was supported by *The S.C. Centre for Research and Medicinal Plant Processing "PLANTAVOREL" S.A.* Piatra Neamt, ROMANIA **References:** [1] Dobre T., Floarea O., (2000), Separarea compusilor chimici din produse naturale, Ed. Matrix, Bucuresti. [2] Mestroni G., Calliagris M., (1989), Progress in Clinical Biochemistry and Medicine, 10: 72.

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In vitro macrophage activation by organic waste materials

Teichmann K[1], Klimitsch A[1], Schatzmayr G[1]
[1]BIOMIN Research Center, Technopark 1, A-3430 Tulln, Austria

The EU-wide ban on antibiotic growth promoters in animal production has stimulated research on functional feed additives that improve animal health and performance in the related industries. Organic wastes from industries like pharmaceutical or food production often have to be disposed at high costs and sometimes also cause environmental problems. The potential further use of such plant residues or by-products as value-added products in animal or human nutrition is currently evaluated in the course of the EU-funded project SAFEWASTES. Here we report on possible immunomodulating activity of several organic wastes by *in vitro* activation of a chicken macrophage cell line. Macrophages are important elements of the innate, non-specific and specific immune reaction of vertebrates. They represent a first-line defence against invading pathogens by phagocytosis, cytokine and nitric monoxide (NO) production and antigen presentation. *In vitro* activation of a chicken bone marrow-derived macrophage cell line (HD-11) was measured by NO production in a cell culture assay. Active substances were checked for lipopolysaccharide (LPS) contaminations caused by gram-negative bacteria that could lead to false-positive results. 52 extracts prepared from 18 different organic waste materials were investigated. Nine water extracts showed high macrophage activation comparable to the positive control, a bacterial cell wall preparation. Among them, willow bark water extract had no detectable amounts of LPS while they probably accounted for some activity of the other extracts. Bioassay-guided fractionation was done to further identify the active principles of the materials. *Acknowledgements:* The SAFEWASTES project is funded by the 6th Framework Programme of the European Union.

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Histopathological evaluation of honey's effect in treatment of experimental wounds in dogs

Esmaeelian B[1], Kamrani YY[1], Naderi MM[2], Amanlou M[1]
[1]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Medical Sciences/University of Tehran, P.O.Box: 14155 – 6451, Tehran, Iran; [2]Department of small animal internal medicine, faculty of veterinary, science & research of Tehran Azad university, Tehran, Iran

Honey has been generally used as traditional medicine for the treatment and healing of wounds. In this study, we evaluated the histopathological effect of unboilded honey in the management of experimental wounds [1]. The experiment was performed on 20 mix breeding dogs. Weights of dogs were almost 25 kg. Surgical preparation and anesthesia were carried out and then surgical wounds were produced by a similar pattern (rectangle shape – 25 x 50 mm^2) in the thoracolumbar region. Wounds were divided in two groups: treatment group on the left side and control group on the right side. Postoperative treatment in the honey group included daily wound irrigation with normal saline and then topical application of honey. In the control group only irrigation with normal saline was performed. Dogs were euthanized at 7, 14, 21 and 28 days after operation. Sampling and histopathological evaluation were performed in each group at the same time [2]. According to the results of this study, from a histopathological point of view, honey has a significant effect on healing of experimental wounds when compared to the control

group. In the treatment group, especially from the second week on, the compact connective tissues were developing from the base of the wound and sepsis was observed in some superficial points. In addition, less neutrophilic chemotaxis was found in the treatment group than in the control group. Honey can be used as a natural substance to accelerate the healing process of skin wounds. *Acknowledgements:* 1. Tehran University of medical Sciences and Pharmaceutical Sciences Research Center 2. Dr. Sanaz Rahmani **References:** [1] Lusby P.E. et al. (2002) J. Wound Ostomy 22(6): 273 – 274. [2] Efem S.E. (1988) J. Surg 75(7): 679 – 681.

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Effects of vegetable by-products on basic parameters of porcine caecal microbial metabolism in a modified Colon Simulation Technique (COSITEC)

Mader A[1], Zentek J[2]
[1]Institute of Nutrition, Veterinary University of Vienna, Veterinärplatz 1, 1210 Vienna, Austria; [2]Institute of Animal Nutrition, Free University Berlin, Brümmerstraße 34, 141195 Berlin, Germany

Aim: Semi-continuous colon simulation technique [1] was used as an experimental model to study potential effects of various organic wastes (OW) from the food and pharmaceutical industry after their technological processing. Method: For *in vitro* incubation the fluid and solid phase of the caecum content (CC) of slaughtered finisher pigs, kept on conventional diets, were used. The 7-day equilibration period, adding 1 g freeze-dried CC/day, was followed by OW exposure (100 mg OW and 900 mg freeze-dried CC/day for 1 day) and equilibrating (1 g freeze-dried CC/d for 1 day) in turn. Basic parameters like pH, redox potential and samples for NH_3, lactate and short-chain fatty acids (SCFA) were determined 0, 2, 4, 6, 8, 12 and 24 hours after substrate addition. This standardized *in vitro* technique allows measuring quantitatively the direct influences of OW on the microbial metabolism. **Results:** The influence on the basic parameters was strongest 4 hours after substrate addition. Some OW (i.e. water extracts of mango and blueberry peels) resulted in significant decreases of the pH. An influence on the redox potential could not be found. The ammonia concentration increased with i.e. larch sawdust (raw material) and decreased with i.e. grape seeds (raw material) significantly. A significant influence of the OW was detectable for following SCFA: i- and n- butyric acid and i-valeric acid, but not for total SCFA concentration. Conclusion: From the results it can be speculated that some OW are fermented by the caecal bacteria indicating "prebiotic" substrate effects. We could not detect antimicrobial properties under these conditions and further research on the impact on the physiological gut flora is currently performed. **References:** [1] Dreyer J. (1990) In vitro-Untersuchungen mit der Colon-Simulations-Technik (Cositec) zum mikrobiellen Stoffwechsel im Dickdarm von Schweinen. Diss. sc. agr., Göttingen.

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Murciano-Granadina goat feeding with aromatic plant by-products. Effect on the milk production and presence of polyphenols in "Al Vino" Murciano goat cheese

Jordán MJ[1], Martínez C[1], Moñino MI, López MB[2], Ferrandini E[2], Lafuente A[1], Sotomayor JA[1]
Murcian Institute of Investigation and Agricultural Development (IMIDA) C./ Mayor s/n 30150 La Alberca (Murcia) Spain; Food TechnologyDepartment. Faculty of Veterinary. University of Murcia. Campus de Espinardo 30071 Espinardo (Murcia) Spain

The principal goals of feeding goats with rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus zygis* subsp. *gracilis*) by-products from plant steam distillation are to improve the animal production characteristics and to transfer their antioxidant properties to the milk produced. For our study, thirty six goats were randomly assigned into three homogeneous groups. One group was fed with unifeed as a control and the diet of the other two groups was modified by substituting 10% and 20% of the control diet (respectively) with distilled leaves. For this, a pellet composed for a mixture of unifeed and distilled leaves was made. Milk production was not affected by the introduction of aromatic plants in the animal diet. Annual milk production for goats fed with rosemary leaves was (1.17 ± 0.263) control; (1.33 ± 0.24) 10% and 20% (1.37 ± 0.27) L/day. When thyme leaves were added, the dairy yields were (0.99 ± 0.26) control; 10% (1.23 ± 0.34) and 20% (1.16 ± 0.47) L/day. Polyphenolic components present in the cheese were extracted from 1.5 g of lyophilized sample using methanol as extracting solvent. For the chromatographic analysis (HPLC), a method adapted from Zheng and Wang [1] was used. 11 polyphenolic components were detected in the goat cheese. Among these components, in both experimental groups (10 and 20%, respectively), only rosmarinic acid with a ratio of concentration of 1.20 – 1.20 ($p < 0.001$) respectively, and carnosol (1.42 – 1.58) ($p < 0.05$) increased in concentration with respect to the control group. For the thyme, rosmarinic acid (1.20 – 1.20) ($p < 0.001$) and carnosic acid (1.14 – 1.23) ($p < 0.05$) were the components detected at higher concentrations when compared to the control group. From these results it can be concluded that the administration of by-products- from rosemary and thyme- in the diet of goat does not modify the yield of animal production and favours the presence of polyphenols with antioxidant capacity in the cheese. *Acknowledgment:* We thank the INIA, for providing the project SC00 – 052-C7 – 3RTA04 – 077-C2, under which this work has been accomplished. **References:** [1] Zheng, W., Wang, S.Y. (2001). J. Agric. Food Chem. 49: 5165 – 5170.

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Segureña sheep feeding with the distillate from rosemary (*Rosmarinus officinalis* L.). Polyphenolic components in lamb meat and their effect on the meat stability

Moñino MI, Martínez C, Sotomayor JA, Lafuente A, Gamaza AM, Jordán MJ
Murcian Institute of Investigation and Agricultural Development (IMIDA) C./ Mayor s/n 30150 La Alberca (Murcia) Spain

The aim of the present work is to study whether the supplementation of rosemary plant by-products from the plant steam distillation in the daily animal feeding allows the transfer of antioxidant active components to the meat without detriment to the animal productivity. For that, thirty six Segureña ewes were randomly assigned into three homogeneous groups. One group was fed with unifeed as a control and the diet of the other two groups was modified by substituting 10% and 20% of the control diet (respectively) with distilled rosemary leaves. For this, a pellet composed for a mixture of unifeed and distilled leaves was made. The introduction of rosemary leaves in the animal diet did not modify the lambs daily weight gain values: control (0.18 ± 0.08); 10% (0.18 ± 0.09); 20% (0.19 ± 0.07) kg. Concerning the presence of polyphenolic components in lamb meat (muscles from the front legs (FL) and the abdominal wall (AW)), the methanolic extraction and the subsequent chromatographic analysis (HPLC) allowed the identification of 11 polyphenols. Results from both experimental groups 10 – 20% revealed that among these polyphenols, only rosmarinic acid (in FL meat) with a ratio of concentration of 2.17 – 2.09 respectively ($p < 0.001$), carnosol (FL: 2.14 – 1.97 ($p < 0.05$); AW: 1.57 – 2.08 ($p < 0.001$)) and carnosic acid (FL: 3.36 – 2.6; AW: 2.95 – 2.68) ($p < 0.001$) were detected at higher concentrations when compared to the control. In order to study the antioxidant stability of the lamb meat, the radical scavenging activities of the methanolic extracts (1.5 g of dry meat/ml) were measured according to the method described by Brand-Williams et al. [1]. An increment in the decoloration power of samples (% $DPPH^{\cdot}$ decoloration) from the 10% (FL: 52.3 ± 1.93; AW: 52.1 ± 2.18) ($p < 0.05$) and 20% (FL: 52.1 ± 2.18; AW: 52.46 ± 2.45) ($p < 0.001$) groups was stated when compared to the control group (FL: 46.0 ± 3.37; AW: 46.3 ± 1.89). From these results it can be concluded that the administration of rosemary leaves does not modify the animal yield and improves the antioxidant stability of the lamb meat. *Acknowledgment:* We thank the INIA, for providing the project

SC00 – 052-C7 – 3RTA04 – 077-C2, under which this work has been accomplished. **References:** [1] Brand-Williams, W. et al. (1995) Lebensm.-Wiss. Technol. 28: 25 – 30.

P 323

Identification of polyphenolic components in Murciano-Granadina goat milk and suckling goat kid plasma

Martínez C, Jordán MJ, Moñino MI, Lafuente A, Quílez M, Sotomayor JA
Murcian Institute of Investigation and Agricultural Development (IMIDA) C./ Mayor s/n 30150 La Alberca (Murcia) Spain

The principal goals of feeding goats with rosemary by-products from the plant steam distillation are to improve the animal production characteristics and transfer their antioxidant properties to the milk and at the same time to the suckling goat kids. For that, thirty six Murciano-Granadina goats were randomly assigned into three homogeneous groups. One group was fed with unifeed as a control and the diet of the other two groups was modified by substituting 10% and 20% of the unifeed respectively with distilled rosemary leaves. For this purpose, a pellet composed of a mixture of unifeed and distilled leaves was made. Polyphenols present in goat milk, were extracted with methanol according to the method described by Ternes and Schwarz [1]. The chromatographic analysis of the methanolic extracts [2] allowed the identification of 11 major polyphenolic components. Among these components, and considering both experimental groups (10 – 20%), only rosmarinic acid (with a ratio of concentration of 1.21 – 1.21 ($p < 0.001$) respectively), carnosic acid ((1.14 – 1.41) ($p < 0.001$)) and carnosol ((1.19 – 1.26) ($p < 0.05$)) increased in concentration with respect to the control group. The introduction of rosemary leaves in the animal diet did not modify the goat kid daily weight gain values: control (0.18 ± 0.03); 10% (0.21 ± 0.03 kg); 20% (0.19 ± 0.03 kg). To determine the presence of polyphenolic components in goat kid plasma, a modified method described by Cerdá et al [3] was used. Once again, rosmarinic acid (with a ratio of concentration of 35.34 – 35.41 respectively ($p < 0.001$)), carnosol (4.07 – 4.17) ($p < 0.001$) and carnosic acid (30.01 – 34.51) ($p < 0.001$) were the components which increased their concentrations in both experimental groups when compared to the control. From these results it can be concluded that the supply of rosemary leaves from the distillate to ewes increases the presence of polyphenols in the goat milk and their transference to suckling goat kids. *Acknowledgment:* We thank the INIA, for providing the project SC00 – 052-C7 – 3RTA04 – 077-C2, under which this work has been accomplished. **References:** [1] Ternes W., Schwarz, K. (1995) Zeitschrift fü Lebensm Unters Forsch. 201: 548 – 550. [2] Zheng W., Wang S.Y. (2001) J. Agric. Food Chem. 49: 5165 – 5170. [3] Cerdá B. et al. (2005) J. Agric. Food Chem. 53: 227 – 235

5. Phytochemistry and structure elucidation of natural products

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Impact of analysis method on anthocyanin content of elderberry products

Chrubasik C[1], Maier T[2], Luond M[1], Schieber A[2]
[1]Institute of Forensic Medicine, University of Freiburg, Albertstr. 9, 79104 Freiburg, Germany, [2]Institute of Food Science and Biotechnology, University of Hohenheim, August-von Hartmann-Str. 3, 70599 Stuttgart, Germany

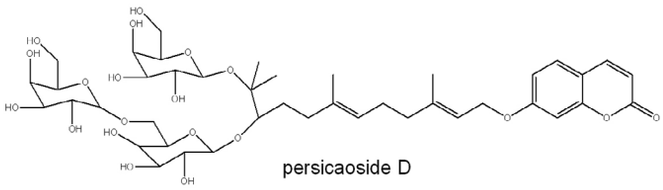
Knowledge of the anthocyanin content is required to pre-estimate the potential antioxidative or antiaging effects of food supplements. **Aim** of this investigation was to compare the anthocyanin contents of two elderberry products by using different analytical methods. **Methods:** We used (A) a commercial dietary supplement based on elderberry juice concentrate prepared from 120 g fresh berries and supplemented with elderflower juice and extract based on 3.9 g dried flowers per 200 ml and (B) an elderberry juice mother concentrate (prepared in a ratio of 6.5:1 by Wild (Eppelheim, Germany)) and employed (i) the spectrophotometric method described in the French Pharmacopoeia (1), (ii) the pH-differential spectrophotometric technique (2) and, (iii) HPLC analysis with mass spectrometric identification of the cyanidin derivatives (3,4). **Results:** The anthocyanin contents were (i) 762 mg/L at 528 nm, (ii) 85,4 mg/L at 513 nm and (iii) 4 mg/L (minute amounts of cyanidin-3-glucoside (0.6 mg/L), cyanidin-3-sambubioside (2.2 mg/L) and cyanidin-3-sambubioside-5-glucoside (1.2 mg/L) in A and (i) 6400 mg/kg at 528 nm, (ii) 17200 mg/kg at 513 nm and (iii) 15430 mg/kg (13705 mg/kg cyanidin-3-sambubioside, 1725 mg/kg cyanidin-3-sambubioside-5-glucoside) in B. **Conclusion:** Our results show that the results obtained from the determination of anthocyanins are strongly affected by the method employed. Hyphenated techniques such as HPLC combined with diode array and mass spectrometric detection appear to be most promising. Less sensitive methods consider more or less also degradation products. If food supplements claim for any clinical effects associated with anthocyanins, they must provide correct values. **References:** [1] Anonymous (2000) French Pharmacopoeia 10th Edition. Monograph Folia Vitis vinifera. [2] Giusti, M., Wrolstad, R.E. (2000) www.does.org/masterli/facsample.htm. [3] Kammerer, D. et al. (2004) J. Agric. Food Chem. 52, 4360 – 4367. [4] Chandra, A. et al. (2001) J. Agricult. Food Chem. 49, 3515 – 3521.

P 325

Persicaosides A-D, polar secondary metabolites of *Ferula persica* roots

Iranshahi M[1], Schneider B[2], Mojarab M[1], Sadeghian H[3], Hanafi-Bojd MY[1]
[1]Department of pharmacognosy and Biotechnology, Mashhad University of Medical Sciences, Mashhad, Iran; [2]Max-Planck-Institute for Chemical Ecology, Beutenberg Campus, Hans-Knöll-Str. 8, D-07745 Jena, Germany; [3]Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

The exclusively old world genus *Ferula* belongs to the family Umbelliferae with about 130 species distributed throughout the Mediterranean area and central Asia, specially in the former USSR and neighboring countries such as Iran. In spite of many phytochemical investigations carried out on *Ferula*, there are only a few reports about polar secondary metabolites of this genus. On the other hand, some species of *Ferula* have been used in traditional medicine. Therefore, identification of polar compounds of this genus, especially of medicinal species, can be worthwhile. Up to now, various secondary metabolites including sesquiterpenes [1], sesquiterpene coumarins [2], flavonoids and sulphur containing compounds [3] have been reported from *Ferula persica*. In present work, we wish to report four new sesquiterpene coumarin glycosides, named persicaoside A-D, together with other known compounds. The structures of these compounds were elucidated by extensive spectroscopic methods including 1D-(^{1}H and ^{13}C) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC, and ROESY) as well as CI-MS analysis.



persicaoside D

References: [1] Iranshahi, M. et al. (2003) Pharm Biol 41: 431 – 433. [2] Iranshahi, M. et al. (2004) Pharm Biol 42: 440 – 442. [3] Iranshahi, M. et al. (2003) Phytochemistry 63: 956 – 966.

P 326

Isolation, structure elucidation, cytotoxicity and anti-adhesive properties of proanthocyanidins from *Rumex acetosa* L

Anke J[1], Petereit F[1], Hensel A[1]
[1]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

Rumex acetosa L. (Polygonaceae) was investigated concerning structural features of proanthocyanidins. An acetone/water extract was purified via Sephadex LH20. Further separation was achieved by MLCCC, MPLC (MCI®, RP-18), preparative TLC and HPLC (RP-18, diol). In total, 3 monomers, 16 dimers, 5 trimers, 2 tetramers and a polymer fraction were obtained. All compounds isolated consist of epicatechin, catechin or epiafzelechin as flavan-3-ol monomers. Stereochemical investigations indicated the presence of (+)-catechin and (-)-catechin. A- and B-type interflavan-linkage was found as well as substitution with gallic acid. Structure elucidation was performed using 1D- and 2D- NMR, ESI-MS, CD and optical rotation methods. For structure-effect-relation *in-vitro* toxicity towards HepG2-cells was investigated (MTT test). Epicatechin and catechin did not show any toxicity up to 3,5 µmol/ml (48 h incubation). Epicatechin-3-O-gallate (IC_{50} 0,19 µmol/ml), gallic acid (IC_{50} 0,16 µmol/ml) and epicatechin/gallic acid mixture (IC_{50} 0,17 µmol/ml) showed high toxicity, indicating significant toxicity of gallic acid itself and as a substituent of proanthocyanidins. Epicatechin was cytoprotective when cells were pre-treated with H_2O_2 (2,5 mM). The polymer fraction revealed toxic effects at concentrations higher than 50 µg/ml while lower concentrations showed proliferative effects. Despite being toxic towards eukaryotic cells, none of the tested proanthocyanidins showed toxicity towards *E. coli*. Anti-adhesive effects of oligomeric proanthocyanidins on adhesion of *E. coli* to human bladder cells (T24) were shown.

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Chemical Investigation of *Opuntia tuna* Mill growing in Egypt

Tawfik WA, Shahat AA, Hassan NM, Abdelazim NS, Isma SI, Hammouda FM
Phytochemistry Dept., National Research Centre, 12311, Dokki, Egypt

The fruit of the genus *Opuntia* is of great economic value as it is used to some extent as forage. It is also cultivated for its edible fruits. *Opuntia tuna* is wildly growing in Egypt and is also cultivated for its edible properties [1]. *Opuntia tuna* was subjected to chemical investigation in this work for the first time. We report here on the flavonoids of the flowers and the mucilage and the betalaines of the fruits. Isolation and purification processes were carried out by applying successive chromatographic techniques (column chromatography, TLC, PPC and HPLC). Identification of the isolated compounds was carried out by spectroscopic analysis (UV, MS, ^{1}H-NMR and ^{13}C-NMR). Quantitation of betalaines was established by TLC densitometry. The isolated flavonoids were identified as isorhamnetin, isorhamnetin-3-O-glucoside and isorhamnetin-3-O-rhamnoglucoside [2]. The mucilage study revealed the presence of L-rhamnose, L-arabinose, D-galactose, fructose D-glucuronic acid and D-galacturonic acid [3]. Indicathanthin, betanin and isobetanin were identified [4]. **References:** [1] Watt, J. M. and Breyer Brandwijk, M. G. (1962) " The Medicinal and Poisonous Plants of Southern and Eastern Africa" 2nd ed., E. and S l-evinston Ltd., Edinburgh, U.K. [2] Abdel Shafeek, K. A., et al. (2000) J. Nat. Prod., 63: 845 – 847. [3] Nazif, N. N. (2001) Bull. Fac. Pharm., Cairo Univ., Vol. 39, No. 1. [4] Forni, E., et al. (1992) J. Chromatogr. 593: 177 – 83.

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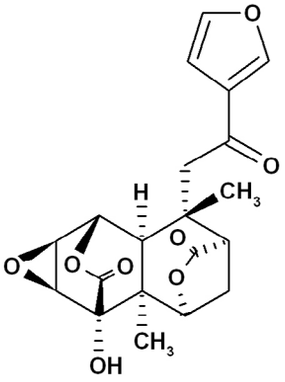
Spiciflorin: a novel clerodane from *Cleidion spiciflorum* (Euphorbiaceae)

Kijjoa A[1], Naengchomnong W[2], Herz W[3]
[1]Instituto de Ciências Biomédicas de Abel Salazar – CIIMAR, Universidade do Porto, 4099 – 003 Porto, Portugal; [2]Department of Chemistry, Faculty of Science, Burapha University, Bangsaen, Chonburi 20131, Thailand; [3]Department of Chemistry and Biochemistry, The Florida State University, Tallahassee, FL 32306 – 4390, USA

In the continuation of our work on bioactive secondary metabolites from the plants of the family Euphobiaceae from Thailand [1], we have isolated a new clerodane diterpene which we have named spiciflorin (1) and the known clerodane columbin (2), besides 3, 3' 4-*O*-trimethyl ellagic acid (3), scopoletin (4), *trans*-4-propenylphenol glycoside (5), 5-hydroxymethylfurfural (6) from the roots of *Cleidion spiciflorum* (Burm.f.) Merr., collected from Northern Thailand. The structure of the new compound was established by ^{1}H, ^{13}CNMR, DEPTs, COSY, HSQC, HMBC, NOESY as well as by HRMS.



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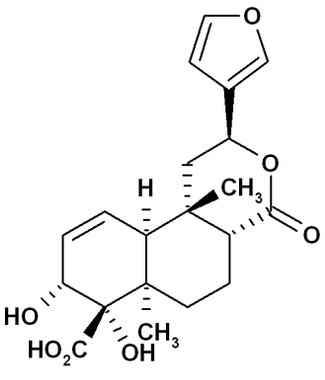

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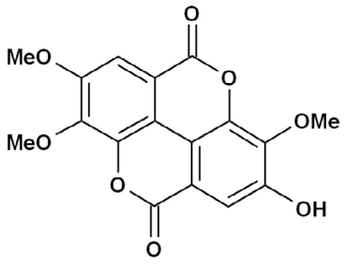

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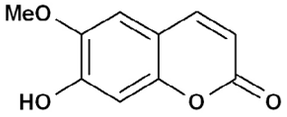

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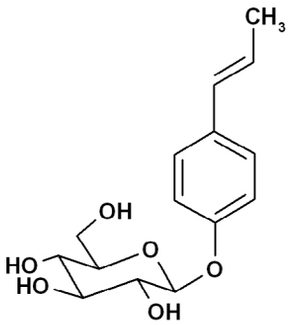

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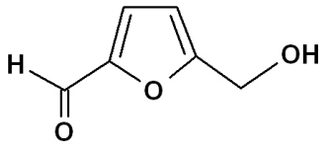

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Spiciflorin (1) was found to be inactive when evaluated, by the SRB method, for its capacity to inhibit the *in vitro* growth of three human tumour cell lines: MCF-7 (breast), NCI-H-460 (lung) and SF-268 (brain). *Acknowledgements:* This work was supported by Fundação para a Ciência e Tecnologia (Unidade de I&D 226/96), POCTI (QA III), FEDER and CIIMAR Plurianual **References:** [1] P. Pinho et al. (2006) The 10th International Congress Phytoparm 2006 "Actual Problem of creation of new medicinal preparations of natural origin". St. Petersburg, Russia, 27 – 30 July 2006.

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Mosquito larvicidal activity of essential oils of *Cymbopogon citratus* and *Thymus vulgaris* grown in Cameroon

Nkouaya Mbanjo EG[1], Tchoumbougnang F[1], Jazet Dongmo PM[1], Sameza ML[1], Amvam Zollo PH[1], Menut C[2], Bessière JM[2]
[1]Laboratoire de Phytobiochimie, Faculté des Sciences, Université de Douala, BP 24157 Douala, Cameroun; [2]Ecole Nationale Supérieure de Chimie de Montpellier, 34296 Montpellier Cedex 5, France

The chemical composition and the larvicidal activity of the essential oils obtained by hydrodistillation of fresh leaves from *Cymbopogon citratus* and *Thymus vulgaris* growing in Cameroon were investigated. The yields of extraction indicate that leaves of *Thymus vulgaris* were richer (0.95%) in essential oil than those of *Cymbopogon citratus* (0.67%). By means of GC and GC/MS, the main compounds were found to be for *Cymbopogon citratus*: geraniol (31.95%), geranial (29.25%), sabinene acetate (15.57%) and myrcene (14.0%); and for *Thymus vulgaris*: thymol (40.11%), β-phellandrene (23.39%) and γ-terpene (15.15%). Biological testing revealed that both essential oils possess remarkable larvicidal properties as they could induce 100% mortality in the larveae of *Anopheles gambiae* at the concentration of 100 ppm for *Cymbopogon citratus* and at 200 ppm for *Thymus vulgaris*. The essential oil from *C. citratus* was found to be most toxic to the larvae.

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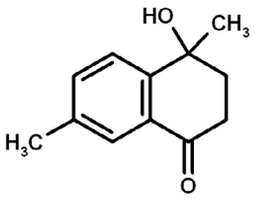
Constituents of *Polyalthia jucunda* and their cytotoxic effect on human cancer cell lines

Mondranondra IO[1], Suedee A[1], Kijjoa A[2], Pinto M[3], Nazareth N[3], São José Nascimento M[3], Herz W[4]
[1]Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand; [2]CIIMAR-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4099 – 003 Porto, Portugal; [3]Centro de Estudo de Química Orgânica, Fitoquímica e Farmacologia de Universidade do Porto (CEQOFFUP), Faculdade de Farmácia, Rua Aníbal Cunha 164, 4050 – 047-Porto, Portugal; [4]Department of Chemistry and Biochemistry, The FloridaState University, Talahassee, Fl32306 – 4390, USA

During our search for bioactive constituents from Thai plants, we have investigated the constituents of *Polyalthia jucunda* (Piere) Finet & Gagnep, collected from the North East of Thailand. Besides 4-hydroxy-4,7-dimethyl-α-tetralone (**1**) and 4,5-dihydroblumenol A (**2**), N-trans-feruloyltyramine (**3**) and 24-methylenelanosta-7, 9(11)-dien-3-β, 15α-diol (**4**) have been isolated from its stem bark. The structure of the compounds was established by spectral analysis (^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY) as well as HRMS. All the isolated compounds were tested for their *in vitro* effect on growth of four human tumor cell lines: ER (+) MCF-7, ER (-) MDA-MB-23, SF 268 and NCI-H460), as well as of the non-tumor cell line MRC-5. Only compound **4** exhibited a dose-dependent growth inhibitory effect against both tumor and non-tumor cell lines but with less effect on the latter. Using the TUNEL assay, it was found that the inhibitory effect of compound **4** on NCI-H460 cells was probably caused by apoptosis.



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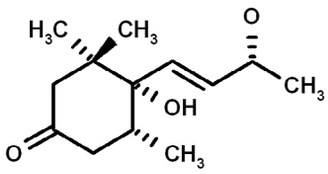

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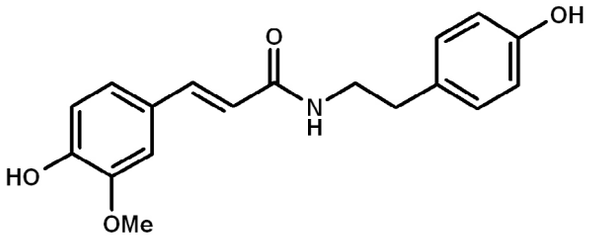

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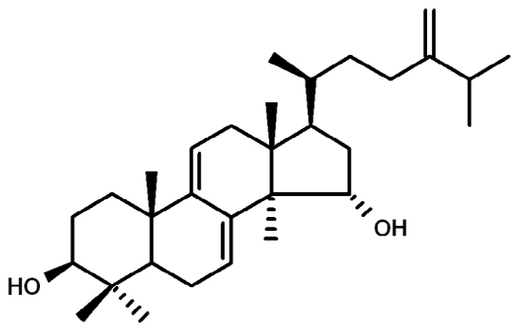

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Acknowledgements: This work was supported by Fundação para a Ciência e Tecnologia (Unidade de I&D 226/96), POCTI (QA III), FEDER and CIIMAR Plurianual

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Phytochemical and bioactivity investigations of *Macfadyena unguis-cati* L. (Bignoniaceae)

Aboutable EA[1], Hashem FA[2], Sleem AA[3], Maamoon AA[2]
[1]Pharmacognosy Department, Faculty of Pharmacy, Cairo, Egypt.;
[2]Pharmacognosy Department, National Research Centre, Cairo, Egypt;
[3]Pharmacology Department, National Research Centre, Cairo, Egypt

GC/MS analysis of the volatile components of the aerial parts of *Macfadyena unguis-cati* L. (Bignoniaceae) revealed the presence of 74 compounds, 52 of them (representing 75.97%) were identified [1]. The major compound is n-decane (21.21%) followed by phytol (12.19%). The saponifiable fraction of the petroleum ether extract showed 21 fatty acids identified as methyl esters. 37 compounds were identified in the unsaponifiable fraction; representing 93.26%. β-amyrin, squalene, β-sitosterol and 3α', 5-cyclo-ergosta-7,22-dien-6-one were identified in the USM. Determination of LD_{50} of different extracts showed that the total ethanol extract is the safest one (LD_{50} 4.9 g/kg) followed by the petroleum ether extract, (LD_{50} 4.5 g/kg) and the ethyl acetate extract which has the lowest LD_{50} (3.1 g/kg). The total ethanol extract was shown to be the most potent as an antipyretic, followed by the ethyl acetate extract. The ethanol extract, as well as a coumarin containing fraction also exhibited significant analgesic activity. **References:** [1] Adams, R.P. (1995) Identification of Essential Oil Components by Gas Chromatography Mass Spectroscopy. Allured Publishing Corporation, Carol Stream. Illinois USA.

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Seasonal changes of glucosinolates in *Isatis* leaves, and effect of harvest regimen and post-harvest treatment

Suter K[1], Mohn T[1], Hamburger M[1]
[1]Institute of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Woad, *Isatis tinctoria* L. (Brassicaceae) is an ancient indigo dye and medicinal plant, which has been used and cultivated in Europe since antiquity. The anti-inflammatory potential of *Isatis tinctoria* had been analyzed in a broad-based pharmacological profiling, and inhibitors of cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), human neutrophil elastase, and histamine release were identified [1]. Animal pharmacological studies and a clinical pilot study corroborated the anti-inflammatory potential of lipophilic *Isatis* extracts [2]. We observed that the phytochemical profile of woad undergoes profound changes during post-harvest treatment. For example, the indigo precursors are largely transformed, whereas the dual COX-2/5-LOX inhibitor tryptanthrin is formed only during the drying process [3]. The precursors of tryptanthrin are presently unknown, but the tetracyclic ring system contains at least one indole unit. We believe that some additional alkaloids with indole moieties may also be formed only during the drying process. Indole glucosinolates occur in rather high concentration in fresh *Isatis* leaves and are thus potential precursors for such alkaloids. As parts of a comprehensive metabolite profiling, we investigated the seasonal fluctuation of these glucosinolates of five *Isatis tinctoria* and *Isatis indigotica* accessions (first year, rosette stage) grown on field plots under identical conditions. The effects of repeated harvesting during the growth season on glucosinolate concentrations, and the influence of post-harvest processing were studied. Analysis of the non-derivatized glucosinolates was carried out with a recently developed and validated PLE (pressurized liqiud extraction) protocol and ion-pair HPLC coupled with ESI-MS detection in the negative ion mode. **References:** [1] Hamburger, M. (2002) Phytochem. Rev. 1: 333 – 344. [2] Recio, C. et al. (2006) Planta Med. 72: 715 – 720. [3] Oberthür, C., Hamburger, M. (2004) Planta Med. 70: 642 – 645.

P 333

Elicitation of tropane alkaloid synthesis in root culture of Hyoscyamus by hydrogen peroxide and ascorbic acid

Zayed R
Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, 44519 Zagazig, Egypt

In the present work, the role of hydrogen peroxide (H_2O_2) and ascorbic acid (AS) as activators of secondary metabolite biosynthesis was studied on root cultures of Hyoscyamus. Root cultures of four species of Hyoscyamus (*H. muticus*, H. *albus*, *H. aureus* and *H. deserteroum*) were treated with different concentrations of both elicitors. This treatment revealed that tropane alkaloid biosynthesis was induced in a dose-dependent manner. Root cultures were treated with elicitors in concentrations ranging from 0.01% to 1% of H_2O_2 and 0.5 mM to 2 mM of AS for 24 h. Both elicitors increased the cellular alkaloid content of tropane alkaloids depending on the elicitor type, the dose employed and the plant tissues. The content of hyoscyamine, which represents the main tropane alkaloid in Hyoscyamus was increased 5 – 6 fold in *H. albus* and *H. aureus* with 0.01% H_2O_2 and with 0.1% in *H. muticus*. In contrast, ascorbic acid elicited the accumulation of approximately 15 – 20 fold amounts of cuscohygrine compared to the untreated control in *H. muticus* and *H. aureus*.

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Phytochemical and biological investigation of *Echium sericeum* (Vahl) growing in Egypt

Radwan HM[1], Shams KA[1], Mohamed WA[2], Ismail IA[2]
[1]Phytochemistry Dept. National Research Centre, Dokki, Cairo, Egypt, 12311; [2]Pests and Plant Protection Dept. National Research Centre, Dokki, Cairo, Egypt, 12311

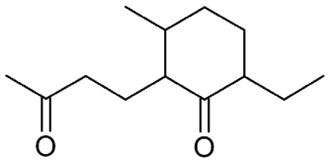
The genus Echium is represented in Egypt by seven species [1]. A number of naphthoquinones have been isolated from certain Echium species [2],[3]. The flavonoid constituents of certain Echium ssp. have been studied and a number of flavonoids were identified [4], as well as several pyrrolizidine alkaloids [5]. From the medical point of view, many Echium species are used in traditional Iranian medicine as tonic, tranquillizer, diaphoretic, cough suppressant and as a remedy for sore throat [6]. The present work deals with the study of the flavonoids of *Echium sericeum* and the evaluation of their insecticidal activity against *Spodoptera littoralis* (Boised) as well as the investigation of the anti-oxidant activity of both the total extracts and the isolated compounds, using the DPPH free radical assay. About 1 kg of the aerial parts of *E. sericeum* were dried, powdered and extracted with petrol ether and then with 80% ethanol. After partition with chloroform, ethyl acetate and n-butanol the ethanolic extract yielded crude extracts containing flavonoids. These extracts were subjected separately to preparative paper chromatography (3MM, developed with 15% acetic acid) and the main flavonoid bands were cut and eluted separately with 90% methanol. The eluted fractions were further purified on a Sephadex LH-20 column. The isolated flavonoids were identified as apigenin, luteolin-7-O-rutinoside, apigenin-7-O-rhamnoside and quercetin-3-O-rhamnoside. Their identity was proven by TLC, PC, m.p., UV, ^{1}H-NMR and FAB-MS. The ethyl acetate and n-butanol fractions as well as the four flavonoids isolated from the plant showed a strong antioxidant activity in the DPPH assay. The petrol ether extract of *E. sericeum* showed the strongest insecticidal activity against *S. littoralis*, causing 71.5% mortality, whereas the butanol and 80% alcoholic fractions also had a marked effect causing 62.5% mortality at a concentration of 2.5%, respectively. **References:** [1] Täckholm, V.(1974) "Students Flora of Egypt" 2nd Ed. The Anglo Egyptian Bookshop, Cairo University [2] Tabata, M.et al. (1982) Planta Med. 44: 234. [3] Fedoreev, et al. (1979) Khim. Prir Soedin, 62: 5. [4] Bandyukova, et al. (1970) Tr. Vses. Sezda Farm., 1st Ed.: 253. [5] Culvenor, C.C.J (1956) Aust.J.Chem 9: 512. [6] Amin, Gh.(1991) Popular Medicinal plants of Iran. Bulletin Iranian Research Institute of Medicinal Plants, Tehran: 80

P 335

A new decomposition product of dihydroartemisinin

Dhooghe L[1], Van Miert S[2], Jansen H[2], Vlietinck A[1], Pieters L[1]
[1]Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium; [2]Dafra Pharma NV, Slachthuisstraat 30/7, B-2300 Turnhout, Belgium

Artemisinin is a natural product with antimalarial properties that originates from the plant *Artemisia annua*. It can chemically be reduced to dihydroartemisinin, which was reported to be more active than the parent compound and which serves as a precursor for the antimalarial drugs artesunate and artemether [1,2]. After prolonged storage, samples of dihydroartemisinin were found to contain a decomposition product with a molecular weight of 238, the formation of which was accelerated by heating at 60 °C for several days. This decomposition product was isolated and identified as 2-(3-oxobutyl)-3-methyl-6-(2-propanal)-cyclohexanon, known to be formed by thermolysis of dihydorartemisinin at 190 °C [3]. However, during work-up of this compound a hitherto unknown decomposition product of dihydroartemisinin with a molecular weight of 210 was obtained and identified by ^{1}H, ^{13}C and 2D NMR spectroscopy as 2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanone. This new degradation product can be formed by oxidation and subsequent decarboxylation of the former decomposition product.



2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanon

References: [1] Sriram, D. et al. (2004) Nat Prod Research 18: 503 – 527. [2] Meshnick, S.R. et al. (1996) Microbiol Rev 60: 301 – 315. [3] Lin, A.J. et al. (1986) Tetrahedron 42: 2181 – 2184.

P 336

Identification of TLC markers and quantification by HPLC-MS of various constituents in Noni fruit powder and commercial Noni-derived products

Potterat O, von Felten R, Dalsgaard P, Hamburger M
Institute of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Since the approval of Noni juice as a novel food by the European Commission in 2003, products derived from Noni fruit (*Morinda citrifolia*, Rubiaceae) are becoming increasingly popular as food supplements [1]. While the knowledge on constituents of Noni fruit has considerably increased over recent years, quantitative data on Noni secondary metabolites remain scarce and the chemical composition of commercial products distributed mainly via internet is poorly established. In the present study, TLC profiles of commercial Noni juices and capsules were compared. Chromatographic markers such as 3-methyl-1,3-butanediol typically found in Noni juices were identified. The presence of sorbic acid (E200) was also revealed in one Noni juice declared as additive-free. In order to obtain quantitative data on the composition of Noni products, an HPLC-MS method has been developed and validated, which enables the quantification of various Noni constituents, including iridoid glucosides, scopoletin, rutin, fatty acid glucosides and anthraquinones. The separation is performed on a C-18 column with a gradient of acetonitrile in water containing 0.1% formic acid. Detection is carried out with ESI-MS in the negative ion mode. The method was applied to the analysis of various commercial juices and capsules. Significant differences were observed between the samples. Asperulosidic acid, deacetylasperulosidic acid and rutin were present in all products analysed, but their concentrations differed greatly between the products. The fatty acid glucosides noniosides B and C [2], as well as scopoletin, present in the fruit powder, were only detected in some commercial preparations. The mutagenic anthraquinone alizarin which has been reported from roots and leaves was not detected in the investigated samples. **References:** [1] Potterat, O., Hamburger, M. (2007) Planta Med. 73: 191. [2] Dalsgaard, P.W. et al. (2006) Planta Med. 72: 1322.

P 337

Phytochemical and biological investigations on *Halocnemum strobilaceum* (Chenopodiaceae)

Radwan HM, Shams KA
Chemistry of Medicinal Plants Dept., National Research Centre, Dokki, Cairo, Egypt, 12311

The genus *Halocnemum* is represented in Egypt by only one species [1], [2] and little information is available about its constituents. The lipid content of *Halocnemum strobilaceum* growing in Egypt was studied [3]. The unsaponifiable fraction was identified by GLC. A series of hydrocarbons ranging from C_{17} - C_{31} were identified in addition to campesterol, stigmasterol, β-sitosterol and α-amyrin. GLC analysis of the fatty alcohol fraction revealed the presence of 7 fatty alcohols, and the analysis of the fatty acid fraction revealed the presence of 10 fatty acids, mainly palmitic acid (39.26%). The flavonoids isolated from the chloroform and the ethyl acetate fractions of the aqueous alcoholic extract of *Halocnemum strobilaceum* were identified as chrysoeriol, luteolin 7-O-galactoside, quercetin 7-O-rhamnoside, luteolin [4], [5], and a coumarin, namely scopoletin, was also identified [6]. Their identity was proven by m.p., TLC, PC, UV, ^{1}-H-NMR and MS analysis. This is the first record of these flavonoids in *Halocnemum strobilaceum.* GLC analysis of the volatile oil of the plant resulted in the identification of 23 compounds representing 63% of the oil. The oil is characterized by a high content of hydrocarbons (55.9%), while the oxygenated hydrocarbons make up 33.8% of the identified components. In addition, the sesquiterpene hydrocarbons and oxygenated sesquiterpene compounds were presented by 2.6% and 8.2%, respectively. The radical scavenging effects of the extracts and isolated compounds were studied using the DPPH free radical assay [7]. The ethyl acetate extract had a strong antioxidant activity, as well as the isolated flavonoids, compared to Trolox (standard antioxidant compound). The different extracts and isolated compounds of the plant exhibited no cytotoxic activity against the Ehrlich-ascitis carcinoma cell line at the tested concentrations, except the volatile oil which showed moderate activity [8]. **References:** [1] Täckholm, V. (1974) Student's Flora of Egypt 2nd ed., Cairo University. [2] Rizk, A.M. (1986) The Phytochemistry of the Flora of Qatar, Scientific and Applied Research Centre, University of Qatar. [3] Radwan, H.M. et al. (1997) Bull. Fac. Pharm. Cairo Univ. 35: 1. [4] Mabry, T.J. et al. (1970) The Systematic Identification of Flavonoids. Springer Verlag, Berlin. [5] Mabry, T.J., Markham, K.R. (1975) The Flavonoids. Chapman and Hall, London. [6] Murray, R.G.H. et al. (1997) The Natural Coumarins. Wiley, Chichester,. [7] Chen, J.H. and Ho, C.T. (1997) J. Agric. Food Chem. 45: 2374 – 2378. [8] El-Hossary, MM., et al. (2000) Bull. Fac. Pharm. Cairo Univ. 38: 1.

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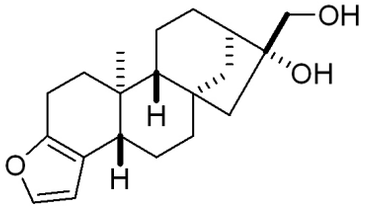
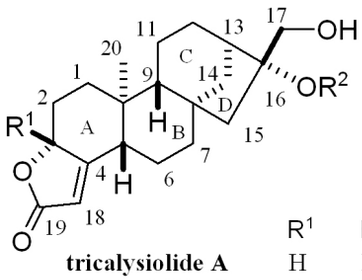
New Diterpenoids from *Tricalysia dubia*

Takeya K[1], Nishimura K[1], Hitotsuyanagi Y[1], Fukaya H[1], Aoyagi Y[1], Hasuda T[1], Kinoshita T[2]
[1]School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432 – 1 Horinouchi, Hachioji, Tokyo, 192 – 0392, Japan; [2]Faculty of Pharmaceutical Science, Teikyo University, 1091 – 1 Suarashi, Sagamikocho, Sagamihara, Kanagawa, 199 – 0195, Japan

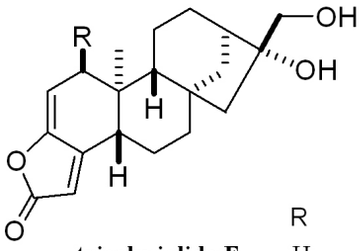
Tricalysia dubia (Rubiaceae), an evergreen shrub or tree, is widely distributed in Taiwan, the southern parts of China and Japan. From the leaves of this plants, unique rearranged *ent*-kaurane glycosides A – G, and the *ent*-kaurane glycosides H – O, have been isolated. In our presentation, six rearranged *ent*-kaurane diterpenes, tricalysiolides A – F [1], tricalysiamides A – D [2], tricalysiolide H, tricalysiones A and B [3], and a known diterpene, cafestol from the wood of this plant are reported, especially their cytotoxic activity against P388 leukemia cells in mice as well as the structure elucidation using spectral analyses and chemical methods.



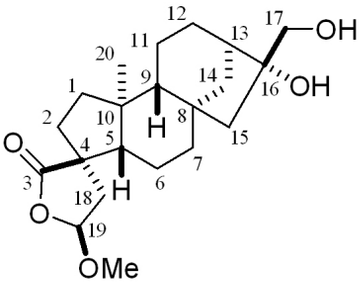
cafestol



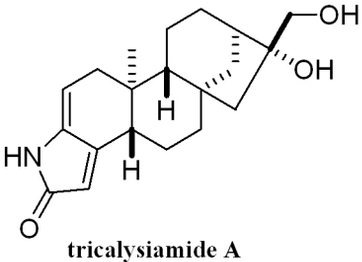
	R^1	R^2
tricalysiolide A	H	H
tricalysiolide B	OH	H
tricalysiolide C	OMe	H
tricalysiolide D	H	Me



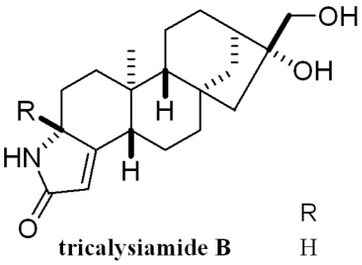
	R
tricalysiolide E	H
tricalysiolide F	OH



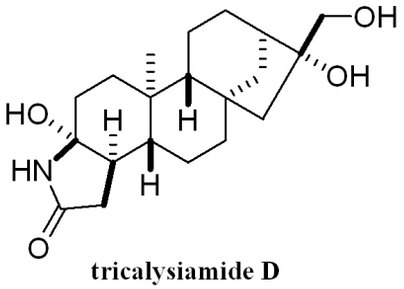
tricalysiolide H



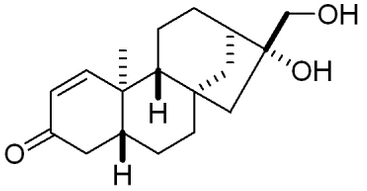
tricalysiamide A



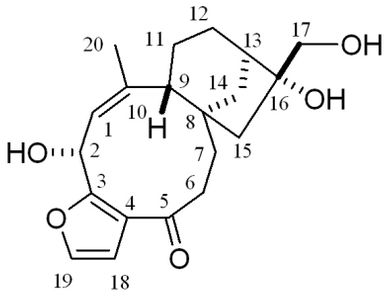
	R
tricalysiamide B	H
tricalysiamide C	OMe



tricalysiamide D



tricalysione A



tricalysione B

References: [1] Nishimura, K. et al. (2006), Tetrahedron, 62, 1512. [2] (2007) J. Nat. Prod., in press. [3] (2007) Tetrahedron, in press.

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Psychotropic constituents of *Mentha aquatica* L.

Van Staden J[1], Stafford GI[1], Almqvist JP[1,2], Vangsøe SAK[1,2], Olsen HT[1,2], Christensen SB[2], Adsersen A[2], Jäger AK[2]

[1]Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa; [2]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, 2 Universitetsparken, 2100 Copenhagen O, Denmark

Mentha aquatica L. is used in Zulu traditional medicine as treatment against colds, respiratory problems and as protection against evil spirits[1]. It is a perennial herb growing in marshes and damp places from the South-western Cape to tropical Africa and Europe. Six extracts of varying polarity of *Mentha aquatica* L. were tested in a photometric peroxidase linked MAO bioassay. The 70% ethanol extract gave highest inhibitory activity. *(S)*-Naringenin was isolated from the extract by bioassay guided fractionation using VLC and preparative TLC. The structure of the compound was verified by ^{1}H, ^{13}C and DEPT NMR and measurement of the optical rotation. The IC_{50} values for MAO inhibition by naringenin were 342 ± 33 μM for the rat liver mitochondrial fraction, 955 ± 129 μM for MAO-A and 288 ± 18 μM for MAO-B. The ethanolic leaf extract has previously shown strong affinity to the GABA-benzodiazepine receptor[1]. Viridiflorol from the essential oil and (*S*)-naringenin from an ethanolic extract was isolated by bioassay-guided fractionation using the ^{3}H-Ro 15 – 1788 (Flumazenil) GABA-benzodiazepine receptor bin-

ding assay. Viridiflorol had an IC_{50} of 0.19 M and (*S*)-naringenin of 2.6 mM. **References:** [1] Pooley E. (2005) A field guide to wild flowers of KwaZulu-Natal and the Eastern Region. Natal Flora Publications Trust. Durban, p. 424. [2] Stafford GI et al. (2005) J Ethnopharm. 100: 210 – 215.

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Sensitive and selective quantification of ruscogenin and neoruscogeninby liquid chromatography with mass spectrometry detection

Vlase L[1], Balica G[2], Crişan G[2], Tămaş M[2], Leucuta SE[1]
[1]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu", Emil Isac 13, 400023, Cluj-Napoca, Romania; [2]Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu", Emil Isac 13, 400023, Cluj-Napoca, Romania

A new sensitive and selective liquid chromatography-mass spectrometry (LC/MS/MS) method for quantification of ruscogenin and neoruscogenin in hydrolyzed extracts from *Ruscus aculeatus* L. (Liliaceae), was developed and validated. Ruscogenin and neoruscogenin were separated on a reversed phase column (Zorbax SB-C18, 100 mm x 3.0 mm I.D., 3.5 μm, preceded by a 0.5 μm online filter) under isocratic conditions using a mobile phase of 70/30 (v/v) acetonitrile/0.1% (V/V) formic acid in water containing 10 μM sodium acetate. The flow rate was 1 ml/min and the column temperature 45 °C. In these chromatographic conditions, the retention times were 2.2 minutes for ruscogenin and 1.8 minutes for neoruscogenin respectively. The detection of both analytes was in multiple reaction monitoring mode using an ion trap mass spectrometer with electrospray positive ionisation. In electrospray MS spectra, only sodium adducts of dehydrated molecules of ruscogenin and neoruscogenin were observed, at m/z 431 and 429 respectively. For quantification purposes, the ion transitions monitored were 431→(269+287) for ruscogenin and 429→(269+287) for neoruscogenin. Calibration curves were generated over the range of 2 – 1000 ng/ml for both ruscogenin and neoruscogenin, with values for coefficient of determination greater than 0.993 and by using a weighted (1/y) linear regression. This is the first reported method for analysis of ruscogenin and neoruscogenin using LC/MS/MS. It is also the most sensitive assay reported to date, with a quantification limit of 2 ng/ml for both compounds. The developed analytical method has been successfully applied to a phytochemical study of ruscogenin and neoruscogenin from *Ruscus aculeatus* hydrolyzed extracts. *Acknowledgements:* This work was supported by the project ET 3263/2005 financed by CNCSIS Romania.

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Composition of the essential oil of *Origanum vulgare* L. ssp. *vulgare* from various countries

Raal A[1], Arak E[1], Orav A[2]
[1]Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia; [2]Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

Variations in the essential oil composition of *Origanum vulgare*, cultivated in Estonia, were determined in our previous investigation [1]. In this work**,** a comparative study of the essential oil composition of additional samples of *O. vulgare* from Estonia and from various other countries was carried out. Plants (*Origani herba*) were cultivated in Estonia (n = 15) or obtained from retail pharmacies from different countries (Scotland, Italy, Moldova, Ukraine, Russia) in 1999 – 2006. The essential oils were isolated by hydrodistillation and analyzed by capillary gas chromatography as described in [2]. A total of 66 compounds were identified in the essential oils from 20 *O. vulgare* samples, representing over 95% of the total oil. The major constituents of the essential oil from Origani herba were found to be carvacrol (0 – 58.1%), caryophyllene oxide (0.3 – 34.3%), thymol (0.1 – 32.6%), *(E)*-β-caryophyllene (0.7 – 29.3%), germacrene D (0 – 17.4%), sabinene (0 – 15.3%), *(Z)*-β-ocimene (0 – 13.8%), terpinen-4-ol (0.5 – 10.9%), p-cymene (0.1 – 10.7%), and *(E)*-β-ocimene (0 – 10.0%). The sum of carvone and geranial varied between 0 and 52.0%. Also the content of α-cadinol (0 – 7.5%), bicyclobermacrene (0 – 5.8%), γ-terpinene, 2,6-dimethyl-*p*-cymene (both 0 – 5.6%), T-muurolol (0 – 5.2%), β-bisabolene (0 – 5.0%) was remarkable in some samples. The sample from Italy was characterized by a high content of thymol (32.6%) and carvacrol (20.2%), the content of carvacrol was the highest (58.1%) in Origani herba from Scotland. The content of thymol in the samples cultivated in Estonia was extremely low (0.1 – 1.2%), and carvacrol was not found in 14 samples. The results of this study confirm the wide variations in the chemical composition of the essential oils of *O. vulgare*. *Acknowledgements:* Estonian Science Foundation (grant No. 4332). **References:** [1] Ivask K. et al. (2005) J. Essent. Oil Res. 17: 384 – 387. [2] Orav A. et al. (2006) Nat. Prod. Res. 20: 1082 – 1088.

P 342

Biosynthesis of hyperforins from amino acid precursors in *Hypericum perforatum*

Karppinen K[1], Hokkanen J[2], Tolonen A[3], Mattila S[2], Hohtola A[1]
[1]Department of Biology, University of Oulu, P.O. Box 3000, FIN-90014 Oulu, Finland; [2]Department of Chemistry, University of Oulu, P.O. Box 3000, FIN-90014 Oulu, Finland; [3]Novamass Analytical Ltd, Medipolis Center, Kiviharjuntie 11, FIN-90220 Oulu, Finland

Hypericum perforatum L. (St. John's wort) is a medicinal plant widely used for treatment of depression [1]. Polyprenylated acylphloroglucinol derivatives, hyperforin and adhyperforin contribute to the antidepressant activity of *H. perforatum* [1,2]. The biosynthetic pathways for hyperforins are not clear, but polyketide-type pathways have been proposed [3,4]. In this study, we fed shoot cultures of *H. perforatum* with L-[U-^{13}C5]valine, L-[U-^{13}C6]isoleucine and L-[U-^{13}C6]leucine to examine the involvement of branched-chain amino acids in the biosynthesis of hyperforin and adhyperforin. The incorporation of ^{13}C labels from amino acids into hyperforin and adhyperforin was subsequently monitored by high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS). The *H. perforatum* shoot cultures were also fed with unlabelled L-valine, L-isoleucine, L-leucine and L-threonine to explore the possibility to enhance the production of hyperforin and adhyperforin through biotransformation of amino acid precursors. L-[U-^{13}C5]valine and L-[U-^{13}C6]isoleucine, upon administration to the shoot cultures, were incorporated into the acyl side chain of hyperforin and adhyperforin, respectively. Feeding the shoot cultures with unlabelled L-isoleucine at a concentration of 2 mM induced a significant, 3.7-fold increase in the production of adhyperforin. The addition of 3 mM L-threonine, a precursor of isoleucine, stimulated a significant, 2.0-fold increase in the accumulation of adhyperforin. The administration of L-valine at concentrations of 0 – 5 mM had no stimulating effect on the hyperforin production in *H. perforatum* shoot cultures [5]. Our results show that it is possible to increase adhyperforin concentration but not hyperforin concentration in *H. perforatum* shoot cultures through biotransformation of amino acid precursors. **References:** [1] Medina, M.A. et al. (2006) Life Sci. 79: 105 – 111. [2] Jensen, A.G. et al. (2001) Life Sci. 68: 1593 – 1605. [3] Adam, P. et al. (2002) J. Med. Chem. 45: 4786 – 4793. [4] Klingauf, P. et al. (2005) Phytochemistry 66: 139 – 145. [5] Karppinen, K. et al. (2007) Phytochemistry 68: 1038 – 1045.

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Essential oil from fruits and leaves of *Schinus areira* L. with acetylcholinesterase inhibitory activity

Vela Gurovic MS[1], *Murray AP*[1], *Ferrero AA*[2]
[1]*Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, Bahía Blanca, 8000, Argentina;* [2]*Departamento de Biología, Bioquímica y Farmacia, San Juan 670, Bahía Blanca, 8000, Argentina*

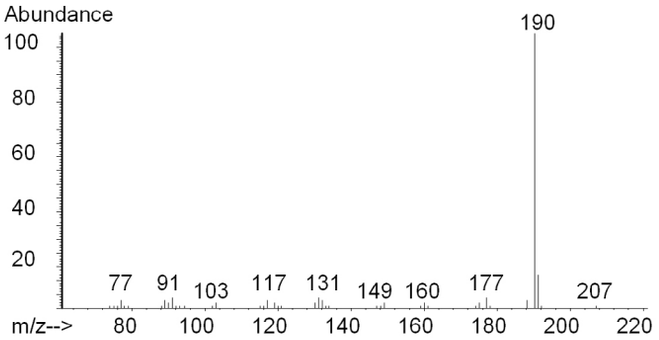
Currently available drugs for the symptomatic treatment of Alzheimer's disease (AD) are based on the inhibition of the enzyme acetylcholinesterase (AChE). Recently, essential oils as well as terpenoids have been shown to inhibit AChE in *in vitro* assays [1], [2]. In the present communication we are reporting the evaluation of the essential oils from fruits and leaves of *Schinus areira* L (Anacardiaceae), extensively used in folk medicine in South America [3], for their activity towards AChE. The enzymatic activity was evaluated using an adaptation of a previously described method [4]. The composition of the essential oils was determined by GC and GC-MS [5]. The essential oil from fruits showed a 29.00 ± 4.84% AChE inhibition while the essential oil from leaves showed a 13.67 ± 0.67% AChE inhibition under the same conditions (0.1 µL/mL). Chromatographic fractionation of the essential oils guided by the bioassay led to the isolation of the active fractions. GC-MS analysis of those fractions revealed the presence of sesquiterpenes like β-eudesmol and elemol, which have been reported as compounds that may have potential therapeutic use against amnesia-inducing diseases like AD [6], [7]. *Acknowledgements: CONICET, ANPCYT, SECYT-UNS.* **References:** [1] Perry, N.S.L et al. (2003) Pharmacol Biochem Behav 75: 651 – 659. [2] Miyazawa M., Yamafuji C. (2005) J. Agric. Food Chem. 53: 1765 – 1768. [3] Gupta M.P. (1995) 270 Plantas Medicinales Iberoamericanas. Ed. CYTED, D.C. Colombia. [4] Rhee I. K. et al. (2001) J. Chromatogr. A 915: 217 – 223. [5] Murray A. P. et al. (2005) Z. Naturforsch. 60C: 25 – 29. [6] Obara Y. et al. (2002) J. Pharmacol. Exp. Ther. 301: 803 – 811 [7] Kim K et al. (2006) Biosci Biotechnol Biochem. 70: 1821 – 1826.

P 344

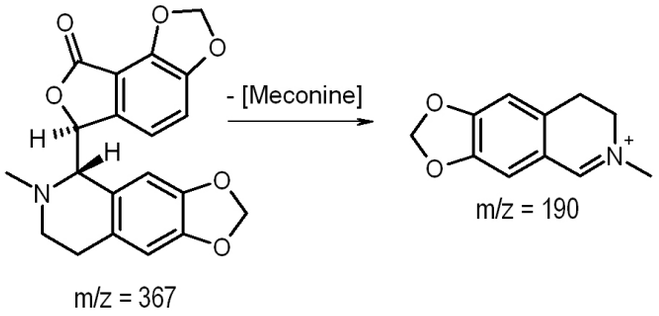
Identification of alkaloids from fumariaceous plants

Meyer A, Relius K, Imming P
Institut für Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06120 Halle (Saale), Germany

The majority of alkaloids from fumariaceous plants belong to protopine, protoberberine, phthalideisoquinoline, spirobenzylisoquinoline and aporphine alkaloids [1]. Protopine, protoberberine and aporphine alkaloids were assigned by GC-MS. Even constitutional isomers could be separated by GC and showed little but distinct variation of MS spectra. Although the molecular peak of phthalideisoquinoline alkaloids is missing in regular EI-MS, it appears with ESI-MS. Their distinctive EI-MS spectra contribute to structure elucidation as their fragmentation is quite characteristic [2]. The NMR spectra reveal aromatic substitution patterns and conformational preferences. We present results from an analytical study of various alkaloids from fumariaceous plants covering their GC-MS, TLC, UV and NMR data with an emphasis on spectral details that enable assignment to an isoquinoline alkaloid subclass.



EI-MS spectrum and fragmentation pattern of **adlumidine**, as example for phthalideisoquinoline alkaloids



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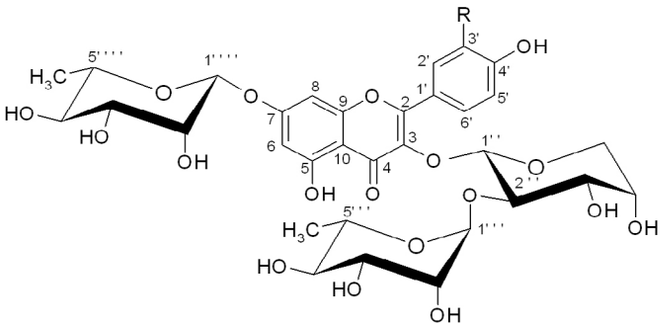
New flavonoid triglycosides from *Anthyllis hermanniae*

Halabalaki M, Paschali A, Mitakou S, Skaltsounis AL
Laboratory of Pharmacognosy and Natural Products Chemistry, School of Pharmacy, University of Athens, Panepistimioupolis, Zografou, 15771 Athens, Greece

Anthyllis hermanniae L. (Leguminosae) is a shrub with tortuous, woody branches, typical of the Mediterranean region. Information concerning the phytochemical content of the genus *Anthyllis* is limited and even more so for the species *A. hermanniae* [1]. The plants of the genus are rich in acids, flavonoids and isoflavonoids. The methanol extract of *A. hermanniae* yielded two novel flavonoid triglycosides characterized by an *O*-linked branched trisaccharide together with other flavonoid glycosides, isoflavones, coumarins, sterols and phenolic acids. The structures of the novel glycosides were determined to be quercetin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside (**1**) and kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-α-L-rhamnopyranoside (**2**). Flavonol glycosides with an arabinosyl unit on position-3 of the aglycone and (1→2) interglycosidic linkage within the disaccharide residue are generally very rare in nature.



1 R = OH Hermannioside A
2 R = H Hermannioside B

Mass spectrometry, UV-Vis and NMR spectroscopy (1 and 2D) were employed for the structural elucidation of the isolated constituents. Preparative HPLC was performed for the isolation of hermannioside A and B using a reversed phase column (Supelcosil SPLC-18, 5 µm, 10 mm i.d. x 25 cm) with a flow rate of 3 mL/min and a detection wavelength at 365 nm. The mobile phase consisted of H_2O containing 2% acetic acid (solvent A) and CH_3CN containing 2% acetic acid (solvent B) in a gradient elution mode. **References:** [1] Pistelli L. et al. (1996) Phytochemistry 42: 1455 – 1458.

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HPLC-MS-Analysis of flavonoid-C-glycosides in the Mongolian medicinal plant *Dianthus versicolor*

Obmann A, Radovic T, Kletter C, Glasl S
Department of Pharmacognosy University of Vienna, Althanstraße 14, 1090 Vienna, Austria

Dianthus versicolor is used in Traditional Mongolian Medicine against liver diseases together with other Mongolian plants. Until now chrysoeriol-C-glycosides were found in the plant [1]. Our first investigations of an aqueous extract (OWE) by TLC indicated the occurrence of further flavonoids. For preparation of the OWE the plant material was powdered and extracted with water (pH 2, trifluoroacetic acid) at 40 °C for 1 h by shaking gently. To purify the extract and concentrate the flavonoids Solid Phase Extraction (SPE) was used as appropriate method. This fraction increased the bile flow in the perfused isolated rat liver [2]. For further investigations an analytical HPLC-method had to be developed to provide a separation technique which may be coupled to MS. HPLC-analyses were performed on a Shimadzu liquid chromatograph (LC-20 AD) using RP-18e, Luna Phenyl-hexyl and Aquasil C_{18} as stationary phases. Aquasil C_{18} showed the best separation of the seven main flavonoids. HPLC-ESI-MS experiments were carried out with a Perkin Elmer liquid chromatograph (Series 200) coupled to a PE Sciex API 150 EX single quadrupole instrument. According to retention time, mass- and UV-spectra, one flavonoid was supposed to be apigenin-6,7-*O*-diglucoside (saponarin). To confirm this hypothesis a small amount of this compound was isolated by semipreparative HPLC and compared to pure saponarin by TLC and UV-experiments using shift reagents ($AlCl_3$/HCl and $NaOCH_3$). The isolated substance showed the same R_f-value and fluorescence colour after detection with natural compound reagent A at UV 366nm. The reaction to UV-shift reagents was the same as the pure substance, therefore the compound was identified as apigenin-6,7-*O*-diglucoside. **References:** [1] Boguslavskaya, L.I. et al. (1983) Khim. Prir. Soedin. 6: 783 – 4; [2] Glasl S., Tsendayush D. et al. (2007) Planta Med. 73: 59 – 66

P 347

The new "green" methodology for isolation of natural products from medicinal plants utilizing a mechanochemical approach

Pankrushina N[1,3], Lomovsky O[2], Boldyrev V[2,3]
[1]Novosibirsk Institute of Organic Chemistry, Acad. Lavrentjev Ave. 9, Novosibirsk, 630090, Russia; [2]Institute of Solid State Chemistry and Mechanochemistry, Kutateladze 18, Novosibirsk, 630128, Russia;[3]Novosibirsk State University, REC, Pirogova 2, Novosibirsk, 630090, Russia

Natural products from medicinal plants are known to be the basis for many pharmaceuticals. The development of synthetic natural products is difficult and unprofitable, so these substances are extracted from plant raw material. This traditional method consumes a high volume of organic solvents, and therefore is environmentally unsafe. The aim of our research is to develop an effective and environmentally safe mode of isolating natural products from medicinal plants using a mechanochemical approach. This approach is realized through the mechanical treatment of plant raw material and solid substances of an appropriate chemical nature [1] in a special ball mill, where the natural products effectively interact with the solid substances. We then treat the resulting reaction mass with a minimal quantity of organic solvent or water to obtain the desired product in a concentrated solution, which can easily be used for isolation, purified or dried. We have successfully demonstrated the application of mechanochemical "solvent free extraction" in many examples: isolation of the alkaloid lappaconitine from *Aconitum septentrionale* roots, of the alkaloid berberine from *Berberis sibirica* roots, of the alkaloid theobromine from *Theobroma cacao* pod and of sesquiterpene lactones from *Inula helenium* roots. Natural products were obtained in greater yield and in some cases with high selectivity [2].

Natural product	Traditional method, yield %	Mechanochemical approach, yield %
Lappaconitine	0.30	1.30
Berberine	0.77	2.00
Theobromine	0.49	1.27
Sesquiterpene lactones	2.00	5.00

References: [1] Pankrushina, N. et al. (2001) Patent RU N 2176919 C2., Inventions, useful models, 35, N 1: 159. [2] Goncharov, A., et al. (2006) Chem. Nat. Comp. 3: 274 – 276.

P 348

Multidisciplinary approach to the colour of pumpkin seed oil

Kreft S[1], Janež D[1], Kreft M[2]
[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana, Aškerèeva 7, 1000 Ljubljana, Slovenia; [2]Lab. Neuroendocrinology-Molecular Cell Physiology, Inst. Pathophysiology, Faculty of Medicine, University of Ljubljana, Založka 4, & Celica Biomedical Center, Proletarska 4, 1000 Ljubljana, Slovenia

Pumpkin (*Cucurbita pepo*) seeds are used in phytotherapy for treating benign prostatic hyperplasia. In some regions of central Europe (Slovenia and Austria) salad dressing is made preferably with pumpkin seed oil which has a strong characteristic nut-like taste [1] and a characteristic colour. The colour of the pumpkin seed oil was previously described as brownish yellow [1], dark green [2,3], dark green to red ochre [4], dark reddish brown to light yellow green [5] or green-red [6]. We found, that its hue changes with changing the layer thickness and we explained this phenomenon by the help of physical chemistry and human colour perception physiology and psychology [7]. Furthermore, phytochemical analyses elucidated the chemical aspects of pumpkin oil colour. A selective liquid-liquid extraction of lipophilic pigments from the lipophilic matrix was developed. We tested extraction with methanol, ethanol, 2-propanol, 1-butanol, acetone, ethyl acetate, petrolether, DMSO, acetic acid and DMF. DMF was able to extract over 90% of the colour from 0,5 ml of pumpkin seed oil after threefold extraction with 1 ml of DMF. The pigments were than analysed by TLC. Optical properties of

the pigments were measured and compared to chlorophyll. Finally, the origin of the pigments in the pumpkin seed was investigated by microscopical study of morphology and anatomy. The pigment was localised in the thin layer in the lowest part of the testa by microfluorimetry. A thin section of pumpkin seed was observed through the fluorescent microscope (excitation light: 458 nm), and the spectra of emitted light was measured at various parts of the tissue and compared to the emission spectra measured in isolated compounds. **References:** [1] Younis, YM. et al. (2000) Phytochemistry 54: 71 – 5. [2] Teppner, H. (2000). Phyton, 40, 1 – 42. [3] Murkovic, M et al. (1996) Z Lebensm Unters Forsch. 203: 216 – 9. [4] Murkovic, M, et al. (2004) Food Chemistry 84: 359 – 365. [5] Axtell, BL. et al. (1992) FAO Agricultural Services Bulletin No. 94. [6] Ministry of Agriculture, Forestry and Food of R Slovenia (1999) Ur.l. RS, žt. 56/1999. [7] Kreft, M. & Kreft, S. (2007) Naturwissenschaften, in press.

P 349

Cytotoxic activity of aqueous extract from *Holodiscus discolor* (Pursh) Maxim. leaves

Mrižová M[1], Laciková L'[1], Haladová M[1], Eisenreichová E[1], Granèai D[1], Ficková M[2]
[1]Dept. Pharmacognosy and Botany, Pharmaceutical Faculty, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovakia; [2]Institute of Experimental Endocrinology SAS, Vlárska 3, 833 06 Bratislava, Slovakia

Holodiscus discolor (Pursh) Maxim. (Rosaceae) is a shrub used in traditional medicine of Native Americans for the treatment of viral and skin diseases. Antifungal [1], antibacterial [2] and cytotoxic [3] activities of leaf extracts have been described. Based on these data, time (24, 72 h) and dose (1 – 150 µg/ml) dependent toxic effects of the leaf extract were investigated *in vitro* in human skin carcinoma cells A431. Cytotoxicity was measured by MTT assay for cell viability, and LDH leakage was used for determiniation of membrane integrity. Significant correlation in time was observed, when proliferation was inhibited in lower extract concentrations than cell lysis arose. The aqueous extract of *Holodiscus discolor* leaves has been analyzed for cytotoxic activity for the first time. Table 1: ED_{50} and maximal effects for MTT and LDH toxicity tests. The values are mean ± SE of 4 separate experiments performed in duplicates. Statistical significance was calculated by Student's t-test, when $^{***}p < 0{,}001$; (24 h vs. 72 h)

	MTT		LDH	
	ED_{50} (µg/ml)	max. inhibition (%)	ED_{50} (µg/ml)	max. effect (%)
24 h	41,2 ± 1,12	45,3 ± 3,19	90,4 ± 1,10	21,4 ± 4,56
72 h	31,9 ± 1,30***	26,2 ± 4,43***	71,9 ± 1,03***	17,7 ± 0,28

Acknowledgements: Institute of Experimental Endocrinology SAS, Grant Agency VEGA SR No. 1/4289/07 and Comenius University Grant UK/77/06. **References:** [1] McCutcheon, A.R. et al. (1994) J Ethnopharmacol 44: 157 – 169. [2] Jantová, S. et al. (2000) Phytother Res 14: 601 – 603. 3. Jantová, S. et al. (2001) Phytother Res 15: 22 – 25.

P 350

Optimized isolation and structural characterization of biologically active sulfated polysaccharides from the red alga *Delesseria sanguinea* (Hudson) Lamouroux

Grünewald N, Alban S
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

With the objective of increasing the fishing value of the Baltic Sea of coastal Mecklenburg-Vorpommern, a man-made large-scale subaqueous reef has been installed near Rostock, Germany, in recent years. These structures are populated by some macroalgae, whereby the red seaweed *Delesseria sanguinea* (D.s.) (Ceramiales) dominates with more than 80% of the biomass. Therefore, a project was initiated to evaluate the economic usability of D.s. from the Baltic Sea reef. The presented study focuses on its sulfated polysaccharides (SP). Initial water extracts of D.s. confirmed the content pf SP possessing an elastase inhibitory activity stronger than that of heparin, but a lower anticoagulant activity. To optimize the isolation procedure, several batches of D.s. were extracted by modifying various parameters such as extraction solvent, temperature, extraction time, dialysis conditions, and manual pre-purification of the alga. Guiding parameters were yield of SP, degree of sulfation (DS), protein contamination, elastase inhibitory and anticoagulant activity and batch-to-batch variability of the extraction method. The extraction with water at 85 °C showed to be the most suitable procedure. The resulting SPs have a DS of 0.48 as determined by conductimetric NaOH titration (PhEur 5.0) and elementary analysis. They also contain 3.4% of uronic acids according to the Blumenkrantz' method. Acetylation and methylation analysis suggest a xylogalactan structure with low amounts of mannose and glucose. SEC with RI- and MALLS-detection revealed a high polydispersity and a mean MW of 180 kDa. Contamination with proteins was less than 0.5% as determined by the Bradford-assay. In summary, D.s. from the Baltic Sea reef contains biologically active SP which can be isolated in a highly reproducible quality by using a specific extraction procedure. Thus, an important prerequisite for a potential economic use is fulfilled. *Acknowledgements: this project is financed by the EU (FI-AF/EFF) and the LFALF Mecklenburg-Vorpommern*

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A simple HPLC-method for the separation of oligofructans

Breit U, Blaschek W
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

Oligofructans are functional food ingredients with a great potential to improve the quality of food. They are used as low calory sweeteners and as prebiotics, they e.g. stimulate growth of Bifidobacteria in the gastrointestinal tract or enhance calcium absorption [1]. Oligofructans mainly consist of β(2→1) linked fructose units [2]. A starting glucose moiety normally is present, but may be lost during processing. There are different methods for separation and characterisation concerning the degree of polymerisation (DP) like HPLC, GLC, high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and SEC [3]. The methods often require derivatisation, expensive equipment or are time consuming. Therefore the aim of this work was to develop a cheap and feasible method for the separation of oligofructans. Oligofructans show different solubility in acetonitrile-water mixtures depending on their DP. With increasing proportion of acetonitrile, the solubility of oligofructans with higher DP decreases. Thus, commercially available fructan preparations containing oligo- and polysaccharides with a wide range of DP were used for controlled precipitation with acetonitrile-water mixtures to obtain oligofructan preparations. With these oligofructans good separation up to DP of approximately 15 could be achieved by HPLC on a Luna Amino 5 µm NH2 100A 250 x 4.6 mm column (Phenomenex), RI-detection and a mobile phase mixture of 62% acetonitrile and 38% water. This method allows reproducible analysis within 30 minutes. **References:** [1] Roberfroid MB et al. (1998) Ann. Rev. Nutr. 18: 117 – 143 [2] Crittenden RG et al. (1996) Trends Food Sci. Technol. 7: 353 – 361. [3] Sangeetha PT et al. (2005) Trends Food Sci. Technol. 16: 442 – 457

P 352

Characterization of the carbohydrate moiety of arabinogalactan-proteins from *Avena sativa* L., *Secale cereale* L. and *Triticum aestivum* L., isolated with Yariv reagent

Göllner EM, Classen B, Blaschek W
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

The nonstarch polysaccharides, although minor constituents in common oat (*Avena sativa* L.), rye (*Secale cereale* L) and wheat (*Triticum aestivum* L.), are important for growth of plant tissue. Arabinogalactan-proteins (AGPs) from wheat induce embryogenesis in microspore culture [1] and play a role in breadmaking [2]. Already in 1975 [3], it has been claimed that in contrast to most other AGPs, those of *Avena sativa* L. and *Triticum aestivum* L. show no precipitation with Yariv reagent. Therefore, up to now it is common praxis to isolate AGPs from cereals by stepwise ethanol precipitation. This includes the disadvantage of contamination of AGP-preparations with other polysaccharides. Our investigations show for the first time that AGPs from *Avena sativa* L., *Secale cereale* L. and *Triticum aestivum* L. can be isolated by precipitation with β-glucosyl Yariv reagent after extraction with water, starch removal by centrifugation, protein denaturation and dialysis. Specific interaction of the isolated AGPs with Yariv could also be demonstrated by a gel-diffusion test. In size-exclusion-chromatography the isolated AGPs elute as individual peaks with a molecular mass specific for poacean AGP. Methylation analysis was performed to characterize the carbohydrate moiety of the AGP which mainly consists of 1-Ara*f*, 1,3-Gal*p* and 1,3,6-Gal*p*. **References:** [1] Letarte J et al. (2006) Plant Cell Reports 25(8):877. [2] Schrøder M et al. (1999) J.Agric. Food Chem. 48: 1334 – 1343. [3] Jermyn M, Yeow Y (1975) Aust. J. Plant Physiol. 2: 501 – 531.

P 353

Butenolide ring formation in cardenolide biosynthesis: Spontaneous cyclization of pregnane-21-*O*-malonyl hemiesters

Pádua RM[1], Waibel R[2], Kuate SP[1], Riedl PK[1], Gmeiner P[2], Kreis W[1]
[1]*Friedrich-Alexander-Universität, Lehrstuhl für Pharmazeutische Biologie, Staudtstr. 5, D-91058 Erlangen, Germany;* [2]*Friedrich-Alexander-Universität, Lehrstuhl für Pharmazeutische Chemie, Schuhstr. 19, D-91052 Erlangen, Germany*

Cardiac glycosides are valuable drugs in the medication of patients suffering from cardiac insufficiency [1]. Although features of the biosynthetic pathway of cardenolides have been elucidated with the use of labelled and unlabelled precursors, its biosynthesis still not fully understood [2]. The incubation of a 21-hydroxy-20-oxo-pregnane precursor with malonyl-coenzyme A in the presence of cell-free extracts from cardenolide-producing plants leads to the formation of its 21-*O*-malonyl hemiester [3]. To investigate the next step in cardenolide biosynthesis, namely the butenolide ring formation, it is necessary to have mg quantities of the respective substrates. We developed a simple, fast and efficient chemical method for the synthesis of 21-hydroxypregnane-21-*O*-malonyl hemiesters and applied 1D and 2D NMR techniques for their complete ^{1}H and ^{13}C assignments. The incubation of 21-hydroxypregnane-21-*O*-malonyl hemiesters in the presence of cell-free extracts obtained from cardenolide-producing plants leads to the hydrolysis of the 21-*O*-ester linkage of the substrate. On the other hand, spontaneous butenolide ring formation of pregnane 21-*O*-malonyl hemiester was observed in buffer (100 mM KPi buffer, pH 7.5) and in the mixture of buffer + DMSO at 60 °C. The reaction was dependent on temperature and substrate concentration. Butenolide ring formation from the 21-hydroxypregnane-21-*O*-malonyl hemiester with the hydroxyl group at the position C-14β (typical of cardenolide aglycone) was higher than from compounds without this particular group. Although only spontaneous butenolide ring formation has been observed so far, the presence of a butenolide ring cyclase in cardenolide containing-plants can not be ruled out. *Acknowledgments:* We are grateful to the German Academic Exchange Service (DAAD) for the doctoral fellowship to R.M.P. **References:** [1] Wasserstrom, AJ. et al. (2005) Am. J. Physiol. Heart Circ. Physiol. 289: 1781 – 1793. [2] Kreis, W. et al. (1998) Planta Med. 64: 491 – 499. [3] Stuhlemmer U. et al. (1996) Tetrahedron Lett. 37: 2221 – 2224.

P 354

Arabinogalactan-proteins in roots of *Echinacea purpurea*: Structural investigations and microscopic localization by immunofluorescent labelling

Bossy A, Blaschek W, Classen B
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76 – 78, 24118 Kiel, Germany

Arabinogalactan-proteins (AGPs) are glycoproteins which are supposed to be cell wall associated and belong to the putative active compounds of Echinacea preparations (1). $(\beta\text{-D-Glc})_3$Yariv phenylglycoside (Yariv) specifically binds to most plant-AGPs and was used to isolate AGPs from the high molecular weight fraction (> 30.000Da) of an aqueous extract of root material from *Echinacea purpurea* (L.) Moench (Asteraceae). These AGPs with a carbohydrate-moiety consisting predominantly of Gal and Ara (2:1, > 90%) have been quantitatively and structurally characterized. Methylation analysis detected as main components 1,6-Gal*p*, 1,3-Gal*p*, 1,3,6-Gal*p* and in the side chains 1,5-Ara*f* and terminal 1-Ara*f*. Determination of the acidic compounds showed the presence of 1-GlcA*p*. For microscopic localization, a polyclonal antibody (generated in rabbit directed against *Echinacea* AGP) (2) in combination with a secondary FITC-labelled anti-rabbit antibody was used for an immunofluorescent labelling of AGPs on thin sections (16 µm) of root material of *Echinacea purpurea*. Furthermore, a Yariv-antibody (generated in rabbit directed against albumin-coupled Yariv) was used to label a Yariv-treated thin section of the same roots. After addition of the FITC-conjugated secondary antibody, the sections were observed with a Confocal Laser Scanning Microscope (CLSM). In both cases the labelled AGP was located in the cell walls, often in contact with the cytoplasma membrane. Confirming results on tomato roots (3), especially xylem tracheary elements showed very strong immunolabelling of the secondary wall. **References:** [1] Classen B et al. (2006) Phytomed 13: 688 – 694 [2] Classen B et al. (2005) Planta Med. 71: 59 – 66 [3] Gao M et al. (2000) Planta 210: 865 – 874

P 355

Efficient extraction of essential oils from fruits of *Pimpinella anisum* L. with milk

Bossy A, Blaschek W
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76 – 78, 24118 Kiel, Germany

Essential oils are a composite of many lipophilic and volatile aromatic components. The concentration of essential oils in drugs adds up to 1 – 2%, in exceptions up to 20%. Their aromatic compounds normally consist of mono- and sesquiterpenes and/or allylbenzenes [1]. The essential oil from fruits of *Pimpinella anisum* L. mainly consists of the allylbenzene trans-anethol with a concentration of about 80%. Brewing a tea infusion with water seems at first view, due to their lipophilic character, improper to dissolve the essential oils. Looking at milk as a solvent, there are both: an aqueous component and a fatty component, capable of solubilizing any lipophilic parts of the essential oil. In our investigations [2] we produced infusions out of 3 g of fruits from *Pimpinella anisum* L. with water, with low-fat milk (1.5%) and full cream milk (3.5%). After adding carvone as an internal standard and distillation with a Neo-Clavanger-distiller, we measured the amount of essential oil in the infusions with gas-liquid chromatography (GLC). Comparing the aqueous extract with the full cream milky extract, we nearly doubled the concentration of essential oil in the infusion. Further experiments

with weighted samples of 1 g to 15 g of drug material show saturation-effects corresponding to the net weight, with linearly increasing concentrations of solubilized trans-anethol. Especially for children, milky preparations of fruits from *Pimpinella anisum* or similar drugs like fruits from *Foeniculum vulgare* may lead to better acceptance in addition to the desired higher amount of ingested essential oils. **References:** [1] Wichtl M (2002) Teedrogen und Phytopharmaka. Wiss.Verlagsges. Stuttgart. [2] Bossy A et al. (2005) DAZ 46: 42 – 50

P 356

Amount of antioxidative compounds in herbs of *Calluna vulgaris* (L.) HULL originating from three different altitudes

Rieger G[1], Müller M[2], Guttenberger H[2], Bucar F[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitätsplatz 4/1, A-8010 Graz, Austria; [2]Institute of Plant Sciences, Department of Plant Physiology, University of Graz, Schubertstrasse 51, A-8010 Graz, Austria

Traditional folk and experiental medicine uses herbs of *C. vulgaris* (L.) HULL against various diseases, such as renal and urinary tract disturbances, gastrointestinal, respiratory and sleep disorders [1], [2]. In literature anti-inflammatory and antioxidant activity of polar extracts is documented [3], [4]. However, there is only limited information about the variation of compounds contributing to these capacities in *C. vulgaris* (L.) HULL growing at different altitudes. For our investigation, we selected the Naturpak Sölktäler in Upper Styria (Austria) in order to have comparable climatic conditions. The variation of the main hydroxycinnamic acid 5-*O*-caffeoylquinic acid, the dihydroflavonol taxifolin-3-*O*-glucoside, the flavonol-3-*O*-glycosides (quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside) and four flavones were investigated in two consecutive vegetation periods. The air-dried herbs obtained from samples collected at 800 m, 1000 m and 1500 m above sea level were subjected to accelerated solvent extraction (ASE) and subsequently analysed using RP-HPLC/PDA and LC-MS. Concerning these two substances the samples yielded 0.39% – 1.36% of 5-*O*-caffeoylquinic acid and 0.49% – 1.63% of taxifolin-3-*O*-glucoside. We found no significant differences between the three groups investigated. Among the six remaining flavonoids in our study, five appeared in significantly higher values in the samples from 1500 m above sea level in comparison to both the specimen originating from 1000 m and 800 m in both years. Antioxidant capacity was assessed by reaction with the stable radical DPPH (diphenylpicrylhydrazyl) [4] with IC_{50} values ranging from 4.9 to 11.5 µg/mL. *Acknowledgements:* Faculty of Natural Sciences/University of Graz, Gandolph-Doelter Foundation, Dr. Heinrich Jörg Scholarship for financial support. **References:** [1] Länger R., Kubelka W. (2001) Phytokodex. Krause & Pachernegg GmbH. Gablitz. [2] Königshofer I. (2004) Erhebung von Heilkäutern im Naturpark Sölktäler, Diploma thesis, University of Graz. [3] Tunon, H. et al. (1995) J Ethnopharmacol. 48: 61 – 76. 4. Calliste, C-A. et. al. (2001) J. Agric. Food Chem. 49: 3321 – 27.

P 357

Seasonal variations of biologically active sulfated polysaccharides extracted from the red alga *Delesseria sanguinea* (Hudson) Lamouroux from the Baltic Sea

Grünewald N, Alban S
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

The red seaweed *Delesseria sanguinea* (D.s.) is the dominating macroalga populating a man-made large-scale reef, which has been installed near Rostock, Germany. D.s. showed to contain biologically active sulfated polysaccharides (SP) with an interesting activity profile. Important prerequisites for a potential economic use of these SP are the availability of sufficient amounts and a reproducible high quality. To evaluate this, D.s. has been monthly harvested over the course of more than one year and the SP were isolated using two optimized extraction procedures, one with water, the other one with 0.1 M NaOH at 85 °C. The only parameter showing a clear seasonal variation is the dry mass of D.s., ranging form 12% in spring up to 23% in late autumn. In general, water extraction leads to ~50% lower yields (1.9 ± 0.5% of fresh algae) than the alkaline extraction (3.1 ± 0.9%), but the obtained SP have a higher degree of sulfation (DS) (0.48 vs. 0.40), a lower protein contamination (0.5% vs. 1.4%), and ~50% higher elastase inhibitory and anticoagulant activities. Moreover, the quality of the SP extracted with water is highly reproducible, whereas the SP extracted with NaOH show considerable batch-to-batch variation. This partly tends to be dependent on the season, as the yield increases from spring to autumn, whereas the DS decreases. Main reason seems to be the coextraction of starch-like polysaccharides by NaOH, which are accumulated over the year and contribute to the increasing dry mass of D.s. This has been confirmed by acetylation analysis of the SP, indicating a glucose content rising from spring to autumn. In conclusion, by using an optimized extraction procedure, SP of reproducible and high quality can be isolated from D.s.. Compared to NaOH, the extraction with water is more specific and allows an extraction of D.s. batches harvested throughout the whole year. *Acknowledgements: this project is financed by the EU (FIAF/EFF) and the LFALF Mecklenburg-Vorpommern*

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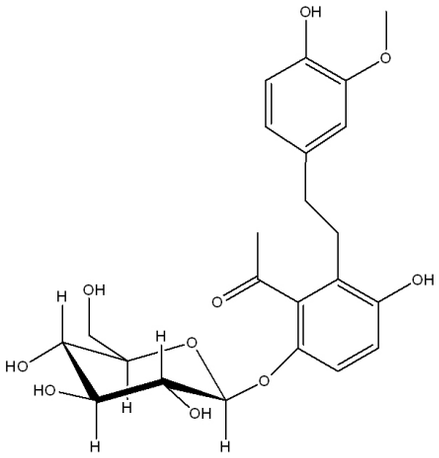
Study on chemical constituents of the Mongolian medicinal plant *Scorzonera radiata*

Wang Y[1], Edrada RA[1], Tsevegsuren N[2], Müller WEG[3], Wray V[4], Lin W[5], Proksch P[1]
[1]Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, Geb.26.23, 40225 Düsseldorf, Germany; [2]Department of Organic and Food Chemistry, Faculty of Chemistry, National University of Mongolia, Ulaanbaatar, Mongolia; [3]Institut für Physiologische Chemie und Pathobiochemie, Johannes-Gutenberg-Universität, Duesbergweg 6, 55128 Mainz, Germany, [4]Helmholtz-Zentrum für Infektionsforschung GmbH, Inhoffenstr.7, 38124 Braunschweig, Germany, [5]State Key Laboratory of Natural and Biomimetic Drugs, Peking University, 100083 Beijing, P. R. China

Scorzonera radiata occurs in the mountainous regions of Mongolia. The root part is used in Mongolian traditional medicine for the treatment of many diseases, such as for curing fever, carbuncle, and mastitis. An extract of the aerial parts of the Mongolian medicinal plant *Scorzonera radiata* afforded two new stilbenes, as well as 13 known compounds including three flavones and nine phenolic acid compounds. Six of the phenolic compounds are quinic acid congeners. The structures of the isolated compounds were clearly elucidated on the basis of NMR (^{1}H, ^{13}C, COSY, HMBC), mass spectrometric data and chemical methods. The biological activities of the new stilbene derivatives **1** and **2** have been assessed. Compound **1** exhibited higher antioxidative activity in the DPPH assay when compared to its dimethoxylated congener. However, both compounds did not show any cytotoxicity towards L5178Y mouse lymphoma cell line.



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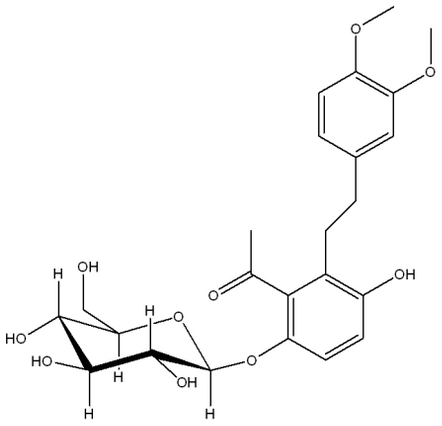

2

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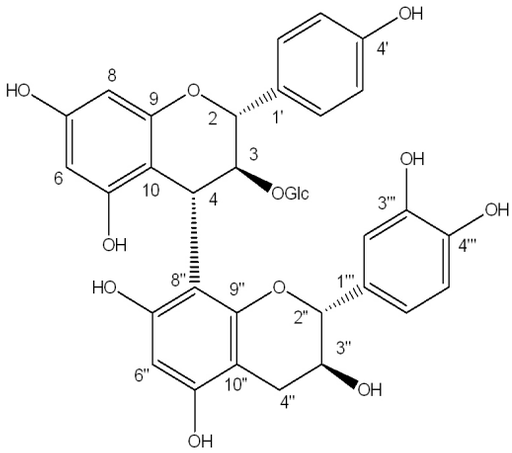
A novel dimeric proanthocyanidin 3-*O*-glucoside from *Quercus ilex* L. (Fagaceae)

Karioti A[1], *Bilia AR*[2], *Skaltsa H*[1]

[1]*Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece;* [2]*Department of Pharmaceutical Sciences, University of Florence, Ugo Schiff 6, Polo Scientifico, Sesto Fiorentino, 50019, Florence, Italy*

This work has been conducted in the framework of a general project aiming at understanding plant-insect interactions of *Quecus ilex* in the Mediterranean ecosystem. Chromatographic investigations on the MeOH extract of leaves of *Quercus ilex* led to the isolation and identification of an unusual dimeric proanthocyanidin glucoside (**1**) together with catechin and several flavonoids, namely quercetin-3-*O*-glucopyranoside, isorhamnetin-3-*O*-glucopyranoside, kaempferol-3-*O*-(6''-*E*-p-coumaroyl)-glucopyranoside, kaempferol-3-*O*-(6''-*Z*-p-coumaroyl)-glucopyranoside and kaempferol-3-*O*-(2'',6''-di-*E*-p-coumaroyl)-glucopyranoside. Leaves of *Q. ilex* were extracted with solvents of increasing polarity at room temperature in order to deactivate any enzymes and avoid polymerizations and oxidations. Final extraction was carried out using MeOH and MeOH/H_2O 8/2. A fast fractionation of the residue was done using VLC over silica gel followed by repeated cc over Sephadex LH-60 and Sephadex LH-20. Fractionations were monitored with HPLC-DAD-MS. The structures of the isolated compounds were established by means of 1D & 2D NMR.



(**1**)

Acknowledgements: This project is co-financed within Op. Education by the ESF (European Social Fund) and National Resources.

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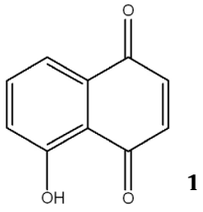
Secondary metabolites from the pericarps of *Juglans regia*

Kavroulaki E[1], *Karioti A*[1], *Bilia AR*[2], *Skaltsa H*[1]

[1]*Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece;* [2]*Department of Pharmaceutical Sciences, University of Florence, Ugo Schiff 6, Polo Scientifico, Sesto Fiorentino, 50019, Florence, Italy*

It is well documented [1], [2] that naphthoquinones in walnut pericarps play an important role in the protection of walnuts against fungal attacks. In search of new antifungal agents of plant origin, the pericarps of *Juglans regia* were investigated for their chemical content. Nut pericarps were extracted with solvents of increasing polarity at room temperature. Chromatographic investigations of both non-polar and polar fractions including VLC, CC over both silica gel and Sephadex-LH-20 afforded a series of phenolic constituents, namely, caffeic, ferulic, dimethoxycaffeic and vanillic acid as well as the previously isolated naphthoquinones, juglone (**1**), regiolone (**2**) and sclerone (**3**). The latter two seem to be the main secondary metabolites of the plant.



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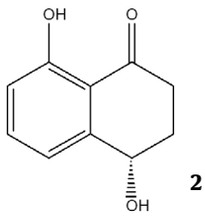

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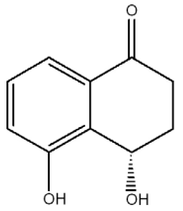

3

References: [1] Mahoney, N. et al. (2000), J. Agric. Food Chem. 48: 4418 – 4421. [2] Mahoney, N. et al. (2004), J. Agric. Food Chem. 52: 1882 – 1889.

P 361

Antimicrobial activity of sesquiterpene lactones from *Centaurea pullata* L., growing wild in Algeria

Djeddi S[1], Karioti A[1], Sokovic M[2], Stojkovic D[2], Skaltsa H[1]
[1]Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece; [2]Department of Plant Physiology, Mycological Laboratory, Institute for Biological Research, 29 Novembar 142, 11 000 Belgrade, Serbia

The genus *Centaurea* contains more than 300 species [1]. It is represented by 45 endemic taxa in Algeria [2]. Isolation of sesquiterpenes from *C. pullata* was carried out according to Bohlmann [3].The plant afforded, in addition to five known sesquiterpene lactones, i.e. 11β,13-dihydrocnicin,11β, 13-dihydro 19-desoxycnicin, 8α-hydroxy-dihydro-onopordaldehyde, 11β,13-dihydrosalonitenolide, and melitensin, a new germacranolide, i.e. 8α-*O*-(4-acetoxy-5-hydroxy-angeloyl)-11β,13-dihydrocnicin, and two new eudesmanolides, i.e. 8α-*O*-(4-hydroxy-2-methylene-butanoyloxy)-11β,13-dihydro-sonchucarpolide and 8α-*O*-(4-hydroxy-2-methylene-butanoyloxy)-11β,13-dihydro-4-*epi*-sonchucarpolide. The structures were mainly elucidated by 1D and 2D NMR spectroscopy and HR-MS. The *in vitro* antimicrobial activity of all isolated sesquiterpene lactones was tested using the micro-dilution method [4], [5] against the Gram-negative bacteria *Escherichia coli* (ATCC 35210), *Pseudomonas tolaasii* (isolated from *Agaricus bisporus*), *Salmonella enteritidis* (ATCC 13076), the Gram-positive bacteria *Bacillus subtilis* (ATCC 10907), *Micrococcus flavus* (ATCC 10240) and *Staphylococcus epidermidis* (ATCC 12228), as well as against the fungi *Aspergillus niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *A. flavus* (ATCC 9643), *Penicillium ochrochloron* (ATCC 9112), *P. funiculosum* (ATCC 36839), *Trichoderma viride* (IAM 5061), *Fusarium tricinctum* (CBS 514478) and *Alternaria alternata* (DSM 2006). All compounds tested showed greater antibacterial and antifungal activities than the positive controls. *Acknowledgements: The authors wish to thank the Algerian ministry of high education for financial support.* **References:** [1] Wagenitz, G. and Hellwig, F. H. (1996) Proceedings of the International Compositae Conference, Royal Botanic Gardens, Kew.1: 491 – 510. [2] Quezel, P. et Santa, S. (1963) Nouvelle flore de l'Algérie et des régions désertiques méridionales. Ed. Centre national de la recherche scientifique, p. 1019. [3] Bohlmann F. et al. (1984), Phytochemistry 23: 1979 – 88. [4] Booth, C. (1971). Fungal Culture Media. In: Norris, J. R. and Ribbons, D. W. (Eds.), Methods in Microbiology, Academic Press, London & New York, pp. 49 – 94. [5] Hanel, H. and Raether, W. (1988). Mycoses 31: 148 – 154.

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Sesquiterpene lactones of *Centaurea grisebachii* (Nyman) Heldr. ssp. *grisebachii* from Greece

Djeddi S, Karioti A, Skaltsa H
Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece

The genus *Centaurea* comprises approximately 300 species, indigenous in Europe, the Mediterranean area and Asia [1]. In continuation of our phytochemical investigations on *Centaurea* species of the Greek flora [2], [3], [4], [5], [6], [7], we studied *C. grisebachii* ssp. *grisebachii* belonging to the section Acrolophus (Cass.) DC from Greece. The aim of the present study is the isolation and identification of its secondary metabolites and the subsequent evaluation of their biological activities. We report here the isolation and structure elucidation of the sesquiterpene lactones: cnicin, 11β, 13-dehydromelitensin, 8α-*O*-(3,4-dihydroxy-2-methylene)-11β, 13 dehydromelitensin, 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-sonchucarpolide, 8α-*O*-(3,4-dihydroxy-2-methylene-butanoyloxy)-4-*epi*-sonchucarpolide, as well as the flavonoids jaceosidine, salvigenine, 6,7,3',4'-tetramethoxyflavone, and the lignan arctigenin. The isolation has been carried out by chromatographic methods of separation, i.e. TLC and CC and their structures were established mainly by means of 1D and 2D NMR (^{1}H-NMR, ^{13}C-NMR/DEPT, ^{1}H-^{1}H COSY, NOESY, HSQC, HMBC). These preliminary data show that *C. grisebachii* ssp. *grisebachii* is particularly rich in active secondary metabolites and therefore its investigation is still in progress. *Acknowledgements:The authors wish to thank Dr Th. Constantidinis for the identification of the plant.* **References:** [1] Dostál, J. (1976) Flora Europea: Plantaginaceae to Compositae and Rubiaceae. Edited by: Tutin, T.G. et al., 4: 274 p. [2] Skaltsa, H. et al., (1999) Plant. Med. 65: 393. [3] Skaltsa H. et al., (2000) Phytochemistry 55: 903 – 908. [4] Gousiadou C. and Skaltsa H. (2003) Biochem. Syst. Ecol. 31: 389 – 396. [5] Koukoulitsa, E. et al., (2002) Planta Med. 68: 647 – 649. [6] Karioti et al. (2002), Z. Naturforsch. 57c: 75 – 80. [7] Saroglou, V. et al., (2005) J. Nat. Prod. 68: 1404 – 1407.

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Phytochemical investigation of the endemic species *Marrubium thessalum* Boiss. & Heldr.

Argyropoulou C, Karioti A, Skaltsa H
Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece

The genus *Marrubium* comprises approximately 30 species, indigenous in Europe, the Mediterranean area and Asia [1]. In continuation of our phytochemical investigations into *Marrubium* species of the Greek flora [2], [3], [4], [5] we studied the Greek endemic species *M. thessalum*, used in folk medicine since antiquity. The aim of the present study is the isolation and structure elucidation of its secondary metabolites and the subsequent evaluation of their biological activities. Previous investigations of two other Greek endemic species, *M. velutinum* Sibth. & Sm. and *M. cylleneum* Boiss. & Heldr., showed that they are rich in bioactive compounds. The genus has especially been studied for its diterpenes, due to their medicinal properties. We report here the isolation and identification of five secondary metabolites from the dichloromethane extract of the aerial parts of *M. thessalum*. One new diterpene, 7-α-hydroxymarrubiin, was isolated along with two known ones, preperegrinine and peregrinine, one oxygenated sesquiterpene, caryophyllene oxide, and one flavonoid, ladanein. The isolation has been carried out by chromatographic methods of separation, i.e. TLC and CC, and structures were established mainly by means of 1D and 2D NMR (^{1}H-NMR, ^{13}C-NMR/DEPT, ^{1}H-^{1}H COSY, NOESY, HSQC, HMBC). These preliminary data show that *M. thessalum* is particularly rich in active secondary metabolites and therefore its investigation is still in progress. **References:** [1] Mabberley, D.J. (1997) The Plant Book. Cambridge University Press. Cambridge. p. 440. [2] Michelis, F. et al. (2002) Pharm. Biol. 40: 245 – 248. [3] Karioti, A. et al. (2003) Phytochemistry 64: 655 – 660. [4] Karioti, A. et al. (2005) Z. Naturforsch. B 60b: 1 – 5. [5] Karioti, A. et al. (2005) Phytochemistry 66: 1060 – 1066.

P 364

Serratula wolffii, as a source of new ecdysteroids

Liktor-Busa E[1], Simon A[2], Báthori M[1]
[1]Department of Pharmacognosy, University of Szeged, Szeged, Eötvös utca 6, H-6720 Hungay; [2]Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Szt. Gellért tér 4, H-1111, Hungary

Ecdysteroids were discovered as steroid hormones of arthropods. They regulate moulting, metamorphosis, reproduction and diapause of insects. [1] The functional analogues of ecdysteroids were used as selective pest control agents. Phytoecdysteroids are structurally related to the main insect hormone ecdysone. The levels of ecdysteroids in plants are generally between 0.1 – 3% of the dry weight. Their easy availability in plants allowed pharmacological studies, which demonstrated that they influence many physiological functions in a positive way and they are not toxic to mammals. Their most pronounced effect on mammals is a stimulation of protein synthesis without adverse androgenic, antigonadotropic, thymolytic side ef-

fects. Ecdysteroid-inducible gene expression system is a new line of biomedical application of ecdysteroids [2]. We now report the isolation and structure determination of five new ecdysteroids from the roots of *Serratula wolffii*. The isolation of compounds from the methanol extract involves simple cleanup using precipitation and combined chromatographic procedures, including CC on polyamide and C_{18}, RPC and preparative HPLC. The newly discovered ecdysteroids are as follows: 14,15α-epoxy-(20R,22R,)-2β,3β,20,22,25-pentahydroxy-5β-cholesta-7,14-dien-6-one, (20R,22R)-2β,3α,20,22,25-pentahydroxy-5β-cholesta-7-en-6-one, 22-methylene-2β,3β,11α, 14α,25-pentahydroxy-5β-cholesta-7-en-6-one, 2β,3β,14α,25-tetrahydroxy-5β-cholesta-7,20(22)-dien-6-one, 1β,2β,3β,14α,25-pentahydroxy-5β-cholesta-7,20(22)-dien-6-one. The structures of compounds were elucidated by 1D and 2D NMR spectroscopy and mass spectrometry. **References:** [1] Dhadialla, T.S. et al. (1998) Annu. Rev. Entomol. 43: 545 – 569. [2] Lafont, R. et al. (2003) J. Insect Sci. 3: 7:1 – 30.

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Estrogenic properties of traditional Cameroonian medicinal plants

Vollmer G[1], Njamen D[2]
[1]Molekulare Zellphysiologie & Endokrinologie, Technische Universität Dresden, Zellescher Weg 20b, 01217 Dresden, Germany; [2]Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1, P.O. Box 812 Yaounde, Cameroon

Medicinal plants of tropical and subtropical areas of Cameroon which are traditionally used to treat female ailments were investigated towards potential estrogenic activities. By a screening procedure with the yeast estrogen receptor assay on 33 extracts from 18 plants five extracts with estrogenic activities could be identified (ethyl acetate extracts of the stem bark of *Millettia conraui* and *Millettia drastica*, the methanol extracts of the leaves of *Bridelia ferruginea*, the roots of *Pseudarthria hookeri* and the roots of *Nauclea latifolia)*. Results were verified by alkaline phosphatase induction in Ishikawa cells at 10 mg/mL and 100 mg/mL concentrations. Treatment with the pure antiestrogen fulvestrant abolished the extract induced stimulatory activity. In conclusion, a screening on 33 extracts of 18 medicinal plants yielded the identification of five extracts with estrogenic activity. Further, the ethyl acetate extract of the stem bark of *Erythrina lysistemon* also proved to be estrogenic in two *in vitro* assays. Therefore, the estrogenic activity of this extract was investigated *in vivo* in young ovariectomized female Wistar rats after a 7-day treatment. The oral administration of 200 mg/kg BW/d of *Erythrina lysistemon* extract in comparison to vehicle treated ovariectomized rats significantly increased the vaginal epithelial height by 47% and induced a weak increase of uterine epithelial height by around 7%. Both effects were not as pronounced as those elicited in positive control of 100 µg/kg BW/d of ethinylestradiol given orally. Overall our results suggest that the extract of *Erythrina lysistemon* has week estrogenic activity.

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Hairy roots of *Ruta graveolens* L. – a rich source of coumarins and furanocoumarins

Sidwa-Gorycka M[1,3], Orlita A[2], Maliński E[2], Bugalska A[1], Lojkowska E[1], Królicka A[1]
[1]Department of Biotechnology UG & AMG, Kladki 24, 80 – 822 Gdansk, Poland; [2]Department of Environmental Analytics, University of Gdansk Faculty, Sobieskiego 18/19, 80 – 952 Gdansk, Poland; [3]Gdynia Innovation Centre, Laboratory of Pomeranian Scientiffic & Technology Park, Al. Zwyciestwa 96/98, 81 – 645 Gdynia, Poland

Ruta graveolens L. (Rutaceae) is one of the richest natural sources of coumarins (i.a.: umbelliferone) and linear furanocoumarins (i.a.: psolaren, bergapten, xanthotoxin). *Ruta* has been used in the treatment of leucoderma, vitiligo and psoriasis diseases. The aim of our work was to obtain hairy root cultures of *R. graveolens* after transformation of explants of *in vitro* grown plant with *Agrobacterium rhizogenes*. Hypocotyls were transformed with 2 strains of *A. rhizogenes* (A4, LBA9402) using 2 methods. Axenic cultures derived from a single root tip were established after 3 – 5 weeks of subculture in an Erlenmeyer flask containing B_5 medium, 3% sucrose, claforan and carbenicillin (500 mg l^{-1}). Hairy roots were maintained on a liquid B_5 medium without antibiotics (16/8 h photoperiod or in the darkness), on a rotary shaker at 110 rpm. Subcultures were made every 4 weeks. The transformation of *Ruta* tissue was confirmed on molecular level using PCR tests with primers based on the sequence of *rolB* and *rolC* genes of *A. rhizogenes*. Quantitative and qualitative determination of coumarins and furanocoumarins in chloroform and methanol extracts was performed by using a Hewlett- Packard Model 5890 gas chromatograph coupled with mass spectrometer TRIO 3000. Separations were performed on a capillary column BP-1 (30 m x 0,25 i.d., 0, 25 µm film thickness). The growth index (t_{30}/t_1) for control shoots grown *in vitro* amounted to 5.5 and for hairy roots 4.8. In comparison to control shoots of *R. graveolens* in hairy roots growing in the dark we detected 2 times more bergapten; 16.5 times more izopimpinellin and 4 times more rutacultin isomer. In addition new compounds were identified, like osthol, 3-(1,1-dimetylallylo)scopoletin, 2,5-dimetyl-7-hydroxychromon, 5-(3-metyl-2-butenyl)-8-metoxypsolaren and chalepin. The level of these compounds in hairy roots growing under a 16 h photoperiod was on average 2 – 3 times lower than in roots growing in the dark. The obtained results indicate that hairy roots of *R. graveolens* grown in the dark are a rich source of coumarins and furanocoumarins. *Acknowledgements:* State Committee for Scientific Research, Grant No PBZ-KBN 112/P06/2005

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Protein fractions in some Macedonian edible Boletaceae mushrooms

Bauer Petrovska B[1], Kulevanova S[1], Ugrinova L[1], Kirovska Cigulevska O[2]
[1]Faculty of Pharmacy, Vodnjanska 17; [2]Institute of Public Health, 3th Macedonian brigade 18; 1000 Skopje, R. Macedonia

In this study the proteins of Macedonian edible Boletaceae mushrooms were separated and the individual fractions studied in detail. The relative proportion of each fraction in mushrooms strongly affects the nutritional quality of the total mushroom protein. The protein distribution in each of fifteen species of Macedonian edible Boletaceae (of *Boletus*, *Suillus* and *Leccinum* genera) mushrooms was established by the Landry and Moreaux fractionation scheme [1]. The investigated mushroom samples contained relatively high total protein content (TPC) (22.31 – 41.31%, dry basis). The protein fractions of all fifteen investigated mushroom samples were as follows: albumin (25.32%), globulin (15.29%), glutelin (10.12%), prolamin (7.14%), prolamin-like material (6.31%) and glutelin-like material (4.74%). Boletaceae mushrooms, when compared to other food sources (Table 1), contain greater total protein quantity and represent a good protein source food. Investigated Boletaceae mushroom proteins are composed of a very low proportion of prolamins and glutelins and a high proportion of albumins and globulins. The presence of large amounts of residual non protein nitrogen, gave a protein converting factor of 4.31. Table 1 Protein fractions (average values) in mushrooms as compared to other food expressed in % on dry mass basis

source	TPC (N x 6.25)	albumins	globulins	prolamins	glutelins	residue
maize [1]	9.82	8.3	4.6	41.8	37.3	8.0
wild rice [2]	16.1	10	10	1	79	
sorghum [3]	11.62	23.4	11.6	51.2	13.7	
mushrooms	30.98	25.32	15.29	13.45	14.86	31.03

Acknowledgements: Institute of Biology, Faculty of Natural Sciences, Prof. Dr. Mitko Karadelev **References:** [1] Landry, L. et al. (1980) J Agric Food Chem 28: 1186 – 1191. [2] Wang, HL. et al. (1978) J Agric

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Prenylated flavonoids with antioxidative activity from *Epimedium koreanum* Nakai

Kim ES[1], Kang HK[2], Park YI[2], Dong MS[2], Chung HS[1]
[1]College of Natural Sciences, Duksung Women's University, Seoul 132 – 714, Korea; [2]School of Life Sciences and Biotechnology, Korea University, Seoul 136 – 701, Korea

Epimedium koreanum Nakai (Berberidaceae) has long been used to stimulate hormone secretion, to treat asthmatic attacks and menstrual irregularity in Korean folk medicine. Evaluation of antioxidant components using a DPPH free radical scavenging assay in *Epimedium koreanum* has led to the isolation of prenylated flavonoids through repeated column chromatographic fractionation. Chemical structures of isolated components were identified on the basis of spectroscopic methods, particularly high resolution (HR) FABMS and 1D and 2D NMR, as des-*O*-methylhydroicaritin 7-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranoside](**1**), 2"-*O*-Rhamnosylicariside(**2**), hyperoside(**3**), epimedin A(**4**), epimedin B(**5**), epimedin C(**6**), icariin(**7**), hexandraside E(**8**) and epimedoside A(**9**). The HR-FABMS of compound **1** indicated a molecular ion of *m/z* 701.2058. The presence of two two-proton doublets at δ 6.93 ppm(*J*= 9.0 Hz), δ 8.03 ppm(*J*= 9.0 Hz) and a one-proton singlet at δ 6.58 ppm, suggested that the aglycone should be based on kaempferol with a substituent carbon linked at C-8. The characteristic signals of an isopentenyl group were observed at δ 1.59 ppm and δ 1.73 ppm, each as a three-proton singlet, and at δ 3.14 ppm as a two proton multiplet. The anomeric protons of glucose residue at δ 5.18 ppm(*J*= 8.0 Hz) and δ 4.56 ppm(*J*= 7.5 Hz) were observed. Chemical shift of inner glucose at δ 81.2 ppm was connected at C-2" of terminal glucose, since its chemical shift was downfielded. HMBC long range correlations were observed between anomeric proton at δ 5.18 ppm of inner glucose and a carbon at δ 156.9 ppm assigned to C-7, and terminal anomeric proton at δ 4.56 ppm caused a cross peak with a carbon of C-2" at δ 81.2 ppm. The occurrence of compound **1** had not been reported previously.

P 369

The composition of a flavonoid complex from *Larix* wood (Pinaceae). The stereochemistry of dihydroquercetin

Kolesnik YA[1], Demyanovich VM[2], Tashlitsky VN[2], Tikhonov VP[1], Titova EV[1], Shmatkov DA[1]
[1]Diod Co, 11A, Derbenevskaya str., 115114, Moscow, Russia; [2]M.V. Lomonosov Moscow State University, Department of Organic Chemistry, Department of Chemistry of Natural Compounds, Leninskie Gory, 119992, Moscow, Russia

Nowadays flavonoids are widely used as prophylactic and medicinal agents and as natural antioxidants [1]. In Russia *Larix sibirica* Ledeb. and *Larix gmelini* (Rupr.) wood (Pinaceae) are the main sources of flavonoids. However, the exact composition of the bioflavonoid complex (BFC) has not yet been sufficiently studied. Now we present the results of a more detailed investigation of BFC composition by HPLC, MS-MALDI and LC-MS methods. Dihydroquercetin (DHQ) (taxifolin [2], CAS No. 480 – 18 – 2, CHO, 2,3-dihydro-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-*4 H*-1-benzopyran-4-one; 3,5,7,3',4'-pentahydroxyflavonone) is the main component of *Larix* BFC. There are two chiral centers in the DHQ molecule and there exist two pairs of enantiomers: with 2*R*,3*R* and 2*S*,3*S* absolute configuration (*trans*-isomers) and 2R,3*S* and 2*S*,3*R* (*cis*-isomers). We have shown the presence of all mentioned isomers of DHQ in *Larix* wood by HPLC-MS, UV- and IR-spectroscopy and ^{1}H-NMR (1D-NOE) methods. The enantiomeric composition of *cis*- and *trans*-isomers of DHQ was studied in two modes: dynamic modification of the mobile phase by β-cyclodextrin and enantioselective HPLC with the use of a chiral sorbent with a triacetylcellulose and Eurocel 01-column. The 2*R*3*R* enantiomer of the *trans*-isomer of DHQ was shown to be the predominant one in *Larix* wood which is in accordance with the literary data about other sources of DHQ [3, 4]. The 2*R*3*S* enantiomer of DHQ predominates in the minor *cis*-isomer. 2*R*3*R* configuration of studied enantiomer of DHQ was confirmed by chiroptical methods ($[\alpha]_D$ and CD- spectra). The 2*R*3*R*-enantiomer of DHQ is characterized by melting point, $[\alpha]_D$ (MeOH), UV-, IR-, ^{1}H- and ^{13}C-NMR- and CD-spectra. **References:** [1] Ed. Cathrin A. Rice-Evans, Lester Packer (1998) Flavonoids in Health and Desease, Marcell Dekker Inc. [2] Antonova G., Tjukavkina N. (1993) Chim. Drev., 2: 39 – 46. [3] Sakashima A. et al. (2002) Nat. Prod. Lett.., 16(6): 383 – 387. [4] Kiehlmann E. et al. (1995) J. Nat. Prod., 58: 450 – 455.

P 370

Analysis of buckwheat (*Fagopyrum esculentum*) aroma compounds with GC-MS

Janež D[1], Kantar D[2], Prosen H[2], Kreft S[1]
[1]Faculty of Pharmacy, University of Ljubljana, Ažkerèeva 7, 1000 Ljubljana, Slovenia; [2]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ažkerèeva 5, 1000 Ljubljana, Slovenia

Buckwheat herb is used in phytotherapy for the treatment of venous insufficiency. Its grains have high nutritional value because of a favourable amino acid composition, minerals, dietary fibre and flavonoids. They are used as a cereal in some parts of the world (Central and Eastern Europe, Japan) for the preparation of different dishes like noodles, bread, kasha etc. Buckwheat has a strong characteristic aroma, but its phytochemical background was not yet fully elucidated [1,2]. The aim of our work was to identify and quantify individual compounds responsible for buckwheat aroma. Volatiles from freshly ground buckwheat flour were extracted with different methods: direct extraction (extracting solvents methanol, petroleum ether and pentane were used), distillation with Clevenger apparatus and headspace solid-phase microextraction method (HS-SPME). The extracts were analysed by gas chromatography- mass spectrometry (GC-MS) with electron ionisation (EI). Compounds were identified on the basis of their EI spectra, and by comparison of their retention times with standards. Direct extraction with methanol with subsequent concentration of the extract and distillation proved to be very efficient. In those extracts 24 and 33 compounds were identified, respectively. Only 2 compounds occurred in both extracts (salicylaldehyde and phenylacetaldehyde). The compounds with highest contribution to the aroma (odour activity value, OAV > 20) are those with oxygen-containing functional groups: aldehydes (*trans,trans*-2,4-decadienal, *trans*-2-nonenal, phenylacetaldehyde, salicylaldehyde, decanal), phenols (guaiacol, 2-methoxy-4-vinylphenol) and furane derivatives (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 2-pentylfurane). **References:** [1] Yajima, I. et al. (1983) Agric. Biol. Chem., 47: 729 – 738. [2] Mazza, G. et al.(1999) J. Food Qual. 22: 341 – 352.

P 371

Compounds from *Salvia ringens* Sibth. et Sm. with cytotoxic activity

Janicsák G[1], Hohmann J[2], Nikolova M[3], Genova E[3], Zupkó I[4], Forgo P[5], Máthé I[1,2]
[1]Institute of Ecology and Botany of the Hungarian Academy of Sciences, Alkotmány str. 2 – 4., Vácrátót, H-2163, Hungary; [2]Department of Pharmacognosy, University of Szeged, Eötvös str. 6., Szeged, H-6720, Hungary; [3]Department of Applied Botany, Institute of Botany, Bulgarian Academy of Sciences, G. Bonchev str., bl. 23, Sofia, 1113, Bulgaria; [4]Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös str. 6., Szeged, H-6720, Hungary; [5]Department of Organic Chemistry, University of Szeged, Eötvös str. 6., Szeged, H-6720, Hungary

The genus *Salvia* (Lamiaceae) is a rich source of non-volatile compounds (e.g. flavonoids, phenolics, di- and triterpenes) [1]. These

components have several beneficial biological effects [2,3,4]. Our previous study revealed a promising cytotoxic activity *of Salvia ringens* Sibth. et Sm. Literature provides only a few data on this sage species, so it was decided to investigate it for its bioactive compounds. The plant material was collected in Bulgaria (Shumensko Plato National Park). An acetone extract of the roots was subjected to multiple chromatographic separations under the guidance of antiproliferative assay on HeLa cells using MTT assay. From the most active fractions seven abietane diterpenes were isolated by preparative column and thin-layer chromatographic purification. Until now, royleanone, horminone, 7-*O*-methylhorminone and 7-acetylhorminone were unambiguosly elucidated. The structures were established by ESI-mass spectroscopy and advanced two-dimensional NMR methods, including ^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC experiments. All the elucidated compounds were evaluated for cytotoxic activity and displayed marked concentration-dependent effects. **References:** [1] Kintzios, S.E. (2000) Sage. The genus Salvia. Harwood Academic Publishers, Amsterdam. [2] Miliauskas, G. et al. (2004) Food Chem. 85: 231 – 237. [3] Ulubelen, A. (2003) Phytochemistry 64: 395 – 399. [4] Chen, X. et al. (2002) J. Nat. Prod. 65: 1016 – 1020.

P 372

Unusual cystine lyase activity of alliinase isolated from *Allium sativum*: direct formation of polysulphides

Keusgen M[1]

[1]*University of Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany*

Since ancient times, garlic (*Allium sativum* L.) and related species have been used as foods, spices, and herbal remedies in many parts of the world. Sulphur containing flavour compounds are responsible for the characteristic smell and taste of members of the onion family (Alliaceae). These volatile flavour substances, *e.g.* allicin (allyl-2-propenethiosulfinate), are formed by the action of alliinase (EC 4.4.1.4) on cysteine sulphoxides, *e.g.* alliin (S-(+)-allyl-L-cysteine sulfoxide). Additionally, alliinase catalyses the C-S lysis of cystine in a manner of a cystine lyase. Ammonium, pyruvate and elementary sulphur but not cysteine could be detected as reaction products. The ratios between cystine, ammonium and pyruvate are 1:1.9:1.9 suggesting a new type of reaction mechanism (Figure). Elementary sulphur is probably formed from enzymatically produced persulphide. Thiocysteine and disulphine were assumed as intermediates. Moreover, parallel incubation of cystine and alliin gave mainly allyl-(poly)sulphides as reaction products instead of allicin. These substances were not observed as direct enzymatic products until now. Thus, the significance of alliinase and its enzymatic products has to be newly considered in terms of ecological, pharmacological, and biochemical aspects.

Acknowledgements: Thanks are due to Mrs. J. Glodek and Dr. I. Krest, Institute for Pharmaceutical Biology, Bonn, for technical assistance. This research was supported be the Fonds der Chemischen Industrie and the DPhG-Stiftung as part of the Stifterverband für die Deutsche Wissenschaft.

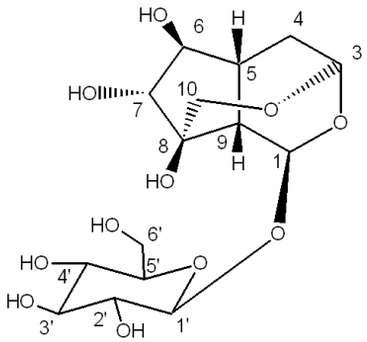
P 373

Iridoids from *Scutellaria albida* ssp. *albida*

Gousiadou C[1], *Karioti A*[1], *Heilmann J*[2], *Skaltsa H*[1]

[1]*Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, 157 71, Athens, Greece;* [2]*Institute of Pharmacy, Department of Pharmaceutical Biology, University of Regensburg, Universitätsstrasse 31, D-93053, Regensburg, Germany*

Scutellaria albida L. ssp. *albida* (Lamiaceae) is an herbaceous perennial plant, distributed from Northern Italy to the Balkan peninsula and Crimea [1]. Several species of the genus *Scutellaria* present antispasmodic, diaphoretic and febrifuge properties and are used in folk medicine [2]. The methanolic extract of the aerial parts of *S. albida* ssp. *albida*, after being successively chromatographed on silica gel columns and RP-HPLC, yielded four new iridoid glycosides, namely scutelloside (**1**), 6'-*O*-*E*-*p*-coumaroylgardoside, 6'-*O*-*p*-*E*-coumaroyl-8-*epi*-loganic acid and 6'-*O*-*E*-*p*-caffeoyl mussaenosidic acid, along with an anomeric mixture in equilibrium of one iridoid aglycone, dihydrocatalpogenine (C-1) α-epimer/β-epimer, nine known iridoid glycosides, i.e. catalpol, albidoside, picroside III, dihydrocatalpol, 10-descinnamoylglobularinin, globularin, gardoside, 8-*epi*-loganic acid, macfadyenoside, four known phenylethanoid glycosides, i.e. martynoside, isomartynoside, deacyl-martynoside, acteoside, and six known phenolic derivatives, i.e. *E*-*p*-coumaric acid, *E*-caffeic acid, *E*-ferulic acid, *E*-*p*-coumaroylglucoside, vanilloloside and benzyl-β-glucopyranoside.



(**1**)

References: [1] Bothmer, R. (1985). Nord. J. Bot. 5: 421 – 439. [2] Duke, J. A. (1986). Handbook of Medicinal Herbs, CRC Press..Inc p. 440.

P 374

The phytochemical evaluation of some extracts of *Ocimum* sp.

Gille E[1], *Danila D*[1], *Stanescu U*[2], *Hancianu M*[2]

[1]*"Stejarul" Biological Research Centre/INCDSB Bucuresti, Alexandru cel Bun Street, 610004, Piatra Neamt, Romania;* [2]*University of Medicine and Pharmacy "Gr.T.Popa", 16, Universitatii Street, 700115, Iasi, Romania*

The work presents the results obtained from analyses of *Ocimum* species cultivated in Romania: *O. basilicum* L., *O. basilicum* var. *citriodora* and *O. sanctum* L. Out of the numerous medicinal species to be used in anti-aging treatments, plants such as those from the genus *Ocimum* are of great importance due to their richness of active principles with antioxidant, adaptogene and antistress actions helping the organism to fight the free radicals linked to illness and the aging process [1]. The volatile oils from the vegetal raw materials were obtained by hydrodistillation [2] and analysed by GC-MS. We selected samples of *O. sanctum* (O.s). and Herba Basilici (O.b.w. – variety with white flowers; O.b.r – variety with red flowers; O.b.c. - *citriodora* var.). The O.b.w. sample contains the greatest quantity of volatile oil (0.61 ml/100 g d.w.). The major components identified are: linalool, estragol, geraniol, germacrene D and β-elemene; in the O.b.c. sample we found *Z*- and *E*-citrals. The volatile oils contained the same groups, i.e. monoterpenes, aromatic

compounds and sesquiterpenes. In O.b.w. we additionally found aliphatic components (2-dodecanone, n-eicosane). The O.b.c. sample was characterised by its monoterpene pattern (citrals, geranyl acetate, linalool, etc.); linalool is the dominant component of the analysed samples: 38.93% (O.s.), 30.33% (O.b.w.), 29.28% (O.b.r.), 13.89% (O.b.c.). These differences of chemical composition registered in the analysed samples was also reflected in their biological activity. O.b.w. is more active against gram negative bacteria and O.b.c. is active against gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). *Acknowledgments:* The work is sustained in the CEEX-BIOTECH program financed by the Romanian Government – The Ministry of Ed. and Research. **References:** [1] Archana R., Namasivayam A. (2000) J.Ethnopharmacol**,** 73**:** 81 – 85. 2. (1998) Farmacopeea Romana, Ed. X, Ed. Med. Bucharest

P 375

Anti-inflammatory and gastroprotective activity of *Hypericum perforatum* oil extracts

Zdunić G[1], Gođevac D[2], Milenković M[3], Šavikin K[1], Petrović S[4]
[1]Institute for Medicinal Plant Research "Dr. Josif Panèić", T. Kožćužka 1, 11000 Belgrade, Serbia;[2]Institute of Chemistry, Technology and Metallurgy, Njegoževa 12, 11000 Belgrade, Serbia; [3]Institute of Microbiology and Immunology, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade, Serbia; [4]Institute of Pharmacognosy, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade, Serbia

Oil extracts of flowering tops of *Hypericum perforatum* are popular remedies in traditional medicine of many countries. They are used externally for the treatment of wounds, first-degree burns, acute and contused injuries, myalgia and internally for dyspeptic complaints [1]. We prepared *H. perforatum* oil extracts in three different ways: by maceration of the fresh flowering tops in sunflower oil exposed to sunlight for 40 days (extract A) [2], by maceration of dried flowering tops with 96% EtOH followed by evaporation of the solvent in the presence of sunflower oil (extract B) [3], and by digestion of dried flowering tops in sunflower oil for 3 h (extract C) [4]. Carrageenan-induced rat paw oedema test has been used as an experimental model for screening the anti-inflammatory activity according to the modified method of Oyanagui and Sato [5]. The extracts were administered p.o. in a dose of 1.25 ml/kg to rats and compared with indomethacin, which was used as a reference at a dose of 8 mg/kg p.o. Statistical analysis was performed by the Mann-Whitney exact test, and $P < 0.05$ was considered as significant. The obtained anti-inflammatory effect was 88%, 96% and 77% for extract A, B and C, respectively. Indomethacin had an anti-inflammatory effect of 81%, but large gastric lesions were detected. When the investigated extracts (1.25 ml/kg p.o.) were concomitantly given with indomethacin (8 mg/kg p.o.), the gastric lesions were significantly reduced. Extract B showed the highest gastroprotective activity. According to the HPLC profiles, purified oil extract B had higher content of I-3,II-8-biapigenin than purified oil extract A, while in purified oil extract C, this compound has not been detected. **References:** [1] Blumenthal, M. et al. (eds) (1998) The Complete German Commission E Monographs. American Botanical Council. Austin. [2] Tucakov, J. (1996) Leèenje biljem. Rad. Beograd. [3] Shikov A. et al. (2004) Rastitel'nye masla i maslyanye ekstrakty: tehnologiya, standardizatsiya, svoistva. Ryskii vratch. Moskva. [4] Shass E. Yu. (1952) Fittoterapiya, An SSSR. [5] Oyanagui, Y., Sato, S. (1991) Arzneim.-Forsch./Drug Res. 41: 5.

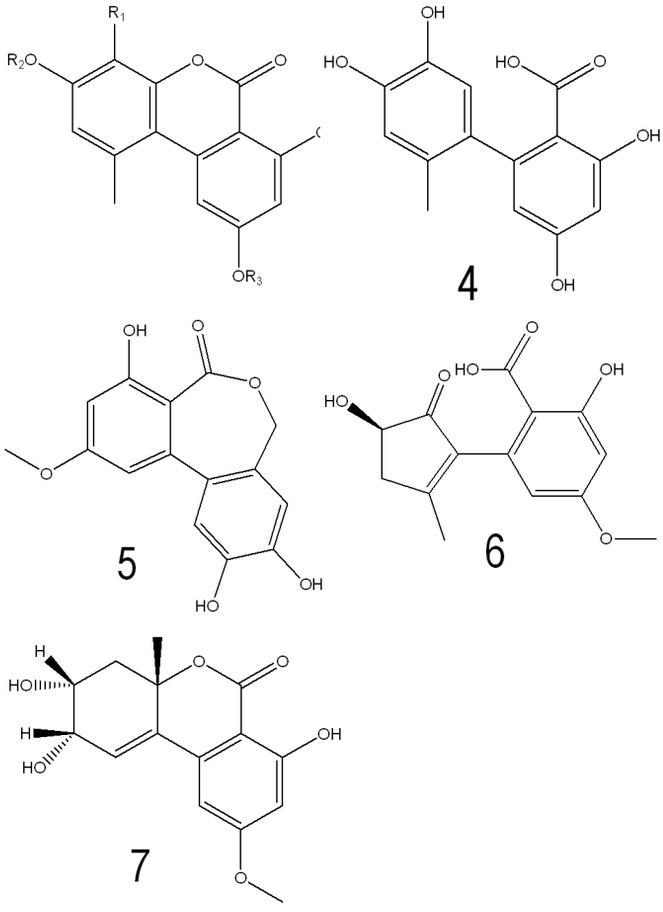
P 376

Bioactive metabolites from the fungal endophyte *Alternaria* sp. and their detection in the host plant *Polygonum senegalense*

Aly AH[1], Edrada RA[1], Wray V[2], Müller WEG[3], Proksch P[1], Ebel R[1]
[1]Heinrich-Heine-Universität, Institut für Pharmazeutische Biologie und Biotechnologie, Universitätsstrasse 1, Geb. 26.23, D-40225 Düsseldorf, Germany; [2]Helmholtz Centre for Infection Research, Inhoffenstraße 7, D-38124 Braunschweig, Germany; [3]Johannes-Gutenberg-Universität, Institut für Physiologische Chemie und Pathobiochemie, D-55099 Mainz, Germany

In this study we investigated the natural products produced by a hitherto undescribed endophytic fungal strain of the genus *Alternaria* when grown in liquid medium and on solid rice medium. From extracts of the fungus grown in liquid culture we obtained new sulphated derivatives of alternariol (1) and its monomethyl ether (2) as well as the known compounds alternariol and its monomethyl ether, altenusin, 2,5-dimethyl-7-hydroxychromone, tenuazonic acid and altertoxin I. When grown on solid rice medium the fungus yielded four new compounds (3 – 6) in addition to alternariol and its monomethyl ether, altenusin, talaroflavone and altenuene. Furthermore, we isolated a new altenuene isomer that was given the trivial name 4'-epialtenuene (7). The structures of the compounds were unambiguously established on the basis of NMR spectroscopic and mass spectrometric data. The crude extracts and the isolated compounds exhibited moderate to strong toxicity toward L5178Y (mouse lymphoma) cell line. Altenusin, alternariol and its monomethyl ether were also unambiguously identified in *Polygonum senegalense* thereby proving that this fungal endophyte contributes to the chemical composition of the host plant.

Compound	R_1	R_2	R_3
1	H	H	SO_3H
2	H	SO_3H	CH_3
3	OH	H	CH_3

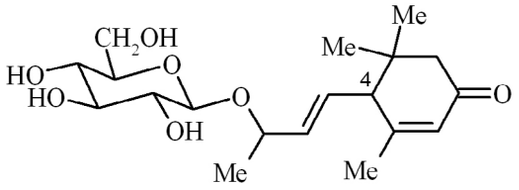


P 377

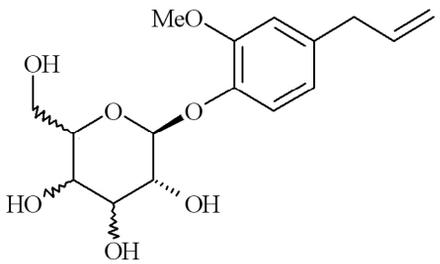
Two novel glycosides from *Morus alba* L. – a new antidiabetic constituent of the plant?

Hunyadi A[1], Báthori M[1], Simon A[2], Szendrei K[1]
[1]Department of Pharmacognosy, University of Szeged, Szeged, Eötvös str. 6. H-6720, Hungary; [2]Department of Inorganic and Analytical Chemistry, Budapest Universiy of Technology and Economics, Budapest, Szt. Gellért tér 4. H-1111 Hungary

The leaf of *Morus alba* L. and to a lesser degree its root bark, are popular constituents of antidiabetic teas. According to the constituents published from the plant, four major groups of compounds may be involved in the antidiabetic effect: iminosugars, ecdysteroids, phenolic compounds and glycoproteins. Iminosugars have α-glycosydase inhibitory activity; Miglitol, the known oral antidiabetic agent is also an iminosugar derivative. Chinese researchers isolated two ecdysteroids (20-hydroxyecdysone and inokosterone) from *M. alba*; in Japan there are ecdysteroid-based products available for the treatment of type II diabetes. The flavonoid concentrate of the root bark of *M. alba* showed protective effect on pancreatic B-cells against streptozotocine, and two benzofuranes from the leaf of a related species, *M. insignis*, were effective in experimental diabetic rats. The root bark of *M. alba* contains two insulin-like glycoproteins as well.



1.



2.

Here we report the isolation and structure elucidation of a megastigmane (1) and a phenyl-propane (2) glycoside from mulberry leaf. We also prove the presence of 20-hydroxyecdysone in the Hungarian mulberry. The 50% methanolic extract was pre-purified by fractionated precipitation, and the isolation procedure was carried out through consecutive steps of chromatographic methods with different selectivity. After solid phase extraction on polyamide, vacuum reversed-phase column chromatography was used, which was followed by a further fractionation step using rotation planar chromatography on silica. The final purification was accomplished by the use of NP-HPLC. For structure elucidation, 1 and 2D NMR spectroscopy was used. Both compounds are new constituents in the *Morus* genus. According to Khan et al., the 4-hydroxy derivative of compound 1. shows significant insulin-releasing effect on rat pancreatic isles [1]. **References:** [1] Khan, S.K. et al. (2003) Pharm. Biol. 41: 512 – 515.

P 378

Isolation of unique ecdysteroids from the *Ajuga reptans* var. *reptans*

Báthori M[1], Hunyadi A, Simon A[2], Tóth G[2], Polgár L[3], Máthé I[1]
[1]Department of Pharmacognosy, University of Szeged, Szeged, Eötvös utca 6, H-6720 Hungary; [2]Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Szt. Gellért tér 4, H-1111, Hungary, [3]Department of Ecotoxicology, Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Herman Otto út 15, H-1525, Hungary

Ecdysteroids represent biologically active steroids which attract great scientific interest because of their perspective use in the conventional health improvement methods and they have a tremendous potential in the most modern therapies (gene-switch systems), too. Several studies proved the activity of ecdysteroids to increase the rate of protein synthesis in mammalian tissue without any hormonal consequences. Because of their anabolic activity, ecdysteroids and ecdysteroid- containing preparations are widely advertised on the Internet as growth promoters. The use of ecdysteroids as inducers is also promising in genetics. The *Ajuga* species are inexhaustible sources of the known as well as new ecdysteroids. This presentation gives an account of the discovery of three new and the isolation of two known ecdysteroids from *Ajuga reptans* var. *reptans*. A sophisticated isolation procedure was used for the preparative-scale separation of pure ecdysteroids. This process includes a simple clean-up using solvent-solvent distribution, precipitation and solid-phase extraction followed by multi-step chromatographic methods such as column chromatography on silica or alumina and octadecyl silica and at last preparative HPLC. The isolation of ecdysteroids was improved by the use of rotation planar chromatography. The optimized combination of preliminary purification and chromatographic separation resulted in 3 new ecdysteroids, namely 25-deoxy-20,23-dihydroxy-24-ethyl-ecdysonoic acid 23,26-lactone, 24-hydroxy-25-methyl-makisterone C 30-methylate and 23-hydroxy-capitasterone. Structure elucidation was performed using 1D and 2D NMR and MS.

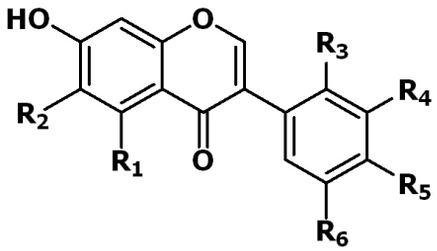
P 379

Isoflavones with estrogenic activity from *Dalbergia parviflora* Roxb.

Monthakantirat O[1], Umehara K[3], Matsushita A[3], Terada E[3], De-Eknamkul W[2], Miyase T[3], Warashina T[4], Noguchi H[3]
[1]Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand; [2]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand; [3]School of Pharmaceutical Sciences, University of Shizuoka, 52 – 1 Yada, Shizuoka 422 – 8526, Japan; [4]Institute for Environmental Sciences, University of Shizuoka, 52 – 1 Yada, Shizuoka 422 – 8526, Japan

Dalbergia parviflora Roxb. (Leguminosae) is a woody climber found in the Southeast Asian peninsula, especially in Thailand [1]. During our investigation of the chemical constituents of the heartwood of this plant, five new isoflavones, khrinone A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) along with 12 known isoflavones, 7-demethylrobustigenin (**6**), biochanin A (**7**), 2′-methoxybiochanin A (**8**), tectorigenin (**9**), pratensein (**10**), 2′-methoxyformononetin (**11**), formononetin (**12**), 3′-methoxydaidzein (**13**), calycosin (**14**), theralin (**15**), genistein (**16**) and bowdichione (**17**) were isolated. The structures of these compounds were determined by extensive spectroscopic studies by and comparison with previously reported data. These isolates were then evaluated for their estrogenic activity by measuring the cell proliferation on MCF-7 and T-47D human breast cancer cell lines [2]. The compounds **7**-**12**, **15**-**16**, **3** and **4** and **16** showed stimulatory effect on both cell lines whereas the compounds **5**, **13** and **17** showed their estrogenic activity against only MCF-7 cells. Among these, compound **16** showed the highest activity (EqE10 value against MCF-7:

0.01 μM, EqE100 value against MCF-7: 0.11 μM, EqE10 value against T-47D: < 0.01 μM, EqE100 value against T-47D: 0.08 μM).



	R_1	R_2	R_3	R_4	R_5	R_6
1	H	H	OH	H	OMe	OH
2	OH	H	OH	H	OMe	OH
3	OH	H	OMe	OH	OMe	H
4	OH	H	OMe	H	- OCH_2O-	
5	H	H	OMe	OH	OMe	H

Acknowledgements: 1. The Royal Golden Jubilee Ph.D. Program from The Thailand Research Fund. 2. The Central Analytical Laboratory of University of Shizuoka for MS measurements. **References:** [1] Niyomdham, C. (2002). Thai For. Bull. (Bot.) 30: 124 – 166. [2] Soto, A.M. et al. (1995) Environ. Health. Perspect. 103: 113 – 122.

P 380

Chemical investigation of some *Capparis* species growing in Egypt and their antioxidant activity

Hammed AR, Abdel-Azim NS, Ismail SI, Hammouda FM
Phytochemistry Department, National Research Centre, El Bohoth str., 12311, Dokki, Egypt

The family Capparaceae comprises 39 genera and 650 species distributed through warm regions. The genus *Capparis* comprises 250 species including shrubs, trees and woody climbers. This genus is represented in Egypt by six species. Dioscurides recommended the use of the leaves and roots of *Capparis spinosa* L. to reduce swelling and calm the pain of teeth. *Capparis cartilaginea* and *C. deserti* growing in Egypt were investigated for their glucosiolates and rutin content. From *Capparis cartilaginea* four isothiocynates were isolated and identified using GC and EI/MS techniques. These compounds were butyl isothiocyanate (1), 6-methylsulphonylhexyl isothiocyanate (2), 7-methylsulphonylheptyl isothiocyanate (3) and 5-benzylsulphonyl-4-pentenyl isothiocyanate (4). In addition to compounds 1 and 2, two other compounds were isolated and identified from *Capparis deserti*. These compounds are 3-methylthiopropyl isothiocyanate (5) and [11-(2-butenylthio) 6-undecenyl isothiocyanate] (6). Compounds 1, 2, 5 and 6 are reported in this study for the first time from *Capparis deserti*. The main flavonoid component in the studied species was isolated and identified as rutin by comparing the data with those reported. Also, quantitative evaluation of rutin in the two species was carried out by TLC-densitometric analysis. The antioxidant activity different fractions of both species was determined using diphenylpicrylhydrazyl (DPPH) radical scavenging method. The butanol fraction from *C. cartilaginea* and *C. deserti* showed the highest antioxidant properties. **References:** [1] Boulos, L. (1999). Flora of Egypt 1st ed., vol. 1, Al Hadara publishing, Cairo, Egypt, pp 177. [2] Täckholm, V. (1974). Students Flora of Egypt, 2nd ed., Cairo University Cooperative Printing Company, Beirut, pp. 162. 3. Gunther, R. (1959). Cited in "The Greek Herbal of Dioscorides". Hafner Publishing Co., New York, pp. 215.

P 381

Secondary metabolites from *Eremostachys laciniata*

Çalış I[1], Güvenç A[2], Armağan M[3], Koyuncu M[2], Gotfredsen CH[4], Jensen SR[4]
[1]Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, TR-06100 Ankara, Turkey; [2]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, TR-06100 Ankara, Turkey; [3]Yüzüncü Yıl University, Education Faculty, Department of Science Education, Zeve Campus, TR-65080 Van, Turkey; [4]The Technical University of Denmark, Department of Chemistry Building 201, DK-2800 Lyngby, DENMARK

Eremostachys is represented by three species in Turkey, namely *E. moluccelloides* Bunge, *E. laciniata* (L.) Bunge and *E. glabra* Boiss. ex Benth. [1]. Our previous chemotaxonomic studies on plants belonging to Lamiaceae resulted mainly in the isolation of iridoid and phenylethanoid glycosides. In this respect, we have studied several taxa from the subfamilies of Lamioideae (*Galeopsis, Lamium, Leonurus, Marrubium, Phlomis, Sideritis, Stachys*), Scutellarioideae (*Scutellaria*) and Teucrioideae (*Teucrium*) on the genus or species level. As a part of our ongoing phytochemical study on the members of the Lamiaceae growing in Turkey, we have now investigated *E. laciniata*. From the aerial parts of the title plant, thirteen iridoid glucosides: sesamoside (**1**), 5-desoxysesamoside (**2**), 6β-hydroxy-7-*epi*-loganin (**3**), chlorotuberoside (**4**), 5-deoxypulchelloside I (**5**), and lamalbide (**6**), lamalbidic acid (**7**), phloyosides I (7-*epi*-phlomiol) (**8**), and II (**9**), phlomiol (**10**), shanzhiside (**11**), shanzhiside methyl ester (**12**), 8-*O*-acetylshanzhiside methyl ester (**13**), four phenylethanoid glycosides: forsythoside B (**14**), verbascoside (**15**), leucosceptoside A (**16**) and martynoside (**17**) and six flavone derivatives: apigenin (**18**), luteolin (**19**), apigenin 7-*O*-glucopyranoside (**20**), luteolin 7-*O*-glucopyranoside (**21**), luteolin 7-*O*-(6''-*O*-apiofuranosyl)-glucopyranoside (**22**), apigenin 7-*O*-(6''-*O*-*p*-coumaroyl)-glucopyranoside (**23**) were isolated. The structures of all metabolites in three different classes have strongly supported that the genus *Eremostachys* is closely related to the genus *Phlomis*. The structures of the metabolites were elucidated by spectroscopic (UV, IR, 1D- and 2D-NMR, and ESI-MS) evidence as well as their specific optical rotation values. **References:** [1] Edmondson, J.R. (1982) Eremostachys Bunge, in Flora of Turkey and the East Aegean Islands, Ed. Davis, P.H., University Press, Edinburgh, Vol. 7, p. 100.

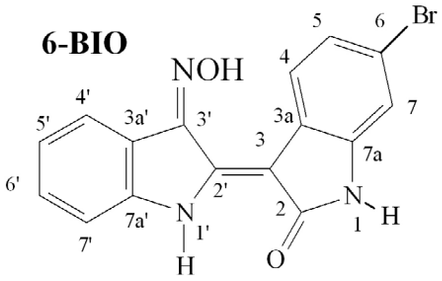
P 382

Natural and synthetic indirubins as potent and selective inhibitors of the protozoan parasite *Leishmania donovani*

Xingi E[1], Smirlis D[1], Bisti S[1], Myrianthopoulos V[2], Magiatis P[2], Meijer L[3], Mikros E[2], Skaltsounis AL[2], Soteriadou K[1]
[1]Laboratory of Molecular Parasitology, Department of Microbiology, Hellenic Pasteur Institute, 127 Bas. Sofias Ave., 1521 Athens, Greece [2]Laboratories of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens 15771, Greece; [3]C.N.R.S., Cell Cycle Group, Station Biologique, B.P. 74, 29682 Roscoff cedex, Bretagne, France

Indirubins constitute an important class of natural products with kinase inhibitory activity [1]. A series of natural and synthetic indirubins known to target mammalian cyclin-dependent kinases (CDKs) and/or glycogen synthase kinase (GSK-3) were tested for their antileishmanial activity. 6-Br-indirubin-3'-oxime (6-BIO) was the most potent inhibitor of *Leishmania donovani* promastigotes and amastigotes growth. Promastigotes-treatment with 6-BIO induced a sub-G0/G1 phase accumulation and morphological/biochemical changes which lead to induction of cell death exhibiting characteristic features of apoptosis. Taking into account that in mammalian cells the 6-Br substitution greatly enhances the selectivity for GSK-3 over CDKs, we cloned and expressed in *E. coli* the leishmanial GSK-3β homologue (LGSK-3β) which displays 49% identity and 68% similarity with human GSK-3β. LGSK-3β, a 40 kDa protein, was detected in both promastigotes and amastigotes. The indirubins, that were found to significantly inhibit the growth of *L. donovani* promastigotes and intracellular amastigotes turned out to be

potent inhibitors of rLGSK-3β. Structural studies confirmed the level of homology of LGSK-3β with human GSK-3 but also predicted the existence of functional/structural differences in their active site that are sufficient to explain the lower inhibitory activity of these compounds towards LGSK-3β compared to its mammalian counterpart. In conclusion, LGSK-3β appears to be a potential drug target for combating leishmaniasis and could be exploited for the development of selective inhibitors like indirubins.



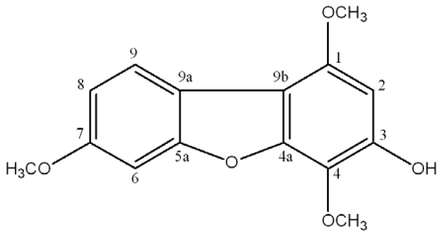
References: [1] Meijer, L. et al. (2003) Chem. Biol. 10: 1255 – 1266.

P 383

Isolation and characterization of a new dibenzofuran from *Hypericum revolutum* ssp. *revolutum* Vahl

Ka Po Shiu W[1], Gibbons S[1]
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, UK

The phytochemistry of *Hypericum perforatum* has been widely studied owing to its anti-depressant activity. The phytochemical data on other species of the *Hypericum* genus are, on the other hand, relatively limited. This study is part of an ongoing project to investigate the phytochemistry and anti-staphylococcal activity of the *Hypericum* genus [1]. The aim was to isolate and characterize compounds from *Hypericum revolutum* ssp. *revolutum*, of which little chemistry is known. The dichloromethane extract of the aerial parts of the plant was fractionated by vacuum-liquid chromatography over silica gel, Sephadex LH-20 size-exclusion chromatography, solid-phase extraction and purified by preparative thin-layer chromatography to yield the new 3-hydroxy-1,4,7-trimethoxy-dibenzofuran (1). This compound was identified by extensive 1- and 2D NMR spectroscopy. Dibenzofurans have been isolated from the *Mespilus* species previously and were found to be the major phytoalexins formed in the sapwood when challenged by a pathogenic fungus [2]. This is the first report of a dibenzofuran natural product being isolated from the *Hypericum* species.



(1)

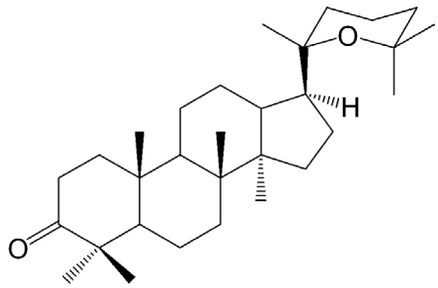
References: [1] Gibbons, S. et al. (2002) Fitoterapia 73: 300. [2] Tetsuo, K., et al. (1995) Phytochemistry 39: 1039.

P 384

New Dammarane Triterpenes from *Gardenia aubryi* VIEILL.

Grougnet R[1,3], Magiatis P[1], Mitaku S[1], Skaltsounis AL[1], Cabalion P[2], Tillequin F[3], Michel S[3]
[1]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens 15771, Greece; [2]Laboratoire des Substances Naturelles Terrestres et Savoirs Traditionnels, Centre IRD de Nouméa, 98800 Nouméa, New Caledonia, [3]Laboratoire de Pharmacognosie de l'Université René Descartes, UMR/CNRS 8638, Faculté de Pharmacie, 4 Avenue de l'Observatoire, F-75006 Paris, France

Gardenia aubryi Vieill. (Rubiaceae) is an endemic plant of New Caledonia. The leaves and flower buds of the plant are covered with an exudate that is used locally for chewing. In a continuation of our phytochemical investigation of *G. aubryi* [1], and of other plants from New Caledonia [2], we report here the isolation of three new dammarane triterpenoids. Gardaubryones A-C (**1**-**3**) were isolated from the gum collected on the aerial parts, together with the known hydroxydammarenone II, ocotillone, cabraleone, and hollongdione. The structures of the novel compounds were established on the basis of mass spectrometry, NMR experiments and chemical correlation reactions.



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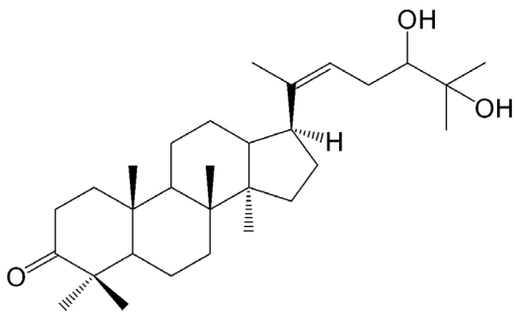

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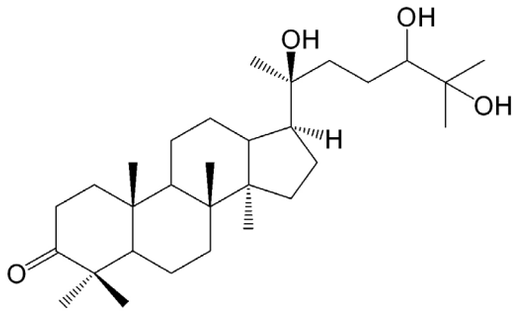

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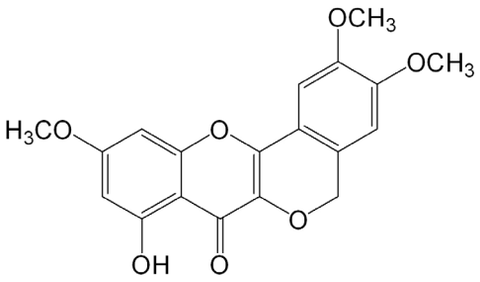
References: [1] Grougnet, R. et al. (2006) J. Nat. Prod., 69, 1711 – 1714. [2] Grougnet, R. et al. (2005) J. Nat. Prod., 68, 1083 – 1086.

P 385

New peltogynoids from *Ficus sycomorus* L.

Ahmadu A[1,2], Grougnet R[2,3], Magiatis P[2], Skaltsounis AL[2], Tillequin F[3]
[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria 810261, Nigeria, [2]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens 15771, Greece; [3]Laboratoire de Pharmacognosie de l'Université René Descartes, UMR/CNRS 8638, Faculté de Pharmacie, 4 Avenue de l'Observatoire, F-75006 Paris, France

The genus *Ficus* is the only one included in the tribe Ficeae of Moraceae. It comprises some 850 species of trees, shrubs, epiphytes, and root-clinging lianas including stranglers, widely distributed in the warm and tropical regions of the World. *Ficus sycomorus* L., the sycamore of the Bible or mulberry fig, occurs in Tropical Africa and has been brought into cultivation in the Mediterranean area for its edible fruits. The latex of this species is locally used in West Tropical Africa to cure various ailments, including skin diseases. We report here the isolation and the structure elucidation of two new peltogynoids, sycoficol I (**1**) and II (**2**), together with known triterpenes from the latex of *Ficus sycomorus* L. collected in Nigeria. The structures of the novel compounds were established on the basis of mass spectrometry and NMR experiments.



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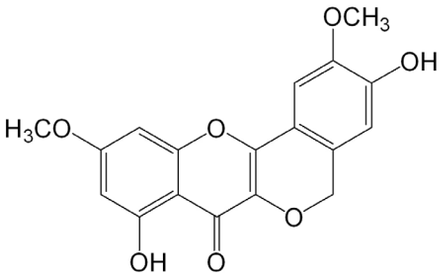


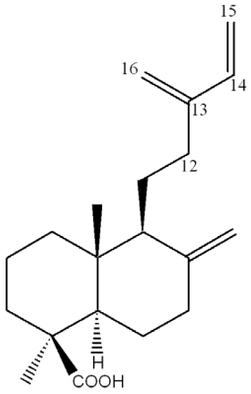
2

P 386

Myrceocommunic acid – a labdane diterpene with activity against *Staphylococcus aureus* and *Propionibacterium acnes*

Smith E[1], Gibbons S[1]
[1]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London WC1N AX, UK

As part on an ongoing study investigating rare conifer species for antimicrobials, the known labdane diterpene myrceocommunic acid (1) **[1]** was isolated from the bark of *Callitris oblonga*. The compound was tested in minimum inhibitory concentration (MIC) assays against two effluxing strains of *S. aureus*: clinical isolate XU212 which has the TetK pump specific for tetracyclines, and strain SA1199B which overexpresses the multidrug-resistance NorA pump. Activity against EMRSA-15, one of the major methicillin-resistant strains found in UK hospitals (2), and against *P. acnes* standard strain ATCC 6919 was also investigated.



Myrceocommunic acid showed good activity with an MIC of 2 μg/ml against *P. acnes* and a more modest activity of 16 μg/ml against resistant strains of *S. aureus*. The compound is very similar to the labdane *trans*-communic acid, which instead of a C13 – 16 vinyl group, has the double bond positioned between C12 and C13 with a methyl at C16. We have previously shown *trans*-communic acid to be active against these same strains of *S. aureus* with MICs of 8 – 16 μg/ml (3), therefore the difference in the position of the double bond at C13 does not appear to affect activity. Although myrceocommunic acid has previously been isolated (1), its antibacterial activity has not been reported. With an activity similar to that of *trans*-communic acid, which has known anti-staphylococcal and anti-acne activities (3,4,5), these results highlight the value of conifer labdane diterpenes in the search for new antibacterials. *Acknowledgements:* We thank Stiefel International R & D Ltd for funding this study. **References:** [1] Atkinson, P. et al. (1970) Tetrahedron 26: 1935; [2] Johnson, A. et al. (2001) J. Antimicrob. Chemother. 48: 143 – 144; [3] Smith, E. et al. (2007) Phytochemistry 68: 210 – 217; Muhammad, I. et al. (1995) Phytother. Res. 9: 584 – 588; 5. Ko, J. et al. (2006) patent (KR 2006067255).

P 387

An *ent*-kaurane diterpene from *Mikania laevigata* (Asteraceae)

Baratto L[1], Schenkel EP[1], Falkenberg M[1]
[1]Department of Pharmaceutical Sciences, Federal University of Santa Catarina, CIF/CCS/UFSC, Campus Trindade, 88.040 – 900 Florianópolis, Brazil

The genus Mikania contains more than 450 species and around 150 of them are found in Brazil [1]. This genus is divided in two groups of species, one of them produces preferently sesquiterpene lactones and the other, diterpenic acids. The late compounds seem to play an important role in defense system of plants [2]. *Mikania laevigata* Schultz Bip. ex Baker ("guaco") is the most abundant *Mikania* species in Southern Brazil [3]. The leaves are used mainly to treat respiratory diseases, but antiseptic, antiasthmatic and antirheumatic properties have been described. The characteristic smell of *Mikania* species is due to coumarins and some of their properties are also attributed to these compounds [4]. The plant material was cultivated at Federal University of Santa Catarina. The dried and powdered leaves (281 g) were macerated with ethanol during 7 days. The crude extract was treated with MeOH:H_2O (9:1), and extracted with petrol (PE), CH_2Cl_2, ethyl acetate and n-butanol. PE fraction was fractionated by flash chromatography (gradient of PE:CH_2Cl_2) followed by silica gel column chromatography (PE:ethyl acetate gradient) and Sephadex LH-20 (MeOH), yielding 21 mg of compound 1. The NMR-H^1, NMR-C^{13}, IR and UV data suggested compound 1 to be ent-15β-benzoyloxy-kaur-16(17)-en-19-oic acid. This compound has been isolated also from other *Mikania* species [5,6], but it has never been found in *M. laevigata*, therefore being a new compound of this species. **References:** [1] Hind, D.J.N. (1993) Kew Bull. 48: 245 – 77. [2] Lobitz G.O. et al. (1997) Phytochem. 46: 161 – 4. [3] Ritter, M.R. et al. (1992) Flora Ilustrada do Rio Grande

do Sul. Porto Alegre. [4] Fabbri et al. (1997) Biochem. System. Ecol. 25: 563 – 4. [5] Veneziani et al. (1999) Biochem. System. Ecol. 27: 99 – 102.

P 388

Antioxidant activity of polyphenolic compounds isolated from the bark of *Trichilia catigua*

Resende FO[1], Rodrigues-Filho E[2], Petereit F[3], Mello JCP[1]
[1]Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Av. Colombo, 5790, BR-87020 – 900, Maringá, Brazil, [2]Departamento de Química, Universidade Federal de São Carlos, Rod. Washington Luiz, km 235, BR-13905 – 905, São Carlos, Brazil, [3]Institut für Pharmazeutische Biologie und Phytochemie, Universität Münster, Hittorfstrasse 56, DE-48149, Germany

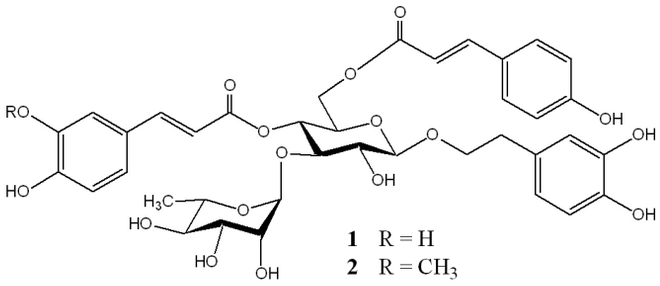
Trichilia catigua A. Juss. (Meliaceae) is popularly known as "catuaba" and widely distributed in Brazil. Its bark is mainly used as stimulant, and has a high concentration of tannins. An ethyl acetate (EAF) and an aqueous (AQF) fraction were obtained from the crude extract (CE) of the "catuaba" bark. The aim of this study was to evaluate the chemical composition and the antioxidant capacity of the CE and the EAF and AQF fractions. The total polyphenol content (TP) and total tannin content (TT), assessed by a pharmacopoeia test [1], were, PT = 10.51 ± 0.17 (RSD%= 1.62) and TT = 6.95 ± 0.11 (RSD%= 1.57). The TT was also evaluated in CE, EAF and AQF, giving values of 38.89 ± 1.14 (RSD%= 2.93); 69.36 ± 0.49 (RSD%= 0.70) and 33.80%± 0.30 (RSD%= 0.89), respectively. EAF was fractionated by column chromatography (Sephadex® LH-20) and 32 subfractions were obtained. The following compounds were isolated and identified: epicatechin (EP), procyanidins B_2 (PB_2), B_4 (PB_4) and C_1 (PC_1), cinchonains Ia (CIa), Ib (CIb), IIb (CIIb) and the new compounds cinchonain IIc (CIIc) and apocynin E (ApE) [2]. The in vitro antioxidant capacity of the CE, EAF and AQF and isolated compounds was evaluated using the $DPPH^{\cdot}$ scavenging method and Fe^{3+}-Fe^2 reduction capacity. The values obtained by the $DPPH^{\cdot}$ method for the extracts were IC_{50} ($\mu g.ml^{-1}$): CE = 5.44 ± 0.18 (RSD%= 3.31); EAF = 3.85 ± 0.09 (RSD%= 2.35) and AQF = 8.76 ± 0.15 (RSD%= 1.68); for the isolated compounds the values were IC_{50} (μM): EP = 10.12 ± 0.24 (RSD%= 2.43); PB_2= 7.95 ± 0.04 (RSD%= 0.51); CIa = 7.87 ± 0.05 (RSD%= 0,63); CIb = 7.67 ± 0.23 (RSD%= 2.98); CIIb = 5.05 ± 0.05 (RSD%= 0.98); CIIc = 5.15 ± 0.08 (RSD%= 1.61) and PC_1= 4.08 ± 0.01 (RSD%= 0.28). Concerning the reduction capacity, the extracts had higher activity than Trolox, but lower than vitamin C. All the isolated compounds were more effective than vitamin C and Trolox; procyanidin C_1 had the highest reduction capacity. *Acknowledgements:* CNPq and CAPES, Brazil. **References:** [1] Farmacopéia Brasileira. 4th. ed., 2002. p.176. 2. Resende, F.O. (2007) M.Sc. Thesis, Maringá, Brazil. 167 p.

P 389

New phenylethanoid glycosides from *Globularia alypum*

Kırmızıbekmez H[1], Piacente S[2], Bassarello C[2], Çalış I[3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, TR-34755, Kayışdağı, İstanbul, Turkey; [2]Department of Pharmaceutical Sciences, University of Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy, [3]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100, Ankara, Turkey

Globularia alypum L. (formerly Globulariaceae, now "new" Plantaginaceae) is a shrub distributed to the Mediterranean area [1]. It is widely utilized in indigenous systems of medicine in some Mediterranean countries, especially in Morocco as a hypoglycaemic agent, laxative, cholagogue, and stomachic [2]. Recent researches on this plant have resulted in the isolation of various types of iridoid glucosides, phenylethanoid glycosides and flavonoids [3, 4]. As a part of our continuing phytochemical investigation of members of the genus *Globularia* growing in the flora of Turkey, we have investigated the chemical constituents of *G. alypum*. Our current investigation on the MeOH extract of the leaves led to the isolation and identification of two new phenylethanoid glycosides (**1** and **2**), in addition to two known phenylethanoid glycosides, calceolarioside A and verbascoside. Eight iridoid glucosides (lytanthosalin, catalpol, globularicisin, globularin, globularidin, globularinin, globularimin, and alpinoside), a flavon glycoside (6-hydroxyluteolin 7-*O*-sophoroside), a lignan glycoside (syringaresinol 4′-*O*-β-D-glucopyranoside), and a phenylpropanoid glycoside (syringin) were also obtained and characterized. The structures of the isolates were elucidated on the basis of 1D and 2D NMR experiments as well as HR-MS. Compounds **1** and **2** are rare examples of phenylethanoid glycosides bearing two aromatic acyl units.



Acknowledgements: This work was supported by the Hacettepe University Scientific Research Unit (Project No: 02 G 076) **References:** [1] Edmondson, J.R. (1982) Globularia L., in: Flora of Turkey and the East Aegean Islands. Vol. 7 (Ed. Davis P.H.), University Press, Edinburgh. [2] Bellakhdar, J. et al. (1991) J. Ethnopharmacol, 35: 123 – 143. [3] Es-Safi, N-E. et al. (2005) J. Nat. Prod. 68: 1293 – 1296. [4] Es-Safi, N-E. et al. (2006) Chem. Pharm. Bull: 54: 85 – 88.

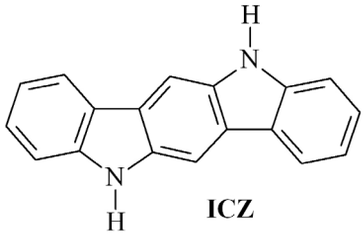
P 390

Identification of the AhR ligand indolo[3,2-b]carbazol as a metabolite of *Malassezia furfur* strains isolated from diseased skin

Stathopoulou K[1], Magiatis P[1], Gaitanis G[2,3], Alexopoulos E[4], Velegraki A[2], Bassukas I[3], Skaltsounis AL[1]
[1]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens 15771, Greece; [2]Mycology Laboratory, Department of Microbiology, Medical School, University of Athens, Athens, Greece;[3]Department of Skin and Venereal Diseases, University Hospital of Ioannina, University of Ioannina, Medical School, Ioannina, Greece; Department of Public health, Medical School, University of Patras, Greece

Pityriasis versicolor (PV), dandruff (DF) and seborrheic dermatitis (SD) are common skin diseases with significant economic burden, that are unequivocally associated with *Malassezia* yeasts. The aim of this study was to compare *M. furfur* isolates from healthy and diseased skin (PV, SD, DF) for the production of indole derivatives, analyze the HPLC pattern and subsequently isolate and characterize substances that are preferentially produced by *M. furfur* strains isolated from diseased skin. Investigation of the ethyl acetate extracts of *M. furfur* strains grown on L-tryptophan agar, using preparative HPLC, led to the isolation and structure elucidation by NMR and MS of several known indole derivatives [1], [2] such as malassezin, pityriacitrin, malassezindol A, indol-3-carboxaldehyde, and indolo[3,2-*b*]carbazol (ICZ). ICZ, which is a known highly potent ligand of Aryl hydrocarbon receptor (AhR), is described for the first time as a metabolite of *M.furfur*. All the isolated compounds were used as standards for the development of an analytic methodology using HPLC-DAD. HPLC analysis of the extracts from 15 reference and clinical strains (7 healthy, 8 diseased) revealed for the first time the preferential *in vitro* production of malassezin and ICZ only by *M. furfur* strains isolated from diseased skin lesions and not from healthy ones. The selective production of compounds which are active AhR ligands offers indirect, statistical significant evidence

for a candidate mechanism for the development of PV, SD and DF and the variability of pathogenicity among *M. furfur* strains.



ICZ

References: [1] Wille G (2002) Bioorg. Med. Chem 9: 955 – 960 [2] Krämer HJ (2005) Chem. Bio. Chem. 6: 860 – 865.

P 391

The effect of the extraction and processing methods on the alkaloid composition of roots of *Aconitum carmichaeli*

Csupor D[1,3], Strömberg M[2], Bohlin L[2], Woelkart K[3], Wenzig EM[3], Hohmann J[1], Bauer R[3]
[1]Department of Pharmacognosy, University of Szeged, Szeged, Eötvös u. 6., 6720, Hungary; [2]Department of Medicinal Chemistry, Division of Pharmacognosy, Uppsala University, Uppsala, BMC Box 574, S – 751 23, Sweden; [3]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens University Graz, Graz, Universitätsplatz 4.,8010 Austria

Preparations of certain *Aconitum* species native to Asia are indispensable materials in Eastern medicine. The tubers and roots of *Aconitum* species are applied after cautious processing (usually boiling) in order to reduce their toxicity. The processed drugs are typically used as painkillers and antirheumatic agents. The unprocessed roots are too toxic for internal use, but are used for external application as anaesthetics. Aconitine, the main alkaloid of several species, including *Aconitum carmichaeli,* is one of the most toxic natural products. However, poisoning with *Aconitum* preparations of Oriental medicine are not common. Since the high toxicity is characteristic of esterified alkaloids, it is assumed that during processing the reduction of the total alkaloid amount and/or hydrolysis of the ester groups decrease the toxicity [1]. As part of our ongoing work on the analytical characterization of Chinese herbal medicines, we analyzed the diterpene alkaloids of the roots of *Aconitum carmichaeli* by means of TLC, HPLC, LC-MS methods. Since the standardization of *A. carmichaeli* roots is usually based on the quantitative determination of aconitine, a reproducible and efficient extraction method is essential for the reliable quality control of the drug. The influence of different extraction methods (extraction in neutral, acidic or alkaline medium, solvent partitioning at different pH) on the diterpene alkaloid content of *Aconitum carmichaeli* root extracts was analyzed. To assess the efficiency of the methods, the amount of aconitine was determined by HPLC. Extraction in neutral medium seems to be the most appropriate method, since changing the pH resulted in the formation of artifacts. We also examined the effect of processing on qualitative and quantitative change of the alkaloid content. The alkaloid composition of the crude and the processed drug was analyzed with LC-MS. *Acknowledgements:* The financial support of Aktion Österreich-Ungarn, ARGE Alpe-Adria and Hungarian State Eötvös Fellowship is gratefully acknowledged. **References:** [1] Bisset, N. G. (1981) J Ethnopharmacol 4: 247 – 336

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C_{18} diterpene alkaloids from *Aconitum toxicum* Rchb.

Csupor D[1], Forgo P[2], Csedő K[3], Hohmann J[1]
[1]Department of Pharmacognosy, University of Szeged, Szeged, Eötvös u. 6., 6720, Hungary; [2]Department of Organic Chemistry, University of Szeged, Dóm tér 8., 6720, Hungary; [3]Department of Pharmacognosy, University of Medicine and Pharmacy, Targu Mures, Gh. Marinescu 38., 4300, Romania

For centuries, *Aconitum* species have been used by various civilizations as sources of both poisons and medicines, due to their marked physiological effects. Because of their noteworthy activity on the cardiovascular system and the central nervous system, the diterpene alkaloids of *Aconitum* species are targets of structure-activity experiments, and regarded as lead compounds for the design and synthesis of new drug molecules. The increasing pharmacological significance of the compounds motivated us to investigate the alkaloids of the phytochemically poorly examined *Aconitum* species native to the Carpathian Basin [1,2,3]. As a continuation of this comprehensive work, we carried out the phytochemical analysis of *Aconitum toxicum*. To minimize the risk of acidic or alkaline hydrolysis, an isolation procedure in neutral medium was proposed to obtain the alkaloids from the roots of *A. toxicum*. Initially, CC using a polyamide stationary phase was applied to remove polyphenolic compounds from the crude extract. After extensive chromatographic purification (including CC, VLC, GFC, PLC and CPC) with the use of SiO_2, Al_2O_3 and Sephadex LH-20 and different solvent systems, five compounds were isolated. The structures of the compounds were determined by means of mass spectrometry and 1D and 2D NMR experiments (^{1}H NMR, JMOD, ^{1}H,^{1}H-COSY, HSQC, HMBC and NOESY). From the plant one C_{19} (neolinine) and four C_{18} diterpene alkaloids (delavaconitine, dolaconine, aconosine, acotoxicine) were identified, which represent a series of biogenetically related alkaloids. Dolaconine and aconosine contain only 4 oxygen functions, which is very rare among C_{18} diterpene alkaloids. All the compounds are reported from this species for the first time, and acotoxicine is a new natural product. *Acknowledgements:* The financial support of the Gedeon Richter Centenary Foundation is gratefully acknowledged. **References:** [1] D. Csupor, P. Forgo, I. Máthé, J. Hohmann (2004) Helv Chim Acta 87: 2125 – 30. [2] D. Csupor, P. Forgo, I. Zupkó, P. Szabó, J. Hohmann (2007) Z Naturforsch B 62: 135 – 41. [3] D. Csupor, P. Forgo, K. Csedő, J. Hohmann (2006) Helv Chim Acta 89: 2981 – 6.

P 393

Obtaining the bioactive compounds from *Geranium robertianum* and *Viscum album L.* in a concentrate form by ultrafiltration

Paun G[1], Neagu E[1], Moroeanu V[1], Gatea F[1], Radu GL[2]
[1]National Institute for Research-Development of Biological Sciences, 296 Spl.Independentei, PO Box 17 – 16, 060031, Bucharest 6, Romania; [2]Faculty of Applied Chemistry and Materials Science, Politehnica University of Bucharest, 313 Spl.Independentei, 060042, Bucharest, Romania

This work aims at obtaining purified and concentrated bioactive compounds from aqueous and hydroalcoholic extracts of *Geranium robertianum* and *Viscum album* by using membrane processes which work at ambient temperature. The extract processing was effected by micro- and ultrafiltration (MW = 20kDa) and the bioactive compounds characterization has been performed by UV-VIS spectroscopy and HPLC. The following polyphenolic compunds were identified in *G. robertianum* extracts by the HPLC method: caffeic acid, chlorogenic acid, rutin, cumaric acid and ferulic acid, while for the *V. album* extracts proteins (such as lectine) have been followed up and analyzed [1,2]. The recovery degrees of the total polyphenols were over 71% for *G. robertianum* aqueous concentrated extract and over 75% for the hydroalcoholic extract. The levels of the protein recovery were over 90% for *V. album* aqueous extracts and over 94% for hydroalcoholic extracts. All experiments were carried out with the same batches of extract concentrates. **References:** [1] Leucuta S.,

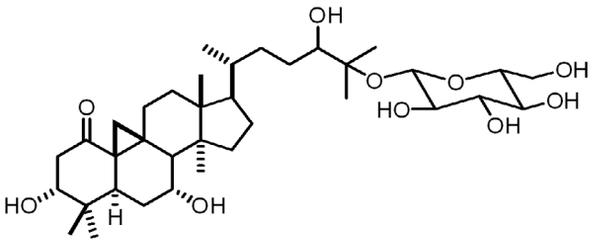
Vlase L., Gocan S., Radu L., Fodorea C. (2005) J. Liq. Chrom. Relat. Tech. 28: 3109 – 3117. [2] Paun-Roman G., Batrinescu Gh., Garganciuc D, Popescu G. (2006) The membrane processes used for the biological active compounds separation and concentration from the liquids media, Eds. Cartea Universitara, Bucharest

P 394

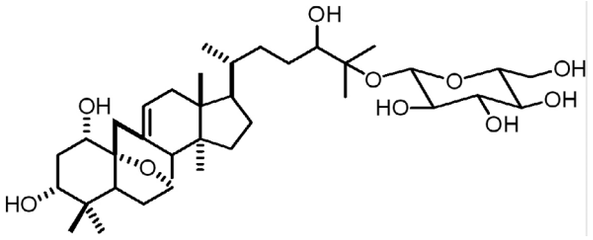
Phytochemistry of *Sutherlandia microphylla*, an important South African medicinal plant

Olivier DK[1], Albrecht CA[2], van Wyk BE[2], Van Heerden FR[3]
[1]Department of Chemical Technology, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, Johannesburg, South Africa; [2]Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 526, Auckland Park 2006, South Africa; [3]School of Chemistry, University of KwaZulu-Natal, Private Bag X101, Scottsville 3209, South Africa

Sutherlandia microphylla R. Br. ex W.T. Aiton (Fabaceae, tribe Galegeae), commonly known as 'cancer bush', is an important medicinal plant in South Africa, yet little is known on the phytochemistry of this plant. It has been reported that extracts of *S. microphylla* have antioxidant [1,2], hypoglycemic [3], analgesic [3] and anti-inflammatory [3] effects. Extracts also inhibit phorbol ester-induced COX-2 expression in mouse skin [4,5], have antiproliferate effect on several human cell lines [2], can induce apoptosis [6] and decrease the corticosterone response to chronic stress. Inhibition studies on HIV reverse transcriptase indicated that although organic extracts of the leaves did not show any activity, inhibition was observed by an aqueous extract. In this contribution, the structural elucidation and the biological significance of the major novel triterpenoids (SU1 and SU2) and flavonoids of *S. microphylla* will be discussed.



SU1



SU2

Acknowledgements: National Research Foundation (South Africa) **References:** [1] Fernandes, A. C. et al. (2004) J. Ethnopharmacol. 95: 1 – 5. [2] Tai, J. et al. (2004) J. Ethnopharmacol. 93: 9 – 19. [3] Ojewole, J.A.O. (2004) Meth. Findings Exp. Clin. Pharm. 26: 409 – 416. [4] Na, H.-K. et al. (2004) Biofactors, 21:149 – 153. [5] Kundu, J.K. et al. (2005) Canc. Lett., 218: 21 – 31. [6] Chinkwo, K. A. (2005) J. Ethnopharm., 98: 163 – 170.

P 395

Anti-HIV activity of *Euphorbia hirta* L.

Vasas A[1], Gyuris Á[2], Minárovics J[2], Hohmann J[1]
[1]Department of Pharmacognosy, University of Szeged, Szeged, Eötvös u. 6, 6720, Hungary; [2]Johan Béla National Center for Epidemiology, Microbiological Research Group, Budapest, Pihenő út 1, 1529, Hungary

Many research programs aimed to search anti-HIV agents for developing countries from local natural products, especially of botanical origin, which can play a role in the management of HIV-1 infection and AIDS. Recent antiviral screenings have demonstrated that some Euphorbiaceae species are effective against virus infections.[1,2,3] With the aim to find new antiviral compounds from the family, *Euphorbia hirta* (syn. *E. pilulifera*) was studied. This plant is used in the traditional medicine in many parts of Africa and Asia for the treatment of gastrointestinal disorders (diarrhoea, dysentery) bronchial and respiratory diseases (asthma, bronchitis) and ocular diseases (conjunctivitis). The present paper reports the antiretroviral activity of the extracts of *E. hirta*, which were investigated *in vitro* on MT4 human T lymphocyte cell line. The cytotoxicity of the extracts in uninfected cells was determined by MTT cell proliferation assay. First the direct effect of the aqueous extract on SIV_{mac251}, HIV-1 and HIV-2 reverse transcriptase was determined. A dose-dependent inhibition of viral replication was observed in all three assays. Thereafter the HIV-1 inhibitory activity of aqueous (A) and 50% methanolic extracts (B) were compared, and found that the alcoholic extract showed higher antiretroviral effect (ID_{50}= A: 9 μg/ml, B: 5 μg/ml). The 50% methanolic extract was then subjected to liquid-liquid partition with cyclohexane, dichloromethane and ethyl-acetate. Only the aqueous phase, remaining after extraction with organic solvents, exhibited significant antiviral activity, the lipophilic extracts revealed to be inactive. After removal of the tannins from the aqueous extract, the viral replication inhibitory effect was remarkably decreased (ID_{50}= 81 μg/ml), therefore it was concluded that most probably tannins are responsible for the high antiretroviral effect. **References:** [1] Ahn, MJ. et al. (2002) Planta Med. 68: 457 – 459. [2] Mucsi, I. et al. (2001) Planta Med. 67: 672 – 674. [3] Lam, WY. et al. (2006) J. Cell Biochem. 97: 795 – 812.

P 396

Biological activity and novel cytotoxic curcuminoid from *Pleuranthodium racemigerum* – an Australian Zingiberaceae

Wohlmuth H[1], Deseo MA[2], Brushett DJ[2], MacFarlane G[3], Waterman PG[2], Stevenson LM[2], Leach DN[2]
[1]Department of Natural and Complementary Medicine, Southern Cross University, PO Box 157, Lismore NSW 2480, Australia; [2]Centre for Phytochemistry and Pharmacology, Southern Cross University, PO Box 157, Lismore NSW 2480, Australia; [3]School of Molecular and Microbial Sciences, University of Queensland, Brisbane QLD 4072, Australia

The Zingiberaceae species *Pleuranthodium racemigerum* (F. Muell.) R. M. Sm. (syn. *Alpinia racemigera* F. Muell.) is native to the tropical parts of north-eastern Australia. As part of a larger screening program of Zingiberaceae species, an ethanolic extract of the dried rhizome was tested for anti-oxidant activity in the Oxygen Radical Absorption Capacity (ORAC) assay and for inhibition of PGE_2 production by calcium ionophore-stimulated 3T3 mouse embryo fibroblast cells in an enzyme immuno-assay. The extract displayed dose-dependent anti-oxidant activity in the range 1 – 10 μg/mL. At 10 μg/mL, the activity was equivalent to Trolox 10 μM. The same extract dose-dependently inhibited the production of PGE_2 by stimulated 3T3 cells in the range 10 – 100 μg/mL. At 100 μg/mL, the inhibition was equivalent to that of Aspirin (1 mM). The extract was subject to activity-guided fractionation by preparative HPLC resulting in an isolated compound. Structural elucidation was performed by NMR using a Bruker AVANCE DRX500 (^1H at 500 MHz; ^{13}C at 125 MHz; 5 mm QNI probe) spectrometer with XWin-NMR software. ^1H NMR, J-modulated ^{13}C NMR, COSY, HSQC and HMBC experiments were conducted. Accurate mass was determined by high-resolution mass

spectroscopy. The isolated compound had a molecular weight of 296.40 and a λ_{max} of 203 nm. NMR experiments revealed a novel curcuminoid compound, 1-(4"-methoxyphenyl)-7-(4'-hydroxyphenyl)-2-heptene, with the molecular formula $C_{20}H_{24}O_2$. Using the PerkinElmer ATPlite kit, the compound was found to be cytotoxic against 3T3 cells with EC_{50}= 16 µg/mL after 3 hours.

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Modulation of multidrug resistance of cancer cells by sesquiterpene esters from *Euonymus* species

Hohmann J[1], Engi H[2], Molnár J[2]
[1]Department of Pharmacognosy, University of Szeged, Szeged, Eötvös u. 6, 6720, Hungary; [2]Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Dóm tér 10, 6720, Hungary

Multidrug resistance (MDR) is one of the main reasons of failure in malignant tumor chemotherapy. This resistance is associated with a reduced intracellular drug accumulation due to increased cellular drug efflux. A primary mechanism of efflux-pump activity is the overexpression of the energy dependent P-glycoprotein, which is encoded by mdr1 gene. In the past few decades extensive studies have been performed with the aim of developing effective resistance modulators from natural sources. Previous investigations revealed that sesquiterpenes isolated from Celastraceae are promising modulators of MDR in several human cancer cell lines and *Leishmania* strains [1,2]. The aim of our study was to find further efficient MDR modifiers among Celastraceae sesquiterpenes in order to contribute to structure-activity relationship studies. In our experiments 20 dihydro-β-agarofuran polyesters were investigated, which were isolated from *Euonymus verrucosus* Scop., *E. japonicus* Thunb., *E. sachalinensis* (F. Schmidt) Maxim., *E. nanus* M. B. and *E. kiautschovicus* Loes. The mdr-reversal effect was evaluated on mouse T-lymphoma cell line transfected with human mdr1 gene using rhodamine exclusion test. Verapamil was used as positive control (fluorescence activity ratio R = 3.5 in 4 µg/ml concentration), and the fluorescence of cell population was measured by flow cytometry. Almost all of the tested compounds were able to increase the drug accumulation and display a significant concentration-dependent effect. Especially, penta- and hexaesters of 1β,2β,6α,9α,15-pentahydroxydihydro-β-agarofuran and 1β,2β,6α,8β,9β,15-hexahydroxydihydro-β-agarofuran exhibited high activity in reversing MDR (fluorescence activity ratios R = 29.4 – 96.8 in 4 µg/ml). These results provided further information for characterisation the structural requirements of dihydro-β-agarofuran sesquiterpenes as resistance modifier. **References:** [1] Cortes-Selva, F. et al. (2005) Curr. Pharm. Des. 11: 3125 – 3139. [2] Munoz-Martinez, F. et al. (2004) Cancer Res. 64: 7130 – 7138.

P 398

Comparative analyses on fatty acid composition of bioactive organic extracts from different anatomical organs of five Turkish *Rhododendron* species

Tasdemir D[1], Cartagena M[2], Carballeira NM[2]
[1]Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK; [2]Department of Chemistry, University of Puerto Rico, San Juan 00931 – 3346, Puerto Rico

Rhododendron species (Ericaceae) are common garden plants with glossy, evergreen leaves and large, showy flower displays. The genus *Rhododendron* is represented by six species in the flora of Turkey, one of which (*R. smirnovii*) is endemic to the country. We have previously reported a wide range of biological activities (antimicrobial, antioxidant, acetylcholinesterase inhibitory and antiprotozoal) from the organic extracts of Turkish *Rhododendron* plants without any significant toxicity on mammalian cells [1,2]. In this study, we analyzed the fatty acid (FA) composition of the non-polar extracts (hexane) obtained from different anatomical organs of five Turkish *Rhododendron* species (*R. luteum, R. ponticum, R. smirnovii, R. sochadzeae and R. ungernii).* A total of 33 different FAs, ranging from C_{12} to C_{30}, were detected and quantified by GC-MS after methylation. Palmitic acid (16:0) was the most abundant FA detected in most extracts investigated, particularly in *R. sochadzeae* fruits (73%). With the exception of two extracts (*R. sochadzeae* leaves and *R. ponticum* flowers), all leaf and flower extracts contained significant portions (> 40%) of C 18 unsaturated FAs (18:1, 18:2, 18:3). There were notable differences in the FA profile between species and anatomical organs. Another interesting point observed was the presence of very long chain FAs (>C20) in almost all extracts in significant amounts. Particularly, *R. sochadzeae* leaves (24%), and the flowers of *R. ponticum* (22.3%) and *R. ungernii* (16%) had high portions of these less common FAs. This is the first study describing the detailed FA composition of *Rhododendron* sp. Fatty acids might partly be responsible or involved in the reported biological potentials of these extracts. **References:** [1] Tasdemir, D. et al. (2004) Pharm. Biol. 42: 374 – 383. [2] Tasdemir, D. et al. (2005) Phytotherapy Res. 19: 162 – 166.

P 399

Composition of essential oil of the fruits and roots of *Prangos denticulata* Fisch. & Mey. (Umbelliferae) growing in Turkey

Kılıç CS[1], Coşkun M[1], Duman H[2], Demirci B[3], Baser KHC[3]
[1]Ankara University Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Ankara, Turkey; [2]Gazi University Faculty of Science and Letters, Department of Biology, 06500, Ankara, Turkey; [3]Anadolu University Faculty of Pharmacy Department of Pharmacognosy, 26470, Eskişehir, Turkey

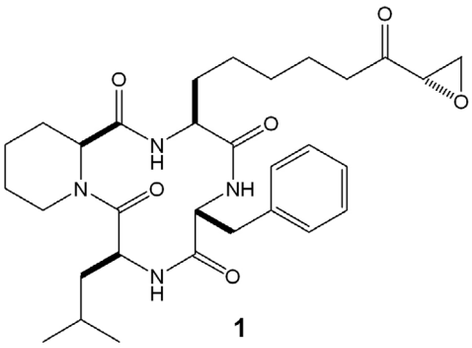
The genus *Prangos,* belonging to the family Umbelliferae (Apiaceae) has 13 species and altogether 14 taxa growing in Turkey; 7 of which are endemic [1 – 3]. Turkish local names for these species are "Çakşır" or "Çağşır" just like species of the genera *Ferula* and *Ferulago* [4] and though some *Prangos* species are reported to have antibacterial and antioxidant effects [5 – 7], they are mostly known as aphrodisiacs [4]. Essential oil compositions of *Prangos* species are intensively studied, however, this is the first report on the essential oils of the fruits and roots of *P. denticulata*. Water-distilled essential oils were analysed by GC-MS and the fruit oil yielded 121 components representing 95.2%; the root oil yielded 70 components representing 88.1% of the oil, respectively. The major constituents of the oils were determined as sabinene (26.1%) and p-cymene (19.7%) for the fruit and δ-3-carene (49.3%) and Z-3,5-nonadiyne-7-ene (20.4%) for the root. It is interesting for this species to contain nonadiyne derivatives since 3,5-nonadiyne, isolated from rhizome of *Cachrys ferulacea* (L.) Calestani was reported to inhibit nitric oxide release by rat peritoneal macrophages [8]. Fruit anatomy of the species was also studied and it was observed that aerenchyma tissue was present as the characteristic feature of the genus *Prangos*. **References:** [1] Davis, P. H. (1972) Flora of Turkey and the East Aegean Islands Volume 4, Edinburgh University Press, Edinburgh, UK. [2] Davis, P. H. (1988) Flora of Turkey and the East Aegean Islands Volume 10, Edinburgh University Press, Edinburgh, UK. [3] Güner, A. (2000) Flora of Turkey and the East Aegean Islands Volume 11, Edinburgh University Press, Edinburgh, UK. [4] Baytop, T. (1999) Therapy with Medicinal Plants in Turkey – Past and Present, 2nd Edn. Nobel Tıp Basımevi, İstanbul, Turkey. [5] Durmaz, H. et al. (2006) Afr. J. Biotechnol. 5: 1795 – 1798. [6] Sagun, E. et al. (2006) Int. J. Food Prop. 9: 255 – 260. [7] Mavi, A. et al. (2004) Biol. Pharm. Bull. 27: 702 – 705. [8] Dokovic, D.D. (2004) Chem. Pharm. Bull. 52: 853 – 854.

P 400

New cytotoxic α-pyrone derivatives from the sponge-derived fungus *Petriella* sp.

Ebel R[1], *Riebe F*[2], *Proksch P*[2], *Schulz B*[3], *Müller WEG*[4]
[1]*University of Aberdeen, Department of Chemistry, Meston Walk, Aberdeen AB24 3UE, U. K.,* [2]*Heinrich-Heine-Universität Düsseldorf, Institut für Pharmazeutische Biologie und Biotechnologie, Universitätsstr. 1, 40225 Düsseldorf, Germany,* [3]*Technische Universität Braunschweig, Institut für Mikrobiologie, Spielmannstr. 7, 38106 Braunschweig, Germany,* [4]*Johannes-Gutenberg-Universität Mainz, Institut für Physiologische Chemie und Pathobiochemie, Duesbergweg 6, 55128 Mainz, Germany*

During a systematic evaluation of fungal strains obtained from the Mediterranean sponge *Suberites domuncula* collected at different locations in the Adriatic Sea near Rovinj, Croatia, an isolate of a previously undescribed *Petriella* sp. caught our attention due to the striking cytotoxic activity of its crude extract. Chemical analysis revealed the presence of the known cyclic tetrapeptide WF-3161 [1] (**1**) and three new α-pyrone derivatives (**2**, **3** and **4**) related to infectopyrone which had previously been described from *Alternaria infectoria* [2]. When tested for their cytotoxic properties, compound **2** exhibited pronounced activity against the L5178Y cell line, while congeners **3** and **4** only showed moderate activity. WF-3161 (**1**) displayed exceptionally strong activity with an ED_{50} value < 0.1 μg/ml.



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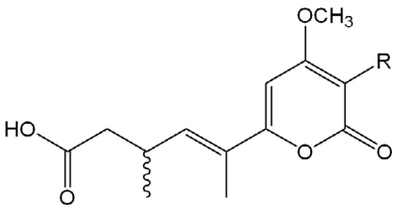
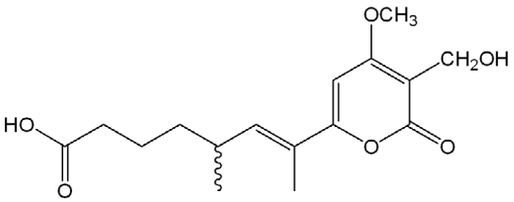


2 (R = CH_3)
3 (R = CH_2OH)



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References: [1] Umehara, K. et al. (1983) J. Antibiotics 36: 478 – 483. [2] Ostenfeld Larsen, T. et al. (2003) Tetrahedron Lett. 44: 4511 – 4513.

P 401

Antimicrobial, Antinociceptive and Anti-inflammatory Activity Studies on *Pseudevernia furfuracea*

Güvenç A[1], *Küpeli E*[2], *Yıldız S*[3], *Çalış I*[4]
[1]*Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, TR-06100 Ankara, Turkey;* [2]*Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, TR-06330 Ankara, Turkey;*[3]*Ankara University, Faculty of Pharmacy, Department of Microbiology, TR-06100 Ankara, Turkey;* [4]*Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, TR-06100 Ankara, Turkey*

Lichens are a large group of organisms which are represented by approximately 18 000 species worldwide. Lichens form an interesting group of lower plants featuring algae and fungi symbiotically combined. Aliphatic, cycloaliphatic, aromatic and terpenic compounds were reported as their secondary metabolites. Lichens and their metabolits have some biological activities such as antimicrobial, antitumor, antioxidant, anti-inflammatory, analgesic and antipyretic, enzyme inhibiting and cytotoxic. *Pseudevernia furfuracea* (L.) Zopf (Parmeliaceae) is a common epiphytic lichen in the coniferhardwood forest of Anatolia. This lichen is used in traditional medicine as a treatment for wound, eczema and hemorrhoids in Turkey. In the present study, methanolic extract from thallus of *P. furfuracea* and its fractions were investigated for its *in vitro* antimicrobial, and *in vivo* anti-inflammatory and antinociceptive activities. Significant antimicrobial activities were observed against Gram (+) microorganisms and *Candida krusei*. For the anti-inflammatory activity assessment, carrageenan-induced hind paw edema, 12-*O*-tetradecanoyl-13-acetate (TPA)-induced mouse ear edema and for the antinociceptive activity, *p*-benzoquinone-induced abdominal constriction tests were used. Methanolic extract of the plant was found to possess significant inhibitory activity on the carrageenan-induced hind paw edema model in mice whereas the other fractions did not show any activity. While CH_2Cl_2 and EtOAc fractions showed notable anti-inflammatory activity on carrageenan-induced hind paw edema model without inducing any apparent acute toxicity or gastric damage, these fractions did not show against 12-*O*-tetradecanoyl-13-acetate (TPA)-induced mouse ear edema model and antinociceptive activity. 2,4-dihydroxy-3,6-dimethyl-benzoic acid methyl ester (= atraric acid) [**1**], methyl hematommate [**2**], and methyl chlorohematommate [**3**] were isolated from the active fractions as the main compounds. The structures of **1 – 3** were elucidated by spectroscopical (NMR, MS) means.

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Composition of the volatiles from flowers of *Bystropogon maderensis* Webb by SPME

Castilho PC[1]
[1]*Centro de Química da Madeira, Departamento de Química, Universidade da Madeira, Campus da Penteada, piso 0, 9000 – 390 Funchal, Portugal*

The genus *Bystropogon* (Lamiaceae) is endemic to the Macaronesian Islands and represents the best-known example of a putative phytogeographic connection between these islands and the New World. Canary Islands have 5 endemic subspecies and in Madeira Archipelago there are two: *Bystropogon maderensis* and *Bystropogon punctatus*. The two can be differentiated by the shape of the leaves and the intensity of their fragrance. Both species are used in traditional medicine in Madeira Island, in the form of infusion of fresh leaves, as sedative and somnofacient. The composition of the essential oils of the leaves of *B. maderensis*, collected at several locations in Madeira island, was studied by GLC and GLC/MS and the results are in good consistency to the only previous study on this species, with pulegone being the main compound, > 90%. In the present study we analyzed the composition of the volatiles emitted by the flowers of *B. maderensis*, that folk medicine parishioners are so careful to remove before making an infusion and for a good reason: while leaves have a very pleasant minty fragrance the flowers are foul smelling. Hydrodistillation of flowers was not possible since it resulted in a

viscous brownish liquid forming in the Clevenger apparatus. A dynamic headspace solid-phase microextraction (HS-SPME) and gas chromatography coupled to ion trap mass spectrometry (GC-ITMS) method was thus employed. Flowers were crushed and placed in a vial containing warm water and the fibre was exposed to the volatiles. Best results were obtained using a 75 μm CAR/PDMS fibre during headspace extraction at 40 °C, stirring at 750 rpm for 15 min. The main components found were, in descending amounts, benzyl benzoate, methyl acetate, caryophyllene, methyl salicylate and α-coapene. *Acknowledgements:* CQM is funded by Fundação para a Ciência e Tecnologia (FCT) and FEDER. This work had financial support from programme INTERREG, project BIOPOLIS **References:** [1] Trusty JL, Olmstead RG et al. (2004) Systematic Botany, 29: 702 – 715. [2] Economou D, Nahrstedt A. (1991) Planta Med. 57: 347 – 351

P 403

Martynoside, forsythoside B, ladanein and 7-α-acetoxy-royleanone from *Ballota nigra* L

Tóth E[1,2], Tóth G[3], Máthé I[1,2], Blunden G[4]
[1]Institute of Ecology and Botany of The Hungarian Academy of Sciences, Alkotmány u. 2, 2163 Vácrátót, Hungary;[2]Department of Pharmacognosy, University of Szeged, P.O. Box 121, H-6720 Szeged, Hungary; [3]Department of General and Analytical Chemistry, Budapest University of Technology and Economics H-1521 Budapest, Hungary; [4]School of Pharmacy and Biomedical Sciences, 'University of Technology', St Michael's Building, White Swan Road, Portsmouth, Hampshire PO1 2DT, UK

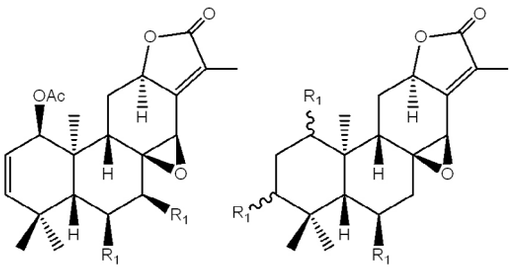
We have isolated β-sitosterol; two phenylethanoids, martynoside and forsythoside B; a flavonoid, ladanein; and a diterpene, 7-α-acetoxy-royleanone from *Ballota nigra*. The dried aerial parts of *Ballota nigra* (500 g) were extracted three times with MeOH (3 l) at room temperature. The extracts were evaporated to dryness and extracted successively with diethyl ether, $CHCl_3$, EtOAc, and *n*-BuOH. Fractions containing ladanein (6-hydroxy apigenin 7,4'-dimethyl ether), 7-α-acetoxy-royleanone and β-sitosterol were chromatographed on Merck Kieselgel 60 F_{254} layers using $CHCl_3$-MeOH (19:1) as the development solvent and 50% methanol-sulphuric acid as the locating reagent. The phenylethanoid glycosides, martynoside and forsythoside B were obtained from the EtOAc fraction of the plant extract. The structures of the isolated compounds were elucidated by NMR spectroscopy. The UV, ^{1}H-, and ^{13}C- NMR spectroscopic data obtained were identical with those previously described for these compounds in the literature (1). Martynoside, ladanein and 7-α-acetoxy-royleanone have not previously been reported from *Ballota nigra*. Phenylethanoids, including forsythoside B (2), have been found in other *Ballota* species, but this is the first record of martynoside for the genus. Labdane and clerodane type diterpenoids have been isolated from *Ballota* species (2,3), but 7-α-acetoxy-royleanone is the first quinnoid-type diterpene to be reported for the genus. *Acknowledgement:* thanks the OTKA grant (T043148) for financial support. T046127. **References:** [1] Citoglu, G., Tanker, M. 1999. Pharm. Biol. 37, 158. [2] Seidel, V., Bailleul, F. 1999. Recent Res. Devel. Phytochem. 3, 27. [3] Ahmad, V.U., Farooq, U. 2004. Chem. Pharm. Bull. (Tokyo) 52/4, 441.

P 404

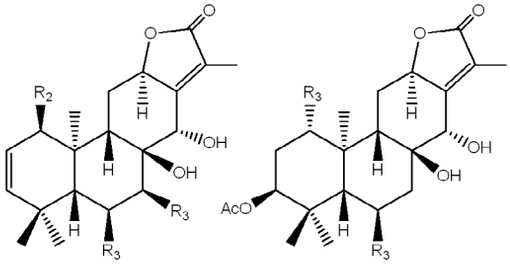
Cytotoxic abietane diterpenes from *Gelonium aequoreum*

Chang FR[1], Lee CL[1], Hsieh PW[1], Chiang MY[2], Wu CC[1], Huang ZY[1], Lan YH[1], Chen M[1], Wu YC[1]
[1]Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan [2]Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 804, Taiwan

Fourteen new *ent*-abietane diterpenes, gelomulides K-X (**1**-**14**), along with three known compounds were isolated from the leaves of *Gelonium aequoreum*. Their structures were elucidated by spectroscopic and chemical methods, including NMR, MS, UV, IR, CD, and Mosher's method. The *in vitro* cytotoxic activity of the isolated compounds was determined. Only compounds **1** and **3** showed moderate cytotoxicity against lung (A549), breast (MDA-MB-231 and MCF7), and liver (HepG2) cancer cell lines [1].



R_1= OAc or OH or H



R_2= OAc or OH or carbonyl
R_3= OAc or OH or H

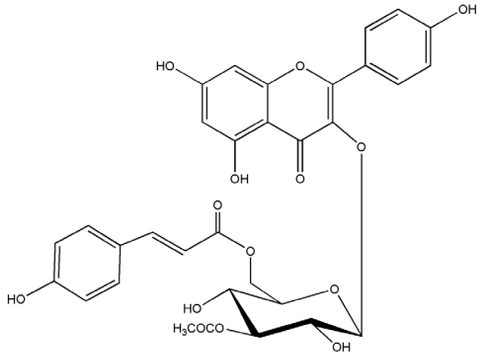
Acknowledgements: 1. The Office of National Science and Technology Program for Biotechnology and Pharmaceuticals, Taiwan. 2. National Science Council, Taiwan **References:** [1] Lee, C.L. et al. (2007) Phytochem. Accepted for publication.

P 405

Chemical constituents from *Scabiosa hymettia* Boiss. & Spruner

Christopoulou C, Graikou K, Chinou IB
Dept. of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens,-Zografou, 15771, Athens, Greece

Various species belonging to the genus *Scabiosa* L. *s. l.* have been used in traditional medicine against tumours [1] and consumed as salad vegetable, as well as appetizers and tonics. *Scabiosa hymettia* is an endemic plant, growing in the central, southern Greece and northern Aegean region [2] and has never been studied phytochemically before. From the methanolic extract of its aerial parts, nine known compounds have been isolated (astragallin, vanillin, vanillic acid, scopoletin, 5-hydroxymethylisochroman-1-one, erythrocentaurin, loganin, loganic acid and swertiamarin) as well as one new natural flavonoid **1** [kaempferol-3-*O*-(6-*E*-*p*-coumaroyl-3-acetylglucoside)]. Their structural elucidation was determined by modern spectral means (1D-, 2D-NMR and MS experiments) and literature data. Loganin and loganic acid characterize Dipsacaceae family and could be of value as chemotaxonomic markers of the family [3], swertiamarin has been reported just once before in the family [4] while all the other constituents are reported for the first time in the genus as well as in the family. The biological activities (antimicrobial, antioxidant and cytotoxic ones) of all isolated compounds are under research.



1

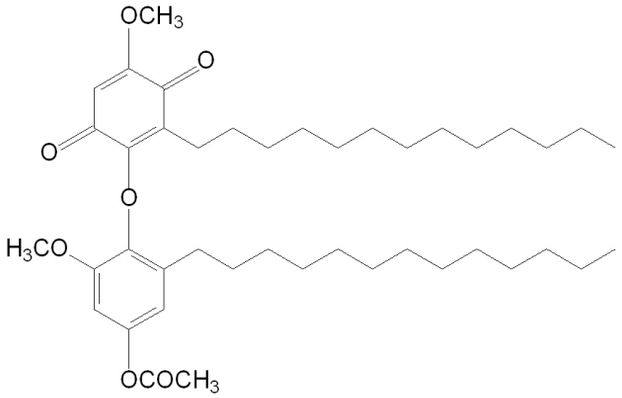
Acknowledgement: The financial support from a PAVET project (70/9075) and Korres Natural Product S.A.. **References:** [1] Hartwell, J.L. 1982 Plants used against cancer; a survey. Massachusetts: Quarterman Publ., Inc. [2] Boratynski A., Zielinski J. 1989 New data to the distribution of Scabiosa hymettia. Arboretum Kornickie 34: 65. [3] Jensen, S.R. et al. 1979 Phytochem. 18: 273. [4] Papalexandrou, A. et al. 2003 Bioch. Syst. Ecol. 3: 91.

P 406

Two novel compounds from *Ardisia punctata* Lindl.

Chun L[1], Brantner AH[2], You-fu S[1]
[1]Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences 1,16#Nanxiao street, Dongzhimen Beijing, 100700, China; [2]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz4/1, A-8010 Graz, Austria

Bioactivity-guided fractionation of the petroleum ether extract of *Ardisia punctata* (Myrsinaceae) root has led to the isolation of one new alkylbenzoquinone derivative and one new alkylphenol derivative, designated as belamcandaquinone F (**1**) and ardisiphenol D (**2**) [1,2], together with 2-methoxy-6-tridecyl-1,4-benzoquinone, glutinol, 24-ethyl-5α-cholesta-7, 22(E)-dien-3-one and tetratriacontanoic acid. The antioxidant activities of the two new compounds have been assessed using DPPH spectrophotometric assays. Ardisiphenol D was the more active antioxidant compound and showed IC_{50} values of 42.7 μM.



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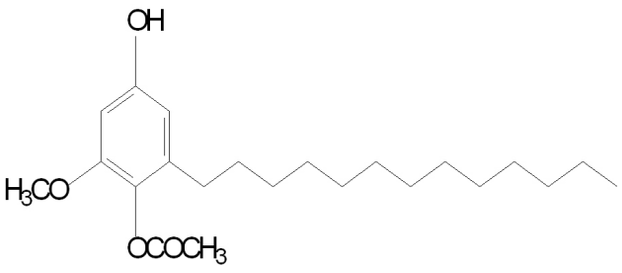

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Acknowledgements: The authors would like to thank Prof. Dai Bin of Institute of Guangxi Folk Medicines for collecting and identifying the plant material. **References:** [1] Li Chun. et al. (2006) Acta Pharmaceutic Sinica 41: 830 [2] Megumi Sumino.et al. (2001) Chem. Pharm. Bull 49: 1664

P 407

Resveratrol oligomers from *Vitis Vinifera* L. stems

Amira-Guebailia H[1], Richard T[2], Rouaiguia S, Waffo Tueguo P[2], Delaunay JC[2], Mérillon JM[2]
[1]Laboratoire de Chimie Appliquée, Université du 08 Mai 1945, Guelma, 24000, Algérie; [2]Groupe d'études des Substances Végétales à Activité Biologique, EA 3675, UFR Sciences Pharmaceutiques, Université Victor Segalen Bordeaux II, 33076, Bordeaux CEDEX, France

Stilbenes an important polyphenolic subclass are widely distributed in the family Vitaceae and Leguminosae, they are reported to decrease the incidence of coronary heart disease [1], reduce platelet aggregation and provide antioxidative and carcinogenic protection [2], moreover, stilbenes are known for their beneficial effect for the plant itself as they are produced in response to fungal infection or abiotic stresses such as heavy metal ions or UV light [3]. In this survey, we report the isolation of *trans* scirpusin A, a resveratrol dimer for the first time, from stems of *Vitis vinifera*, *along* with known stilbenes, trans resveratrol, (+)trans-ε-viniferin, and resveratrol tetramers, hopeaphenol and isohopeaphenol. Centrifugal Partition Chromatography was used for fractionation. Final purification was achieved using semi-preparative HPLC. Identification of the compounds was performed using 1 H NMR, 2D correlations and mass spectrometry. **References:** [1] A. St Leger et al. (1979) Lancet 1: 1017 – 1020. [2] E. Frankel et al. (1993) Lancet 341: 454 – 457. [3] P. Jeandet et al. (1997) Anal. Chem. 69: 5172 – 5177

P 408

Flavonoids from *Sonchus erzincanicus* Matthews

Özgen U[1], Sevindik HG[1], Kazaz C[2], Yiğit D[3], Kandemir A[3], Seçen H[2]
[1]Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, 25240, Turkey; [2]Atatürk University, Faculty of Arts and Science, Department of Chemistry, Erzurum, 25240, Turkey; [3]Erzincan University, Faculty of Education, Erzincan, 24030, Turkey

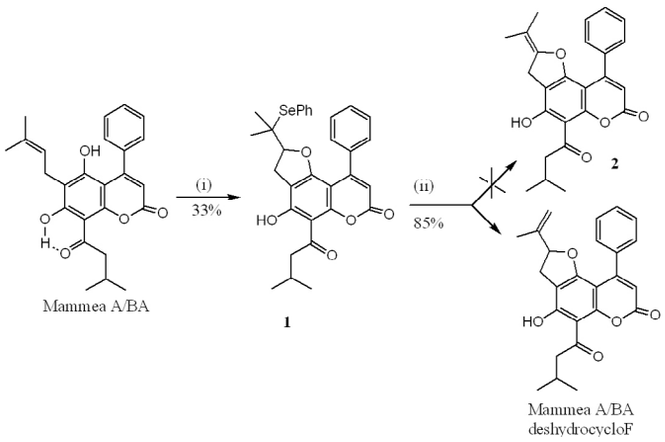
Sonchus erzincanicus (Compositae) is an endemic species for Turkey and six *Sonchus* species grow in Turkey [1]. *Sonchus* species are known as "sütlük, kuzu gevreği, eşek marulu" in Turkey [2]. It has been found that some *Sonchus* species contain sesquiterpene lactone glucosides, flavonoids, triterpenes and steroids [3,4]. No phytochemical study has so far been carried out on *S. erzincanicus*. Phytochemical studies were performed on aerial parts of that plant. The isolation of the compounds was carried out using several and repeated chromatographic techniques from chloroform, ethyl acetate and aqueous phases that were partitioned from the methanol extract obtained from the plant. 3′,4′,5,7 tetrahydroxy 3-methoxy flavone and quercetin 3-*O*-β-D-glucoside were isolated from the ethyl acetate phase; luteolin 7-*O*-β-D-glucoside, luteolin 7-*O*-glucuronide, apigenin 7-*O*-glucuronide were isolated from aqueous phase. The structures of the compounds were elucidated by means of ^{1}H-NMR, ^{13}C-NMR and 2D-NMR (COSY, HMQC, HMBC). **References:** [1] Matthews, V.A. (1975) Sonchus L. Flora of Turkey and the East Aegean Islands, University Pres. Edinburgh, Volume: 5, pp. 690 – 696 (edited by Davis, P. H.). [2] Akartürk, R., (2001) Şifalı Bitkiler Flora ve Sağlığımız, Orman Genel Müdürlüğü Mensupları Yardımlaşma Vakfı (in Turkish). [3] Helal A. M., et al. (2000) Phytochemistry 53, 473 – 477. [4] Devkota, K. P. (2005). Ph. D. Thesis, Research Institute Of Chemistry/University of Karachi

P 409

Induced proliferation arrest of a non-small cell lung carcinoma cell line (NSCLC-N6) by a new 4-phenylcoumarin

Moreau D[1], Jacquot C[1], Chinou I[2], Tomasoni C[1], Jugé M[1], Séraphin D[3], Duval O[3], Guilet D[4], Richomme P[3], Roussakis C[1]
[1]ISOMer (Institut des substances et organismes de la mer), Lab. de Pharmacologie Marine, School of Pharmacy, 44035 Nantes, France; [2]Univ. of Athens, Div. of Pharmacognosy and Chemistry of Natural Products, Panepistimiopolis Zografou, Athens 15771, Greece; [3]USONAS, UFR des Sciences Pharmaceutiques, Bd Daviers, F- 49100 Angers, France; [4]Université Claude Bernard Lyon-1, Lab. de Pharmacognosie, Faculté de Pharmacie, Lyon, France

In spite of our growing insight into carcinogenesis, treatment of tumors, especially non-small cell lung cancer (NSCLC), remains limited and it is urgent to develop strategies that target tumor cells and their genetic features. In this regard, our work was concerned with genetic modifications arising in an ***in vitro*** NSCLC cell line after treatment with the newly synthesized 4-phenylcoumarin Mammea A/BA deshydrocyclo F, regioisomer of Mammea A/AA deshydrocyclo F isolated from the aerial part of the tropical plant ***Calophyllum dispar***. First, it has been shown that Mammea A/BA deshydrocyclo F induces arrest of proliferation by blocking cells in G1 phase of the cell cycle. Then "differential display" strategy has been used to clarify the genetic mechanisms involved in this arrest of proliferation. Therefore, these data provide new insights about mechanisms participating in arrest of proliferation of tumour cells.



P 410

Potent antimicrobial compounds from the Australian medicinal plant *Eremophila duttonii* (F. Muell.) (Myoporaceae)

Jones G, Smith J, Watson K, Tucker D
University of New England, School of Biological, Biomedical and Molecular Sciences, Armidale NSW, 2351, Australia

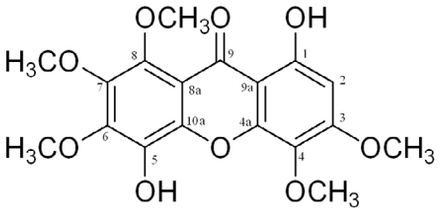
The Australian medical ethnobotanical literature reveals a number of citations concerning the arid adapted genus *Eremophila* describing traditional uses suggestive of antimicrobial activity, e.g. in the topical treatment of minor wounds, ocular and otonasopharangeal complaints [1]. In broad based screening programmes examining antibacterial activity of native indigenous medicinal plants, extracts of the species *Eremophila duttonii* (F. Muell.) (Myoporaceae) have been consistently shown to produce the greatest levels of activity amongst all plants studied, both by this group and elsewhere [2;3]. The genus is characterised phytochemically by the acumulation of structurally and stereochemically unusual terpenoids, unique to the plant kingdom [1]. Here we report on the isolation and identification of three compounds from a petroleum extract of *Eremophila duttonii* exhibiting antibacterial activity against *Staphylococcus aureus* and *Candida albicans*. Active compounds were detected and isolated using a combination of TLC, bioautography and flash column chromatography. Structural assignments for active compounds were performed using 2-dimensional ^{13}C and ^{1}H NMR spectroscopy. Major active compounds were identified as the serrulatane diterpenes, (serrulat-14-en-7,8,20-triol [I] and serrulat-14-en-3,7,8,20-tetraol [II]) and a novel furanosesquiterpene (11-hydroxy ngaione [III]). Minimum inhibitory concentrations (MIC) of the isolated compounds were determined for inhibition of three gram positive bacteria commonly associated with dermal and upper respiratory tract infections. [I] produced the lowest MIC (23 µg/ml) against *Streptococcus pneumoniae*. **References:** [1] Richmond, G.S., Ghisalberti, G. (1994) Econ. Bot. 48: 35 – 59. [2] Palombo, E., Semple, S.J. (2001) J. Ethnopharm. 77: 151 – 157. [3] Palombo, E., Semple, S.J. (2001) J. Basic Microbiol. 42: 444 – 448.

P 411

Securidacaxanthones B and C, xanthones from *Securidaca longepedunculata* (Polygalaceae)

Meli AL[1], Ngninzeko FN[1], Castilho PC[2], Wansi JD[3], Kuete V[4], Lontsi D[1], Beng VP[4], Choudhary MI[5], Sondengam BL[1]
[1]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, PO Box 812, Yaounde, Cameroon; [2]Centro de Quimica da Madeira, Dept. Quimica, Univ. Madeira, Campus Universitario da Penteada, 9000 – 390 Funchal, Portugal;[3]Department of Chemistry, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon; [4]Department of Biochemistry, Faculty of Science, University of Yaounde I, PO Box 812, Yaounde, Cameroon; [5]International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan

New compounds 1,5-dihydroxy-3,4,6,7,8-pentomethoxyxanthone (**1**) and 5-*O*-prenyl-1-hydroxy-,3,4,6,7,8-pentamethoxyxanthone (**2**), together with seven known compounds: 2-hydroxy-1,7-dimethoxyxanthone (3), 1,7-dihydroxyxanthone, 1,7-dihydroxy-4-methoxyxathanthone, β-sitosterol, 3-*O*-β-D-glucopyranosyl-β-sitosterol, quercetin-3-*O*-β-galactopyranoside and 3-hydroxy-6-methoxysalicylic acid, were isolated from the ethyl acetate fraction of the root part (whole root) of *Securidaca longepedunculata* Fresen. The structures of these compounds were established by means of spectroscopic (NMR) methods. Some of the isolated xanthones showed moderate antimicrobial activity.



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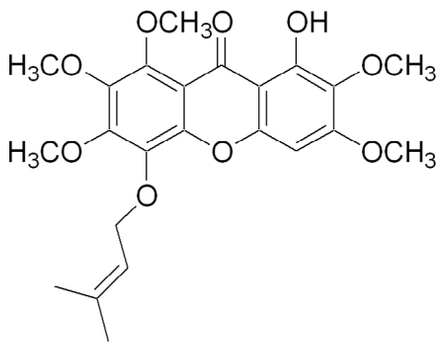

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Acknowledgements: CQM is funded by Fundação para a Ciência e Tecnologia (FCT) and FEDER. This work had financial support from programme INTERREG, project BIOPOLIS. A. Meli Lannang, is thankful to the TWAS for financial support for research and advanced training at the H.E.J Research Institute of Chemistry, International Center of Chemical Sciences, Pakistan. **References:** [1] Meli AL,

Lontsi D, Ngounou FN, Sondengam BL, Nkengfack AE, Heerden FR, Assob JCN, (2006) Fitoterapia 77: 199 – 202.

P 412

Labdane-Type Diterpenes from *Cistus creticus* L.

Güvenalp Z[1], Demirezer LÖ[2], Kuruüzüm-Uz A[2], Kazaz C[3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University, 25240, Erzurum, Turkey; [2]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey; [3]Department of Chemistry, Faculty of Art and Science, Atatürk University, 25240, Erzurum, Turkey

The genus *Cistus* (Cistaceae) is represented by 4 species in Turkish flora [1]. Different parts of *Cistus* species are commonly used in Turkish folk medicine for the treatment of a variety of diseases due to their emmenagogue, astringent, expectorant, antiseptic and sedative effects [2]. Diterpenes are the most common substances in the genus *Cistus*. They might play an important role for chemotaxonomic classification. In this study, we report the isolation and structure elucidation of 5 labdane-type diterpenes; ent-3β-acetoxy manoyloxide, ent-3β-hydroxy-13-epi-manoyloxide, ent-3β-acetoxy-12β-hydroxy-13-epi-manoyloxide, ent-3β,12β-dihydroxy-13-epi-manoyloxide, ent-3β,12α-dihydroxymanoyl oxide, from the aerial parts of *Cistus creticus* L. The structures of the compounds were elucidated by high field 1D and 2D NMR and EI-MS techniques. **References:** [1] Coode, M.J.E. (1978) In: Davis, P.H. (Ed.), Flora of Turkey and East Aegean Islands, Vol: 1. University Press, Edinburgh, pp. 506 – 508. [2] Baytop, T. (1985) Therapy with Medicinal Plants in Turkey (Past and Present), 2nd ed., Nobel Tıp Kitabevleri, İstanbul, p 316.

P 413

Fibroblast Growth Stimulation by Henna Extract

Hacımüftüoğlu A[1], İkbal M[2], Özgen U[3], Doğan H[4], Sevindik HG[3], Göçer F[1]
[1]Atatürk University, Faculty of Medicine, Department of Pharmacology, Erzurum, 25240, Turkey; [2]Karadeniz Technical University, Faculty of Medicine, Trabzon, 61080, Turkey; [3]Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, 25240, Turkey; [4]Atatürk University, Faculty of Medicine, Department of Genetics, Erzurum, 25240, Turkey

Henna consists of the dried leaves of *Lawsonia inermis* (Lythraceae), a shrub cultivated in India, Ceylon and North Africa including Egypt. Henna contains a colouring matter, lawson (a hydroxynaphthoquinone), various coumarins, luteolin and its 7-*O*-glucoside, fats, resin and henna-tannin [1]. Henna has been used for centuries in many cultures, mainly as a dye for hair and nails as well as for decorative body painting. It is used both as a cosmetic agent to stain the hair, skin and nails and is applied to body on lesions in the treatment of seborrheic dermatitis or fungal infections [2]. In this study, human amnion fibroblasts (HAFs) were used for cell culture study. The Neutral Red Assay was performed for this research. The absorbance was read at 550 nm. The absorbance values are correlated with the number of surviving cells [3]. The methanol extract of henna was investigated for its ability to stimulate the growth of HAFs. HAFs were taken with routine amniocentesis procedure from pregnants. A range of concentrations was studied and the extract was found to stimulate the growth of HAFs *in vitro* at 5 μg/ml, 1 μg/ml, 0.5 and 0.1 μg/ml.

Concentration (μg/ml)	100	50	10	5	1	0.5	0.1
Sample absorbance/Control absorbance	< 1	< 1	< 1	1.16	1.2	1.16	1.14

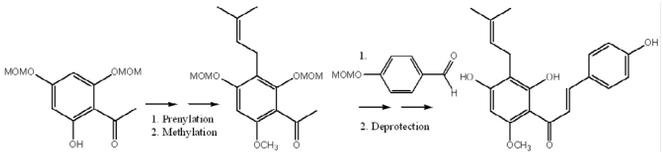
Cytotoxicity was observed at 100 μg/ml, 50 μg/ml and 10 μg/ml. This means that Henna extract can stimulate fibroblast growth and can therefore be useful for some dermatological diseases. Further studies are required for understanding which chemical compounds produce this effect. **References:** [1] Evans W.C. (1989) Trease and Evans' Pharmacognosy, The Alden Press, Oxford. [2] Kök A.N., et al. (2005) J Emerg Med, 29: 343 – 344. [3] Ozgen, U. et al. (2006) J Ethnopharmacol 104: 100 – 103.

P 414

Synthesis of prenylated hop chalcones and evaluation of their cytotoxic activity

Vogel S, Heilmann J
Institute of Pharmaceutical Biology, University of Regensburg, Universitaetsstrasse 31, 93040 Regensburg, Germany

Xanthohumol is the main prenylated chalcone in the cones of *Humulus lupulus* L. and has been recently investigated for its cytotoxic and chemopreventive activity [1]. Besides xanthohumol, hop cones also contain other prenylated chalcones like desmethylxanthohumol and xanthohumol B-E [2], but due to the lower content the availability of these compounds is limited and in contrast to xanthohumol pharmacological data are scarce. Therefore, we developed the first general strategy for the synthesis of xanthohumol and other related prenylated chalcones. The synthesis starts with protection of the hydroxyl groups of 2,4,6-trihydroxybenzophenon with MOM and leads to xanthohumol via prenylation, methylation, coupling with a protected 4-hydroxy-benzaldehyde and deprotection as key steps.



The cytotoxic activity of xanthohumol was evaluated against a HeLa cell line and showed in accordance with literature a remarkable activity with an IC_{50} value of ~ 10 μM, whereas desmethylxanthohumol and xanthohumol C were less active. **References:** [1] Gerhauser C. et al. (2002) Mol. Cancer Ther. 11: 959 – 969. [2] Stevens J.F. et al. (2000) Phytochemistry 53: 759 – 775

P 415

KIOM-79 prevents apoptotic cell death and AGEs accumulation in the retina of diabetic db/db mice

Kim YS, Sohn EJ, Lee HY, Kim CS, Lee YM, Kim HJ, Kim JS
Dept. of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, 461 – 24 Jeonmin-dong, Yuseong-gu, Daejeon 305 – 811, Republic of Korea

KIOM-79, a mixture of extracts obtained from *Pueraria lobata, Magnolia officinalis, Glycyrrhiza uralensis* and *Euphorbia pekinensis*, inhibits the formation of advanced glycation end-products (AGEs) *in vitro* and is more effective than aminoguanidine. KIOM-79 reduces AGEs accumulation in the kidney and retards the development of diabetic nephropathy in an animal model for type 1 and 2 diabetes [1 – 3]. In this study, we evaluated whether KIOM-79 could prevent apoptotic cell death and AGEs accumulation in the retina of diabetic db/db mice. The mice were treated orally with KIOM-79 once a day for 12 weeks. In the retina, the number of terminal dUTP nick end labeling (TUNEL) immunoreactive cells increased in the ganglion cell layer and AGEs accumulated. KIOM-79 reduced the number of TUNEL immunoreactive retinal cells and prevented AGE accumulation. These data show that KIOM-79 could prevent apoptosis in neuronal cells, AGEs accumulation in the retina, and retard the development of diabetic retinopathy. *Acknowledgements:* This research was supported by a grants [L06010] from the Korea Institute of Oriental Medicine. **References:** [1] Kim, JS. et al. (2002) Korean J. Pharmacognosy. 33: 308 – 311. [2] Kim, YS. et al. (2006) Korean J. Pharmacognosy. 37: 103 – 109. [3] Kim, C-S. et al. (2007) J Ethnopharmacol. Feb 23 (available online).

P 416

Phytochemical study of the aerial parts of *Zygophyllum geslini*

Smati D[1,2], Mitaine-Offer AC[1], Miyamoto T[3], Hammiche V[2], Lacaille-Dubois MA[1]

[1]Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique (UMIB), UPRES-EA 3660, Faculté de Pharmacie, Université de Bourgogne 1, 7 Bd Jeanne D'Arc, BP 87900, 21079 Dijon Cedex, France; [2]Laboratoire de Botanique Médicale, Faculté de Médecine, Université d'Alger 2, 18 Av Pasteur, 16000 Alger, Algeria; [3]Graduate School of Pharmaceutical Sciences, Kyushu University 3, Fukuoka, Japan

Zygophyllum geslini Coss. (Zygophyllaceae), traditionally used as antidiabetic [1], was collected in the Algerian Central Sahara. From a methanolic extract of the aerial parts, a crude saponin mixture was obtained and submitted to successive solid/liquid preparative chromatographic methods, i.e. VLC, MPLC over silica gel and RP-18. Five saponins were isolated and purified, and their structures were established mainly by 600 MHz 2D NMR techniques (COSY, TOCSY, NOESY, HSQC, HMBC) and mass spectrometry. Three new triterpene glycosides were identified as 3-*O*-α-L-rhamnopyranosyl-(1(→2)-α-L-arabinopyranosyl-(1(→2)-β-D-glucuronopyranosyl-urs-20(21)-en-28-oic acid-28-*O*-(2-*O*-sulfo-β-D-glucopyranosyl) ester, 3-*O*-(2-*O*-sulfo-β-D-glucuronopyranosyl)-urs-20(21)-en-28-oic acid-28-*O*-(2-*O*-sulfo-β-D-glucopyranosyl) ester, and 3-*O*-(2-*O*-sulfo-β-D-glucopyranosyl)-quinovic acid, together with the known zygophylosides E and G. Comparing the results obtained with different *Zygophyllum* species [2], the presence of the ursane-type aglycone and the sulfonyl moiety in these molecules may represent chemotaxonomic markers of this genus. **References:** [1] Smati, D. et al. (2004) J. Ethnopharmacol. 95: 405 – 407. [2] Safir, O. et al. (1998) J. Nat. Prod. 61: 130 – 134.

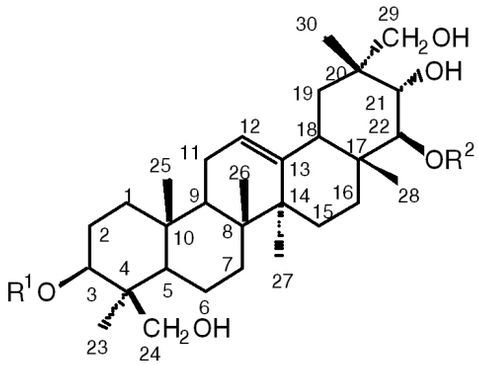
P 417

New oleanane-type saponins from *Astragalus flavescens*

Avunduk S[1,2], Mitaine-Offer AC[1], Miyamoto T[3], Alankus-Caliskan O[2], Lacaille-Dubois MA[1]

[1]Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique (UMIB), UPRES-EA 3660, Faculté de Pharmacie, Université de Bourgogne 1, 7 Bd Jeanne D'Arc, BP 87900, 21079 Dijon Cedex, France; [2]Department of Chemistry, Faculty of Science, Ege University 2, Bornova, Izmir, 35100 Turkey; [3]Graduate School of Pharmaceutical Sciences, Kyushu University 3, Fukuoka, Japan

Six new oleanane-type saponins were isolated by solid/liquid preparative chromatography, from a methanolic extract of the roots of a Turkish plant of the Fabaceae family, *Astragalus flavescens* Boiss. While mainly cycloartane-type saponins were found in the genus *Astragalu*s [1], their aglycone was identified as (21α,22β,24β,29α)-21,22,24,29-tetrahydroxyolean-12-ene mainly by 600 MHz 2D NMR techniques and mass spectrometry. Two heterosidic linkages were established at the C3 and C22 positions of this aglycone. At The C3 position, either of two oligosaccharidic chains were found, α-L-rhamnopyranosyl-(1(→2)-β-D-xylopyranosyl-(1(→2)-β-D-glucuronopyranosyl and α-L-rhamnopyranosyl-(1(→2)-β-D-glucopyranosyl-(1(→2)-β-D-glucuronopyranosyl, and at the C22 position, the hydroxyl group can be either free or substituted with a β-D-glucopyranosyl unit or an α-L-arabinopyranosyl unit.



References: [1] Verrota, L., El-Sebakhy, N.A. (2001) Studies in Natural Products Chemistry, Vol. 25. Atta-ur-Rahman (Ed.). Elsevier. Amsterdam.

P 418

Ecdysteroids from *Dioscorea dumetorum*

Sautour M[1], Canon F[1], Miyamoto T[2], Dongmo A[3], Lacaille-Dubois MA[1]

[1]Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique, UMIB UPRES- EA 3660, Faculté de Pharmacie, Université de Bourgogne, 7, Bd. Jeanne d'Arc, BP 87900, 21079 Dijon Cedex, France; [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812 – 8582, Japan; [3]Laboratoire de Biologie des Organismes Animaux, Faculté des Sciences, Université de Douala, Cameroun

Amoung the yam species commonly grown and consumed in Cameroon, *Dioscorea dumetorum* (Kunth) Pax (Dioscoreaceae) is the most important one [1]. The tuber of this plant is used in traditional medicine for the treatment of diabetes and crude extracts of *D. dumetorum* have been shown to posses a hypoglycemic effect in fasted normal rats and rabbits [2]. As a part of our ongoing studies of the plants from Dioscoreaceae [3,4], a phytochemical investigation of the rhizome of *D. dumetorum* has led to the isolation of a new ecdysteroid, 5,11,20-trihydroxyecdysone (**1**), and two known ecdysteroids, kerkesterone (**2**) and ajugasterone C (**3**), by several chromatographic steps on normal and reversed phase silica gel. Their structures were determined by spectroscopic methods including 1D- and 2D-NMR (COSY, TOCSY, HSQC and HMBC). This is the first report on the occurrence of phytoecdysteroids in the Dioscoreaceae family. These compounds were devoid of antifungal activity against three *Candida* species (*C. albicans*, *C. glabrata* and *C. tropicalis*, MIC > 200 μg/ml). **References:** [1] Agbor-Egbe, T. et al. (1995) J. Food Comp. Anal. 8: 274 – 283. [2] Undie, A.S. et al. (1986) J. Ethnopharmacol. 15: 133 – 144. 3. Sautour, M. et al. (2004) Planta Med. 70: 90 – 92. 4. Sautour, M. et al. (2004) Chem. Pharm. Bull. 52: 1235 – 1237.

P 419

Isolation and identification of neuroprotective compounds from *Machilus thunbergii*

Ma CJ[1], Lee KY[1], Yang HK[1], Yoon JS[1], Kim YC[1], Sung SH[1]

[1]College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151 – 742, South Korea

The CH_2Cl_2 fraction of the bark of *Machilus thunbergii* Sieb. et Zucc. (Lauraceae) significantly protected primary cultures of rat cortical cells exposed to the excitotoxic amino acid, L-glutamate. Through the activity-guided isolation of the CH_2Cl_2 fraction, (+)-9'-hydroxygalbelgin (**1**), isogalcatin B (**2**), (7*S*,8*S*,8'*R*)-3',4'-dimethoxy-3,4,-methylenedioxylignan-7-ol (**3**), 1-hydroxy-7-hydroxymethyl-6-methoxyxanthone (**4**), 5,7-dimethoxy-3',4'-methylenedioxyflavan-3-ol (**5**), (+)-(3*S*,4*S*,6*R*)-3,6-dihydroxypiperitone (**6**), protocatechuic acid methyl ester (**7**) and tyrosol (**8**) were obtained. Among them, compound **3**, **5**, **7**, **8** had significant neuroprotective activities against glutamate-induced neurotoxicity in primary cultures of rat cortical

cells at concentrations ranging from 0.1 μM to 10.0 μM. At a concentration of 10.0 μM, neuroprotection against glutamate-induced toxicity of compounds **3**, **5**, **7** or **8** were 25.1, 31.1, 37.9 or 40.1 % ($p < 0.01$), respectively. *Acknowledgements:* This research was supported by a grant (M103KV010024 – 06K2201 – 02410) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, the Republic of Korea.

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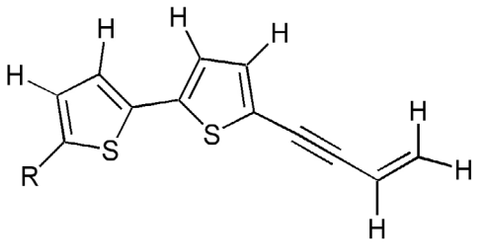
Phytochemical investigation of *Dracopis amplexicaulis*

Blunder M[1], Schühly W[1], Kunert O[2], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University, Universitätsplatz 4, 8010 Graz, Austria; [2]Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, 8010 Graz, Austria

Dracopis amplexicaulis (Vahl) Cass. (Clasping Coneflower, syn. *Rudbeckia amplexicaulis* Vahl.; Asteraceae) is an annual herb growing in Eastern North America. The plant belongs to the tribe Heliantheae, which also contains related genera such as *Echinacea* and *Ratibida* ("Coneflowers"). *Dracopis* is known for its content of thiophene derivatives and polyacetylenes [1]. Aerial plant material was subjected to a phytochemical analysis using chromatographic methods such as VLC, MPLC (Sephadex LH20) prep TLC and prep HPLC. Isolated compounds were structurally elucidated using UV, NMR (1- and 2-dimensional) and ESI-LC-MS. From the dichloromethane extract, a variety of secondary constituents such as thiophene derivatives (**1**) (e.g. R = CHO, 5-but-8-en-6-ynyl-[2,2']bisthiophenyl-5'-carbaldehyde; R = CH_2OH, (5-but-8-en-6-ynyl-[2,2']bisthiophenyl-5'-yl)-methanol) and the sesquiterpene lactone (**2**) (11-β-methyl-(11α,13-dihydrotomentosin), which has been reported to this genus for the first time, were identified.



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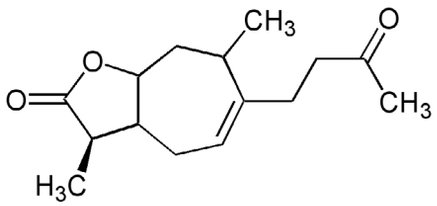

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Acknowledgements: The authors thank Dr. Lowell Urbatsch, Lousiana State University Herbarium (LSU, Baton Rouge) for recollecting plant material. **References:** [1] Bohlmann, F. et al. (1967) Chem. Ber. 100: 2518 – 22.

P 421

A new flavonoid compound isolated from *Fagonia taekholmiana* and its carcinogenic evaluation

Lamyaa F, Ali E
Department of Phytochemistry and Plant Systematics, National Research Centre, Dokki, Giza, Egypt

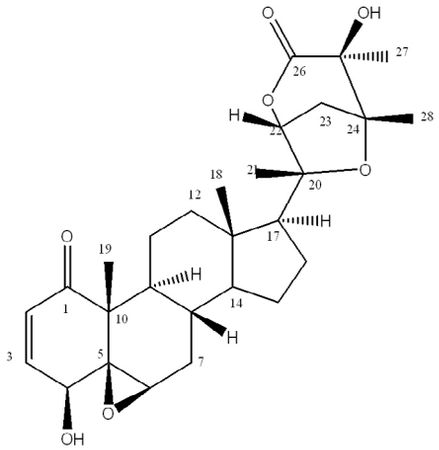
A new flavonoid trioside and six known flavonoids were isolated from the EtOH extract of *Fagonia taekholmiana* (Zygophyllaceae) for the first time. The new compound was identified as kaempferol-3-*O*-β-L-arabinopyranosyl-(1 4)-O-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside and the six known compounds were apigenin-7-*O*-glucopyranoside, quercetin-3-*O*-glucopyranoside, kaempferol-3,7-di-*O*-rhamnopyranoside and the aglycones apigenin, quercetin and kaempferol. Their structures were elucidated by spectroscopic methods including UV, FABMS, ^1H, ^{13}C and 2D-NMR [1 – 3]. The cytotoxic activity against some human cancer cell lines was determined [4]. **References:** [1] Mabry T. J., Markham K. R., and Thomas M. B. (1970) The Systematic Identification of Flavonoids, Spring. Verlag, Berlin. [2] Harborne J. B. (1994) The Flavonoids, Advances in Research since 1986, Chapman and Hall, London. [3] Markham K. R. (1982) Techniques of Flavonoid Identification, Academic Press, London. [4] Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenny S., and Boyd M. R. (1990) J. Natl. Cancer Inst., 82: 1107.

P 422

Screening of some Solanaceae plants for cytotoxic activity, and isolation and structure elucidation of a new steroid from the active fraction of *Physalis divarivata* D. Don

Namjooyan F[1], Azemi ME[1], Mosaddegh M[2], Cheraghali A[3], Kobarfard F[2], Porzel A[4]
[1]Pharmacognosy Department, School of Pharmacy, Jondishapour University of Medical Sciences, Ahwaz, Iran; [2]Pharmacognosy Department, School of Pharmacy, Shaheed Beheshti, University of Medical Sciences Tehran, Iran; [3]Baghiatallah University of Medical Science; [4]Institute for Plant Biochemistry, IPB, Halle/Saale, Germany

Most Solanaceae species show various biological effects such as cytotoxic, antimicrobical and antifungal activity [1] In this study, eight plants belonging to the genera *Solanum* and *Physalis* of this family were screened for cytotoxic activity against cancer cells (Vero, HeLa, CHO and HT- 29 cell lines). Plants were extracted with methanol – H_2O (9:1). Exposure of cell lines to methanolic extracts resulted in a dose-dependent growth inhibition evaluated by the modified MTT method. The results indicated significant cytotoxic activities with IC_{50} = 4.85, 7.04, 15.24 and 9.56 μg.ml^{-1} for the methanolic extract of *Physalis divaricata* on cancer cells, respectively. Fractionation of the methanolic extract was carried out using *n*-hexane, EtOAc, and chloroform. The cytotoxic activity of each fraction was examined. The EtOAc fraction showed significant cytotoxic activity on Vero, HeLa, CHO and HT- 29 cells wit IC_{50} of 4.62, 7.79, 14.02 and 10.98 μg ml^{-1}, respectively. Withanolides are natural C-28 steroidal lactones mainly produced by plants of 12 genera of Solanaceae with antimicrobial, antitumor, antiinflammatory, hepatoprotective, immunomodulatory activity and insect antifeedent properties [2], [3], [4]. A withanolide with a new skeleton was isolated and purified by RP-HPLC from the EtOAc fraction. The structure of this new withanolide was determined by UV, IR, HR-MS and different NMR techniques (1 H NMR, 13C NMR, COSY, HMQC, HMBC and ROESY). The systematic name for the compound is 4-hydroxy-17-(4-hydroxy-4,5,7-trimethyl-3-oxo-2,6-dioxa-bicyclo[3.2.1]oct-7-yl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-4 H-20-oxa-cyclopropa[5,6] cyclopenta [α]phenanthren-1-one (see figure).



References: [1] Graham, J. G. et al, (2000). J. Ethnopharmacol., 73: 347 – 77. [2] Metin, D. (2001) Pharm. Biol. 39: 346 – 350. [3] Minguzzi et al. (2002) Phytochemistry 59: 635 – 41. [4] Dinan, L.N. et al. (1997) Phytochemistry 44: 509 – 12. [5] Ahmed, S. et al. (1999) Phytochemistry 50: 647 – 51.

P 423

Eucalyptus species in Montenegro. Chemistry and antioxidant activity

Jovin E[1], *Beara I*[1], *Mimica-Dukić N*[1], *Grbovic S*[1], *Bugarin D*[1], *Balog K*[1]
[1]*Department of Chemistry, University of Novi Sad, Trg Dositeja Obradovica 3, 21000 Novi Sad, Serbia*

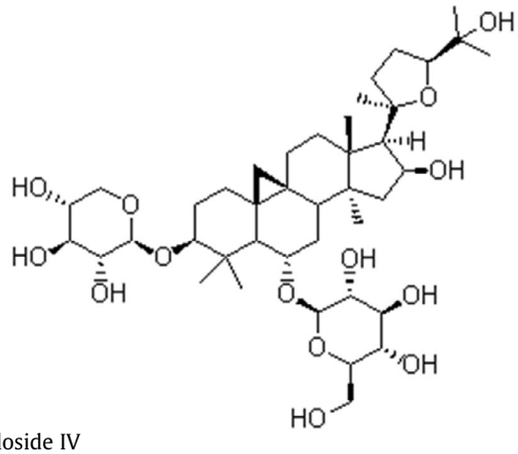
Although native to Australia, more than 700 eucalyptus species wildly grow in many parts of the world. Used for centuries as a traditional Aboriginal herbal remedy, eucalyptus leaves and their essential oils have many uses in everyday life due to their antiseptic and anti-inflammatory properties. More than 10 species of the genus Eucalyptus have been introduced to the coastal area of Montenegro at the beginning of the last century. In this study we have examined the chemical composition (HPLC-DAD) and antioxidant activity (free radical scavenger capacity, RSC and effect on lipid peroxidation, LP) [1], [2], [3] of methanolic extracts obtained from *Eucalyptus camaldulensis* Dehn. (syn. *E. rostrata* Schl.) and *Eucalyptus gunnii* Hook. F. collected from six localities on the Montenegro coastline. Whereas the differences in the HPLC-profile between the two eucalyptus species were evident, no qualitative variation among *E. camaldulensis* samples was found. In *E. gunni* phenylpropanoids and flavonoids were present in a significantly higher amount than in *E. camaldulensis*, whereas the latter was rich in ellagic acid and its derivatives. The highest RSC was obtained for *E. gunnii* extracts regarding to both DPPH (IC_{50}= 2.05 µg/ml) and OH (IC_{50}= 92.90 µg/ml) radicals, respectively. In addition, this species exhibited the highest LP inhibition (IC_{50}= 77.55 µg/ml). Comparing these results with those of synthetic antioxidants (BHA-butylated hydroxyanisole, BHT-butylated hydroxytoluene, and PG-propyl gallate), one can conclude that the methanolic extract of *E. gunnii* is a better antioxidant in all performed assays than BHA and BHT, but slightly less active than PG. The most potent methanolic extract from *E. camaldulensis* samples was that obtained from plant material collected near H. Novi. **References:** [1] Soler-Rivas C. et al. (2000) Phytochem. Anal. 11: 330 – 338. [2] Fukuzawa, K., et al. (1981) Arch. Biochem. Biophys. 206: 173 – 180. [3] Halliwell B et al. (1987) Anal Biochem 165: 215 – 219.

P 424

Determination of Astragaloside IV in *Astragalus mongholicus* using liquid chromatography electrospray ionization mass spectrometry

Woelkart K[1], *Wenzig EM*[1], *Heydel B*[1], *Heubl G*[2], *Bomme U*[3], *Bauer R*[1]
[1]*Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Austria;* [2]*Systematic Botany and Mycology, University of Munich, Germany;* [3]*Bavarian State Research Center for Agriculture, Institute of Plant Cultivation and Breeding (IPZ 3 d), Freising, Germany*

Astragaloside IV (AGS-IV) is an active constituent of *Astragalus mongholicus* Bunge (Leguminosae), a plant used in many Traditional Chinese medicines. It is a characteristic constituent whose presence forms part of the quality assurance of Radix Astragali and products containing it [1]. A sensitive and specific assay using liquid chromatography electrospray ionization mass spectrometry has been developed for the quantification of AGS-IV for quality control in *Astragalus* extracts, since UV-detection had shown to be inapplicable due to a lack of chromophase of AGS-IV. Samples were analyzed using a reversed-phase column (Zorbax Narrow Bore C18) as a stationary phase and acetonitrile and water containing 0.1% formic acid under gradient conditions as the mobile phase. Under the applied MS conditions, AGS-IV, a cycloartane-type triterpene glycoside, with a molecular ion of m/z 785 accumulates to a dimer which is actually detected (m/z 1570). The content of AGS-IV in 60 analyzed samples from a cultivation project at the Bavarian State Research Center for Agriculture ranged from 0.002 to 0.017%.



Astragloside IV

Acknowledgement: The project was funded by the Bavarian Ministry of Agriculture. **References:** [1] The Chinese Pharmacopoeia (Beijing, China) 1 (2005) 249.

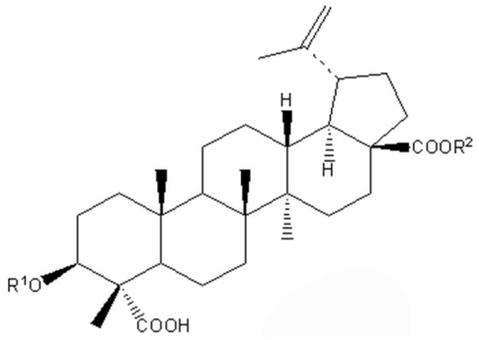
P 425

Identification of three new saponins in *Bupleurum fruticescens*

Díaz AM[1], *Vegue E*[1], *Pont E*[1], *Bertoldi L*[1], *Bernabé M*[2]
[1]*Departamento de Farmacología, Lab. De Farmacognosia, Facultad de Farmacia, Universidad de Alcalá de Henares. Carretera Madrid-Barcelona, Km-33,600, 28871-Alcalá de Henares, Madrid, España;* [2]*Departamento de Química Orgánica Biológica, Instituto de Química Orgánica, C.S.I.C. Juan de la Cierva, 3. 28006-Madrid, España*

Several *Bupleurum* species have been used in Traditional Chinese Medicine for over 2000 years. Additionally, some *Bupleurum* species are officially indexed drugs in the Chinese Pharmacopoeia [1]. *B. fruticescens* is a species that grows in Spain. Some compounds from *B. fruticescens* have shown anti-inflammatory activity [2,3]. In this work, the butanolic extract from *B. fruticescens* has been studied and

three new compounds have been identified by chromatographic and spectroscopic techniques:



	R^1	R^2
1	Glc*p*-(1→	Glc*p*-(1→
2	Glc*p*-(1→6)-Glc*p*-(1→	H
3	Glc*p*-(1→6)-Glc*p*-(1→	Glc*p*-(1→

Acknowledgements: Proyecto FISS (Rf:PI060119) & Ayudas a la Creación y Consolidación de Grupos de Investigación CAM-UAH (Rf:CG06-UAH/SAL-0672) **References:** [1] Sanchez, S., Diaz Lanza, AM. et al. (2002). Studies in Natural Products Chemistry, Vol 27, Bioactive Natural Products. (Part H). Attaur-Rahman/Ed., Elsevier Science Publishers, 659 – 696. Netherlands [2] Martin, S. et al. (1993) Planta Med. 59: 533 – 6. [3] Prieto, J.M. et al. (2004) Fitoterapia 75: 179 – 86.

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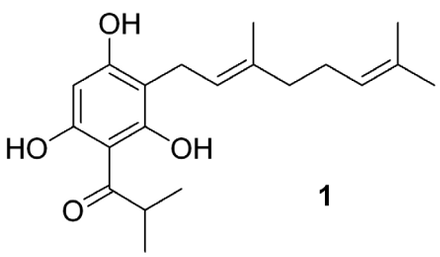
Phloroglucinol Derivatives from Two Mediterranean *Hypericum* L. (Clusiaceae) Species

Crockett SL[1], Kunert O[2], Bauer R[1]

[1]*Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, 8010 Graz, Austria;* [2]*Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-University Graz, 8010 Graz, Austria*

The flowering plant genus *Hypericum* L. (St. John's Wort, Clusiaceae) is the subject of considerable interest due to the use of *H. perforatum* extracts as treatment for mild to moderate depression. Extensive phytochemical and pharmacological research on this species has revealed many bioactive substances, including flavonoid glycosides, naphthodianthrones (hypericin) and phloroglucinol derivatives (hyperforin) [1]. The latter class of substances is interesting both chemically and pharmacologically [2]. As part of our continuing research on phytochemistry of the genus *Hypericum*, two species – *H. polyphyllum* Boiss. & Balansa (section *Olympia*) and *H. empetrifolium* Willd. (section *Coridium*) – occurring in Mediterranean region were phytochemically studied. A phloroglucinol derivative (**1**) previously isolated from *H. jovis* and *H. stypheloides* was isolated from the DCM extract of *H. empetrifolium* fruits. Two new flavones, one major (**2**) and one minor one, were isolated from the DCM extract of *H. polyphyllum* fruits.



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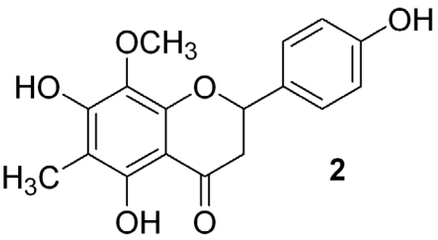

2

Acknowledgements: The Graz Botanical Garden is thankfully acknowledged for their help with cultivation of *H. polyphyllum*. We are grateful to Dr. Norman K. B. Robson for allowing the collection of *H. empetrifolium* from his garden in Great Britain. **References:** [1] Ernst, E. (Ed.) (2003) Hypericum: The Genus Hypericum, Taylor & Francis, London/New York.

P 427

Essential oils analysis of wild populations of *Achillea wilhelmsii* Koch. from two ecological conditions of Iran

Azizi M[1], Ghani A[1], Hassanzadeh Khayyat M[2], Pahlavanpoor AA[3]

[1]*Ferdowsi university of Mashhad, Azadee Sq. Wakeelabad Avevue, Mashhad, Iran;* [2]*Mashhad Medical Science University, Azadee Sq. Wakeelabad Avevue, Mashhad, Iran;* [3]*Research Institute of Forest and Rangeland of Iran-Fars province*

Achillea wilhelmsii Koch. is an aromatic plant belonging to the Asteraceae family and is distributed in different parts of Iran. In this study, we compared essential oil content and constituents in two wild populations of two different ecological conditions (Fars and Khorasan Provinces, South West and North East of Iran, respectively). The blooming herbs were collected and after drying, essential oils of the samples were extracted in a Clevenger apparatus. Essential oil constituents were determined by GC and GC-MS. Our results show that "Khorasan" population of *A. wilhelmsii* contains more essential oil than "Fars" population (0.65 and 0.20% v/w, respectively). Thirty components of "Khorasan" population representing 96.94% of the oil were successfully determined, as the main compounds camphore (19.06%), cembrene (10.00%), 1,8-cineole (8.78%), α-pinene (8.06%) and linalool (7.47%) could be identified. Thirty-four components (91.98%) of "Fars" wild population were identified including the main components isopentyl-isovalerate (9.46%), α-pinene (8.75%), 1,8-cineole(8.70%), 10-*epi*-γ-eudesmol (5.56%) and spathulenol (4.94%). In conclusion there are differences between these two populations concerning essential oil content and constituents. *Acknowledgements:* Pharmaceutical Faculty of Medical Science University of Mashhad

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Allelopathy of saffron – a biological control of *Phalaris minor*

Astaraei AR

College of Agriculture -Ferdowsi University of Mashhad 9177948974 – 1163. Mashhad-Iran

Weeds can inhibit the growth and yield of many agronomic plants by release of phytotoxins to the plant environment. *Phalaris arundinacea* has less effect on seed germination but reduces growth, causes twisted roots, inhibits root development and kills the roots of test plants. Obviously, allelopathy can be used as biological weed control. Many species of medicinal plants have pronounced allelopathy effects as well. The present study evaluates the allelopathy effect of saffron (*Crocus sativus* L.) in a germination bioassay using *Phalaris minor* Retz (Poaceae). Leaf, corm and soil attached to corm (SAC) was assumed as the main sources of allelopathy. Water extracts of these sources at five concentrations (0, 0.5, 1, 3 and 5%) were used in 3 x 5 factorial arrangement with three replications. Plumule and radicle fresh weight of *P. minor* were reduced signifi-

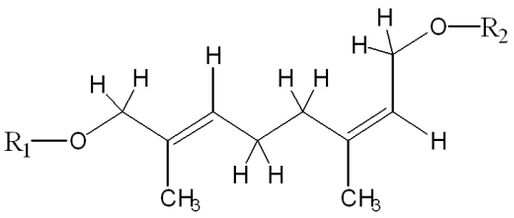
cantly by SAC treatment by -47% and -49.9%, respectively, compared to treatments with water extracts of leaf and corm. Both plumule and radicle lengths showed significant reduction by treatments with water extracts of leaf and corm. Water extracts at 3% and 5% concentration reduced germination by 27% and 55% compared to control [1]. Plumule and radicle fresh weights were reduced at 0.5% water extract by 51 and 50%, respectively, when compared to their controls. Plumule and radicle lengths were reduced significantly at 0.5% (-39%) and 1% (-44%) concentrations respectively compared to their control [1,2]. There was a significant source by concentration interaction for all germination traits. Leaf water extract at all concentrations had more negative effects on plumule and radicle lengths compared to water extracts of corm and SAC. **References:** [1] Hosseini M, Rizvi S J H. (2003). Third National Symposium on Saffron. Iran. PP. 173 – 178. [2] Singh H P, el al.(2002). Critical Review in plant Science. 18: 757 – 772.

P 429

New compounds from *Fadogia agrestis* (Rubiaceae)

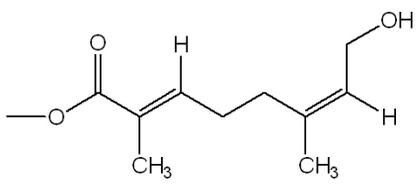
Díaz AM[1], Anero RC[1], Ollivier E[2], Balansard G[2], Bernabé M[3]
[1]Departamento de Farmacología, Lab. De Farmacognosia, Facultad de Farmacia, Universidad de Alcalá de Henares. Carretera Madrid-Barcelona, Km-33,600, 28871-Alcalá de Henares, Madrid, España; [2]Laboratoire de Pharmacognosie-Homeopathie, Faculté de Pharmacie, Université de la Méditerranée 27 Bd Jean Moulin, 13385 cedex 5 Marseille, France; [3]Departamento de Química Orgánica Biológica, Instituto de Química Orgánica, C.S.I.C. Juan de la Cierva, 3. 28006-Madrid, España

Fadogia agrestis Schweinf. ex Hiern (Rubiaceae) is a shrub found from Guinea to Sudan. In African traditional medicine the decoction of this plant is extensively used as a febrifuge which could be associated with its use as an antimalarial drug. This shrub is also used as a diuretic and in the treatment of kidney pain and convulsions [1]. We report on the isolation and structure elucidation of two new monoterpene glycosides isolated from leaves of *F. agrestis*. These compounds have been isolated and identified by chromatographic and spectroscopic techniques [2 – 5] as:



Inresanturoside II: R_1: α-L-Rha*p*-(1→
R_2: α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→
Inresanturoside III: R_1: β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→
R_2: α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→

Acyl B



B

Acknowledgements: Proyecto FISS(Rf:PI060119) & Ayudas a la Creación y Consolidación de Grupos de Investigación CAM-UAH(Rf:CG06- UAH/SAL-0672) **References:** [1] Adjanohoun, E.J. et al. (1989) Contribution aux études ethnobotaniques et floristiques en République populaire du Bénin. Médecine traditonnelle et pharmacopée 458 – 461. [2] Bock, K. et al. (1983) Adv. Carbohydr. Chem. Biochem. 41: 27 – 66. [3] Bock, K. et al. (1974) J. Chem. Soc., Perkin Trans. II, 293 – 297. [4] Iwagawa, T. et al. (1990) Phytochemistry 29: 1913 – 1916. [5] Jaensch, M. et al. (1990) Phytochemistry 29: 3587 – 3590.

P 430

Furostanol glycosides from the roots of *Chlorophytum borivilianum*

Acharya D[1], Mitaine-Offer AC[1], Kaushik N[2], Miyamoto T[3], Lacaille-Dubois MA[1]
[1]Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique (UMIB), UPRES-EA 3660, Faculté de Pharmacie, Université de Bourgogne, 7 Bd Jeanne D'Arc, BP 87900, 21079 Dijon Cedex, France; [2]TERI, Habitat Place, Lodhi Road, New Delhi, 110 003, India; [3]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812 – 8582, Japan

Although chemical investigations on *Chlorophytum* species are rare, several furostane and spirostane glycosides have been reported from a few Chlorophytum species in recent years [1]. *Chlorophytum borivilianum Sant & Fern* (Liliaceae), commonly known as 'safed Musli' is traditionally used in Ayurvedic and Unani medicine in India as a general tonic and immunomodulating agent [2]. From this species, only polysaccharides were characterized [2] and no work on the chemistry of saponins has been done so far. In the course of our search for bioactive saponins from plants [3], we describe here the isolation, structure elucidation and biological properties of saponins from *C. borivilianum*. The crude saponin mixture obtained from the n-BuOH layer of an ethanol extract of the roots was submitted to several chromatographic steps (VLC, flash, MPLC) over silica gel and RP-18. Four new furostane-type saponins were isolated and their structures were established mainly by 600 MHz 2D NMR techniques (COSY, TOCSY, HSQC, HMBC) and mass spectrometry. These compounds were tested for insecticidal and cytotoxic activities. *Acknowledgements:* The authors are grateful to IFCPAR-CEFIPRA, New Delhi, India, for financial support. **References:** [1] Kaushik, N. (2005) Phytochemistry Reviews 4: 191 – 196. [2] Narasimhan S. et al. (2006) Planta Med. 72: 1421 – 1424. [3] Mitaine-Offer, A.-C. et al. (2005) Helv. Chim. Acta 88: 2986 – 2995.

P 431

Influence of the climatic conditions on the volatile oil and active principles in Romanian wild *Arnica montana* L.

Sand C, Barbu CH, Pop MR, Tanase M
Lucian Blaga University of Sibiu, 10 Victoriei Bv., 550024 Sibiu, Romania

Arnica montana L. is a herbaceous plant from the Asteraceae family, found especially in the sub-alpine area, in wet pastures, on acid or neutral soils [1]. Because in Romania wild arnica is endangered due to its massive and illegal gathering, we have initiated a research for its *in vitro* cultivation. In order to obtain descendants with valuable properties, we have gathered several plants from various places in Romania, evaluating morphological properties (number and weight of flowers per plant, rhizome dimensions etc.), determining also the variability coefficient. Beside this, using gas chromatography – mass spectrometry techniques we have determined the amount of volatile oil in these plants, and the composition of their ethanol – water 45:55 v/v extracts [2]. The highest amount of essential oils in rhizomes and roots was found in plants growing on neutral, sunny slopes, at 600 – 800 m altitude. As these plants also had the largest amount of sesquiterpene lactones in mature flowers we started our *in vitro* cultures using inocula from these plants. It was observed that the higher the altitude, the lower the productivity of the flowers resulted. Soil acidity decreased the amounts of volatile oil and sesquiterpene lactones. The results, consistent to those presented in [3], are shown in the table below:

Altitude (m)	Soil pH	Essential oil amount in roots and rhizomes (%, DW)		Total sesquiterpene lactones amount in mature flowers (%, DW)	
		Mean	SD (±)	Mean	SD (±)
605 (n = 4)	6.84 – 7.15	3.51 – 3.67	0.25 – 0.34	0.68 – 0.78	0.036 – 0.046
710 (n = 2)	6.80; 6.90	3.41; 3.42	0.36; 0.39	0.61; 0.62	0.038; 0.041
770 (n = 2)	6.75; 6.82	3.15; 3.21	0.36; 0.45	0.51; 0.53	0.044; 0.051
920 (n = 2)	6.61; 6.78	2.61; 2.76	0.51; 0.55	0.44; 0.45	0.042; 0.049
975 (n = 2)	6.69; 6.72	2.15; 2.23	0.37; 0.39	0.39; 0.41	0.045; 0.041

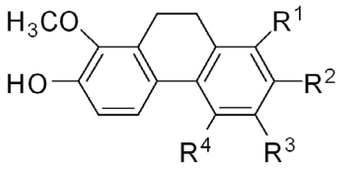
Acknowledgements: The research was performed within the Romanian CNCSIS grant no.114/2006. **References:** [1] Lange, D. (1998) Europe's medicinal and aromatic plants: their use, trade and conservation. TRAFFIC International, Cambridge, Great Britain. [2] Tekko, I. A., et al.(2004), J. Pharm. Pharmacol. 56:204. [3] Spitaler, R. et al. (2006) Phytochemistry. 67(4): 409 – 417.

P 432

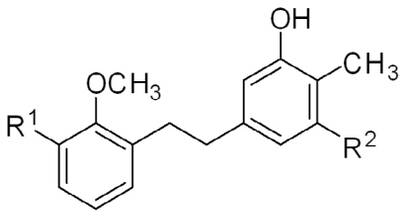
Chemical constituents and biological activity of the extracts from *Stemona pierrei*

Saiai A[1], Jatisatienr A[1,2], Pyne SG[3]
[1]Division of Environmental Sciences, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand; [2]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand; [3]Department of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia

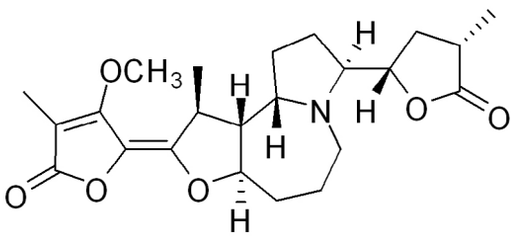
Extracts of *Stemona* roots have been used in traditional Chinese medicine for the treatment of various respiratory diseases and as anthelminthic agents for domestic animals [1]. The investigation of the dichloromethane extracts of *S. pierrei* Gagnep. (Stemonaceae) roots has led to the isolation of five stilbenoids i.e stemanthrene A (**1**), stemanthrene B (**2**), stemanthrene C (**3**), stilbostemin D (**4**), stilbostemin G (**5**) together with a protostemonine (**6**). Their structures were elucidated by spectroscopic methods and NMR spectra were compared with literature data [2]. Compounds **1**, **3**, **4** and **6** were tested for their cytotoxicity using brine shrimp (*Artemia salina* Leach.) lethality test (BST) [3]. The results show that compound **1**, **3**, **4** and **6** were moderately active in the brine shrimp lethality test with LC_{50} values of 42.03, 88.86, 62.10, and > 100 ppm, respectively.



1 R^1= H R^2 = OH R^3 = CH_3 R^4 = OCH_3
2 R^1= H R^2 = OCH_3 R^3 = CH_3 R^4 = OH
3 R^1= CH_3 R^2 = OH R^3 = CH_3 R^4 = OCH_3



4 R^1= H R^2 = OH
5 R^1= OH R^2 = OCH_3



6

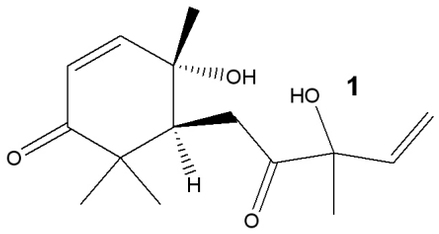
Acknowledgments: We thank the Chiang Mai University (CMU) and University of Wollongong for financial support. **References:** [1] Pilli, R.A., et al. (2000) Nat. Prod. Rep.17: 117 – 127. [2] Kostecki, K.,et al., (2004) Phytochemistry. 65: 99 – 106. [3] Meyer, et al. (1982) Planta Med. 45: 31 – 34.

P 433

Isolation and structure eucidation of novel antimalarial compounds from the Cameroonian spice *Scleria striatonux* De Wild (Cyperaceae)

Dohjinga K[1,2], Ndjoko K[1], Wittlin S[3], Akam M[2], Mbah J[2], Makolo F[2], Wirmum C[4], Efange S[2], Hostettmann K[1]
[1]Laboratoire de Pharmacognosie et Phytochimie, Ecole de Pharmacie Genève, Université de Genève, Quai Ansermet 30, 1211 Genève 4, Switzerland; [2]Pharmacochemistry Research Group, Department of Chemistry, University of Buea, Cameroon; [3]Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland; [4]Medicinal Foods & Plants, Bamenda, Cameroon

The genus *Scleria,* commonly known as nutrush, consists of perennial herbs that grow to around 1 – 1.5 m in height. In Cameroon, there are some 15 species that mostly inhabit swampy areas and forest edges, with a few growing on sandy soils. *Scleria striatonux* De Wild is a perennial herb that grows to 1 m in height. In some parts of Cameroon, the rhizome is used as a spice. Due to the widespread species resemblance and numerous synonyms within this genus and Cyperaceae in general, the genus *Scleria* has not attracted much attention from phytochemists. To the best of our knowledge, the only phytochemical work reported on *S. striatonux*, is that reported by Mve-Mba et al. [1]. The DCM extract of *S. striatonux* exhibited IC_{50}/IC_{90} values of 0.6 µg/ml/1.0 µg/ml (sensitive strain D6) and 0.6 µg/ml/1.1 µg/ml (resistant strain W2). Bioassay guided fractionation afforded sesquiterpenes (**1**, **2, 3**). Their structures were elucidated using rapid LC/UV/ESI-MS/MS with routine 1D and 2D-NMR spectroscopy [2].



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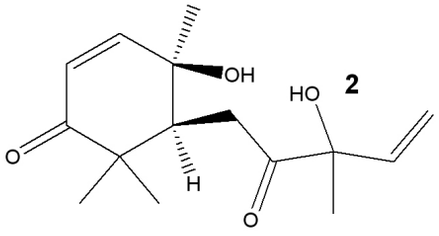

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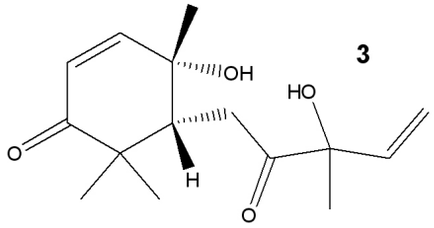


References: [1] Mve-Mba C. E et al. (1996) J. Ess. Oil Res. 8: 59 – 61. [2] Wolfender J.-L. et al. (2001) Phytochem. Anal. 12: 2 – 22.

P 434

Osteoclast Differentiation Inhibitory Activity of Constituents from *Cephalotaxus koreana*

Yoon KD[1], Yang MH[1], Yang HJ[1], Han KR[1], Kim J[1]
College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 151 – 742, Korea

Cephalotaxus koreana Nakai (Cephalotaxaceae), distributed in the southern parts of Korea, has been used to treat wounds by insects [1]. The genus *Cephalotaxus* is well known for cephalotaxus alkaloids such as cephalotaxine, and harringtonine [2,3]. However, there are few publications about the constituents of *C. koreana* [4,5]. Three new flavonoid glycosides, a new hainanolide derivative, and a new dibenzylbutyrolactone type neolignan were isolated from the aerial parts of *C. koreana*, along with twenty known flavonoids. The structures of five new compounds were elucidated as 2*R*,3*R*-6-methylaromadendrin 3-*O*-β-glucopyranoside, apigenin 5-*O*-α-L-rhamnopyranosyl(1'''→2'') [6''-*O*-acetyl]-β-D-glucopyranoside, isoscutellarein 5-*O*-β-D-glucopyranoside, 11-hydroxyhainanolidol, and (3*S*,4*R*,5*S*)-3-[[4-[(3-methoxy-L-rhamnopyranosyl)oxy]-3-methoxyphenyl]methyl]-5-(3,4-dimethoxyphenyl)-3-hydroxy-4-(hydroxymethyl) dihydrofuran-2(3*H*)-one, by spectroscopic evidences. Among twenty five compounds, isoscutellarein 5-*O*-β-D-glucopyranoside, apigenin, luteolin, kaempferol 3-*O*-α-L-rhamnopyranosyl(1'''→6'')-β-D-glucopyranoside, tamarixetin 3-*O*-α-L-rhamnopyranosyl(1'''→6'')-β-D-glucopyranoside, quercetin 3-*O*-[6''-*O*-acetyl]-β-D-glucopyranoside, and quercetin 3-*O*-α-L-rhamnopyranoside showed significant inhibitory activities against osteoclast differentiation at the concentration of 0.1, and 1 μg/mL. **References:** [1] Bae, K.H. (1999) The Medicinal Plants of Korea, Kyohak-Sa, Seoul. [2] Morita, H. et al. (2000) Tetrahedron 56: 2929 – 2934. [3] Takano, I. et al. (1996) Phytochemistry 43: 299 – 303. [4] Sung, J.L. et al. (2005) Chem. Technol. Biotechnol. 80: 1148 – 1153. [5] Lee, M.K. et al. (2006) Bioorg. Med. Chem. Lett. 16: 2850 – 2854.

P 435

Furanocoumarins from *Heracleum crenatifolium* Boissieu

Tosun F, Akyüz Kızılay C
Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Ankara, Turkey

The genus *Heracleum* (Umbelliferae) is represented by fourteen species in the Turkish flora, seven of which are endemic. *Heracleum* species have been reported to have anticonvulsant, analgesic, antispasmodic, antiinflammatory, antibacterial and antiviral activities. *Heracleum crenatifolium* Boiss. is an endemic species distributed in North-East and East Anatolia. This is the first report on furanocoumarins of *H. crenatifolium.* Plant material was collected in the vicinity of Gumushane-Karamustafa (Turkey). Herbarium specimens are preserved at the Herbarium of the Faculty of Arts and Sciences, Gazi University, Ankara, Turkey. Dried and powdered fruits of *Heracleum crenatifolium* were extracted with petroleum ether at room temperature, the solvent was evaporated. By keeping in refrigerator, a coumarin-containing fraction was separated. From this, furanocoumarins were isolated by column chromatography and subsequent preparative thin-layer chromatography. The furanocoumarins isolated from the fruits of *H. crenatifolium* were identified using thin-layer chromatography, melting points and spectroscopic methods (IR, MS and ^{1}H-NMR) as pimpinellin, isopimpinellin, bergapten, isobergapten, sphondin and byakangelicol. *Acnowledgement:* This research was financially supported by the Research Foundation of Gazi University (research grant no. SBE-11 / 2002 – 11)

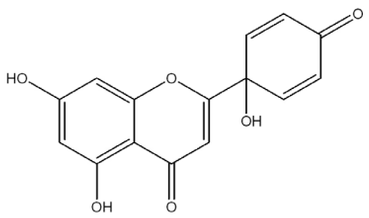
6. Pharmacology, toxicology and clinical studies of natural products

P 436

Anti-cancer agents from Formosan plants

Wu YC, Chang FR, Liaw CC, Lin AS, Wu CC
Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Cancer is the leading cause of death in Taiwan for years. In the current investigation, a herbal extract, WYC-01, from popular fruits, *Annona squamosa* and *Annona muricata*, and a pure compound, WYC-02 (protoapigenone) [1, 2, 3], from the Formosan pteridophyte, *Thelypteris torresiana*, were found to be potent promising candidates. The standardization and activity of WYC-01 was evaluated [4]. The major active components, squamocin and annonacin, were defined by HPLC technology. In addition, the total synthesis and cytotoxicity of WYC-02 and its analogues have been accomplished as well as their mechanism of actions and animal test in the ability of anti- breast cancer had also been evaluated.



WYC-02

Acknowledgements: 1. The Office of National Science and Technology Program for Biotechnology and Pharmaceuticals, Taiwan. 2. National Science Council, Taiwan **References:** [1] Lin A. S. et al. (2005) Planta Med. 71: 867 – 870. [2] Lin A. S. et al. (2007) Chem. Pharm. Bull. 55: 635 – 637. [3] Lin A. S. et al. (2007) J. Med. Chem. Accepted for publication. [4] Wu, Y. C. US patent, USP 7,223,792.

P 437

In vitro screening of extracts from some Egyptian plants for their radical scavenging and oxidant reducing properties

Hamed A[1,2], Soltan MM[2], Zaki AK[2], Shahat AA[2], Fry J[1]
[1]*School of Biomedical Sciences, University of Nottingham, NG7 2UH, United Kingdom;* [2]*Chemistry of Medicinal Plants Department, National Research Centre, Tahrir St., Cairo, Egypt*

The damaging effect of free radicals and oxidative species in a number of disease states has prompted a sustained interest in screening medicinal plants with antioxidant constituents. In the present study, extracts from medicinal plants growing in South Sinai, Egypt, were screened for their antioxidant properties using chemical assays. The study included methanolic extracts of *Alkanna orientalis* (L.) Boiss. roots, *Cucumis prophetarum* Jusl. ap. L, *Cleome droserifolia* (Forssk.) Del., and *Crataegus sinaica* Boiss. (and its ethyl acetate and butanol subfractions). Screening for the radical scavenging was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay [1], whereas the oxidant reducing properties were determined using the ferric reducing antioxidant power (FRAP) assay [2]. Data were expressed as Trolox® equivalents, at an extract concentration

of 62.5 μg/ml. In the DPPH scavenging assay the *Crataegus* and *Alkanna* extracts produced similar strong radical scavenging activity (Trolox equivalents ranging between 37.9 – 38.3 μM), whereas the *Cucumis* and *Cleome* extracts exhibited relatively weak antiradical properties. In the FRAP assay the extracts produced ferric reducing ability in the following order: *Crataegus* (ethyl acetate fraction) > *Crataegus* (butanol fraction) > *Alkanna* > *Crataegus* > *Cleome*, while the *Cucumis* extract was devoid of activity. The antioxidant activity produced by extracts of *C. sinaica* and *A. orientalis* are in agreement with previous reports with these species/genus [3, 4]. These data suggest further evaluation in a suitable cell-based assay to test for the intracellular antioxidant potential of these extracts. *Acknowledgements:* Alison Holmes, Holly Matthews and Tina Shah. **References:** [1] Nara, K. et al. (2006) Biosci. Biotechnol. Biochem.70: 1489 – 91. [2] Benzie I. F., Strain J. J. (1996) Anal. Biochem. 239: 70 – 6. [3] Shahat A A et al. (2002) Planta Med. 68: 539 – 41. [4] Assimopoulou A.N, Papageorgiou V. P. (2005) Phytother Res. 19: 141 – 7.

P 438

Hepatoprotective and Immuno-stimulant Effects of Nutaceutical Compounds from Carteonoid origin

Omara EA[1], Zaharan HG[2], Nada SA[3], Shedeed NA[2]
[1]Department of Pathology; [2]Food Technology Department; [3]Pharmacology Department, National Recherch Centre, El-Tahrir Street, Dokki, Cairo, Egypt

The present study was conducted to evaluate the antioxidant, hepatoprotective, and immuno-stimulant properties of carotenes-derived from the waste of food by-products (tomato peels. mango peels, corn gluten and dill), *in vitro* and *in vivo*. They had been selected for their high superoxide dismutase activity. Free radical scavenging activity (RSA) of carotenes (lycopene, β-carotene, zeaxanthin and lutein) was determined using the DPPH-scavenging activity method (in vitro study). Results revealed that RSA was increased significantly more than by BHA (synthetic commercial antioxidant) after 10 and 20 min in the order lycopene> β-carotene> zeanthin > lutein. Antioxidant and hepatoprotective effects in carbon tetrachloride liver-damaged rats were assayed using oral doses for each carotene (100 mg/kg b.w.) for 7 days prior to carbon tetrachloride administration and 2 days post-carbon tetrachloride treatment. All tested carotenes significantly reduced the elevated values of liver function tests (GGT, ALT & AST) in hepatic damage groups ($p < 0.05$). They also have had immuno-stimulating properties as they increased IgG levels in normal and liver damage rats. Histopathological examination of the liver of CCl_4 administrated rats using Hx. & E. stain revealed drastic alteration in liver-architecture. The hepatocytes were disrupted, vacuolated and lost their polyhedral shape. Vacuolation was most obvious in the centrilobular region which showed widespread necrosis. Fat change was also detected ($p < 0.05$). Studying the DNA content of hepatocytes revealed a decrease in the CCl_4 treated group using an image analyzer system. The liver of rats treated with CCl_4 and carotene showed good recovery from CCl_4 induced liver damage as was evident from the well defined hepatic cords and polyhedral hepatocytes with round nuclei. There was also improvement in DNA content of hepatocytes. In conclusion, carotenes may play an important role as neutraceutical preparations, especially, when obtained from wastes of food by-products, which are of low cost.

P 439

Histomorphometric evaluation of bone tissue exposed to exprimental osteoporosis after treatment with a natural extract

Omara EE[1], Saffie N[1], El-Toumy SAA[2], Aal WA[1]
[1]Department of Pathology, [2]Chemistry of Tannins Department, National Research Center, El-Tahrir Street, Dokki, Cairo, Egypt

Plant-derived phenolic compounds manifested beneficial effects and potential inhibition of several stages of diseases. The aim of this study was to investigate the efficacy of the plant-derived phenolic compounds in the treatment of osteoporosis and their modulatory effects on dexamethasone-induced osteoporosis in rats. About 50 experimental male and female Sprague-Dawely rats (150 gm) were used in this study and divided into 5 groups (for each 10). Group 1 was used as a control group. Group 2 received intraperitoneal injection of dexamethasone at a dose of (20 mg/kg b.w). Group 3 received a hydroalcoholic extract of *Retama raetam* seeds (7:3, CH_3OH: H_2O), (30 mg/kg b.w.). Group 4 received (20 mg/kg b.w) in dose of dexamethasone together with (30 mg/kg b.w.) of *R. raetam* seed extract at the same time, while group 5 received dexamethasone alone and then the plant extract. All these groups were treated for 3 months. Bone samples were decalcified, embedded, sectioned and stained with Hx & E for morphological study and histochemical stain (alkaline phosphatase) for studying the regeneration of diseased bone tissue. All the samples were analyzed using a computer-assisted quantitive system. Our results revealed that dexamethasone can induce histopathological and histochemical changes in bone tissue. The morphometric parameters showed marked decrease in mid-diaphyseal cortical bone thickness in dexamethasone treated rats and an increased bone resorption in treated groups with plant extract [1] ($p < 0.05$). Results of a histochemical study revealed that ALP activity was decreased in dexamethasone treated-rats, and there was improvement in this activity accompanying the treatment with the plant extract ($p < 0.05$). Treatment with the plant extract results in amelioration of the imbalance between bone resorption and formation. These results were more obvious in males than in females. The chemical constituents of *R. raetam* seeds, especially isoflavanoid derivatives present may be involved in the observed antiosteoporosis of the plant extract. **References:** [1] Dontas I, Halabalaki M, Moutsatsou P, Mitakou S, Papoutsi Z, Khaldi L, Galanos A, Lyritis GP. (2006) Maturitas. 53: 234 – 42.

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Response of an insect pest, *Spodoptera littoralis* to the stimulant glucose

Buchwald-Werner S[1], Simmonds MSJ[2]
[1]Cognis Deutschland GmbH & Co KGa, D-40789, Monheim am Rhein, Germany; [2]Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3 AB, UK

Succulent species of plants have become of interest as a potential source for active ingredients to increase satiety and decrease appetite. In our study the efficacy of *Aloe vera* to modulate the response of an insect pest, *Spodoptera littoralis*, to the stimulant glucose was investigated. This insect model was selected as the feeding behaviour of insects and mammals can be modulated by stimulants such as glucose [1]. Initial tests on the insect evaluated whether they could taste extracts of *A. vera* and whether *A. vera* would stimulate or deter feeding. Insects ate about 20% more of the feeding discs treated with *A. vera* than those treated with glucose, although the duration of the first meal taken by the insects was not influenced by *A. vera*. In another experiment the insects were cannulated with either a solution of *A. vera* or a water control and glucose was offered. The time to start the first meal, the latency, was extended by *A. vera*, although the duration of the meal was not influenced. The time to start the second meal was increased by about 15%. The neural response of taste sensilla to glucose was recorded with an electrophysiological bioassay. Neurons in the sensilla respond to compounds in food such as glucose. Cannulation with *A. vera* caused a decrease of

about 20% in neural firings, this suggests that the insects are less responsive to the glucose. The results suggest that *A. vera* modulates the feeding response of insects to feeding stimulants and further investigations are ongoing to determine if *A. vera* can increase satiety or decrease appetite or if both mechanisms are involved. **References:** [1] Simmonds, M. S. J. et al. (1991) J. Exp. Biol. 162: 73 – 90

P 441

Anti-stress, anxiolytic and nootropic activity of roots of *Tylophora indica*

Wakade AS, Kulkarni MP, Juvekar AR
Department of Pharmaceutical Sciences & Technology, University Institute of Chemical Technology, Matunga, Mumbai 400019, India

An aqueous extract [AE] of roots of Tylophora indica [TI] was investigated for its anti-stress activity in chronic cold restraint stress (4 ± 0.5 0 C for 1 h for 7 days) in Wistar rats. The anxiolytic activity of TI [AE] was studied using various behavioral paradigms such as elevated plus maze model, light dark model and open field model. Further nootropic potential of the extract was assessed using Morris water maze test. Stimulation of hypothalamus pituitary adrenal axis in stressful condition alters biochemical levels like plasma corticosterone, glucose, proteins, triglycerides, and cholesterol that were ameliorated by TI [AE] pretreatment (250 mg/kg and 500 mg/kg p.o) in chronic stress model. TI [AE] also restored perturbed neurotransmitter levels in brain, increased adrenal gland weights and atrophy of spleen caused by chronic stress. Histopathological studies of adrenal gland of the stress group revealed degeneration and lesions in the cortex, which was reversed by TI [AE] treatment. The extract at (250 mg/kg and 500 mg/kg p.o) increased time spent in open arm and lit zone in elevated plus maze model and light dark model, respectively. Increased number of squares traversed in open field model reinforced its anxiolytic potential. Further TI [AE] at both doses improved cognitive function with respect to spatial and working memory processes in Morris water maze test. One-way ANOVA followed by Dunnett's or Bonferroni test was applied to test statistical significance. The results indicate that TI [AE] has potential adaptogenic and anti-stress activity along with anxiolytic and nootropic potential. **References:** [1] Juvekar A. R. and Nachankar R. S. (2005) Acta Hort. (ISHS), 680: 49 – 55. [2] Parle M. et al. (2004) Indian J. Pharm. Sci, 66: 371 – 375. [3] Kishore K. et al. (2005) Indian J. Exp. Biol., 43: 640 – 645.

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Ameliorative effect of *Piper longum* fruits against experimental myocardial oxidative stress-induced injury in rats

Wakade AS, Juvekar AR, Kulkarni MP, Shah AS
Department of Pharmaceutical Sciences & Technology, University Institute of Chemical Technology, Matunga, Mumbai 400019, India

The present study was undertaken to investigate the possible protective effect of a methanolic extract of *Piper longum* fruits (PLM) on isoproterenol-induced myocardial infarction in rats. PLM was administered orally to Wistar rats in two different doses (250 mg/kg, 500 mg/kg) for 21 days followed by subcutaneous administration of isoproterenol (85 mg/kg.). Isoproterenol administration was manifested in significant increase in myocardial lipid peroxide levels as well as significant decrease in the activity of the myocardial marker enzymes viz. alanine transaminase, aspartate transaminase, lactate dehydrogenase and creatine kinase with a concomitant increase in their activity in serum. All these levels were restored in PLM treated rats. Isoproterenol administration also had a significant effect on lipid profile as evidenced by increased triglycerides, total cholesterol, LDL cholesterol and VLDL cholesterol levels with a significant decrease in HDL cholesterol levels. These levels were significantly restored by pretreatment with PLM. Activities of heart antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and reduced glutathione were significantly lowered owing to the myocardial infarction in isoproterenol treated rats. PLM also prevented the isoproterenol-induced decrease in antioxidant enzymes in the heart and improved lipid profile. One-way ANOVA followed by Bonferroni test was applied to test statistical significance. The histopathological studies on heart revealed degenerative changes and cellular infiltrations in the isoproterenol group and the pretreatment with PLM reduced the intensity of such lesions in dose dependent manner. The results show that pretreatment with *Piper longum* may be useful in preventing the damage induced by isoproterenol in rat heart. **References:** [1] Sheela, C. S. et al. (2001) Indian J Pharmacol., 32: 198 – 201. [2] Sharma, M. et al. (2001) Mol Cell Biochem, 225: 75 – 79. [3] Saxena, K. K. et al. (1988) Indian J Exp Biol., 26: 235 – 238.

P 443

Antioxidant and cardioprotective effect of *Leptadenia reticulata* against adriamycin-induced myocardial oxidative damage in rat experiments

Wakade AS, Juvekar AR, Hole RC, Nachankar RS, Kulkarni MP
Department of Pharmaceutical Sciences & Technology, University Institute of Chemical Technology, Matunga, Mumbai 400019, India

The present study was intended to investigate the *in vitro* antioxidant effect and plausible cardioprotective effect of a methanolic extract of *Leptadenia reticulata* (LRM) in experimental rats using biochemical and histopathological approaches. LRM was evaluated for *in vitro* antioxidant activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, nitric oxide and hydroxyl radical scavenging activity. LRM exhibited prominent antioxidant activity by scavenging or inhibiting the DPPH, nitric oxide and hydroxyl radical. Further, LRM was investigated for its possible protective effect against adriamycin-induced (ADR) cardiotoxicity in rats. Wistar rats were administered with LRM at doses of 250 mg/kg and 500 mg/kg for 28 days followed by *intra peritoneal* injection of adriamycin (10 mg/kg). Oxidative stress in the myocardium of ADR treated rats was evidenced by decreased levels of the marker the enzymes viz. alanine transaminase, aspartate transaminase, lactate dehydrogenase and creatine kinase in heart homogenate with concomitant increase in these enzyme levels in serum. The basal levels of myocardial antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase were also reduced by ADR. Myocardial reduced glutathione was found to be decreased with consequent increase in lipid peroxidation due to ADR administration. Pretreatment with LRM significantly inhibited the alterations in the aforementioned biochemical parameters. One-way ANOVA followed by Bonferroni test was applied to test statistical significance. The histopathology revealed that LRM pretreatment inhibited adriamycin-induced degenerative changes and cellular infiltrations in heart in dose dependent manner. The results indicated that ADR treatment markedly impaired cardiac function and LRM prevented this toxicity, which might be due to the virtue of its antioxidant activity. **References:** [1] Koima S. et al. (1993) J. Pharmacol. Exp. Ther., 266: 1699 – 1704. [2] Goodman J. et al. (1977) Biochem. Biophys. Res. Commun., 77: 797 – 803. [3] Zhang X. Y. et al. (2005) J. Pharm. Pharmacol., 57: 1043 – 52

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Effects of *Kaempferia parviflora* supplement on semen production and reproductive system of boar

Kupittayanant S[1], Kupittayanant P[2], Lijuan W[1], Buddhakala N[1], Phaopongthai J[3]
[1]Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand; [2]Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand; [3]Institute of Chemical Research, Thanyaburi Rajamangala University of Technology, Thanyaburi, 12120, Thailand

Kaempferia parviflora (KP) grows exclusively in Thailand. The plant is traditionally used in men for its supposed fertility-enhancing properties. However, its effects on improving reproduction in farm animals such as boar have not been investigated. The aim of this study was to investigate 1) the effects of KP supplement on semen quantity and quality, the reproductive organ weight and histology, and sexual behavior of boar and 2) the testosterone-like effect of KP by comparing with the effect of the male reproductive hormone, testosterone. With this purpose, 12 boar at 32 weeks of age were randomly assigned to receive: (1) a control diet; (2) a control diet supplemented with 1% of KP; (3) a control diet with a single injection of testosterone (0.2 mg/kg body wt.), daily for one month. Boar, which received KP had a greater semen volume than the control group and that of the hormone-treated group ($p < 0.05$). Testicular weight of the KP group and the group that received testosterone were lesser than that of the control group ($p < 0.05$). However, there was no singnificant difference in testicular histology among the groups. KP feeding and testosterone injection were likely to increase serum testosterone level. However, this did not reach statistical significance. Neither KP feeding nor testosterone injection increased libido of boar. We conclude that dietary treatment with KP has no adverse effects on the observed reproductive system and that it has no testosterone-like effect on reproduction of boar.

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Antidiabetic and antilipidemic potentials of the aqueous extract of fresh leaves of *Clerodendrum capitatum* in rats

Adeneye AA[1], Adeleke TI[2], Adeneye AK[3]
[1]Department of Pharmacology, Lagos State University College Of Medicine, P.M.B. 21266, Ikeja, Lagos, Nigeria; [2]Department of Pharmacognosy, College of Medicine Of the University of Lagos, Idi-Araba, P.M.B. 12003, Surulere, Lagos, Nigeria; [3]Public Health Division, Nigerian Institute of Medical Research, P.M.B. 2013, Yaba, Lagos, Nigeria

AIM: Diabetes mellitus and obesity, the two most common endocrine disorders of carbohydrate metabolism, are increasing on global scale [1]. Several medicinal plants are employed in the African traditional medical treatment of this condition, one of which is the cold water decoction of *Clerodendrum capitatum* (Willd.) Schumach. et. Thonn. var. *capitatum* (Verbenaceae) (CC). Despite its ancestral use in diabetes treatment, scientific validation of CC's efficacy in the disease treatment is lacking. Thus, the present study was designed to investigate CC's blood glucose and lipids lowering effects in normal Wistar rats. **Methods:** In the current study, hypoglycemic and hypolipidemic effects of fresh leaves aqueous extract of CC were studied in adult Wistar rats weighing 120 – 150 g by administering graded oral dosing (100, 400 and 800 mg/kg/day) for 14 days. Phytochemical analysis of CC was conducted using standard procedures while the preliminary acute oral toxicity study was also conducted using limit dose test of up and down procedure at a limit dose of 5000 mg/kg body weight/oral route [2]. Data were analyzed using two-ways analysis of variance on statistical software program, SYSTAT 10.2. **Results:** Results of the study showed CC to cause significant ($p < 0.05$) dose dependent hypoglycemic and hypolipidemic effects. The hypoglycemic effect of the extract could either be via enhanced insulin secretion or peripheral utilization of glucose. Although, CC did not cause any death up to 5000 mg/kg body weight/oral route, but was associated with transient somatomotor and behavioral toxicities. Phytochemical studies of CC showed the presence of saponins, flavonoids, alkaloids, tannin, glycosides and reducing sugars. The hypoglycemic and hypolipidemic effects of CC could be due to the presence of alkaloids, flavonoids and/or other biological principles it contains in high concentrations. The present result is similar to that reported for *Clerodendrum colebrookianum* Walp [3]. CONCLUSION: The folkloric use of *C. capitatum* in the treatment of suspected type 2 diabetics has a positive correlation with scientific data generated in this study. **References:** [1] Ahima, R.S. (2006), Gastroenterology 131, 991 [2] Acute Oral Toxicity (OECD Test Guideline 425) (2001),http://www.oecd.org/oecd/pages/home/displaygeneral/0,3380,EN-document-524-nodirectorate-no-24 – 6775 – 8,FF.html [3] Sharma, D.K., Bhuyan, S.K. (2006), Planta Med. 72, 1041.

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Protective effect of leaf and seed aqueous extract of *Phyllanthus amarus*

Adeneye AA[1], Benebo AS[2], Agbaje EO[3]
[1]Department of Pharmacolgy, [2]Department of Morbid Anatomy, Lagos State University College of Medicine, PMB 21266, Ikeja, Lagos State, Nigeria; [3]Department of Pharmacology, College of Medicine Of the University of Lagos, Idi-Araba, Surulere, Lagos State, Nigeria

AIM: Various parts of *Phyllanthus amarus* (PA) Schum. and Thonn. (Euphorbiaceae), are attributed with diverse phytotherapeutic properties including hepatoprotective functions [1 – 4]. The present study aimed at investigating the hepatoprotective effect of leaf and seed aqueous extract of PA on alcohol-induced hepatotoxicity in rats. **Methods:** In the present study, 50 – 300 mg/kg of body weight/oral of the leaf and seed aqueous extract of PA was investigated for its hepatoprotective activities on ethanol-induced liver injured, non-hepatectomised adult male Wistar, for 7 days. Of the five groups of six young adult Wistar rats each, four groups had alcohol hepatotoxicity induced by repeated daily oral dosing with 5 g/kg of 50% ethanol for 14 days. Three groups of treated rats were then orally administered 50 – 300 mg/kg/day of PA for additional 7days. Hepatotoxicity enzyme markers consisting of serum transaminases (AST, ALT), alkaline phosphatase (ALP) and serum triglyceride (STG), were assayed for after blood samples were obtained by cardiac puncture under diethyl ether anesthesia. Data were analyzed using two-ways analysis of variance on statistical software, SYSTAT 10.2. **Results:** Oral treatment of rats with 5 g/kg of body weight/day of 50% ethanol for 14 days reliably established ethanol-induced hepatotoxicity as evidenced by significant ($p < 0.05$) elevations in the serum liver enzymes (except ALP which was unaffected) and STG as well as the observed typical ethanol-induced liver injury histopathological lesions. However, oral treatment of rats with PA (50 – 300 mg/kg of body weight/day) significantly ($p < 0.05$) restored hepatic function to normal by bringing the serum levels of AST, ALT and STG back to normal. The hepatoprotective role of PA was also confirmed by histopathological findings. The present study lends support to that reported for the whole plant of *P. amarus* in rats [5]. CONCLUSION: Results of this study validate its folkloric use in the treatment of patients with suspected alcoholic liver disease. **References:** [1] Adeneye, A.A. et al. (2006), Fitoterapia 77: 511 – 514. [2] Odetola, A.A., Akojenu, S.M. (2000), African Journal of Medicine and medical Sciences 29: 119 – 122. [3] Thyagarajan, S.P. et al. (1988), Lancet 2: 764 – 766. [4] Joy, K.L., Kuttan, R. (1998), J. Bioch. Nutr. 24: 133 – 139. [5] Chattopadhyay, P. et al. (2006), International Journal of Pharmacology 2: 426 – 430.

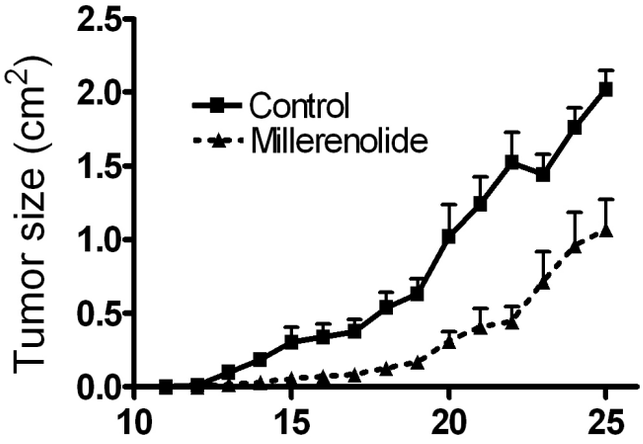
P 447

Evaluation of the anticancer activities of two sesquiterpene lactones, millerenolide and thieleanin, isolated from plants of the Asteraceae family

Dupuy O[1], Bonilla J[2], Murillo R[3], Taylor P[4]
[1]Laboratorio de Fisiología Animal e Inmunobiología Dr. Erich Graetz, Universidad de Panamá, Panamá; [2]Centro para Investigaciones en Biología Celular y Molecular (CIBCM), Universidad de Costa Rica, San José, Costa Rica; [3]Escuela de Química, Universidad de Costa Rica, San José, Costa Rica; [4]Centro de Medicina Experimental, Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 21827, Caracas 1020-A, Venezuela

We studied the anticancer properties of two sesquiterpene lactones, millerenolide (a germacranolide) and thieleanin (a guaianolide) both *in vitro* and *in vivo*. They were isolated from two plants of the Asteraceae family which is known for its anti-inflammatory properties [1]. These lactones showed a similar pattern of cytotoxicity in A549 human lung cancer cells and in 3T3/HER2 cells (3T3 cells transfected with the HER2 oncogene). The parent 3T3 cells and the B16/BL6 mouse melanoma cells were less sensitive to these compounds. Treatment with millerenolide (8 mg/kg, i.p. days 0, 2 and 4 postinoculation) significantly inhibited the growth of subcutaneous B16/BL6 tumors in C57BL/6 mice (50% inhibition at day 25, $P = 0.015$), as well as retarding the appearance of a detectable tumor (millerenolide – day 15.2 ± 0.4 vs. control – day 12.8 ± 0.5, $P = 0.011$). In contrast, treatment with thieleanin neither retarded the appearance of the tumor nor its growth. This is one of the first demonstrations of an anticancer effect of a sesquiterpene lactone *in vivo* and we propose that this effect may be due to mechanisms other than direct cytotoxicity [2].

IC_{50} (μM)	Mill.	Thiel.
B16/BL6	115	> 200
A549	40	32
3T3	54	166
3T3/HER2	28	9



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Antidiabetic activity of a methanol extract of unripe fruits of *Diospyros peregrina* Gurke. (Ebenaceae) in streptozotocin induced diabetic rats

Dewanjee S[1], Maiti A[1], Kundu M[1], Mandal SC[1]
[1]Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India

Diospyros peregrina Gurke. is a moderate sized tree of costal West Bengal, India. The maceration of unripe fruits is successfully employed by traditional people for the treatment of diabetes. The intention of the study is to explore the antidiabetic efficacy of a methanolic extract prepared from unripe matured fruits of *Diospyros peregrina* (MEDP) and also to establish a correlation to reduction of oxidative state associated with diabetes. MEDP was administered orally at doses of 150 and 300 mg/kg b. w. for twelve consecutive days to streptozotocin induced diabetic rats [1]. Fasting blood glucose levels were estimated on day 1, 5, 9 and 12. Serum lipid profiles and pancreatic thiobarbituric acid reactive substances (TBARS) [2] were measured after the animals were sacrificed on day 12. The results showed a statistically significant ($^*p < 0.01$) antidiabetic potential of extract in term of reduction of fasting blood glucose level of diabetic rats and comparable to that of standard drug glibenclamide. Serum lipids, pancreatic TBARS levels were significantly reduced in extract-treated diabetic rats. All results are tabulated hereunder.

	Fasting blood glucose level (mg/dl)				Cholesterol (mg/dl)	Triglycerides (mg/dl)	TBARS (μmol/g)
Group	1st day	5th day	9th day	12th day			
Diabetic control	253.8 ± 5.2	262.7 ± 4.5	272.2 ± 2.9	274.7 ± 2.8	105.8 ± 3.0	99.0 ± 5.6	4.8 ± 0.2
Diabetic + MEDP 150	258.8 ± 4.3	194.7 ± 5.8*	156.7 ± 2.9*	128.3 ± 3.2*	66.2 ± 2.0	74.8 ± 3.1*	3.1 ± 0.2*
Diabetic + MEDP 300	267.2 ± 5.7	179.3 ± 6.0*	146.3 ± 4.8*	118.5 ± 4.2*	61.5 ± 2.5*	64.8 ± 1.7*	2.1 ± 0.2*
Diabetic+ glibenclamide	275.8 ± 5.2	171.3 ± 4.4*	130.7 ± 3.9*	108.7 ± 3.2*	57.5 ± 2.4*	53.2 ± 2.8*	2.2 ± 0.2*

Acknowledgements: The authors are thankful to the World Bank through TEQIP program of Jadavpur University, Kolkata, India for financial assistance. **References:** [1] Siddque, M. et al. (1987) J pharm Sci 76: 341 – 345. [2] Hiroshi, O. et al. (1979) Anal Biochem 5: 351 – 358.

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Enhancement of agrostin toxicity by saponinum album is not based on stimulation of phagocytosis in differentiated U-937 cells as shown by flow cytometry

Weng A, Melzig MF
Free University Berlin, Institute of Pharmacy, Königin-Luise-Str. 2+4, D 14195 Berlin, Germany

Cytotoxicty of agrostin, a ribosome-inactivating protein (RIP) from *Agrostemma githago* L, was shown to be the result of a toxic synergism between agrostin and special triterpenesaponins [1] like saponinum album from *Gypsophila* species with a formyl group attached at position 4. Since latrunculin A from *Latrunculia magnifica* inhibited phagocytosis in macrophages [2] and also hampered synergistic cytotoxicity between agrostin and saponinum album in ECV-304 cells [3], it was hypothesized, that saponinum album stimulates phagocytosis. To examine this assumption we investigated the influence of saponinum album on phagocytosis of fluorescence labelled *Escherichia coli* (*E.coli*, K-12 strain) and 1 μm latex beads in U-937, which were differentiated with IFN-γ (interferon gamma) or PMA (phorbol myristate acetate). Both particles (*E.coli* and latex beads) were either opsonized with IgG or not. Fluorescence of the internalized particles was measured by flow cytometry and cells were examined by fluorescence microscopy. Saponinum album did not enhance the uptake of fluorescence labelled *Escherichia coli* and latex beads in U-937 cells indicating that the saponin has no stimulatory activity on phagocytosis in general. **References:** [1] Hebe-

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Antioxidant activity of extracts of *Cynomorium songaricum* and their neuroprotective effects against cytotoxicity induced by $A\beta_{25-35}$ on SK-N-SH neuroblastoma cells

Yi L[1,2], *Jenett-Siems K*[2], *Melzig MF*[2], *Qingguo W*[1]
[1]*School of basic medicine, Beijing University of Chinese Medicine, 100029, Beijing, China;* [2]*Institut fuer Pharmazie, Freie Universitaet Berlin, 14195 Berlin, Germany*

Cynomorium songaricum Rupr. (Cynomoriaceae) is known as a traditional medicine treating dementia in China. In this study, we investigated the antioxidant activity of different extracts of cynomorii herba as well as their protective effects against $A\beta_{25-35}$ induced toxicity on SK-N-SH cells. Method: *C. songaricum* was extracted with methanol. After evaporation, the residue was partitioned between dichloromethane, ethyl acetate and water, respectively. In order to study the antioxidant activity, we used the xanthine-xanthine oxidase system (XO/XTT) [1]. The reaction was initiated by the addition of XO solution and the absorbance change at 470nm was monitored spectrophotometrically. For investigating possible neuroprotective effects, the SK-N-SH cells were cultured at a density of 1.5×10^4 cells/well in 96-well plates [2]. The cells were treated with the 4 extracts of *C. songaricum* at concentrations of 100 µg/ml, 10 µg/ml, 1 µg/ml and 0.1 µg/ml 4 h before addition of the $A\beta_{25-35}$ (20 µM). Cell viability was quantified using the MTT test after 96hrs. Results and discussion: The methanol as well as the EtOAc- extracts of *C. songaricum* were effective in scavenging superoxide anions generated by the XO/XTT system (IC_{50}= 21.2 µg/ml for the MeOH-extract and 2.8 µg/ml for the EtOAc-extract). But even more important, they significantly protected the SK-N-SH cells against $A\beta_{25-35}$ induced cytotoxicity. As neuronal damage by free radicals as well as direct neurotoxicity of $A\beta_{25-35}$ may play a key role in neurodegeneration observed in Alzheimer dementia, these in vitro results with different extracts of *C. songaricum* might support its traditional use in the AD therapy in China. **References:** [1] Ukeda H, Maeda S. et al. (1997) Anal Biochem 251(2):206 – 9 [2] Ba F, Pang PK (2003) J Neurosci Meth 123(1):11 – 22

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Screening of some Yemeni medicinal plants for inhibitory activity against neutral endopeptidase

Alasbahi R[1], *Melzig MF*[2]
[1]*Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University. P.O. Box 19065, Sana'a, Yemen;* [2]*Institute of Pharmacy, Free University Berlin, Königin-Luise- Str. 2+4. D-14195 Berlin, Germany*

Due to the physiological importance of neutral endopeptidase (NEP) in the modulation of nociceptive and pressor responses as well as intestinal secretory mechanism, there is great interest in searching for inhibitors of NEP as novel analgesic, anti-hypertensive, and antidiarrheal agents [1, 2, 3]. The purpose of our study was therefore to screen 15 extracts of different polarity (dichloromethane, methanol, and aqueous extracts) from 5 Yemeni medicinal plants (*Aspilia helianthoides* leaves, *Ceropegia rupicola* whole plant, *Kniphofia sumarae* whole plant, *Pavetta longiflora* leaves, and *Plectranthus* cf *barbatus* leaves) for inhibitory effect against NEP activity. Enzyme activity was determined according to Bormann and Melzig [4]. A series of concentrations (200, 100, 50, 25, 10, and 1 µg/ml) of each extract were tested. The remaining activity of the enzyme was calculated as a percentage to the control without inhibitor, considering the influence of the solvent (DMSO as solvent for the dichloromethane and methanol extracts), and test extract. Three independent tests with triplicate parallel samples were performed. All values were expressed as mean ± standard deviations. Wilcoxon's U-test was used to test the significance. The IC_{50} values were obtained from dose-effect curves by linear regression. Out of 15 extracts, 4 extracts (methanol extracts of *Ceropegia rupicola*, IC_{50}= 111 µg/ml, *Kniphofia sumarae*, IC_{50}= 141 µg/ml and *Plectranthus* cf *barbatus*, IC_{50}= 139 µg/ml and the aqueous extract of *Pavetta longiflora* IC_{50}= 144 µg/ml) were found able to inhibit the enzymatic activity of NEP. Although the active plants are used traditionally for other purposes e.g. skin and inflammatory diseases and as haemostatic in Yemen [5], the presence of NEP inhibitory constituents in those plants is supported by reports of related species used in other localities as analgesic, antidiarrheal, and antihypertensive agents, and for the treatment of congestive heart failure (6, 7,8). **References:** [1] Noble F., Roques BP. (2007) Expert Opin Ther Targets 11: 145 – 59. [2] Veelken R., Schmieder R E. (2002) J Hypertens. 20: 707 – 14. [3] Farthing, MJ. (2006) Dig Dis 24: 47 – 58. [4] Bormann H., Melzig MF (2000) Pharmazie 55: 129 – 32. [5] Fleurentin J., Pelt J M. (1982) J Ethnopharmacol 6: 85 – 108. [6] Lukhoba, CW. et al. (2006) J Ethnopharmacol 103: 1 – 24. [7] Amos, S. et al. (2003) Biological & Pharmaceutical Bulletin 26: 1674 – 1680. [8] Sukumar, E. et al. (1995) Fitoterapia 66: 403 – 6.

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Studies on anti-proliferative effects of phthalides from *Ligusticum chuanxiong* in hepatic stellate cells

Lee TF[1], *Huang YT*[1], *Lin YL*[2]
[1]*Institute of Traditional Medicine, National Yang-Ming University, 155, Li-Nong Street, Section 2, Taipei, Taiwan;* [2]*National Research Institute of Chinese Medicine, 155 – 1, Li-Nong Street, Section 2, Taipei, Taiwan*

Aims: Suppression of hepatic stellate cell (HSC) growth and activation and induction of apoptosis have been proposed as therapeutic strategies for the treatment and prevention of liver fibrosis. Our previous study showed that a Chinese herb *Ligusticum chuanxiong* (LC) inhibits platelet-derived growth factor (PDGF-BB)-induced HSC proliferation. The present study was designed to investigate the active principles and their action mechanisms. With a bioactivity-directed fractionation approach, DNA synthesis (bromodeoxyuridine (BrdU) incorporation), cell cycle related proteins and apoptosis markers were determined to evaluate the inhibitory effects of active principles of LC. Two phthalides, Z,Z'-6,8',7,3'-diligustilide (**1**) and levistolide A (**2**), from LC significantly abrogated PDGF-BB-induced proliferation in both rat and human HSC lines. These inhibitory effects of compounds **1** and **2** were associated with reduction of α-smooth muscle actin and collagen expressions. The cell cycle promoting proteins, cyclins D1, D2, E, A and B1, were downregulated while the inhibitory proteins p21 and 27 up-regulated. JNK phosphorylation was up-regulated by compounds **1** and **2**. In HSC-T6, the two compounds induced apoptosis through the activation of caspases 9 and 3 with cytochrome *c* release, and downregulation of Bcl-2 and Akt phosphorylation. Moreover, both phthalides did not cause direct cytotoxicity to either HSCs or rat primary hepatocytes under experimental concentrations. Conclusion: These results indicate that two phthalides from LC inhibited PDGF-BB-activated HSC proliferation possibly through cell cycle inhibition and apoptosis mechanisms. They might be potential anti-fibrotic drugs for the treatment and prevention of hepatic fibrosis.

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Study of free radical scavenging activity of extracts of leaves of *Bergenia* by HPTLC-DPPH$^{\cdot}$ method

Ivanova SA[1], *Pozharitskaya ON*[1], *Shikov AN*[1], *Makarov VG*[2]
[1]*St-Petersburg Institute of Pharmacy, 27 – 422, Partizanskaya str, 195248, St.-Petersburg, Russia;*[2]*Interregional Center "Adaptogen",47/5, Piskarevskiy pr, 195067, St-Petersburg, Russia*

Leaves of Siberian plant *Bergenia crassifolia* (L). Fritsch showed antimicrobial, immunostimulating and adaptogen activities [1]. Prominent feature of *Bergenia* is simultaneous presence both green

(young), and black (wintered) leaves on one plant. The aim of this study was to assess the antioxidant properties of green and black *Bergenia* leaves. Total phenols were estimated according to the Folin-Ciocalteu method. The qualitative- quantitative analysis made by HPTLC plates with toluene-ethyl acetate-formic acid-methanol 3:3:0.8:0.2 v/v/v/v as mobile phase. Free radical scavenging activity of methanol extracts of bergenia leaves was examined using HPTLC-DPPH$^{\cdot}$ method [2]. HPTLC-densitometry and post chromatographic derivatization of plates in HPTLC-DPPH$^{\cdot}$ method were developed and validated. The ID_{50} values (the dose of the compounds required to scavenge 50% of DPPH$^{\cdot}$) were calculated (Table). Content and free radical scavenging activity of phenol compounds of bergenia leaves:

Sample	Total phenols, %	%	Arbutin		Gallic acid		Hydroquinone	
		inhibition of DPPH$^{\cdot}$	contents, %	ID_{50}, nmol	contents, %	ID_{50}, nmol	contents, %	ID_{50}, nmol
Green leaves	4.95 ± 0.25	62	7.00 ± 0.60	0.879	0.10 ± 0.01	0.488	not detected	1.227
Black leaves	3.25 ± 0.16	65	not detected		0.50 ± 0.04		1.00 ± 0.08	

No significant association between the total phenolic content and DPPH$^{\cdot}$ radical scavenging for green and black leaves extracts of *Bergenia* was observed. The components arbutin, gallic acid, bergenin and insignificant amount of ellagic acids were identified in green leaves using authentic standards. In black leaves gallic acid, hydroquinone, bergenin and insignificant amount of ellagic acid were detected. All compounds excluding coumarin bergenin were capable to scavenge DPPH$^{\cdot}$ radicals. The radical sceving activity of green leaves extract is due to the high concentration of arbutin and that of black leaves extract to the high concentration of gallic acid. **References:** [1] Popov SV. et al. (2005) Phytother. Res. 19: 1052 – 1056. [2] Pozharitskaya ON. et al. (2007) J Sep Sci. 30; in press.

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Epilobium parviflorum Schreb. – *in vitro* study of biological action

Hevesi TB[1], Houghton PJ[2], Habtemariam S[3], Milligan S[4], Kalita CJ[4], Purohit A[5], Kéry Á[1]

[1]*Department of Pharmacognosy, Semmelweis University, Üllői u. 26., H-1085 Budapest, Hungary;* [2]*Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, 150, Stamford Street, London SE1 9NH, UK;* [3]*Pharmacognosy and Phytotherapy Research Laboratories, Department of Chemical and Pharmaceutical Sciences, Medway School of Science, The University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK;* [4]*Endocrinology and Reproduction Research Group, School of Biomedical Sciences, King' s College, London SE1 1UL, UK;* [5]*Endocrinology and Metabolic Medicine and Sterix Ltd., Faculty of Medicine, Imperial College, St. Mary's Hospital, London W2 1NY, UK*

Epilobium parviflorum Schreb. (Onagraceae) is used for the treatment of benign prostatic hyperplasia (BPH), which is regarded as an endocrine disorder caused by age-related hormone imbalance and increased oxidative damage [1,2,3]. *Epilobium* can moderate the obstructive and the irritative symptoms of BPH [1] but its biological action is not entirely identified. *E. parviflorum* is rich in phytosterols, flavonoids (myricetin, quercetin, kaempferol and their glycosides), phenolic acids, catechins, ellagi- and gallotannins [4]. The potential biological effects of *Epilobium parviflorum* Schreb. have been investigated, in respect to its antioxidant, anti-inflammatory, enzyme-inhibitory and anti-androgenic effect. The whole-plant water extract showed higher antioxidant effect (IC_{50}= 1.65 ± 0.05 µg/mL) in DPPH assay than Trolox or ascorbic acid and inhibited the lipid peroxidation examined in TBA assay (IC_{50}= 2.31 ± 0.18 mg/mL). In concentrations 0.20 – 15.00 µg/mL the extract possessed a protective effect comparable to catalase enzyme (2500 IU/mL), against oxidative damage generated on fibroblast cells. The examination of the COX-inhibitory effect showed that *E. parviflorum* had an anti-inflammatory effect (IC_{50}= 1.38 ± 0.08 µg/mL). Investigation of steroid receptor binding ability and the aromatase enzyme-inhibition showed negative results in the concentration range examined. **References:** [1] Vitalone, A. et al. (2001) Il Farm. 56: 483 – 489. [2] Schalken, J. (2006) Eur. Urol. Suppl. 5: 729 – 736. [3] Aydin, A. et al. (2006) Clin. Biochem. 39: 176 – 179. [4] Hansel R, Rimpler H. (1993) Hagers Handbuch der Pharm. Prax. Springer-Verlag. Germany.

P 455

Anti-ulcer activity of aqueous ethanol extract of *Peperomia pellucida* in *Sprague dawley* rats

Abdul Hamid R[1], Zakaria N[1], Zuraini A[1]

[1]*Department of Biomedical Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

*Peperomia pellucida (*Piperaceae*)*, locally known as 'ketumpangan air' in Malaysia is a herbaceous plant which grows well in loose and humid soils under the shade of trees. In folk medicine, this species is used to treat abscesses, boils and skin wounds. The aerial part of the plant is boiled and the decoction is effective for arthritis and gout problems[1]. Previous studies done on this plant extract have shown prominent activities in anti-inflammatory and analgesic properties [2, 3]. An aqueous ethanol extract of the aerial part of *Peperomia pellucida* (PPE) was tested for anti-ulcerogenic effects in rats, using 0.2 M NaOH, EtOH 80%, 0.6 M HCl, NaCl 25% and 30 mg/kg indomethacin. Administration of 10, 30, 100 and 300 mg/kg doses of PPE was found to provide a dose-dependent protection against the ulcerogenic effects of different necrotizing agents. However, it did not show any dose-dependency for indomethacin-induced ulceration. The extract showed to possess the highest protective effects with 84.8% inhibition against indomethacin-induced ulceration, 99.3% inhibition against HCl-induced ulceration, 99.4% against NaCl-induced ulceration, 98.7% against NaOH – induced ulceration and 96.8% inhibition against ethanol-induced ulceration. The protective effect against indomethacin-induced ulceration of ranitidine 50 mg/kg is as potent as the effects of PPE at 10 mg/kg. Furthermore, at 30, 100 and 300 mg/kg, PPE exhibited similar potency when induced with HCl, NaCl and NaOH. However, it gave a slight difference of inhibitory effects when induced with ethanol. At 300 mg/kg, PPE was almost completely protected from any visible gastric damages. Thus, PPE may possess mucoprotective and antioxidative effects due to its ability to reduce ulcers induced by those 4 necrotic agents. Since it ubiquitously possessed anti-inflammatory and analgesic properties, it is not possible for the plant to also act as COX-2 inhibitor as it reduced the ulcer induced by a common NSAID, indomethacin which gives side effect of gastrointestinal problem in patients after long term consumption. *Acknowledgements:* Research Management Centre, Universiti Putra Malaysia, Vote 531584 **References:** [1]. Bojo, A.C. et al. (2004) Asia Life Sci. 3, 35 – 44 [2]. Aziba, P.I et al. (2001) Fitoterapia 72, 57 – 58 [3]. Arrigoni-Blank et al. (2004) J. Ethnopharmacology 91, 215 – 218

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Aged Garlic Extract Enhances Endothelial Nitric Oxide Release via Increased Tetrahydrobiopterin Bioavailability

Papatheodorou L, Morihara N, Ide N, Koelle P, Weiss N

Medizinische Poliklinik Innenstadt, Klinikum der Universität München, Pettenkoferstr. 8a, D-80336 München, Germany

Introduction: Aged garlic extract (AGE) has been shown to enhance endothelial nitric oxide (NO) production in mice [1] and to restore endothelial dysfunction due to impaired NO bioavailability in humans with acutely elevated homocysteine (hcy) levels [2]. As reduced NO bioavailability during elevated hcy seems to be due to reduction of the NO synthase cofactor tetrahydrobiopterin (BH_4) leading to "uncoupling" of the enyzme [3] we hypothesized that AGE may preserve endothelial BH_4 levels via its antioxidant and thiol-modifying properties, thereby increasing NO release. **Methods:** Human endothelial cells (EA.hy 926) were incubated for 24 h with hypoxanthine, aminopterin, thymidine and methionine (HAT/MET)

to increase cellular hcy levels, and with and without AGE (5 mg/mL). Cellular levels of hcy, BH_4, glutathione, and total thiols were measured by HPLC, and endothelial NO production using the fluorescent probe DAF-2. **Results:** Incubation of endothelial cells with HAT/MET resulted in a significant 2-fold increase in cellular hcy levels (from 0.17 ± 0.03 to 0.35 ± 0.08 μmol/mg), coincubation with AGE had no significant effect on hcy in both control and HAT/MET treated cells. Elevated cellular hcy went along with significantly decreased levels of BH_4 (2.23 ± 0.28 vs. 4.34 ± 0.64 pmol/mg). Incubation with AGE slightly increased BH_4 in control cells (5.33 ± 0.65 pmol/mg), and prevented the decline in BH_4 in HAT/MET treated cells (5.23 ± 0.71 pmol/mg). AGE increased cellular levels of total thiols and glutathione, and prevented HAT/MET induced decrease in endothelial NO release. **Conclusions:** AGE maintains NO bioavailability in endothelial cells even under conditions of elevated hcy levels via increasing cellular BH_4 levels, thereby maintaining normal endothelial function. This may contribute to AGE's antiatherosclerotic properties. *Acknowledgement:* Supported by Wakunaga of America, Mission Viejo, CA., USA. **References:** [1] Morihara N. et al. (2002) Life Sci 71: 509 [2] Weiss N. et al. (2006) J Nutr 136: 750S [3] Topal G et al. (2004) Free Radic Biol Med. 36: 1532.

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Aged Garlic Extract Inhibits CD 36 Expression and Foam Cell Formation in Human Macrophages

Ide N, Morihara N, Paptheodorou L, Stirner R, Weiss N
Medizinische Poliklinik Innenstadt, Klinikum der Universität München, Pettenkoferstr. 8a, D-80336 München, Germany

Introduction: During formation of early atherosclerotic lesions, expression of CD 36, a class B scavenger receptor on macrophages, is crucially involved in the uptake of oxidized low-density lipoprotein (oxLDL) and foam-cell formation. Aged garlic extract (AGE) has been shown to inhibit the progression of coronary calcifications in patients with coronary artery disease [1]. We hypothesized that AGE may inhibit the differentiation of human monocytes (THP-1 cells) into macrophages, the expression of CD 36, and the cellular uptake of oxLDL [2]. **Methods and Results:** THP-1 cells were stimulated for 72 h with phorbol 12-myristate 13-acetate (PMA, 10 nmol/L) in the absence or presence of AGE (5 g/L) to differentiate them into macrophages. CD 36 expression, as measured by flow cytometry, was significantly suppressed by $61.8 \pm 13.9\%$ in AGE treated cells. Dexamethasone (10 nmol/L) was used as a negative, and troglitazone (500 nmol/L) as a positive control. THP-1 cells in the presence of PMA were incubated with or without AGE for 72 h, followed by incubation with DiI-labeled OxLDL (50 mg/L) for 3 h, and the fluorescence intensity was measured by flow cytometry. AGE significantly inhibited DiI-oxLDL uptake into PMA-stimulated THP-1 cells by $85.6 \pm 2.8\%$. As expression of CD 36 on macrophages is at least partly regulated by the peroxisome proliferator-activated receptor-γ (PPARγ) pathway, we performed electrophoretic mobility shift assays to identify the binding of nuclear proteins to a consensus oligonucleotide DNA sequence contained in PPARγ responsive elements (PPRE). AGE inhibited the binding of nuclear proteins to a consensus PPRE sequence compared to PMA-stimulated controls. Troglitazone and GW-9662 (20 μmol/L) were used as the respective positive and negative controls. **Conclusions:** These data indicate that AGE inhibits CD 36 expression and oxLDL uptake in macrophages via modulation of the PPARγ pathway. This suggests a mechanism by which the extract could reduce the formation and progression of atherosclerotic lesions, as shown in a recent clinical trial [1]. *Acknowledgement:* Supported by Wakunaga of America, Mission Viejo, CA., USA. **References:** [1] Budoff, M. et al. (2004) Prev Med 39: 985 – 91. [2] Ide, N. et al. (2006) J Nutr 136: 755S-8S.

P 458

A gamma-glutamyl peptide from onion inhibits the development and activity of osteoclasts in vitro

Langos M, Hofstetter W, Dolder S, Felix R, Mühlbauer RC, Brenneisen R
Department of Clinical Research, University of Berne, Murtenstrasse 35, Berne, CH-3010, Switzerland

Recently we have shown that supplementing the diet of rats with onion powder (*Allium cepa* L.) leads to a significant inhibition of bone resorption [1]. Gamma-glutamyl-propenyl-cysteine-sulfoxide (GPCS) was identified as the active compound by bioassay-guided fractionation [2]. In the present study we further characterized the effects of GPCS on osteoclasts (OC) in vitro. OC development was examined by culturing mouse bone marrow cells (BMC) supplemented with macrophage colony-stimulating factor (M-CSF; 30 ng/ml), receptor activator of NF-κB ligand (RANKL; 5 ng/ml) and varying doses of GPCS. To establish structure-activity relationship and specificity, other dipeptides were tested with the same assay, too. The effect of GPCS on the activity of OC was assessed by determining the number of actin rings in isolated rat OC attached to glass coverslips and by quantifying the number of pits formed by OC cultured on dentin slices. GPCS inhibited osteoclastogenesis from BMC at concentrations ≥ 1 mM ($n = 12$; $p < 0.05$). Sulfur containing compounds with structural similarity to GPCS reduced the number of newly formed OC (gamma-glutamyl-cysteine-ethylester: ≥ 0.3 mM; glycyl-cysteine: 10 mM; allyl-cysteine: 10 mM). Furthermore, compounds without sulfur (aspartyl-phenylalanine-methylester, glycyl-valine, gamma-glutamyl-glycine) did not affect osteoclastogenesis. The number of actin rings formed on glass after pre-incubation for 3 h was not changed by treatment with 2 and 8 mM GPCS for 10 and 25 min, respectively. When 2, 4, and 8 mM of GPCS was added to OC cultured on dentin slices, 8 mM GPCS reduced significantly resorption per OC (number of pits/OC; control: 100 %± 30; 8 mM GPCS: 51 %± 27; mean ± SD, $n = 16$; $p < 0.05$). In conclusion, the data demonstrate that the protective effects of onion on bone may be caused at least in part by a direct effect on the cells of the OC lineage, resulting in an inhibition of osteoclastogenesis and the activity of mature OC. **References:** [1] Mühlbauer RC, Li F (1999) Nature 401: 343 – 344**;** [2] Wetli HA, Brenneisen R et al. (2005) J Agric Food Chem 53: 3408 – 3414

P 459

Ethnopharmacological and phytochemical studies of medicinal plants from Vanuatu

Bradacs G, Heilmann J
Institute of Pharmaceutical Biology, University of Regensburg, Universitaetsstrasse 31, 93040 Regensburg, Germany

Vanuatu is a Melanesian archipelago in the South Pacific with a north-south-length of 1,100 km, spanning different climate zones. The resulting biodiversity of these islands made it a unique target for an ethnobotanical survey during which information about 131 traditionally used plants was collected. Seventeen medicinal plants *Acalypha grandis* Benth. (leaves), *Aidia racemosa* (Cav.) D. D. Tirveng. (leaves), *Allophylus timorensis* (Dc.) Bl. (leaves), *Alpinia sp.* (rhizomes), *Baccaurea stylosa* Lour. (bark and leaves), *Dracontomelon vitiense* Engl. (bark), *Dysoxylum arborescens* (Bl.) Miq. (leaves), *Evodia latifolia* D. C. (leaves), *Grewia inmac* Guillaumin (leaves), *Gyrocarpus americanus* Jacq. (leaves), *Intsia bijuga* (Colebr.) O. Ktze. (leaves), *Macaranga dioica* Muell. Arg. (bark and leaves), *Macaranga tanarius* (L.) Muell. Arg. (leaves), *Macropiper latifolium* (stalks), *Pipturus argenteus* (Forst. f.) Wedd. (bark), *Syzygium malaccense* (L.) Merr. & Perry (leaves) and *Tabernaemontana pandacaqui* Poir. (leaves) were collected for a pharmacological and phytochemical screening based on their ethnomedicinal use. Dichloromethane-, ethyl acetate- and methanol-extracts of these plant parts were produced via an ASE (accelerated solvent extraction) and the extracts are currently under pharmacological investigation. The screening program comprises assays for antifungal, antimicrobial, antiprotozoal and acetylcholi-

nesterase inhibitor potential. Also the MTT cytotoxicity assay is used to screen activity of the extracts against ten human cancer cell lines out of the NCI-60 cell lines panel, which consists of human tumor cell lines representing leukemia, melanoma and cancers of the brain, breast, colon, lung, kidney, ovary and prostate. *Acknowledgements:* The authors are grateful to the government and the Cultural Centre of Vanuatu for their assistance with the field research and we thank our informants for sharing their knowledge with us.

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Effect of safranal from *Crocus sativus* on extracellular hippocampal levels of glutamate and aspartate during kainic acid seizures in anesthetized rats

Hosseinzadeh H[1], Sadeghnia HR[2]
[1]Pharmaceutical Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, P.O. Box: 1365 – 91775, Mashhad, I.R. Iran;[2]Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

An abnormal release of excitatory amino acids (EAA) is involved in the generation and expression of some epileptic seizures. In a previous study [1], we showed that safranal, a constituent of *Crocus sativus*, has a protective effect against both, clonic and tonic phases of pentylenetetrazole (PTZ)-induced seizures in rats. In this study, the effect of safranal pretreatment on concomitant changes in the extracellular hippocampal levels of EAA (glutamate and aspartate) following systemic administration of kainic acid (KA) was investigated in anesthetized rats, using a microdialysis method combined with liquid chromatography (HPLC). Safranal (72.75 mg/kg or 291 mg/kg, ip) was injected 40 min before KA (15 mg/kg, ip). The basal hippocampal concentrations of glutamate and aspartate were estimated to be 0.45 μM and 0.29 μM, respectively. Basal EAA levels were not affected by pretreatment with safranal. Following KA injection, there was a significant increase ($p < 0.001$, Tukey-Kramer test) in the extracellular glutamate and aspartate levels (about 5 times and 3 times, respectively) at 80 min after injection. However, kainite-evoked release of EAA was significantly reduced by safranal (291 mg/kg, ip; $p < 0.001$). The results showed that acute systemic injection of safranal reduces the extracellular concentrations of glutamate and aspartate in the rat hippocampus during seizures induced by KA. **Reference:** 1. Hosseinzadeh, H. Sadeghnia HR. (2007) Phytomedicine 14: 256 – 262.

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Molecular mechanism of action of stilbene compounds from *Cajanus cajan* leaves in cancer cell lines

Ashidi JS[1], Houghton PJ[1], Hylands PJ[1], Sieber S[2], Efferth T[2]
[1]Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom; [2]Pharmaceutical Biology of Natural Products Group (C015), German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

Introduction: Cancer is a major cause of death worldwide. We have earlier reported the *in-vitro* cytotoxicity of two prenylated stilbene components, longistylins A and C, from *Cajanus cajan* (L.) Millsp. leaves, in some solid tumour cell lines [1]. The present work reports the mechanism by which these compounds induce apoptosis in the human T-lymphoblastic leukaemia cell line CCRF-CEM and the multi-drug resistant subline CEM/ADR5000. **Method**: The compounds were isolated and their structures confirmed as described previously [1]. Their anti-proliferative potential was assessed by the XTT assay. Nicoletti assay was used to assess the impact of the compounds on cell cycle after 24 h incubation. A combination of flow cytometry and Western blots was used to unravel their mechanism. Only longistylin A was used for the Western blot assay. **Result**: The $ICBB_{50BB}$ of the compounds tested in the XTT assay ranged between 5 and 10 μM. They did not show any cross-resistance against CEM/ADR5000 cells. Like most stilbenoids, longistylins A and C induced apoptosis of the leukaemia cells by arresting the GBB_{2BB}/M cell cycle, generated significant reactive oxygen species (ROS) and upregulated Fas receptors which consequently led to significant apoptosis. A dose-dependent downregulation of YY1, a transcription factor of extrinsic pathway molecules in apoptosis was observed with longistylin A. There was also significant upregulation of anti-catalase protein. The mitochondria membrane potential ($\Delta\Psi BB_{mBB}$) remains uncompromised. **Conclusion**: Our findings lend support to the local use of *C. cajan* in prevention and therapy of cancer. *Acknowledgement*: Commonwealth Scholarship Commission; King's College London, United Kingdom for financial support of JA. **Reference:** 1. Ashidi J.S. *et al*.(2006), Planta Med.,**72**(11):P016.

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Effects of *Ficus asperifolia* Miq. and *Gossypium arboreum* L. extracts relevant to wound healing

Annan K, Houghton PJ
Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom

Traditional people in Ghana use decoction and poultice prepared from *Ficus asperifolia* Miq. and *Gossypium arboreum* L. to treat a variety of skin ailments, including wounds. Wound healing process involves several steps, including coagulation, inflammation, formation of granulation tissue and remodelling of connective tissue. Fibroblast cells play an important role in majority of these processes. Other factors, like microbial contamination and presence of reactive oxygen species (ROS) may affect the healing process. In this study, *in vitro* methods were used to assess the fibroblast stimulatory, antioxidant and antimicrobial properties of methanol extracts of the two plant species. Using human dermal fibroblast 142BR, *F. asperifolia* and *G. arboreum* extracts (5 μg/ml) were found to increase cell proliferation by 31 and 37% respectively compared to the control (0.5% FBS) [1]. The two plant extracts protected 142BR cell line against hydrogen peroxide-induced damage in an *in vitro* antioxidant assay [1]. At 20 μg/ml, *F. asperifolia* and *G. arboreum* extracts significantly protected 142BR cell line against hydrogen peroxide-induced damage, giving 47% and 58% survival respectively compared to 0% in cells with no extract. *G. arboreum* exhibited antibacterial action against both Gram Positive and Gram Negative bacteria with MIC range 128 – 512 μg/ml in a 96-well microtitre dilution assay [2]. *F. asperifolia* however, showed no antibacterial action. Our findings lend support to the local use of these plants in wound healing. *Acknowledgement:* The Ghana Government for the financial support of KA. **References:** [1] Houghton, P.J. et al. (2005) J. Ethnopharm. 100: 100 – 107. [2] Dickson, R.A. et al. (2006) Phytother. Res. 20: 41 – 45.

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In vitro and *in vivo* wound healing actions of *Paullinia pinnata* L

Annan K[1], Houghton PJ[1], Govindarajan R[2]
[1]Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom; [2]National Botanical Research Institute, Lucknow, 226 001 India

Chronic wounds present a major health burden and a drain on resources worldwide. In developing countries however, many of the indigenes rely on natural products to treat their wounds[1]. *Paullinia pinnata* L. is one of such species used in Ghana. We here report a scientific investigation of this plant in wound healing, using *in vitro* and *in vivo* methods. Human dermal fibroblast cell line 142BR was grown in MEM/15%FBS/1% L-glutamine and maintained at 37°C in a humidified incubator of 5%CO_2:95% air. The effect of different concentrations of *P. pinnata* methanolic extract on cell proliferation was assessed after a 5 day incubation period using the Neutral Red assay

[1]. *In vivo* activity was evaluated using excision and incision wound models in Sprague-Dawley rats following topical application of 30% w/v of *P. pinnata* extract [2]. The plant extract caused a significant increase (94%) in 142BR cell line proliferation at 20 µg/ml compared to the control. There were also a significant increase in tensile strength and hydroxyproline content of healing tissue as well as a decrease in epithelisation period and scar area, compared to the control. *Acknowledgement:* Ghana Government Scholarships Scheme for KA. **References:** [1] Houghton, P.J. et al. (2005) J. Ethnopharm. 100: 100 – 107. [2] Reddy, J.S. et al. (2002) J. Ethnopharmacol. 79: 249 – 251

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Anti-tyrosinase activity of South African plant extracts

Lall N, Mapunya MB[2], Nikolova RV[2], Houghton PJ[3]
[1]University of Pretoria, Department of Botany, 0002, South Africa; [2]University of Limpopo, School of Molecular and Life Sciences, Turfloop campus, Private Bag X1106, Sovenga, 0727, South Africa; [3]Pharmaceutical Sciences Division, Kings College London, 150 Stamford Street, London SE1 9NH, UK

Tyrosinase is known to be the key enzyme in melanin biosynthesis. Melanin is the pigment that is responsible for the colour of eyes, hair and skin in humans. Over-activity of this enzyme leads to dermatological disorders such as age spots, melasoma and site of actinic damage [1]. Ten plants belonging to four families (Asphodelaceae, Anacardiaceae, Olaceae and Rutaceae) were investigated on their effect on tyrosinase using both L-tyrosine and L-DOPA as substrates [1]. Ethanol leaf extracts of *Aloe aculeate, Aloe pretoriensis* and *Aloe sessiliflora* showed 31%, 17% and 13% inhibition of tyrosinase activity respectively at 0.5 mg/ml when L-tyrosine was used as a substrate. Extracts of leaves and bark of *Harpephyllum caffrum* showed inhibition of 90% and 92% respectively at 0.25 mg/ml. *H. caffrum* (leaves) at a concentration of 0.5 mg/ml had an inhibitory effect of 70% on tyrosinase when L-DOPA was used as a substrate. Following the results obtained from the tyrosinase assay, extracts from *H. caffrum* were selected for further testing on their effect on melanin production and their cytotoxicity on melanocytes *in vitro*. Fifty percent inhibitory (IC_{50}) concentration of both extracts was found to be 1.5×10^{-3} mg/ml for melanocyte cells. Bark and leaf extracts of *H. caffrum* showed 26% and 20% reduction respectively in melanin content of melanocyte cells at a concentration of 6×10^{-3} mg/ml. Therefore, both extracts of *H. caffrum* could be considered as anti-tyrosinase agents for dermatological disorders such as age spots, melasoma and pigmentation disorders. *Acknowledgements:* National Research Foundation, University of Pretoria, University of Limpopo. **Reference:** 1. Nerya, O. et al. (2003) J. Agric. Food Chem, 51: 1201 – 1207.

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In-vitro and *in-vivo* wound healing properties of two plants from Ghana

Dickson RA[1], Houghton PJ[1], Govindarajan R[2]
[1]Pharmacognosy Research, Pharmaceutical Sciences Research Division, KCL, 150 Stamford St, SE1 9NH London, UK; [2]Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow 226001, India

Leaves of two plants, namely *Caesalpinia benthamiana (Caesalpiniaceae)* and *Securinega virosa (Euphorbiaceae)*, traditionally used in the treatment of wounds in Ghana, have been assessed using both in-vitro and in-vivo bioassay techniques. Microbial infections and ROS are known impediments to wound healing [1]. Extracts of these two plants, as well as cassane diterpenes isolated from Caesalpinia benthamiana, demonstrated antimicrobial and antioxidant activities as well as modulating the activities of some currently used antibiotics against which resistance has developed. The effect of extract ointments obtained from the two plants using 2% nitrofurazone ointment as standard, in both the excision and incision wound model experiments were assessed by measuring parameters including the period of epithelization, wound or scar area, tensile strength and percentage closure of excision wounds. The results of the in vivo wound-healing assay revealed that the test extract ointments at the selected concentration (100 mg/500mm2), were capable of producing remarkable wound-healing activity on both wound models. The results also revealed that the measurement of healed area and the hydroxyproline content were in agreement, in that the most active extract (total extract of *C. benthamiana*), gave the tissue with highest hydroxyproline content of 12.98 ± 1.86 mg. This extract had the lowest scar area of 31.85mm2 ± 2.78. These findings thus support the traditional uses of the plants in the treatment of wounds. *Acknowledgements:* Commonwealth Scholarship Commission, UK for financial support. **Reference:** 1. Houghton, P.J. et al.(2005). J. Ethnopharmacol, 100: 100 – 107.

P 466

In vitro hypoglycaemic and ACE inhibitory activities of *Marrubium radiatum* Devile ex Benth *and Salvia acetabulosa* L.: two traditional medicinal plants from Lebanon

Rosa Loizzo M[1], Tundis R[1], Menichini F[1], Piccolo V[1], Bonesi M[1], Conforti F[1], Marrelli M[1], Statti GA[1], Saab AM[2], Nicoletti M[3], Houghton PJ[4], Menichini F[1]
[1]Department of Pharmaceutical Science, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, I-87030 Rende (CS) Italy; [2]Chemistry Department, Faculty of Sciences II, Lebanese University, P.O. Box:90656 Fanar, Beirut, Lebanon; [3]Department of Plant Biology, Faculty of Pharmacy, University "La Sapienza", P.le Aldo Moro, 5, 00185 Rome, Italy; [4]Pharmaceutical Sciences Division, King's College London, 150 Stamford Street, London SE1 9NH, UK

Marrubium radiatum Devile ex Benth *and Salvia acetabulosa* L. (Lamiaceae) were used for treatment of hypertension and diabetes in Lebanon traditional medicine. In order to find a scientific validation of their traditional use, *in vitro* assays for α-amylase and α-glucosidase inhibition were performed while the antihypertensive action was analysed by the inhibition of Angiotensin Converting Enzyme (ACE) [1 – 3]. Plant material was extracted using methanol or *n*-hexane as solvent [4]. The ACE inhibitory activity was measured through the cleavage of the chromophore-fluorophore labelled substrate dansyltriglycine by ACE preparation from rabbit lung (EC 3.4.15.1) into dansylglycine, which is quantitatively measured by HPLC. *M. radiatum* showed a IC_{50} of 72.79 and 75.42 µg/mL while *S. acetabulosa* exhibited a IC_{50} of 52.71 and 105.22 µg/mL for MeOH and *n*-hexane, respectively. The glucose absorption, from the intestine to the blood is elated to the α-amylase and α-glucosidase activity. Both *M. radiatum* and *S. acetabulosa* are able to inhibit α-amylase. In particular the MeOH extract exert highest activity with a IC_{50} of 61.12 and 91.16 µg/mL. The ability of *M. radiatum* and *S. acetabulosa* MeOH extract to inhibit α-glucosidase was weak (IC_{50} of 365.95 and 315.89 µg/mL). In conclusion, this study supports the traditional use of these species and further work is necessary in order to identify active principles responsible for the found activities. **References:** [1] Conforti, F. et al. Biol. Pharm. Bull.(2005) 28: 1098. [2] Anonymous, Sigma Tech. Bull No. 510 6/76 (1978). [3] Elbl, G. & Wagner, H. Planta Med. (1991) 57: 137. [4] Choi, H.K. et al. Phytochem. (2004) 65: 857.

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Synthesis and Angiotensin Converting Enzyme (ACE) inhibition activity of chalcone derivatives

Bonesi M[1], Loizzo MR[1], Tundis R[1], Conforti F[1], Menichini F[1], Marrelli M[1], Statti GA[1], Michel S[2], Menichini F[1]

[1]Department of Pharmaceutical Science, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, I-87030 Rende (CS) Italy; [2]Laboratoire de Pharmacognosie de l'Université René Descartes, U.M.R./ C.N.R.S. No. 8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, F-75006 Paris, France

Hypertension is a common and often progressive disorder that poses a major risk for cardiovascular and renal disease [1,2]. It is well recognized that the Renin-Angiotensin System (RAS) has an important role in cardiovascular physiology, water-electrolyte balance, and cell function. Excessive activation of this system has been considered to be a main cause of hypertension. Angiotensin Converting Enzyme (ACE) is the most important regulatory site of RAS [3]. Aldolic condensations of 3,4,5-trimethoxy-acetophenone with appropriately substituted benzaldehydes were carried for synthesize a set of chalcone derivatives with a series of substituents on the B-ring, using commercially available compounds. The *in vitro* ACE inhibitory activity was measured trough the cleavage of the chromophore-fluorophore labelled substrate dansyltriglycine by Angiotensin I-Converting Enzyme preparation from rabbit lung (EC 3.4.15.1) into dansylglycine, which is quantitatively measured by HPLC [4]. The most active compound was **7** (IC_{50} 0.219 mM), it was substituted with amino group in position R_1 and a methoxylic group in position R_2. The high activity was conserved when the amino group was substituted with an hydroxylic group as in **4** (IC_{50} 0.225 mM). Chalcone **5** showed an IC_{50} 0.246 mM on ACE, this compound was characterized by an hydroxylic group in position R_2 and a methoxylic group in position R_1. The absence of hydroxylic group in position R_1 as in **3** cause a reduction of ACE inhibition activity (IC_{50} 0.574 mM) when you compared with **4**. We believe that the current finding would be important start point for design of ACE inhibitor as new therapeutic agent. **References:** [1] Chalmers, J. (1999) Blood Press 8: 9. [2] Odama, U. et al. (2000) J. Clin. Hypertens. 2: 312. [3] Schricker, K. et al. (1994) Hypertension, 24: 157. [4] Elbl, G. & Wagner, H. (1991) Planta Med. 57: 137.

P 468

Comparative chemical composition, antioxidant activity and acetylcholinesterase inhibition of *Citrus medica* L. cv. Diamante and *Citrus bergamia* Risso

Menichini F, Loizzo MR, Tundis R, Bonesi M, Conforti F, Marrelli M, Statti GA, Menichini F

Department of Pharmaceutical Science, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, I-87030 Rende (CS) Italy

Cultivation of *Citrus medica* L. cv. Diamante and *Citrus bergamia* Risso species are limited to Calabria, Southern Italy. The *n*-hexane peel extracts of both species were analysed by GC-MS. The Diamante citron peel extract is characterized by the presence of four monoterpenes as main components: limonene, γ-terpinene, nerol and geraniol. The sesquiterpene fraction was less represented; the major constituents were β-bisabolene and α-bergamotene. The coumarin citropten was also found. *C. bergamia* peel was characterized by the presence of monoterpenes linalool, linalyl acetate, limonene, γ-terpinene, α-pinene and β-pinene and the coumarin bergaptene. Antioxidant activity was measured through the DPPH assay [2]. *C. medica* peel extract showed an IC_{50} of 147 μg/mL while low activity was found when *C. bergamia* peel extract was used. Inhibition of AchE was assessed by a modified colorimetric method of Ellman [4]. *C. medica* L. cv. Diamante peel extract exerted an interesting activity against AchE with an IC_{50} of 621 μg/mL. On the contrary the same extract obtained from *C. bergamia* showed less activity with IC_{50} of 5.02 mg/mL. Limonene, the most abundant compound of *C. medica* L. cv. Diamante peel extract, showed an inhibition of 22.6% at 1.2 mM. **References:** [1] Conforti, F. et al. (2005) Biol. Pharm. Bull. 28: 1791. [2] Perry, N.S. et al. (1992) J. Pharm. Pharmacol. 52: 895.

P 469

Cytotoxic extracts of *Salsola oppositifolia* Desf. (Amaranthaceae) against non-small lung carcinoma (CORL-23) and melanoma (C32) cells

Tundis R, Loizzo MR, Bonesi M, Conforti F, Menichini F, Marrelli M, Statti G, Menichini F

Department of Pharmaceutical Sciences, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, I-87036 Arcavata di Rende (CS), Italy

The search of natural products for cancer therapy represents an area of great interest in which plants have been the most important source. In the continuing search for cytotoxic compounds from plants in the present investigation we reported the cytotoxic activity of extracts from *S. oppositifolia* against CORL-23 and C-32 tumor cell lines. The *Salsola* genus (Amaranthaceae) comprises about 120 species; they are very diffused annual herbaceous plants, especially in the brackish grounds of the moderate and subtropical regions of Europe, Asia, Africa and north America. *Salsola* species are well known in traditional medicine and nowadays have been widely used with a large variety of beneficial effects, such as anticancer, diuretic, anti-hypertensive, and anti-inflammatory [1 – 4]. Dried aerial parts were extracted with MeOH at room temperature. The extract was dissolved in a $MeOH/H_2O$ (9:1) mixture and partitioned with *n*-hexane, dichloromethane, and ethyl acetate. The cytotoxicity was evaluated using the sulforodamine B (SRB) assay [5]. The test is based on the estimation of cell number indirectly by providing a sensitive index of total cellular protein content which is linear to cell density. The most active extracts to inhibit proliferation of non-small lung carcinoma cell line were the dichloromethane and *n*-hexane extracts with IC_{50} values of 31.90 μg/mL and 19.09 μg/mL, respectively. The dichloromethane extract was able also to inhibit proliferation of melanoma cell line with IC_{50} value of 36.29 μg/mL. The chemical composition of the active extracts was reported. **References:** [1] Al-Saleh, F.S., et al. (1993) Fitoterapia LXIV 3: 251 – 256. [2] Nikiforov S.B., et al. (2002) Pharm. Chem. J. 36: 544 – 554. [3] Beloborodova E.I., et al. (2000) Klin. Med. 78: 56 – 9. [4] Fu S. (1959) Zhonghua Nei Ke Za Zhi, 7: 977 – 981. [5] Monks, A. et al. (1991) J. Nat. Cancer Institute, 83: 757 – 66.

P 470

Antioxidant activity of *Capparis ovata* Desf. and *Cynara cardunculus* L. ssp. *cardunculus*

Marrelli M[1], Conforti F[1], Tundis R[1], Loizzo MR[1], Bonesi M[1], Menichini F[1], Vaccaro A[1], Statti G[1], Curini M[2], Menichini F[1]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, I-87030 Rende (CS) Italy; [2]Department of Chemistry and Drug Technology, Faculty of Pharmacy, University of Perugia, 06123 Perugia Italy

Capparis ovata Desf. and *Cynara cardunculus* L. ssp. *cardunculus* are edible plants from Calabria (Italy) traditionally used in local medicine. The antioxidant activity of the samples was carried out using three different in vitro assays. The plant aerial parts were air dried at room temperature and then extracted with 70% aqueous EtOH through maceration. In order to determine the radical scavenging potency the extracts were investigated with the DPPH assay [1]. The antioxidant activity was assessed by the bovine brain peroxidation assay and the ß-carotene bleaching test. The lipid peroxidation activity was evaluated using the thiobarbituric acid (TBA) test [2,3]. Total extracts were tested for their activity against liposomes which were prepared from bovine brain extract in phosphate buffered saline. In the ß-carotene bleaching test the ability of samples to inhibit the linoleic acid oxidation was investigated [4]. As regard the free radical (DPPH) scavenging activity, *Capparis ovata* Desf. extract

showed an IC_{50} of 114 µg/mL while *Cynara cardunculus* L. ssp. *cardunculus* exhibited an IC_{50} value of 72 µg/mL. Using liposomes prepared from bovine brain extract, *Capparis ovata* Desf. and *Cynara cardunculus* L. ssp. *cardunculus* showed a good activity with IC_{50} value of 86 µg/mL and 37 µg/mL respectively. In the ß-carotene bleaching test both *Capparis ovata* and *Cynara cardunculus* showed a good activity (IC_{50} value of 9 µg/mL and 12 µg/mL after 30 min, and IC_{50} value of 16 µg/mL and 15 µg/mL after 60 min of incubation). Total phenolics and flavonoids content was also evaluated and correlated to biological activities [5,6]. **References:** [1] Wang, M. et al. (1998) J. Agric. Food Chem. 46: 4869. [2] Fernandez, J. et al. (1997) Food Chem. 59: 345. [3] Conforti, F. et al. (2002) Fitoterapia. 73: 479. [4] Amin, I. et al. (2004) Food Chem. 87: 581. [5] Singleton, V.L. et al. (1965) Am. J. Enol. Vitic. 16: 144. [6] Quettier-Deleu, C. et al. (2000) J Ethnopharmacol. 72: 35.

P 471

Neuroprotective effects of a butanol fraction prepared from *Opuntia ficus-indica* var. *saboten*

Jin C[1], Jung SY[2], Lee SM[1], Kim HJ[1], Cho J[3], Lee YS[2]
[1]Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, Seoul, 130 – 650, Korea; [2]College of Pharmacy, Kyung Hee University, Seoul, 130 – 701, Korea; [3]College of Medicine, Dongguk University, Gyeongju, 780 – 714, Korea

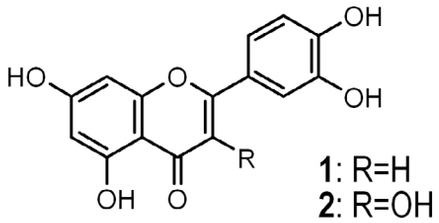
The methanol extract [1] and butanol fraction [2] prepared from *Opuntia ficus-indica* (L.) Mill. *var. saboten* Makino (Cactaceae) and their antioxidative constituents [3] were shown to exert neuroprotective effects against oxidative injuries induced in cortical cell cultures. Given the suggested role of free radicals in neuronal death after ischemic stroke, we attempted to examine neuroprotective effects of the butanol fraction (OFB901) prepared from 50% ethanol extract of the stems in a rat model of transient focal cerebral ischemia. Transient focal cerebral ischemia was induced by occlusion of middle cerebral artery for 2 hr with a silicone-coated 4 – 0 nylon monofilament in male Sprague-Dawley rats under isoflurane anesthesia. OFB901 was administered at a dose of 100, 200, 300 or 500 mg/kg (p.o., twice/day) starting 2 hr after reperfusion for 7 or 14 days. Seven serial coronal slices of the brain were stained with 2,3,5-triphenyltetrazolium chloride and infarct size was measured using a computerized image analyzer. In the 7-day treatment regimen, OFB901 significantly reduced both infarct volume and brain shrinkage only at a dose of 300 mg/kg compared with a vehicle-treated control group, producing neurobehavioral recovery effect. In the 14-day treatment regimen, OFB901 produced significant neuroprotective effects at doses of both 200 and 300 mg/kg, showing enhanced neuroprotective potency than in the 7-day treatment regimen. The results suggest that the butanol fraction prepared from *Opuntia-ficus indica var. saboten* can be developed as a potential oral neuroprotective agent by providing neuroprotection against chronic focal ischemic brain injury. *Acknowledgements:* This research was supported by a grant (PF0320202 – 01) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean Government. **References:** [1] Wie, M.-B. (2000) Yakhak Hoiji, 44: 613 – 619. [2] Cho, J. et al. (2007) Biol. Pharm. Bull. Submitted. [3] Dok-Go, H. et al. (2003) Brain Res. 965: 130 – 136.

P 472

Protective properties of quercetin and luteolin from *Petasites japonicus* leaves against Aβ (25 – 35)-induced neurotoxicity in B103 cells

Jun M[2], Hur JM[1], Yang EJ[1], Lee DG[1], Lee SY[1], Song KS[1]
[1]College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702 – 701, Korea; [2]Division of Food Science, Dong-A University, 840 Hadan-2-dong, Saha-gu, Busan 604 – 714, Korea

Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by progressive dementia that inevitably leads to incapacitation and death [1]. A pathologic hallmark of AD is the formation of extracellular senile plaques composed of 40 – 42 amino acid Aβ peptides, a product of amyloid precursor protein (APP) proteolysis [2]. Aβ fragments have shown to induce oxidative stress and inflammation in the brain, which are postulated to play important roles in the pathogenesis of AD [3]. In the course of screening antidementia agents from natural products, two compounds with the potent protective activity toward Aβ (25 – 35)-induced neurotoxicity were isolated from the ethyl acetate soluble fraction of *Petasites japonicus* leaves. Open column chromatographic separation with silica gel (Merck Art. 7734, CH_2Cl_2-CH_3OH = 10:1 to 4:1) afforded two active principles. By means of ^{1}H-NMR, ^{13}C-NMR and LC/MS spectral analyses, they were identified as luteolin (**1**) and quercetin (**2**). At the concentration range of 1 – 50 µM, **1** and **2** remarkably raised survival rate of the Aβ (25 – 35)-treated B103 neuroblastoma cells in both 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and Hoechst 33342 staining. **1** and **2** also completely inhibited Aβ (25 – 35)-induced reactive oxygen species (ROS) generation in B103 cells at 50 µM. These results suggested that **1** and **2** might be a starting point for rational natural products-based drug design and be useful reagents for studying mechanism of Alzheimer's disease.



References: [1] Sisoida S.S. et al. (1995) FASEB J. 9: 366 – 370.. [2] Vassar R. (2002) Adv. Drug Deliv. Rev. 54: 1589 – 602. [3] Miranda S. et al. (2000) Prog. Neurobiol. 62: 633 – 648.

P 473

Hepatoprotective Action of Moringa Oleifera Lam

Uma NLG[1], Fakurazi S[1], Hairuszah I[2], Mohanambal M[1], Taufik MHB[1], Zulkhairi A[1], Sukardi S[3], Ganabadi S[4], Bahaman AS[5]
[1]Department of Human Anatomy, [2]Department of Pathology, [3]Department of Biomedical Science, Faculty of Medicine and Health Sciences, [4]Department of Preclinical Sciences, Faculty of Veterinary Medicine, [5]Department of Professional Development and Continuing Education, Faculty of Educational Studies, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia

Moringa oleifera Lam. (Moringaceae), commonly known as "Drumstick," is commonly used in Indian folk medicine for the treatment of various illnesses. The leaves of *M. oleifera* are reported to have high antioxidant [1] and hepatoprotective [2] activity. We have conducted a study to evaluate the hepatoprotective effect of an ethanolic extract of *M. oleifera* leaves on hepatocellular damage induced by acetaminophen in rats. Following administration of *M. oleifera* to the rats prior to challenge with acetaminophen, a significant protective action made evident by its effect on the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lipid peroxidation (LP) levels in the liver. Pre-treatment of *M. oleifera* has significantly reduced the level of AST

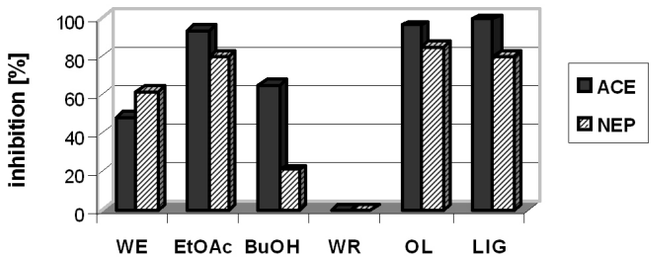
(165.17 ± 8.90U/L; $p < 0.05$) compared to that without the extract (531.85 ± 18.56U/L). ALT level was also significantly reduced (56.47 ± 2.15U/L; $p < 0.05$) compared to that without treatment. The alkaline phosphatase level was also reduced although it was not significant. Meanwhile, pre-treatment of the extract has significantly reduced the lipid peroxidation levels in the pre-treatment group (29.48 ± 0.77nmol/g; $p < 0.05$) compared to the group, which was not pre-treated (36.09 ± 1.01nmol/g). Consequently, the results obtained were supplemented by histopathological examination of liver sections. As a conclusion, *M. oleifera* extracts appear to protect the liver from the damage induced by acetaminophen. **References:** [1] Gupta, R. et al. (2007) Cell Biol Int 31(1): 44 – 56. [2] Ashok Kumar, N., Pari, L. (2003) J Med Food 6(3): 255 – 259

P 474

Dual inhibition of metallopeptidases (ACE and NEP) by iridoids from *Ligustrum vulgare* L. leaf

Kiss AK[1], Derwińska M[1], Melzig MF[2]
[1]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Faculty of Pharmacy, Medical University of Warsaw, 1 Banach St., 02 – 097 Warsaw, Poland [2]Free University Berlin, Institute of Pharmacy, Königin-Luise Str.2+4, 14195 Berlin, Germany

Ligustrum vulgare L. (Oleaceae) is an ornamental plant. In traditional medicine the leaves of privet were used for their anti-inflammatory, anti-rheumatic, diuretic and hypotensive activities. A recent study had confirmed, by anti-complementary test, the anti-inflammatory activity of the methanolic and ethyl acetate extracts and flavonoids from *Ligustrum* [1]. Angiotensin-converting enzyme (ACE) inhibitors are well established as antihypertensive drugs, whereas neutral endopeptidase (NEP) inhibitors prevent degradation of natriuretic peptides and enhance diuresis and natriuresis. Dual ACE/NEP inhibitors were developed as effective and broad-spectrum antihypertensive principles [2]. In order to confirm the traditional use of privet, we investigated the ACE and NEP inhibitory potency of different extracts. Powdered plant material was extracted with water and then with ethyl acetate and *n*-butanol saturated with water. All extracts were evaporated and lyophilised. The ethyl acetate extract showed the highest activity at a concentration of 100 µg/ml with 92 ± 3% and 80 ± 5% of ACE and NEP inhibition, respectively. The bioguided fractionation led to the isolation of two iridoids which were identified by ^{1}H, ^{13}C and HETCOR NMR spectroscopy as oleuropein and ligstroside aglycone. Both compounds are dual ACE/NEP inhibitors with IC_{50} of 20 and 25 µM for ACE and IC_{50} of 35 and 75 µM for NEP, respectively. Oleuropein and ligstroside, as well as flavonoids present in the ethyl acetate extract showed no or slight inhibitory activity. Our results partly support the diuretic and hypotensive activities of common privet.



Metallopeptidases inhibitory activity of extracts and isolated compounds from Ligustum vulgare leaf (100 ug/ml)

WE-aqueous extract, **EtOAc**-ethyl acetate extract, **BuOH**- butanolic extract, **WR**- aqueous residue, **OL**- oleuropein aglycone, **LIG** -ligstroside aglycone **References:** [1] Pieroni, A. et al. (2000) J Ethnopharmacol 70: 213. [2] Croti, R. et al. (2001) Circulation 104: 1856

P 475

Subchronic 90-day oral toxicity study of *Chelidonii herba* in pigs

Kosina P[1], Drabek J[2], Vicar J[1], Vostalova J[1], Ulrichova J[1], Simanek V[1]
[1]Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University in Olomouc, Hnìvotínská 3, 775 15 Olomouc, Czech Republic [2]Clinic of Swine Diseases, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého 1 – 3, 612 42 Brno, Czech Republic

Greater celandine (*Chelidonium majus* L., Papaveraceae) has been traditionally used in a range of conditions including disorders of the liver [1]. On the other hand acute hepatitis cases after a use of preparations with greater celandine were reported [2]. In this study we focused on the safety evaluation of Chelidonii herba (50 ppm) in swine, a model physiologically close to humans. Health status, hematological, biochemical, and histological parameters were monitored. Disposition of alkaloids in plasma and organs was evaluated for sanguinarine (SG) and chelerythrine (CHE), pharmacologically the most active components in *C. majus*. SG and CHE were found mostly in the feces (0.99 and 1.73 µg/g, resp.) and gingiva (0.08 and 0.05 µg/g, resp.). In plasma and various tissues alkaloid content was very low or undetectable. During the 90-day experiment no impairment of the animals' health status was observed. No statistically significant differences between control group and test group of animals were found for the weight gain and feed consumption. No significant differences between experimental and control animals were found by histological examinations in specimens of selected tissues. In plasma, globulin, creatinine, AST, GMT and cholesterol were significantly decreased in test group *vs* control group ($P <$ O.O5). This difference should not be related to the ingestion of SG and CHE as this was ruled out in our previous study, where high doses of SG and CHE were fed to pigs with no effect on above clinical biochemistry findings [3]. Consequently, alteration of clinical biochemistry parameters should originate from other Chelidonii herba components. *Acknowledgements: This work was supported by the Grant Agency of the MSMT (grant No. MSM 6198959216).* **References:** [1] Duke J.A. et al. (2002). Handbook of Medicinal Herbs. CRC Press, Boca Raton, p.168. [2] Stickel F. et al. (2003) Scand. J. Gastroenterol. 38, 565 – 568. [3] Kosina et al. (2004) Food Chem Toxicol. 42, 85 – 91.

P 476

Biological activity of extracts from defatted seeds of *Oenothera paradoxa*

Derwińska M, Kiss AK, Naruszewicz M
Department of Pharmacognosy and Molecular Basis of Phytotherapy, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha St., 02 – 097 Warsaw, Poland

Oenothera sp. (Oenotheraceae) is native to Central and South America and has been used for different medicinal purposes. The oil from seeds of *Oenothera paradoxa*, one species of evening primroses, has been shown to have several pharmacological effects such as anti-hypertension, anti-atherosclerosis, antidiabetic, anti-inflammatory, anti-premenstrual and anti-tumor effects. In the last years interest of this plant has been growing because of its content of polyphenols (flavonoids, phenolic acids and hydrolysable tannins). The aim of our study was to determine the antioxidant activity of an aqueous extract, isopropanolic extract and condensed fraction of polyphenols (CFP) of the defatted seeds of *Oenothera paradoxa*. Antioxidant capacity was assessed according to slightly modified version of the DPPH photometric assay and modified xanthine oxidase assay [1]. We also determined biological effects of mentioned above extracts against activity of enzymes: angiotensin-converting enzyme (ACE), aminopeptidase N (APN) and neutral endopeptidase (NEP) according to Bormann and Melzig [2]. All samples (50 – 1.65ug/ml) showed dose dependent free radical scavenging effect and dose dependent inhibitory effect on xanthine oxidase.

The strongest inhibition of xanthine oxidase activity was demonstrated by CFP (IC_{50}= 3.3 µg/ml) while the highest radical scavenging activity was obtained with isopropanolic extract (IC_{50}= 7.1 µg/ml). Each extract contained gallic acid as main compound. Pure gallic acid demonstrated the strongest antioxidant activity (IC_{50}= 1.1 µg/ml in DPPH trial and IC_{50}= 6.4 µg/ml in xanthine oxidase assay). All investigated extracts significantly inhibited peptidases activity. The strongest effect on ACE activity was exhibited by the CFP with IC_{50}= 26 µg/ml. The highest APN and NEP inhibitions were demonstrated by aqueous extract (IC_{50}= 2.8 µg/ml and IC_{50}= 4.7 µg/ml, respectively) while gallic acid showed a weaker activity against peptidases (IC_{50}> 100 µg/ml). The results of our study demonstrate a high biological activity of the extracts of defatted seeds of *Oenothera paradoxa*. **References:** [1] Choi C. W. et al. (2002) Plant Science 163, 1161 – 68, [2] Bormann H., Melzig M.F. (2000) Pharmazie 55: 129 – 32

P 477

Evaluation of cytotoxicity, phototoxicity and genotoxicity from *Calceolaria chelidonioides* (Scrophulariaceae) flowers ethanol extract

Falcão DQ[1,3], Costa ER[2], Kuster RM[1], Nielloud F[3], Vian L[3], Menezes FS[2,4]
[1]Núcleo de Pesquisas de Produtos Naturais – CCS – UFRJ, 21941 – 590, Brazil; [2]Departamento de Produtos Naturais e Alimentos, Faculdade de Farmácia – CCS – UFRJ, 21941 – 590, Brazil; [3]Faculté de Pharmacie, Université Montpellier I, 14491, France; [4]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland

Calceolaria chelidonioides is an original Brazilian plant belonging to the Scrophulariaceae family, which is used in the Brazilian folk medicine for the treatment of several kinds of cancer. Its cytotoxicity, photoxicity and genotoxicity potential were evaluated in different methodologies *in vitro* using the flowers ethanol extract. The cytotoxicity and phototoxicity were evaluated by the neutral red dye assay using keratinocyte human cells (NCTC 2544). For the phototoxicity evaluation the cell culture containing the compounding test was submitted to UVA radiation (345nm) during 15 minutes. The assays showed the cell viability after the treatment with the extract and its metabolites formed by the UV radiation. The genotoxicity potential was evaluated by two different methods, both of them suggested in the "Genotoxicity: a standard battery for genotoxicity testing of pharmaceuticals" guide [1]. The first one was the Comet assay [2,3] using keratinocyte human cells incubated for 1 hour with the extract, with and without metabolic activation using the S9 mix. The Comet assay is able to detect different kinds of DNA fragmentations caused by the genotoxic agents. The cells which DNA were damaged show an image comet like with a "head" and a "tail" that elongates proportionally to the DNA damages. The second method was the Ames' test [4] which is capable to detect compounds with carcinogenic and mutagenic properties using mutants *Salmonella typhimurium* strains to detect base substitution and frame shift point mutations. These tests were also evaluated with and without metabolic activation. The *C. calceolaria* flowers ethanol extract or even its metabolites didn't show any kind of toxicity in all tested models. *Acknowledgements:* CAPES, CNPq **References:** [1] ICH Harmonised Tripartite Guideline (1997) International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Brussels. [2] Rydberg B., Johanson K.J. (1978) DNA Repair Mechanisms. Academic Press. New York. [3] Östling O., Johanson K.J. (1984) Biochem Biophys Res Commun 123: 291 – 298. [4] Ames B.N., Maron D.O. (1983) Mutation Res 113: 173 – 215.

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Effects of Substance P and Neurotensin on the contractility of ileum of mice *in vitro*: Inhibition by plant extract STW 5 (Iberogast®)

Hagelauer D[1], Kelber O[2], Weiser D[2], Laufer S[3], Heinle H[1]
[1]Institute of Physiology, University of Tuebingen, Gmelinstrasse 5, 72076 Tuebingen, Germany; [2]Steigerwald Arzneimittelwerk GmbH, Scientific Department, Havelstr. 5, 64295 Darmstadt, Germany; [3]Institute of Pharmaceutical Chemistry, 72076 Tuebingen, Germany

Substance P (SP) and Neurotensin (NT) are neuromodulators which are released in the gastrointestinal tract. They influence the contractility of smooth muscle and they are involved in gastrointestinal inflammation. STW 5 (Iberogast®) is a phytotherapeutical drug, made of nine herbal plant extracts: peppermint leafs, camomile flower, liquorice root, angelica root, caraway fruit, milk thistle fruit, lemon balm leafs, greater celandine herb and a fresh herbal extract of Iberis amara. It is used in the treatment of motility disturbances as well in inflammation processes of the gastrointestinal tract. The aim of the present study is to demonstrate, firstly, the effects of substance P and neurotensin on the spontaneous contractility of ileum of mice *in vitro*, and, secondly, the effects of STW 5 on these induced reactions. Longitudinal muscle strips of the ileum are mounted in a perfused organ bath, and isometric contractions are recorded. First, the effects of SP [10, 100 nM] and NT [1, 10, 100 nM] on the spontaneous contractile activity are measured, thus obtaining the standard response, then the application is repeated to the same sample pretreated with STW 5 [10 µl/ml] or butylscopolamin (BSC) [10^{-5} M] for comparison. Transiently, SP provokes in both concentrations an increase of the amplitude P of the spontaneous contractility and a dose-dependent tonic contraction T. STW 5 inhibits P significantly, but not T. BSC exhibits only a blocking of the tonic contraction. NT causes a significant relaxation R of the basale tonus with a transient smaller amplitude P. Application of STW 5 inhibits R while P is persistent reduced. BSC does not show any effects on NT-induced reactions. SP and NT influence the contractility and motility pattern of the ileum significantly. STW 5 inhibits these induced reactions and could help at motility and inflammation disturbances. *Acknowledgement:* Supported by Alfred Teufel-Stiftung, Nagold, Germany

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Acetylcholinesterase inhibitor from *Clitoria ternatea*

Kumar V[1], Mukherjee K[1], Pal BC[2], Houghton PJ[3], Mukherjee PK[1]
[1]School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India; [2]Division of Medicinal Chemistry, Indian Institute of Chemical Biology, Kolkata 700032, India; [3]Pharmacognosy Research, Pharmaceutical Sciences Research Division, King's College London, 150 Stamford St, SE1 9NH London, UK

Clitoria ternatea L. (Fabaceae) is commonly known as 'Butterfly pea'. Extracts of *C. ternatea* have been used as an ingredient in 'Medhya Rasayana' as a rejuvenating recipe used for treatment of neurological disorders and considered as wholesome for intellect. *C. ternatea* has been shown to improve learning, memory and also increase the acetylcholine content of the hippocampus in rats. The aim of study was to isolate triterpenoids from *C. ternatea* and to determine their ability to inhibit acetylcholinesterase (AChE). The alcohol extract *C. ternatea* was fractionated and triterpenoid compounds were purified using column chromatography, IR and ESI-MS were used for the chemical characterization. The structure was elucidated by means of ^{1}H, ^{13}C, Cosy NMR and mass spectrometry. Taraxerol, a pentacyclic compound was tested for *in vitro* and *ex vivo* AChE inhibitory activity. The *in vitro* AChE inhibitory activity of taraxerol was determined by TLC and micro plate AChE assay based on Ellman's method [1]. Taraxerol and physostigmine was found to inhibit AChE activity in TLC assay. Taraxerol showed significant AChE inhibition and found to have IC_{50} values of 69.01 ± 4.26 µM/ml. Physostigmine was used as standard and showed inhibition of AChE with an IC_{50} value of

0.28 ± 0.015 μM/ml. Taraxerol and physostigmine were administered to male Wistar rats and *ex vivo* AChE activity was determined using rat brain homogenate preparations. The results of this *in vivo* treatment indicate that taraxerol and physostigmine caused a dose-dependent inhibition of brain AChE activity, and that physostigmine appeared to be a more potent inhibitor than taraxerol. Since improved learning and memory performance are related to acetylcholine levels, the AChE inhibitory effect of taraxerol from *C. ternatea* may account for its traditional use. *Acknowledgements: The authors wish to express their gratitude to the Department of Biotechnology, Govt. of India, New Delhi, India.* **References:** [1] Mukherjee, P. K., Kumar, V., Mal, M., Houghton, P. J., (2007) Planta Med.73: 283 – 285.

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Cognitive-enhancing activities of iridoid glycosides isolated from *Scrophularia buergeriana*

Jeong EJ[1], Lee KY[1], Kim SH[1], Ma CJ[1], Sung SH[1], Kim YC[1]
[1]College of Pharmacy, Seoul National University, San 56 – 1, Shillim-Dong, Kwanak-Gu, Seoul, 151 – 742, Korea

Cognitive-enhancing activities of two iridoid glycosides, 8-*O*-*E*-*p*-methoxycinnamoylharpagide (MCA-Hg) and *E*-harpagoside (Hs), were measured in scopolamine (1 mg/kg body weight, s.c.)-induced amnesic mice with Morris water maze test. In addition, the effects of MCA-Hg and Hs on memory impairment were evaluated and compared with that of *E*-*p*-methoxycinnamic acid (*E*-*p*-MCA), a potent neuroprotective phenylpropanoid previously reported, using step-through passive avoidance test. Daily oral administration of 1 and 2 mg/kg body weight of MCA-Hg and Hs significantly improved scopolamine-induced memory deficits in Morris water maze test. MCA-Hg exerted a more potent activity on impaired reference memory than Hs in Morris water maze test. Also, the mean latency time, the mean path length and the swimming movement were significantly improved. The activities of MCA-Hg and Hs on memory impairment were higher than that of *E*-*p*-MCA in passive avoidance test. Both MCA-Hg and Hs recovered memory impairment to about 70% of normal control level. On the other hand, *E*-*p*-MCA recovered it only to 30% of normal control level and the efficacy of *E*-*p*-MCA decreased in oral administration as compared to the i.p. injection. These results could be explained by the restriction of intestinal permeation after oral administration. In conclusion, it can be postulated these two iridoid glycosides represent the cognitive-enhancing and neuroprotective activities of *Scrophularia buergeriana in vivo*. *Acknowledgements: This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A050599).*

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In vitro cytotoxicity and p-glycoprotein modulating effects of geranylated furocoumarins from *Tetradium daniellii*

Adams M[1], Mahringer A[2], Fricker G[2], Bauer R[3], Efferth T[4]
[1]Institute of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4053 Basel, Switzerland; [2]Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, INF 366, 69120 Heidelberg, Germany; [3]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria; [4]German Cancer Research Centre, INF 280, 69120 Heidelberg, Germany

Four anti-mycobacterial geranylated furocoumarines isolated from the fruits of *Tetradium daniellii* (Benn.) T. G. Hartley (Rutaceae) [1], were tested in an bioassay using CCRF-CEM leukemia cells and their p-glycoprotein (p-gp) over-expressing subline CEM/ADR5000, to asses their cytotoxicity and effects on cellular efflux pumps. All showed considerable cell proliferation inhibition with IC_{50} values ranging from 0.61 to 3.9 μg/ml against CCRF-CEM and 0.22 to 4.8 μg/ml against CEM/ADR5000, respectively. An assay monitoring the p-gp dependent accumulation of a fluorescent dye in porcine brain capillary endothelial cells was used to study interactions of the test substances with these efflux pumps, where they all were shown to be weak modulators of p-gp. *Acknowledgements: M. Adams was supported by a research grant form the Heinrich Jörg Stiftung (Graz, Austria) to do these studies.* **References:** [1] Adams, M. et al. (2006). Planta Med. 72: 1132 – 1135.

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Immunomodulatory activities of *Ganoderma sinense* and *Ganoderma* spores

Yue GGL[1], Fung KP[2], Leung PC[1], Lau CBS[3]
[1]Institute of Chinese Medicine; [2]Department of Biochemistry; [3]School of Pharmacy; The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

Ganoderma sinense (GS, an allied species of *Ganoderma lucidum*) and *Ganoderma* spores (SP) are widely consumed for health promotion in Asia nowadays. As dietary supplement, the detailed scientific research of GS and SP was seldom reported. In our previous study, different parts of the fruiting body of GS showed differential antiproliferative effects on human breast cancer cells [1]. In the present study, the immunomodulatory effects of a water extract of GS on human peripheral blood mononuclear cells (PBMC) and the immunostimulating effects of SP in Balb/c mice were conducted. The proliferations of PBMC and mouse spleen lymphocytes were determined by thymidine incorporation assay, while the productions of various cytokines were determined by ELISA. The sporoderm-broken SP dispersed in water (1, 2 or 4 g/kg body weight; n = 17) and water treatment (as control) were orally administered to sarcoma-bearing mice for 14 days. The proliferative responses and the cytokine productions of spleen lymphocytes isolated from treated mice were measured. Our results indicated that GS extract (50 – 400 μg/ml) significantly increased the proliferative response of PBMC and IFN-γ production in a dose-dependent manner ($p < 0.05$ at all tested concentrations using Students' t-test). However, the proliferation and the production of IL-2 and IFN-γ in phytohemagglutinin-activated PBMC were significantly ($p < 0.05$) suppressed by GS extract (100 – 400 μg/ml). Besides, our results demonstrated that the proliferative response of spleen lymphocytes on SP (4 g/kg)-treated mice was significantly increased to 5.5-fold ($p < 0.001$) when compared to those of untreated mice. The cytokines (IFN-γ, IL-2, IL-4 and IL-6) produced by spleen lymphocytes were significantly enhanced by SP-treatment, showing the immunostimulating activity of SP in mouse immune system. In conclusion, this is the first scientific report of the immunomodulatory activity of GS and SP on human and murine lymphocytes, respectively. **References:** [1] Yue, G. et al. (2006) J Altern Complement Med. 12(8):777 – 89.

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Free radical scavenging activity of mushrooms from order Polyporales

Kubikova K, Opletal L, Koleckar V, Polasek M, Jahodar L, Rehakova Z, Karlickova J
Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Heyrovskeho 1203, CZ-50005 Hradec Kralove, Czech Republic

Free radical scavenging activity of crude ethanol extracts from the fruiting bodies of 32 taxons of Polyporales mushrooms was evaluated by means of DPPH (2,2'-diphenyl-1-picrylhydrazyl radical) test using SIA (PC-controlled Sequential Injection Analysis system) method developed in our laboratory [1]. The most active samples were tested by means of hydrogen peroxide-induced luminol chemiluminescence assay using SIA [2]. The DPPH radical scavenging activity of the samples was expressed as 50% effective concentration (EC_{50}): *Fomitopsis pinicola* 0.086 mg/ml, *Osmoporus odoratus* 0.276 mg/ml, *Phaeolus schweinityii* 0.297 mg/ml, *Ganoderma lipsiense* 0.379 mg/ml, *Daedaleopsis confragnosa* 0.42 mg/ml, *Albatrellus ovinus* 0.435 mg/ml and *Oligoporus ptychogaster* 0.823 mg/ml, and this va-

lues were compared with known antioxidants: acidum ascorbicum 0,006 mg/ml, and trolox 0,014 mg/ml. The results of the second method was also expressed as EC_{50}: *F. pinicola* 0,124 mg/ml, *G. lipsiense* 0,273 mg/ml, *O. odoratus* 0,393 mg/ml and *P. schweinitzii* 0,573 mg/ml and this values were compared with known antioxidants: trolox 0,025 mg/ml, rutin 0,190 mg/ml and caffeic acid 0,008 mg/ml. The results at this point are promising for further research. *Acknowledgement:* This research was financially supported by Charles University grant 2007 **References:** [1] Polasek, M. et al. (2004) Anal Bioanal Chem 379: 754 – 758. [2] Cheng, Z. et al. (2003) Anal Bioanal Chem 375: 376 – 380

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Natural Product-based Screening for Histone Deacetylase Inhibitors

Krasteva S, Heiss EH, Dirsch V, Krenn L
Department of Pharmacognosy, University of Vienna, Centre of Pharmacy, Althanstraße 14, A-1090 Vienna, Austria

The heart responds to a variety of extrinsic and intrinsic stress stimuli by hypertrophic growth. Modulation of myocardial growth is considered as a potential approach in the prevention and treatment of heart failure [1]. Recent studies have shown that histone acetyltransferases (HATs) and histone deacetylases (HDACs) participate in the regulation of genes that are pivotal for the hypertrophic response of the heart. Type I HDACs hereby favour gene expression by deacetylating histone tails resulting in relaxation of the chromatin structure. *In vivo* studies indicate that pharmacological inhibition of HDAC activity blunts hypertrophic growth. The aim of our study is therefore i) to establish a fast method for the determination of HDAC activity and ii) to identify potent and natural product-based inhibitors of HDAC activity. A fluorimetric enzyme assay was established to investigate HDAC activity and its modulation by different natural compounds. Nuclear extracts from human umbilical vein endothelial cells (HUVECs) and rat vascular smooth muscle cells (VSMCs) served as source of HDAC protein. Natural test compounds were selected according to their structural relationship to known HDAC inhibitors. The initial results showed that compounds such as several isoflavones, anthraderivates and capsaicines have a potential to inhibit histone deacetylase activity, although only at micromolar concentration. IC_{50} values were determined for aloin (150 μM), for sennosides A and B (180 μM and 220 μM) and capsaicine (400 μM). Further approach is to find potent histone deacetylase inhibitors at nanomolar concentration. *Acknowledgements:* We are grateful to Dr. Dan Sorescu, Emory University, Division of Cardiology, Atlanta GA, USA, for helpful discussions. **References:** [1] Frey, N. et al. (2003) Annual Review of Physiology 65, 45 – 79

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Influence of avocado seed fractions (*Persea americana* Mill.) obtained by HSCCC on human skin keratinocytes and fibroblasts: Differences of effects in regard of tested cell types

del R Ramos-Jerz M[1], Villanueva S[2], Deters AM[3]
[1]*University of Braunscheig, Institute for Food Chemistry, Schleinitzstr.20, 30106 Braunschweig, Germany;* [2]*Centro de Investigación y Asistencia en Tecnología y Diseno del Estado de Jalisco (CIATEJ), 44270 Guadalajara, Jalisco, Mexico;* [3]*University of Muenster, Institute for Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, 48149 Muenster, Germany*

The avocado (*Persea Americana*, Mill.) is a tree native to the tropical forests of Mexico. Fruit oil with high contents of unsaturated fatty acids are used in cosmetical preparations to regenerate dry and rough skin, often mixed with crushed seeds in peelings. In our study, avocado seed and cotyledon extracts were separated by high speed counter current chromatography (HSCCC). Hydrophilic and slightly lipophilic crude extracts and fractions were tested on human skin cells for their cell physiology modulating activity. Human skin fibroblast and keratinocytes were incubated with fractions containing polyphenols or polysaccharides for determination of their influence on cell cytotoxicity, proliferation and differentiation. The expression of differentiation and proliferation related genes were tested by qRT-PCR. A possible antioxidant activity was investigated by the DPPH assay. The H_2O-extract had no radical scavenge activity. The MeOH-, MeOH/H_2O- and EtOAc-extract, also related HSCCC fractions reduced the DPPH radical up to 76%. Proliferation and cell viability of keratinocytes were significantly triggered by 10 μg/ml of the H_2O-, EtOAc-extract, and a HSCCC-fraction of the MeOH/H_2O-extract causing up-regulation of proliferation specific genes (EGF-, insulin- and KGF-receptors, STAT6). Keratinocyte differentiation determined by cytokeratin contents was only slightly affected. The EtOAc-extract and HSCCC fractions from MeOH/H_2O-extract reduced fibroblast proliferation and cell viability while the H_2O-extract and other fractions slightly enhanced the proliferation rate though the cell viability of treated cells increased. In conclusion, the dermal fibroblast and epidermal keratinocytes showed different response on the incubation with avocado seed extracts. As side effect, the radical scavenging activity seems not to be equated with enhancement of cell viability or proliferation.

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Evaluation of the antioxidant activity of several naturally occurring coumarins and their synthesized analogues

Rehakova Z[1], Cervenka F[1], Kubikova K[1], Karlickova J[1], Jahodar L[1], Saso L[2]
[1]*Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Kralove, Heyrovskeho 1203, CZ-50005, Czech Republic;* [2]*Department of Human Physiology and Pharmacology "Vittorio Erspamer", University La Sapienza, Roma 00185, Italy*

In present study, the structure-antioxidant activity relationship (SAR) of both natural and synthetic coumarins is discussed. 6 naturally occurring coumarins and their 16 synthesized analogues as well as 2 known antioxidants were measured for their reducing antioxidant activity and radical scavenging activity. The result of FRAP assay (ferric reducing anioxidant power) modified to be used in 96-well microplates [1] are expressed as FRAP values (μM) measured at 4 and 60 minutes. Radical scavenging activity was established by 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) test using sequential injection analysis (SIA) system [2] and the results are expressed as EC_{50} (μM). The results of antioxidant activity suggest that the presence of two dihydroxy groups in *ortho*-position is necessary for reducing activity while *meta*-derivates were only slightly active. It was established, that 7,8-dihydroxy-4-methylcumarins ($FRAP_{value\ 4\ min}$ = 20,3 ± 3,3 μM, $FRAP_{value\ 60\ min}$ = 43,9 ± 5,9 μM, EC 50 = 24,9 μM) and its C3 acetic acid ethylester ($FRAP_{value\ 4\ min}$ = 22,2 ± 1,6 μM, $FRAP_{value\ 60\ min}$ = 45,4 ± 6,2 μM, EC_{50} = 24 μM) and propanoic acid ethylester ($FRAP_{value\ 4\ min}$ = 31,4 ± 5,3 μM, $FRAP_{value\ 60\ min}$ = 71,4 ± 0,8 μM, EC_{50} = 27 μM) derivatives as well as 6,7-dihydroxy-4-methylcoumarins ($FRAP_{value\ 4\ min}$ = 38,3 ± 0,9 μM, $FRAP_{value\ 60\ min}$ = 72,8 ± 5,2 μM, EC_{50} = 74,7 μM) have shown significant ferric reducing and radical scavenging properties compared to known antioxidants trolox ($FRAP_{value\ 4\ min}$ = 19,3 ± 1,2 μM, $FRAP_{value\ 60\ min}$ = 20,2 ± 1,4 μM, EC_{50} = 27.8 μM) and (+)-Catechin ($FRAP_{value\ 4\ min}$ = 20,73 ± 0,3 μM, $FRAP_{value\ 60\ min}$ = 44,1 ± 1,05 μM, EC_{50} = 49 μM) and are perspective for further testing of biological activities. *Acknowledgement:* This research was supported by Charles University grant 2007 **References:** [1] Firuzi, O. et al. (2005) Biochim. Biophys. Acta 1721: 174 – 184. [2] Polážek, M. et al. (2004) Anal. Bioanal. Chem 379: 754 – 758

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Identification of plants with potential anti-diabetic effects by using a screening platform to recognize partial PPARγ agonists

Christensen KB[1], Minet A[2], Svenstrup H[2], Kristiansen K[2], Grevsen K[3], Christensen LP[1]

[1]Department of Food Science, Faculty of Agricultural Sciences, University of Aarhus, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark; [2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark; [3]Department of Horticulture, Faculty of Agricultural Sciences, University of Aarhus, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

Worldwide more than 160 million people suffer from type 2 diabetes (T2D) which signifies that it has reached almost epidemic proportions. T2D is characterized by insulin resistance and the recommended treatment is a change in lifestyle often supplemented by insulin sensitizing drugs. These drugs bind to and activate the Peroxisome Proliferator-Activated Receptor (PPAR) γ, a key regulator of adipogenesis. However, side-effects do occur due to the fact that the drugs are full PPARγ agonists. Hence, partial PPARγ agonists are sought for as evidence suggests these to have less side-effects. Plants have traditionally been used in the treatment of diabetes, making them a potential source of natural products with anti-diabetic effects. A number of plants were selected for testing in a screening platform that makes identification of partial PPARγ agonsits possible. The platform consists of several bioassays which test for PPARγ activation, followed by tests for adipocyte differentiation, glucose uptake, and PPARα and δ activation. Extracts/compounds tested in this row of bioassays will only proceed through by passing each step satisfactory. Partial PPARγ agonists identified in the platform will then be subjected to animal and clinical testing. In the end, this process as a whole can lead to the identification of potential PPARγ activators without the side-effects seen for drugs used in T2D treatment today. Plant material from 23 plants was sequentially extracted with hexane, followed by CH_2Cl_2, CH_3OH and finally H_2O. More than 60% of the tested plants were found to contain partial PPARγ agonists. The most promising anti-diabetic effects were observed in extracts of purple coneflower (hexane), winter savoury (hexane, CH_2Cl_2), elderflower (CH_2Cl_2, MeOH), sage (hexane, CH_2Cl_2), and buckwheat (hexane, CH_2Cl_2). By the use of bioassay-guided chromatographic fractionation it will then be possible to isolate and identify the active principles from the plant extracts.

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Comparative study of the analgesic effect of different phenylethanoid-based fractions from *Plantago lanceolata* L

Armatu A[1], Paraschiv I[1], Ocnaru D[1], Pintilie G[1], Manaila N[1], Rughinis D[1], Segarceanu A[2], Ghita I[2], Nita S[1]

[1]INCDCF – ICCF, 112, Calea Vitan, Bucharest, Romania, 031299, [2]UMF "Carol Davila", Faculty of Medicine, Department of Pharmacology and Pharmacotherapy – 8, Eroilor Sanitari, Bucharest, Romania, 050474

The present study aims to compare the analgesic effect of 3 phenylethanoid-based phytocomplexes obtained by methanol extraction of the herb, liquid-liquid distribution with ethyl acetate and CC on Celite 545, butanol as eluent: phenylethanoid-flavonoidic (PF) fraction (49.5% acteoside, 11.6% luteolin-7-glucoside, free of iridoids), phenylethanoid (P) fraction (46.6% acteoside) and phenylethanoid-iridoidic (PI) fraction (48.8% acteoside, 4.9% catalpol, 7.8% aucubin, free of flavonoids). The compounds were quantified by HPLC and the absence of flavonoids and iridoids was confirmed by negative colour reactions with specific reagents. The potential analgesic activity was evaluated on mice (2 testing groups and one blank control group, 10 mice each for every experiment) using acetic acid-induced writhing test and hot-plate test. The extracts were given to mice at doses of 100 mg and 400 mg/kg i.p and jump and licking parameters were evaluated at 15, 30, 60, 120 minutes, comparative to control group. Statistical analysis was performed with the Student's t-test. The results for PF fraction were statistical significant in the hot-plate test for the jump parameter at 60 min at the dose of 400 mg/kg ($p < 0.05$). The most significant activity was shown in the hot-plate test for P fraction that had a rapid analgesic effect at 15 min at the dose of 100 mg/kg for the jump parameter. The effect was prolonged for 120 min ($p < 0.05$). The analgesic effect was also observed in the writhing test at 30 and 60 min ($p < 0.05$). PI fraction showed an analgesic effect in the hot-plate test for the jump parameter at 30 min and also in the writhing test at 30 and 60 min at a dose of 100 mg/kg ($p < 0.05$).These results show that the phenylethanoid fraction have the most rapid and prolonged analgesic effect comparing to phenylethanoid-flavonoidic and phenylethanoid-iridoidic fractions. The data suggest that flavonoids and iridoids have no positive influence on the analgesic effect induced by phenylethanoids.

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Antioxidant and complement modulating activities of five essential oils

Pérez-Rosés R[1], Risco E[1], Vila R[1], Peñalver P[2], Cañigueral S[1]

[1]Unitat de Farmacologia I Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Avda. Diagonal, 643, E-08028 Barcelona, Spain; [2]Lidervet, S.L. Plaça García Lorca, 17, Baixos. E-43006 Tarragona, Spain

Five essential oils, from clove (CL) (*Syzygium aromaticum* (L.) Merr. & L.M. Perry =*Eugenia caryophyllata* Thunb), lemon (LE) (*Citrus limon* (L.) Burman fil.), rosemary (RO) (*Rosmarinus officinalis* L.), Spanish oregano (SO) (*Thymbra capitata* Griseb.) and thyme (TH) (*Thymus zygis* L.), were investigated for their activity on the complement system and their antioxidant properties by *in vitro* assays. Oils were obtained from commercial sources. Their main components (identified by GC-MS and quantified by GC-FID) were as follow: eugenol (86.2%) for CL; limonene (71.6%), ß-pinene (12.2%) and γ-terpinene (6.9%) for LE; 1,8 cineole (49.7%), α-pinene (9,9%) and camphor (9,6%) for RO; carvacrol (73,5%) for SO, and thymol (56,6%) and p-cymene (28,4%) for TH. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [1]. The activity on classical complement pathway was determined in human serum by hemolytic assay [2]. Positive control was quercetin for both assays, showing an $IC_{50} = 10.5 \pm 4.6$ μg/mL for the DPPH test and an $IC_{50} = 33.7 \pm 4.3$ μg/mL for the assay on the complement system. Only CL showed antioxidant activity in the DPPH ($IC_{50} = 13.2 \pm 2.9$ μg/mL), indicating that this oil has free radical scavenging activity. Results on inhibition of the classical pathway of the complement system, showed that TH and CL have similar weak activity, with $IC_{50} = 61.4 \pm 10,6$ μg/mL and 72.7 ± 4.1 μg/mL, respectively. SO, RO and LE showed no inhibitory effect on the complement system activated by the classical pathway. In conclusion, from the five oils tested, the clove essential showed the best combination of free radical scavenging and complement inhibitory activities. *Acknowledgements:* Thanks are due to Lidervet S.L. (Tarragona, Spain) for the financial support. The work of R. Perez-Roses was supported by the Department of Education and Universities of the Generalitat de Catalunya and the European Social Fund. **References:** [1] Malencic D et al. (2000) Phytother Res 14: 546 – 548. [2] Klerx et al. (1983) J Immunol Methods 63: 215 – 220.

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The transport of $GABA_A$-receptor modulators derived from *Valeriana officinalis* L

Trauner G[1], Neuhaus W[2], Noe CR[2], Kopp B[1]

[1]Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; [2]Department of Medicinal Chemistry, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria

The roots and rhizome of *Valeriana officinalis L.* are therapeutically used due to sedative and sleep enhancing effects. Some of the active compounds found in commonly used extracts are the sesquiterpenic

acids which recently were identified as GABAA-receptor modulators [1]. To interact with this receptor, it is essential that these substances cross the blood-brain barrier (BBB). The aim of our study was to obtain BBB permeability data of these compounds for the first time and to elucidate possible transport pathways across our BBB *in vitro* model. Transport of valerenic acid, acetoxyvalerenic acid and hydroxyvalerenic acid was compared to the permeability of the GABAA-modulator diazepam, which is known to penetrate into the central nervous system transcellularly by passive diffusion. Experiments were carried out with an established Transwell *in vitro* model based on the human cell line ECV304. Results indicated clearly that all three acids permeated significantly slower than diazepam (PEall = 11.65 ± 2.66 μm/min). Valerenic acid (PEall = 0.92 ± 0.22 μm/min) was the slowest followed by hydroxyvalerenic acid (PEall = 2.06 ± 0.36 μm/min) and acetoxyvalerenic acid (PEall = 2.56 ± 0.30 μm/min). The ranking was confirmed in single substance studies as well as in group studies and did not correspond to the according clogP values. To elucidate the contribution of the paracellular transport, studies were performed at different tightness status of the cell layers reflected in different transendothelial electrical resistance (TEER) values. Results showed an exponential correlation between transport and TEER for all three acids, whereas diazepam permeated TEER independently. In summary, it is hypothesised that the investigated compounds from *Valeriana officinalis* L. can only pass the BBB by a still unknown active transport system and not by passive diffusion. **References:** [1] Khom, S. et al. (2006) Neuropharmacology submitted

P 491

Grayanotoxin III Shows Microtubule-Targeted Activity

Popescu R[1,2], Konkimalla VB[3], Rüger B[4], Madlener S[2], Stark N[2], Krupitza G[2], Efferth T[3], Kopp B[1]
[1]Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; [2]Institute of Clinical Pathology, Medical University of Vienna, Währinger Gürtel 18 – 20, A-1090 Vienna, Austria; [3]German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany; [4]Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Währinger Gürtel 18 – 20, A-1090 Vienna, Austria

Grayanotoxins are diterpenes responsible for plant toxicity [1]. They occur in species belonging to the *Ericaceae* family. [2]. Grayanotoxins share structural similarities with paclitaxel a known antineoplastic drug which stabilizes microtubules by tubulin polymerization [3]. Therefore, we studied whether grayanotoxin interacts with microtubules. We used immunofluorescence to visualize alpha- and beta-tubulin in MCF7 breast cancer cells, after exposure to grayanotoxin III. Paclitaxel and colcemid were used as control drugs. While paclitaxel induced the formation of tubulin circumferential bundles and colcemid disassembled the tubulin polymer, grayanotoxin III showed a disorganized microtubule structure, with curled filaments forming a denser reticular network towards the nucleus. Because of the different effects on microtubules, we hypothesized that the binding domain of grayanotoxin III is different from the one of paclitaxel and colcemid. We used the crystal structure of alpha- and beta-tubulin for molecular "blind docking" with grayanotoxin III and paclitaxel. We found that grayanotoxin does not bind to the binding site of paclitaxel on beta-tubulin, but on alpha-tubulin. Subsequently, we took the binding sites of grayanotoxins and paclitaxel for "defined docking" studies. Defined docking provides detailed information on docking properties. Using this approach, we validated that grayanotoxin III binds with high affinity to its own pharmacophore on alpha-tubulin, but not to the paclitaxel pharmacophore on beta-tubulin, and vice versa paclitaxel does not bind to the grayanotoxin binding site. In conclusion, the effect of grayanotoxin III on microtubule organization in immunofluorescence correlated with the molecular docking data indicating the alpha-tubulin as a binding site. **References:** [1] Kakisawa, H., et al. (1965) Tetrahedron 21: 3091. [2] Comroly, J.D., Hill, R.A., (1991) Dictionary of Terpenoids, Vol. 2, Chapman & Hall, Cambridge. [3] Alberts, B., Johnson, A., (2002) Molecular Biology of the Cell. Garland Science. New York.

P 492

The anti-inflammatory extract from leaves of *Isatis tinctoria* (Ze 550) inhibits dose dependently the allergen induced airway-response in sensitized mice

Brattström A[1], Maillet I[2], Schnyder B[2], Moser R[2], Ryffel B[2]
[1]Max Zeller Söhne AG, CH-8590 Romanshorn, Switzerland, [2]Phenotec AG, CH-6304 Zug, Switzerland

The extract Ze 550 obtained by means of supercritical CO2 extraction has been shown in *in-vitro* experiments to inhibit cyxlo-oxygenase 2, 5-lipoxygenase, NO production and to stabilise mast cells (extraction yield 0.8 %). Moreover, in *in-vivo* models the anti-inflammatory and anti-allergic action of Ze 550 was confirmed. Since allergic diseases are accompanied by chronic inflammation the question arose whether or not the extract will also be useful in allergic asthma. A murine model of allergen-induced airways inflammation is well elaborated (6,7) with an increase in IL-4 and IL-5 in the bronchoalveolar lavage fluid (BAL), eosinophil recruitment into the lung tissue indicating that the allergic inflammation is driven by the activation of the T helper (Th) type 2 cells. Therefore the influence of Ze 550 in this model of allergic asthma was investigated. Balb/c mice were immunised subcutaneously twice at weekly intervals with 0.4 ml saline containing 1 μg ovalbumine (OVA) and 1.6 mg alums. Intranasal challenge was repeatedly performed (3 consecutive days) under light i. v. ketamine anaesthesia by applying 50 μl OVA in alum-free saline solution (10 μl) or saline as control. Ze 550 was given at 10 μg, 30 μg and 100 μg intra-nasally (in 40 μl) just before the antigen challenge. The airway resistance was evaluated by whole-body plethysmography. Bronchial hyper-reactivity to aerosolized methacholine (100 mM, 1 min) was investigated 24 h after the last OVA challenge using whole body plethysmography. Ze 550 inhibited the methacholine induced airway hyper-responsiveness (AHR) in a dose dependent manner(10, 30 and 100 μg). AL was performed by cannulating the trachea and washing 4 times with 0.5 ml each of ice-cold PBS 24 h after the last antigen challenge. Ze 550 again inhibited dose dependently and significantly eosinophil recruitment into BAL. Corresponding with the reduced eosinophil recruitment the EPO activity in lung tissue was also decreased. Moreover, mucus hyper-production was dose dependently inhibited by Ze 550 (10, 30, and 100 μg). In BAL IL-4, IL-5 and RANTES were significantly reduced (30 μg). *Conclusion:* Topic administration of Ze 550 into the airway prior to antigen challenge in sensitized mice reduced hyper-reactivity and Th 2 cytokines IL-4 and IL-5.

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Free Radical Scavenging Compounds in *Salvia halophila* Hedge and *S. virgata* Jacq. Extracts Using a Postcolumn Derivatization Method

Kosar M, Göger F, Baser KHC
Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy 26470 Eskisehir, Turkey

Several biochemical reactions generate reactive oxygen species and these are capable of damaging crucial bio-molecules [1]. Free radicals are very important in food products, because oxidative degradation of lipids is one of the main factors limiting their shelf-life [2]. In recent years, natural antioxidants have been focused on due to the harmful effects of synthetic antioxidants [3]. *S. officinalis* L. (Lamiaceae) is an important source of antioxidants and has wider implications for the dietary intake of natural antioxidants [3]. Turkey is an important biogenetic resource for the genus *Salvia* species comprising 88 species. An on-line high-performance liquid chromatography-1,1-diphenyl-2-picrylhydrazyl radical (HPLC-DPPH$^{\cdot}$) method has been improved for the detection of polar and nonpolar radical scavenging compounds in complex plant extracts. Five different ex-

tracts were prepared from *S. halophila* Hedge and *S. virgata* Jacq. using different solvents. After the separation of components by reverse phase chromatography by 10 – 100% methanol with 2% acetic acid as mobile phase, analytes capable of scavenging a citric acid-sodium citrate-buffered 1,1-diphenyl-2-picrylhydrazyl solution were detected by postcolumn derivatization at 517 nm. The HPLC-DPPH· on-line method was applied to qualitative and quantitative analysis of sage extracts. Constituents such as gallic acid, *p*-OH-benzoic acid, caffeic acid, ferulic acid, *o*-coumaric acid, luteolin-7-*O*-glucoside, and luteolin were identified by HPLC-PDA method. These compounds were investigated both on-line and off-line for DPPH· scavenging capacity and statistical correlations and relationships were determined. *Acknowledgements:* This study was supported by a grant from the The Scientific & Technological Research Council of Turkey (TUBITAK-104S603). **References:** [1] Kumaran, A., Joel Karunakaran, R. (2006) Food Chem. 97: 109 – 114. [2] Pizzalle, L. et al. (2002) J.Sci. Food and Agric. 82: 1645 – 1651. [3] Kintzios, S.E. (2000) Sage The Genus Salvia. Harwood academic publishers, 27 – 53 and 185 – 192.

P 494

Pharmacological evidence of the mechanisms of action of *Phoradendron piperoides* Kunth (Viscaceae) aqueous extract

Marçal RM, Almeida DS, Carvalho ACS
Physiology Department, Federal University of Sergipe, Av Marechal Rondon, s/n, São Cristóvão, Sergipe, CEP: 49.100 – 000, Brazil

The infusion from the leaves of *Phoradendron piperoides* is commonly used to soothe abdominal pain in Brazil [1]. We have recently reported that the lyophilized extract from the leaves of *P. piperoides* (AE) did not produce an antinociceptive effect in mice, whereas it produced relaxing responses in the guinea-pig intestinal smooth muscle [1]. Here, we have investigated the effect of the AE on normal intestinal transit in mice and the mechanisms of action in the isolated guinea-pig ileum. AE (200 – 500 mg/kg, p.o.; $p < 0.05$) significantly reduced the intestinal transit in the charcoal meal test when compared to atropine (2 mg/kg; p.o.). In the guinea-pig ileum, the contractions induced by histamine (2 μM), carbachol (2 μM) and $BaCl_2$ (0.03 M) were significantly ($p < 0.01$) reduced in the presence of the AE (1.5 mg/ml). AE (0.05 – 2.0 mg/ml) reduced, in a concentration-dependent fashion, the contractions induced by KCl (60 mM). This effect is probably due to inhibition of calcium influx through voltage-operated calcium (Ca(v)) channels. To confirm this hypothesis, we evaluated their effect on cumulative $CaCl_2$ curves in depolarizing medium nominally without Ca^{2+}. The CE_{50} of $CaCl_2$ concentration-response curves did not change in the presence of AE (1.5 mg/ml). However, AE (1.5 and 3.0 mg/ml) reduced, in a non-reversible fashion, the E(max) of $CaCl_2$ concentration-response curves. Finally, propranolol (5 μM) along with yohimbine (1 μM) and prazocin (1 μM) antagonized norepinephrine (0.3 μM) and AE (1.5 mg/ml) relaxing responses. In brief, the effect of AE is probably due to a blockade of calcium influx through Ca(v) channels and adrenergic receptor activation. *Acknowledgements:* CNPq, FINEP **References:** [1] Dias, K.S. et al. (2007) Braz. J. Pharmacognosy, [accepted].

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Determination of polyphenols and Antioxidant activity of *Melilotus off.* and *Elaeagnus angustifolia* L

Hasan Agha MI[1], Blaschek W[2]
[1]Damascus University, Faculty of Pharmacy Damascus-Syria, P.O. Box 36251; [2]CAU Kiel, Institute of Pharmacy, D-24118 Kiel- Germany

The aim of this study was to determine the relation between the content of polyphenols in *Melilotus officinalis* Desr. and *Elaeagnus angustifolia* L. and the antioxidant activity of their aqueous and methanolic extracts. Both plants are used in folk medicine in Syria [1,2]. Antioxidant activities of aqueous and methanolic extracts of the aerial parts and roots from *Melilotus officinalis* Desr. and leaves and roots from *Elaeagnus angustifolia* L. have been studied by use of the TEAC-assay (Trolox Equivalent Antioxidant Capacity) [3], in which Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carbonic acid, a Vitamin-E derivative) is used as reference compound in a concentration of 1 mmol/l. The content of polyphenols in the extracts was determinend by the folin-ciocalteau method [4]. The extracts in a concentration of 20 mg/ml each had an antioxidant capacity equivalent to approximately 2 – 4 Trolox units. Highest and comparable antioxidant activity could be obseved in aqueous and methanolic extracts of roots from *Melilotus officinalis* and the methanolic extract from *Eleagnus angustifolia*, whereas lowest activity showed aqueous extracts of roots of the latter plant. There was only restricted correlation between the polyphenol content of the extracts and their antioxidant activity indicating that not only the amount of phenolic compounds, but most notably their structure is of great importance. **References:** [1] Ramezani, M. et al. (1992) Ann. Allergy 69: 493 – 496. [2] Melilot – Melilotus officinalis: Pharmacopoeia Francaise. [3] Miller, N.J: et al. (1993) Clin. Sci. (Lond.) 84: 407 – 412. [4] Javanmardi et al. (2003) Food Chem. 83: 547 – 550

P 496

Preliminary phytochemical screening, cytotoxicity and acute toxicity of *Aristolochia albida* ethanolic extract

Kamagaju L[1], Mukazayire MJ[1,2], Nyiligira J[1], Gnoula C[2], Mugiraneza JP[1], Duez P[2]
[1]Centre de Recherches en Phytomédicaments et Sciences de la vie, Institut de Recherche Scientifique et Technologique (I.R.S.T.), B.P. 227 Butare, Rwanda; [2]Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine, Institut de Pharmacie, Université Libre de Bruxelles, CP 205/9, Bd du Triomphe, Bruxelles, Belgique. (32 – 2 – 650.5283)

Aristolochia albida D. (Aristolochiaceae) can be found in Africa, Asia, Europe and in the North and South of America. Its seeds are used in traditional medicine in Rwanda for the treatment of malaria. Phytochemical and biological investigations on the seeds ethanolic extract are still in progress, but the preliminary results show that *A. albida* is rich in alkaloids. A series of seeds ethanolic extract doses (from 0.5 g/kg to 8 g/kg) were administered by oral and peritoneal routes to guinea-pigs which were observed during 24 h; after 24 h some of the animals were sacrificed and the internal organs were observed. No acute toxicological effect could be registered, even at the higher dosage. *In vitro* studies on the cytotoxicity of seeds methanolic extract (doses from 0.01 μg/ml to 100 μg/ml) on U373 MG (human glioblastoma astrocytoma) and A549 (human lung carcinoma) cells (Microculture Tetrazolium Test, MTT) show a promising activity (IC_{50}= 46.4 μg/ml for U373 and IC_{50}= 73.8 μg/ml for A549) worthy of further investigation. **Reference:** Litchifield, J.T. and Wilcoxon, F., 1949, J.Pharmacol. Exptl.Therap., 96:99.

P 497

Protective effect of the flavonoid myricetin on glucose induced oxidative stress in Hep G2 cells

Petlevski R[1], Kostner GM[2], Frank S[2], Juretić D[1], Kalogjera Z[1]
[1]University of Zagreb, Faculty of Pharmacy and Biochemistry, A. Kovaèića 1, 10 000 Zagreb, Croatia; [2]Institut für Medizinische Biochemie und Medizinische Molekularbiologie-Karl-Franzens-Universität, Harrachgasse 21, 8010 Graz, Austria

Myricetin is a naturally occurring flavonol with hydroxyl substitutions at the 3, 5, 7, 3·,4· and 5· positions and has a hypoglycaemic and hypotrigyceridemic effect in diabetes mellitus. Hyperglycemia in diabetes can induce oxidative stress via several mechanisms. These include glucose autoxidation, the formation of advanced glycation end-products (AGE), and activation of the polyol pathway. Other circulating factors such as free fatty acids and leptin, also contribute to increased reactive oxygen species (ROS). The major roles of GSH (γ-glutamiylcysteinylglycine) are to maintain the intracellular redox

balance and to eliminate ROS in cells. The aims of this study were 1) to investigate the effect of the flavonoid myricetin on the concentration of total glutathione (GSH) in Hep G2 cells and 2) to determine whether this flavonoid could protect the cells against glucose-induced oxidative stress. Hep G2 cells was supplemented with 0.5 μM and 1.0 μM of myricetin for 4 hours or 0.5 μM and 1.0 μM of myricetin plus 20 μM glucose for same time. Concentration of GSH in cells was determined by Cayman's GSH assay kit with enzymatic recycling method, using glutathione reductase. Exposure the Hep G2 cells to 0.5 μM of myricetin for 4 hours at 37 ° C resulted in significant increased of the GSH level ($p < 0.05$) when compared with control cells. This data suggest that major features of glucose-induced hepatotoxicity are partially mediated by oxidative stress, and that myricetin at low concentration (0.5 μM) protects Hep G2 cells against glucose toxicity affecting the glutathione level.

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Effect of *Butea superba* on penile erection and sperm production in rats

Jeenapongsa R[1], Tocharus C[1], Smitasiri Y[2]
[1]Faculty of Pharmaceutical Sciences, Naresuan University, Muang, Phitsanulok 65000 Thailand; [2]School of Science, Mae Fah Luang University, Muang, Chiang Rai 57100 Thailand

Butea superba Roxb. (Papilionaceae) has been traditionally used to treat age-related problems including erection disorders. We investigated the effect of an alcoholic extract of *B. superba* on penile erection in aged male Sprague Dawley rats. The animals were pretreated with the extract at the doses of 0.1, 0.5, 1, 10 or 1,000 mg/kg body weight (BW) as a single dose and the cavernous nerves were electrically stimulated. The intracavernous pressure was simultaneously recorded from the beginning. Sperm count was performed using a hemocytometer. Sperm motility was investigated in a modified TCM199 medium. The results showed that *B. superba* extract enhanced the penile erection most effectively at a dose of 1 mg/kg BW. Higher doses did not increase the erection. In addition, long-term treatment with *B. superba* extract (1 mg/kg BW) for six months significantly increased the number of sperm up to about 200% and prolonged the sperm motility of the sperm to about 115% at the sixth hour. These results suggest that *B. superba* is effective in improve penile erection and may be useful in the treatment of erectile dysfunction as well as infertility. *Acknowledgements:* This study was financially supported by Faculty of Pharmaceutical Sciences, Naresuan University and Mae Fah Luang University, Thailand

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Nephroprotective Role of *Retama raetam* Webb & Berthel on Gentamicin-Induced Acute Renal Toxicity in Rats

Farrag ARH[1], El-Toumy SAA[2], Muhamed GS[3]
[1]Department of Pathology, [2]Department of Chemistry of Tannins and Leather Technology, [3]Department of Medical Biochemistry, National Research Center, 12622, Dokki, Cairo, Egypt

The methanol extract of the seeds of *Retama raetam* Webb & Berthel (*Fabaceae*) was studied for its nephroprotective activity in gentamicin-induced renal injury in female albino rats. Twenty four rats were divided into four different groups. Group 1 was served as a control group, Group 2 and 3 were administered a daily dose of gentamicin (40 mg/kg body weight s.c) for 13 days. In group 3, rats were administered with the extract (30 mg/kg body weight) on the 14th day onwards for 10 days. Group 4 were treated with an oral dose of the extract (30 mg/kg body weight) after 2 h s.c. administration of gentamicin for 13 days. Body weight, serum urea and creatinine levels were measured. Moreover, histopathological as well as morphometrical investigations were performed. In the gentamicin model, the methanol extract of *R. raetam* at a dose of 30 mg/kg body wt. reduced blood urea and serum creatinine effectively in the curative and the preventive regimen. Light microscopic examination of the renal tissues from gentamicin-treated rats revealed severe histopathological changes, whereas specimens obtained from extract-treated rats revealed only mild changes. The findings suggest that the methanol extract of the seeds of *R. raetam* counteracts the deleterious effect of gentamicin on renal tubular function.

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Effects of Neurapas® balance on sleep EEG, cognitive performance and mood: A double blind randomised cross-over study in healthy volunteers

Giesler M[1], Thum A[1], Haag A[1], Wartenberg-Demand A[2], McGregor GP[2], Krieg JC[1], Kundermann B[1], Hemmeter U[1]
[1]Clinic of Psychiatry and Psychotherapy, University of Marburg, Rudolf-Bultmann-Strasse 8, D 35039 Marburg, Germany; [2]Pascoe pharmaceutical Preparations GmbH, Schiffenberger Weg 55, D 35383 Gießen, Germany

Sleep disturbance is a major feature of patients with anxiety disorders and depression. Neurapas® balance is a triple herbal combination of extracts of St. John's wort (dry extract (DE) 60 mg), valerian (DE 28 mg) and passiflora (DE 32 mg) that is widely used for treating minor depression in Germany. We tested the effect of Neurapas® balance on mood, sleep-EEG and cognitive performance in order to evaluate its central nervous effects in humans. 20 healthy subjects were examined twice in a double-blind, randomized completely balanced cross-over design and received either placebo or Neurapas® balance (3 days 3 x 2 tabl.). The dependent variables were sleep-EEG parameters, mood (BSKE) and vegetative symptoms (MSKL) and cognitive performance (TAP, ZVT, d2). Neurapas® balance significantly (compared to placebo) improved sleep continuity with a reduction of wakefulness. Non-REM-sleep was reduced in the first and significantly increased in the second sleep cycle and REM-latency was reduced. A positive trend was observed in regard to mental agitation and melancholy of the BSKE and hand-trembling and hand-moisture of the MSKL. No significant differences between Neurapas® balance and placebo were found in the cognitive performance tests. These findings show that Neurapas® balance has central nervous effects as reflected by changes in sleep EEG. It improves sleep continuity and subjective mood without negative effects on cognitive performance. The neurophysiological effects of Neurapas® balance® on the brain and the improved mood of the healthy volunteers is consistent with it having therapeutic effects in anxiety and depression with accompanying insomnia. *Acknowledgement:* This study was supported by Pascoe Pharmaceutische Preparations GmbH, Germany.

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Trypanocidal, Toxicological and Heamatological Evaluation of *M. Ciliata* in *T. Brucei Brucei* infected Rats

Ogbunugafor HA[1], Okochi VI[2], Okpuzor J[3], Esue S[3]
[1]Department of Applied Biochemistry, Nnamdi Azikiwe University. P.M.B 5025, Awka. Nigeria; [2]Department of Biochemistry, College of Medicine, Idiaraba, University of Lagos, P.M.B 12003, Lagos. Nigeria; [3]Department of Cell Biology and Genetics, University of Lagos, Akoka, P.M.B. 12003, Nigeria

The trypanocidal, toxicity and haematological effect of ethanolic root extract of *Mitragyna ciliata Aubrev and Pellerg (Rubiaceae)* was evaluated in *T. brucei brucei* infected rats. Fractions of the extract were administered at 50 mg/kg to *T.b. brucei* infected rats 7 days after infection for 5 days. The butanol fraction had the highest trypanocidal activity with inhibition of parasites' growth of 68.68% compared diaceturate (100%). Administration of 800 mg/kg dose of the extract for 30 days caused decrease ($p << 0.05$) in PCV, ALP, ALT, AST, and creatinine, while there was elevation ($p < 0.05$) in bilirubin. There was a decrease ($p < 0.05$) in PCV, WBC and platelets count in all infected rats compared to uninfected when different fractions of the extract were administrated. Results suggest that trypanocidal activity of *M. ciliata* resides in the butanol fraction and it has a hepatobilliary effect of *M.ciliata.* Data was consistent with charac-

teristics of trypanosomiasis – anaemia [1], leucocytopaenia and thrombocytopaenia [2]. **References:** [1] ILRAD Reports (1984) 2(4)1 – 10. [2] Kagira JM., Thuita JK., Ngotho M., Mdachi R., Mwangangi DM. (2006) Haematology of experimental Trypanosoma brucei rhodesiense infection in vervet monkey. Afr. J.Health Sci.13 (3 – 4)59 – 65.

P 502

An extract from wild green oat improves rat behaviour

Perrinjaquet-Moccetti T[1], Wolffgramm J[2], Wullschleger C[1], Reinhard C[2], Schmidt A[1], Heyne A[2]
[1]Frutarom Switzerland Ltd., Rütiwisstrasse 7, 8820 Wädenswil, Switzerland; [2]medimod pharmacology services GmbH, Aspenhaustrasse 25, 72770 Reutlingen, Germany

Preparations from green oat have traditionally been used to support mental health and cognitive function (1, 2). CNS indications include anxiety, tension, stress, excitation and neurasthenia, although the effectiveness has not been documented so far (3). To investigate the efficacy of the herb for the traditional indications, a water-ethanol extract from wild green oat, Neuravena® (EFLA®955), was tested *in vivo* in rats for its behavioural effects after chronic oral administration via food mixed with extract. 36 male Sprague Dawley rats received (A) standard diet (controls), (B) 10 g/kg extract-admixed food or (C) 100 g/kg extract-admixed food. The following behavioural tests were performed: Elevated plus maze, forced swimming, conditioned avoidance response and tetradic encounter. Body weight, food and fluid consumption were measured and apparent physical appearance was determined twice a week. Apart from a slightly decreased food and fluid intake in the high dose group there were no side effects observed during the treatment. Due to these side effects, the high dose was considered as too high, behavioural effects were not taken into further account. The low dose led to an improvement of active stress response, an enhanced speed of learning as indicated by increased shock avoidances and an improved synchrony in social behaviour that can be interpreted as increased social interest of the extract-treated animals. It may be concluded that the extract is suitable to improve behavioural initiative in different test settings. **References:** [1] Müller, I. (1990) Die pflanzlichen Heilmittel der Hildegard von Bingen. Otto Müller Verlag. Salzburg. [2] Wichtl M (2002). Teedrogen und Phytopharmaka, 4. Auflage. Wissenschaftliche Verlags-Gesellschaft. Stuttgart. [3] Klein J, Blumenthal M (eds) (1998). The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. American Botanical Council. Austin TX.

P 503

Effects of Curcumin Derivatives on Tube-formation of Rat Lymphatic Endothelial Cells and Intracellular Signal Transduction

Boonyarat C[1], Eua-areepichit A[2], Sakurai H[3], Saiki I[3], Vajragupta O[2]
[1]Faculty of Pharmacy, Khonkaen University, 123 Mitraphap Road, Khonkaen 40002, Thailand; [2]Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhya Road, Bangkok 10400, Thailand; [3]Division of Pathogenic Biochemistry, Department of Bioscience, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930 – 0194, Japan

Curcumin and some of its derivatives were known to exhibit a variety of pharmacologic effects including anti-inflammatory, anticancer and anti-metastatic properties [1,2,3]. In the present study, two novel curcumin derivatives, **L01** and **L02**, were synthesized and examined for anti-angiogenic effect and influence on signal transduction pathways. **L01**, **L02,** and curcumin inhibited the tube formation of the rat lymphatic endothelial TR-LE cells in a dose-dependent manner without affecting cell viability. **L01** showed the most potent inhibitory effect with an IC_{50} of 0.9 μM followed by **L02** and curcumin with IC_{50} of 1.35 and 2.9 μM, respectively. To investigate the molecular mechanisms involved, Western blot analysis revealed that all of the test compounds inhibited the phosphorylation of Akt, but not JNK and Erk, in TR-LE cell. **L01**, the most potent compound, completely inhibited phosphorylation of AKT at 3 μM while curcumin and **L02** inhibited phosphorylation of AKT at the higher concentration of 10 μM. The intracellular signal transduction supported that **L01** and **L02** exert anti-lymphangiogenesis action partly through Akt pathway. These results indicate that **L01** and **L02** are the potential lead compounds for the further development of anti-angiogenic drugs. *Acknowledgements:* Japanese-Thai Collaboratetive Scientific Research Fellowship (JSPS-NRCT) 2006 **References:** [1] Maheshwari, R.K. et al. (2006) Life Sci 78: 2081 – 2087. [2] Sharma, R.A., Gescher, A.J., Steward, W.P. (2005) Eur J Cancer 41: 1955 – 1968. [3] Ohashi, Y. et al. (2003) Oncology 65: 250 – 258.

P 504

Influence of the veratrum alkaloid veratridine, menthol and magnesium on catecholamine release at the ductus deferens and adrenal medulla

Jost S[1], Kretschmer N[2], Vierling W[1]
[1]Institute of Pharmacology and Toxicology, Technical University of Munich, Biedersteiner St. 29, 80802, Munich; [2]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens University, Universitätsplatz 4/I, 8010, Graz, Austria

The veratrum alkaloid veratridine, menthol and magnesium influence neuronal ion channels and may thereby modulate neurotransmitter release of the sympathetic nervous system. Therefore we investigated the influence of these substances on catecholamine release by an indirect and direct method, i. e. measuring contraction at the ductus deferens of the rat and measuring catecholamine release voltammetrically with carbon fibre microelectrodes at slices of rat adrenal medulla respectively. In absence of magnesium, already 0.5 μM veratridine augmented electrically induced catecholamine release at the ductus deferens and potassium-induced release at the adrenal medulla. This effect is probably due to the enhancing influence of veratridine on sodium channels [1] causing elevation of intracellular calcium concentration. The veratridine-dependent increase of catecholamine release both at the ductus deferens and at the adrenal medulla could be abolished by magnesium. Menthol and magnesium, known blockers of neuronal calcium channels [2,3] reduced electrically induced catecholamine release at the ductus deferens. The most effective concentration of menthol was 100 μM while the effect of magnesium was especially pronounced in the therapeutically relevant concentration range between 0.6 and 1.2 mM. Direct determination of catecholamine release from adrenal medulla confirmed the magnesium-induced inhibition of release. However, in contrast to the results at the ductus deferens, 100 μM menthol increased potassium-induced release. The results show that, under selected conditions, magnesium and veratridine exert similar effects at the ductus deferens and adrenal medulla. However, for menthol, there seem to be important differences depending on the investigated tissue and the kind of stimulation of release. **References:** [1] Honerjäger, P. (1982) Rev. Physiol. 92: 1 – 74. [2] Swandulla, D., Schäfer, K. (1986) Neurosci. Lett. 68: 23 – 28. [3] Dichtl, A., Vierling, W. (1991) Eur J Pharmacol. 204: 243 – 248

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Preliminary investigation of antiproliferative and antioxidant activity of *Alnus incana* (L.) Moench and *A. viridis* (Chaix) DC.

Šavikin K[1], Zdunić G[1], Stanojković T[2], Juranić Z[2], Janković T[1], Menković N[1]
[1]Institute for Medicinal Plants Research, T. Kožćužka 1, 11000 Belgrade, Serbia; [2]Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia

In folk medicine of Serbia *Alnus incana* and *A. viridis* are used for the treatment of gastrointestinal and skin diseases as well as to gargle mouth and throat. Due to insufficient literature information about

biological activity of these species, the aim of this study was a preliminary investigation of its antioxidant and antiproliferative activity. Plant material (bark, cones and leaves) was collected in 2006 on Èakor mountain in Montenegro (1600 m) and Stara planina in Serbia. Air-dried powdered material was extracted with methanol in a soxhlet apparatus for 24 h. Dry extracts (in different concentrations) were used for experiments. Total phenol content (gallic acid equivalent) was estimated using the Folin-Ciocalteu's reagent. Determination of tannins was done according to Ph Eur V. Antioxidant activity was tested in reaction with $DPPH^{\cdot}$ [1] and in a lipid peroxidation test [2]. Trolox® and ascorbic acid were used as positive controls. Neoplastic HeLa cells were used for the investigation of cytotoxic effects. Cell survival was determined indirectly by measuring total cellular protein by the Kenacid Blue R dye binding method [3]. IC_{50} concentration was defined as concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. All experiments were done in triplicate. The amount of total phenolics varied from 94.8 ± 1.8 to 381.3 ± 3.1 mg GAE/g dry weight of extracts while tannin content was between 1.5 ± 0.3 to 12.2 ± 0.7%. All extracts showed strong antioxidant activity in reaction with $DPPH^{\cdot}$ (IC_{50}= 3.3 ± 0.3 – 18.9 ± 1.4 μg/ml) and an inhibition of lipid peroxidation. All investigated extracts exhibited significant cytotoxic effect, with an IC_{50} values ranging from 26.02 ± 7.17 to 68.52 ± 1.11 μg/ml. Preliminary results show that extracts possess the potential for antiproliferative action against human cervix carcinoma cells *in vitro*. **Referances**: 1. Silva, B. A.. et al. (2005) Food Chem. 90: 157 – 167. 2. Liu F. et al. (1997) Life Sci. 60: 763 – 771. 3. Clothier, R. H. (1995). The FRAME cytotoxicity test. Methods in Molecular Biology, 43, 109 – 118.

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Preliminary investigation of antiproliferative and antioxidant activity of methanolic extracts from leaves and flowers of *Cornus mas* L. and *Cotinus coggygria* L.

Janković T[1], Zdunić G[1], Šavikin K[1], Stanojković T[2], Juranić Z[2], Menković N[1]
[1]Institute for Medicinal Plants Research, T. Kožćužka 1, 11000 Belgrade, Serbia; [2]Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia

The aim of this study was a preliminary investigation of the antioxidant and antiproliferative activity of *C. mas* and *C. coggygria*. Plant material (leaves and flowers) was collected in 2005, on Suvobor and Tara mountains. Air-dried powdered material was extracted with methanol in a Soxhlet apparatus. Dry extracts (in different concentrations) were used for the experiments. Total phenol content (gallic acid equivalent) was estimated using the Folin-Ciocalteu's reagent. Determination of tannins was done according to Ph Eur V. A HPLC method has been developed for the analysis of the extracts. Antioxidant activity was tested in reaction with DPPH radical [1] and in lipid peroxidation test [2]. Trolox® and ascorbic acid were used as positive controls. Neoplastic HeLa cells were used for the investigation of cytotoxic effects. Cell survival was determined indirectly by measuring total cellular protein by the Kenacid Blue R dye binding method [3]. IC_{50} of cell survival was estimated in comparision with a vehicle-treated control. All experiments were done in triplicate. The amount of total phenolics varied from 56.9 ± 1.3 to 515.6 ± 3.3 mg GAE/g dry weight of extracts while tannin content was between 1.6 ± 0.3 to 18.5 ± 0.9%. According to HPLC profiles of the extracts, ellagic and gallic acid, rutin, quercetin, kaempferol, caffeic and chlorogenic acid were detected in *C. mas* while dominant compounds in *C. coggygria* were gallic acid and its derivatives. All extracts showed strong antioxidant activity in reaction with DPPH (IC_{50}= 2.6 ± 0.4 – 7.1 ± 1.1 μg/ml) and an inhibition of lipid peroxidation. All extracts exhibited significant cytotoxic effects toward HeLa cells tested, with an IC_{50} ranging from 19.05 ± 3.91 to 64.05 ± 2.48 μg/ml. Preliminary results from this work show that extracts possess the potential for antiproliferative action against human cervix carcinoma cells *in vitro*. **Referances**: 1. Silva, B. A.. et al. (2005) Food Chem. 90: 157 – 167. 2. Liu F. et al. (1997) Life Sci. 60: 763 – 771. 3. Clothier, R. H. (1995). The FRAME cytotoxicity test. Methods in Molecular Biology, 43, 109 – 118.

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Cytoprotectivity of plant extracts on doxorubicin and irinotecan-treated human peripheral blood lymphocytes

Kopjar N[1], elježić D[1], Kosalec I[2], Bakmaz M[3], Jug M[2]
[1]Institute for Medical Research and Occupational Health, HR-10000 Zagreb, Croatia, [2]Faculty of Pharmacy and Biochemistry University of Zagreb, HR-10000 Zagreb, Croatia, [3]Zagreb City Pharmacy, Rakov Potok, Zagreb, Croatia

Growing clinical, toxicological and biochemical evidence supports the use of different herbs in chemopreventive strategies. It is particularly important in conventional cancer chemotherapy, since majority of antineoplastic drugs are detrimental to non-tumor cells and tissues too. In this study possible cytoprotective and genoprotective effects of plant extracts on doxorubicin and irinotecan-treated human peripheral blood lymphocytes *in vitro* were investigated. The water extracts examined were lemon balm (Melissae folium), green and black tea (Camelliae sinensis folium) and water and ethanolic extracts of purple coneflower (Echinaceae purpureae herba). Total polyphenols was determined using pharmacopoeial Folin-Ciocalteau method. Concentrations of plant extracts were adjusted to 1 and 0.1 mg/mL of crude lyophilized extracts. After 1-hour of pretreatment, lymphocytes were exposed to doxorubicin and irinotecan in their therapeutic concentrations for 2 hours. Assessment of cell viability, apoptosis and necrosis was performed using the fluorescent dye exclusion method. The levels of primary DNA damage were studied simultaneously by the alkaline comet assay. Corresponding negative controls were also included. All plant extracts possess low cytotoxicity and showed cytoprotective potential *in vitro*. The extracts did not induce significant increase of the single strand breaks or alkali-labile sites in pre-treated lymphocytes compared with negative control. Present findings confirm that polyphenolic compounds, as main constituents of extracts tested, are capable to prevent and/or significantly diminish DNA damage produced either directly or indirectly following exposure to both antineoplastic drugs. These preliminary results support further investigations on the possible application of plant extracts as food additives during chemotherapy.

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Investigation of *in vitro* activities of *Crocus sativus* styles extract relevant to Alzheimer's disease

Papandreou M[1], Kanakis CD[2], Polissiou M[2], Efthimiopoulos S[3], Cordopatis P[4], Margarity M[1], Lamari FN[4]
[1]Laboratory of Human & Animal Physiology, Department of Biology, University of Patras, Rion 26500 Greece; [2]Department of Science, Laboratory of Chemistry, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece, [3]Division of Animal & Human Physiology, Department of Biology, University of Athens, Panepistimiopolis, Ilisia, Athens 15784, Greece, [4]Laboratory of Pharmacognosy & Chemistry of Natural Products, Department of Pharmacy, University of Patras, Rion 26500, Greece

Crocus sativus styles are one of the widely known spices (saffron) and consist of unusually polar carotenoids (crocins). Traditionally, saffron has been used for the treatment of cognitive diseases. Alzheimer's disease is characterized pathologically by deposition of amyloid β-peptide (Aβ) fibrils and a diminution of cholinergic activity. The only available drugs for symptomatic therapy of Alzheimer's disease are acetylcholinesterase inhibitors. To identify agents inhibiting the pathogenesis of Alzheimer's disease, we examined *in vitro* the effect on $A\beta_{1-40}$ fibrillogenesis and on the activity of acetylcholinesterase of the water-methanol (50:50, v/v) extract of *C. sativus* styles. The effects on Aβ-aggregation and fibrillogenesis were studied by thioflavine T-based fluorescence assay and by DNA binding shift assay [1]. The extract inhibited Aβ fibrillogenesis in a dose and time-dependent manner. The main carotenoid constituent,

trans-crocin-4, the digentibiosyl ester of crocetin, inhibited Aβ fibrillogenesis at lower concentrations than dimethylcrocetin, revealing that the action of the carotenoid is enhanced by the presence of the sugars [1]. Inhibition of the activity of acetylcholinesterase was studied with a colorimetric assay and the results showed that although the extract exhibited low dose-independant inhibitory values, crocetin and dimethylcrocetin had IC 50 values in the low micromolar range, suggesting that the sugars obstruct their inhibitory activity on acetylcholinesterase. Our findings reveal that *C. sativus* style constituents may have a potential for prevention/treatment of Alzheimer's disease. *Acknowledgements:* This project is co-funded from the European Union by 75% and from the Hellenic State by 25% through the Reinforcement Programme of Human Research Menpower (PENED) 2003/03ED/665 and by GlaxoSmithKline S.A. **References:** [1] Papandreou et al. (2006) J. Agric. Food Chem. 54: 8762.

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Antioxidant activity of methanol extracts of two Spanish Betulaceae species

Muñoz-Mingarro D, Acero N, Dominguez MT, Llinares F
Universidad CEU San Pablo Facultad de Farmacia. Urb. Montepríncipe. 28660 Boadilla del Monte. SPAIN

Extracts from Betulaceae leaves and bark, had been used traditionally in popular medicine for the treatment of several diseases [1]. A special interest for the study of the secondary metabolites of Betulaceae plants had emerged since 1994, due to the ability of betulinic acid against human melanoma [2]. Moreover, the antitumoral, apoptotic, antimetastasic, antioxidant and antimicrobial activity of some Betulaceae extracts had been proved [3]. However, there are no studies about the pharmacological application of extracts or products obtained from Spanish Betulaceae. The antioxidant properties of two Spanish Betulaceae, *Alnus glutinosa* (L.) Gaertn. and *Betula alba* L., were investigated. A methanol extract of leaves and bark of both species exhibited hight 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Moreover, an ABTS assay and their suppressive effects on superoxide (O_2^-) generation using xanthine (XA)-xanthine oxidase (XOD) assay system were evaluated. The extracts of *A. glutinosa* were effective inhibiting both uric acid formation and NTB reduction by the superoxide radical. Determination of the GSH/GSSG ratio is a useful indicator of oxidative stress and can be used to monitor the effectiveness of antioxidant intervention strategies. In this way a GSH assay was performed with three tumoral cell lines, PC-3, HT-29 and HeLa. *A. glutinosa* leaves extract was the most effective. In addition, the content of total phenols in all the extracts was determined with spectrophotometric methods. Results were compared with reference antioxidants (ascorbic and gallic acid). The antioxidant activity was correlated with total phenols extracts content [4]. The results confirm that this type of secondary metabolites may explain the observed antioxidant property. *Acknowledgements:* CEU San Pablo University **References:** [1] Sang, T.K. et al. (2004) Phytother. Res., 18(12): 971 – 975. [2] Joo, S.S. et al. (2002) Arch. Pharm. Res., 25: 493 – 9. [3] Ju, E.M., et al. (2004) Life Sci. 74(8): 1013 – 26. [4] Alasavar, C. (2006) J. Agric. Food Chem. 54: 4826 – 32

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The analysis of the antioxidant compounds of *Melissa officinalis* L. in *in vitro* regenerated variants

Danila D[1], Gille E[1], Gatea F[2]
[1]"Stejarul" Biological Research Centre/INCDSB Bucuresti, Alexandru cel Bun Street, 610004, Piatra Neamt, Romania; [2]National Institute of Biological Sciences Bucharest, 296, Splaiul Independentei, 600031, Bucharest, Romania

The flavons and polyphenols of *Melissae folium*, due to their antioxidant effect, may be bioactive constituents of some phytopreparations [1]. Due to the pharmaceutical and cosmetic importance of *Melissa officinalis*, we considered *in vitro* plant regeneration as a useful and important possibility in the multiplication of phytochemically valuable genotypes. Using the tissue cultures regenerating techniques we had as an aim the micropropagation of valuable genotypes, with well-defined biochemical characteristics [2]. We evaluated the biosynthetic spectrum of the polyphenolic and flavonoidic compounds in alcoholic extracts by means of TLC and RP-HPLC methods (using a linear gradient of acetonitril-sodium acetate brought to pH = 2.65 with acetic acid). The tissue cultures were initiated from stem explants (apexes and nodes), prelevated from experimental field plants. The regenerated neoplantlets went through all the phases of the phenophase after being transfered to the experimental field (Neamt – Romania). In the ethanolic extracts (70%) of the *in vitro* regenerated plants, the content of polyphenols and flavons (μg/mL) was as follows: 1194.86 *rutin;* 157.81 *quercetin,* 1407.69 *caffeic acid, 19171.8 rosmarinic acid,* 65.86 *ferulic acid* and 14.38 *cumaric acid.* The data obtained by HPLC confirm the optimum extraction efficiency – for all the components analyzed, at an ethanolic concentration of 70%. TLC confirms the domination of polyphenols compared to flavons. The *in vitro* plants synthetize and accumulate a smaller quantity of antioxidant compounds yet the biosynthetic spectrum is much diversified, the number of the identified compounds is higher than that of the generatively obtained variants. *Acknowledgments:* The work is sustained in the CEEX-BIOTECH program financed by the Romanian Government. **References:** [1] Istudor V. (2001) Farmacognozie, fitochimie, fitoterapie. Ed. Med., Bucharest, 2: 99 – 101. [2] Meszaros A. et al. (1999) Plant cell tissue organ cult., 57, 2: 149 – 152.

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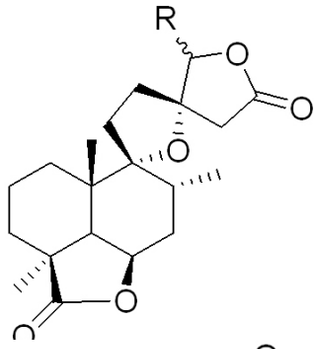
Effect of *Marrubium globosum* ssp. *libanoticum* on intestinal motility

Aviello G[1], Rigano D[2], Borrelli F[1], Formisano C[2], Rosselli S[3], Senatore F[2], Bruno M[3]
[1]Department of Experimental Pharmacology, University of Naples "Federico II", via D. Montesano 49, 80131 Naples, Italy; [2]Department of Chemistry of Natural Products, University of Naples "Federico II", via D. Montesano 49, 80131 Naples, Italy; [3]Department of Organic Chemistry, University of Palermo, Viale delle Scienze, Parco d'Orleans II-90128 Palermo, Italy

In continuation of our phytochemical and pharmacological investigations of *Marrubium* species [1, 2], we report on the antispasmodic activity of a chloroformic extract of *Marrubium globosum* Montbr. et Auch. ex Benth. ssp. *libanoticum* Boiss. (Lamiaceae), as well as on the isolation and identification of diterpenic compounds. *M. globosum* ssp. *libanoticum*, called "hashiashat el kelb" in Northern Lebanon, is a medicinal plant used for urinary tract infections, gastrointestinal ailments and against snake bites [3]. For our study, plant material was collected in August from flowering plants in Lebanon. The powdered plant was sequentially extracted by cold maceration with petroleum ether and $CHCl_3$; (% w/w: petroleum ether 3.7%; $CHCl_3$ 3.2%) the residue was chromatographed on Si gel eluting with petrol and petrol-EtOAc. Further purification by HPLC gave the new natural furolabdane diterpenoids (**1**)-(**7**). Intestinal motility was studied *in vivo* in mice using the charcoal method and *in vitro* on contraction of the isolated mouse ileum elicited by exogenous spasmogen agents. The chloroformic extract of *M. globosum* (MG) significantly inhibited the intestinal transit *in vivo* (% transit: Control 53.8 ± 2.39, MG 0.1 mg/kg 52.4 ± 4.08, MG 0.3 mg/kg 35.6 ± 1.85***, MG 1 mg/kg 32.0 ± 3.30***, MG 3 mg/kg 24.9 ± 4.52***, $^{***}p < 0.001$) and the contractile response elicited by acetylcholine (% inhibition: 1 μg/ml 7.9 ± 2.25; 3 μg/ml 15.8 ± 3.5; 10 μg/ml 24.4 ± 3.1; 30 μg/ml 27.9 ± 3.6; 100 μg/ml 42.6 ± 4.7; 300 μg/ml 64.8 ± 2.6; 1000 μg/ml 89.2 ± 2.6. $p < 0.001$, $n = 6$). and barium chloride (% inhibition: 1 μg/ml 14.1 ± 1.2; 3 μg/ml 18.8 ± 2.1; 10 μg/ml 28.3 ± 1.8; 30 μg/ml 38.1 ± 0.29; 100 μg/ml 62.9 ± 7.0; 300 μg/ml 73.8 ± 5.5; 1000 μg/ml 89.5 ± 0.4. $p < 0.001$, $n = 6$). in the isolated ileum



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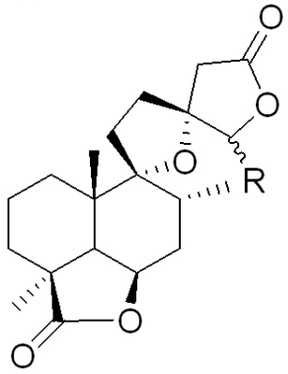
2 R = β-OH



3 R = α-OH
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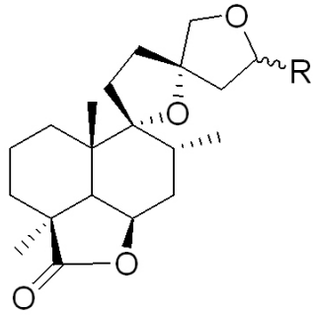

5 R = α-OH
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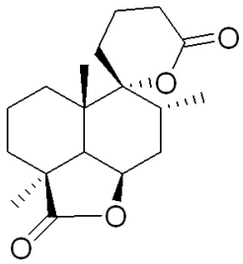

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References: [1] Rigano, D., Grassia, A. et al. (2006) J. Nat. Prod. 69(5):836 – 838. [2] Grassia, A., Senatore, F. et al. (2006) Polish J Chem 80: 623 – 628. [3] Wichtl M., Anton R. (1999) Plantes thèrapeutiques. Tec & Doc. Paris. P. 341.

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Spasmolytic activity of *Phagnalon saxatile* (L.) Cass. on the isolated rat ileum

Aviello G[1], Rigano D[2], Formisano C[2], Capasso R[1], Izzo AA[1], Senatore F[2], Bruno M[3]

[1]Dipartimento di Farmacologia Sperimentale, University of Naples Federico II, via D Montesano 49, 80131 Naples, Italy; [2]Dipartimento Chimica Sostanze Naturali, University of Naples Federico II, via D Montesano 49, 80131 Naples, Italy; [3]Dipartimento di Chimica Organica, Università degli Studi di Palermo, Parco d'Orleans II, I-90128 Palermo, Italy

The genus *Phagnalon* (Asteraceae) is represented by about 30 species, six of which are typical of the Mediterranean region [1]. Different *Phagnalon spp*. are used in traditional medicine: in the Negev desert these plants are used for the healing of burns among the Bedouins [2] and in the Palestinian area to treat asthma, headache and as an analgesic for toothache [3]. *Phagnalon saxatile* (L.) Cass. is one of the five suffruticous chamaephyte species growing wild in Southern Italy [4]. Since the plant is traditionally used in Sicily to treat abdominal spasms and since phytochemical and pharmacological information on this species is limited, we investigated the potential antispasmodic effect of a methanolic extract obtained from the flowering aerial parts of *P. saxatile* (PsE) in the rat ileum. For this study, aerial parts of *P. saxatile* were collected in Capo Zafferano (PA, Southern Italy). Plant material was sequentially extracted with petroleum ether, $CHCl_3$ and CH_3OH (% w/w: petroleum ether 1.89%, $CHCl_3$ 1.98% and CH_3OH 3.67%). The methanolic solution was concentrated obtaining a residue that was chromatographed on a Sephadex column, eluting with CH_3OH. Further purification by reverse HPLC gave pure apigenin 7-*O*-β-D glucopyranoside, luteolin 4'-*O*-β-D glucopyranoside, caffeic acid, chlorogenic acid, methyl chlorogenate, 3,5-di-O-caffeoylquinic acid and two hydroquinone glucosides. Pharmacological assay showed that PsE, at concentrations ranging from 1 to 100 μM, inhibited in a concentration-dependent manner the contractions induced by acetylcholine in the isolated rat ileum (% inhibition: 1 μg/ml 6.3 ± 1.6; 3 μg/ml 26.5 ± 7; 10 μg/ml 35.7 ± 4; 30 μg/ml 59 ± 3; 100 μg/ml 62.2 ± 3. $P < 0.05$ at 3 μg/ml and 10 μg/ml; $P < 0.01$ at 30 μg/ml and 100 μg/ml, $n = 6$). These preliminary results might provide a first pharmacological basis underlying the traditional use of this plant in the treatment of intestinal spasms. **References:** [1] Tutin, T.G., Heywood, V.H. et al. (1976) Flora Europea Vol. 4. Cambridge University Press. Cambridge. p. 133. [2] Friedman J., Yaniv, Z. et al. (1986) J. Ethnopharm. 16: 275 – 287. [3] Ali-Shtayeh M.S., Yaghmour M.R. et al. (1998) J. Etnopharm. 60: 265 – 271. [4] Pignatti, S. (1982) Flora d'Italia Vol. 3. Edagricole. Bologna. pp. 40 – 41.

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Pharmacokinetic evaluation of phytotherapeutic drops with *E. purpurea* extract

Guiotto P[1], Woelkart K[2], Grassi G[3], Campisi B[4], Perissutti B[1], Bauer R[2], Voinovich D[1]

[1]Department of Pharmaceutical Science, University of Trieste, I-34127 Trieste, Italy; [2]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl- Franzens- University Graz, A-8010 Graz, Austria; [3]Department of Internal Medicine, University Hospital of Trieste, Cattinara, I- 34149 Trieste, Italy; [4]DMRN, Via A Valerio 6, University of Trieste, I-34127 Trieste, Italy

Echinacea is a widely used herbal remedy for the prevention and treatment of respiratory upper tract infections due to their immunostimulant properties. Recently, studies on the molecular mode of action of the main lipophilic constituents, the alkamides, have been carried out [1,2]. Due to the high affinity of these components for the buccal membrane, the aim of this research was to prepare drops containing dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides, the major alkamides, in different concentrations (0.07, 0.21 and 0.9 mg). Bioavailability and the pharmacokinetic studies were carried out on these three phytotherapeutic formulations, after administration to 6 healthy volunteers of the three different dosage drops

with one week washout between each other. Liquid chromatography electronspray ionization ion-trap mass spectrometry was used to determine the content of dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides in serum. It was found that, for the 0,07 mg drops, the C_{max} was 0.55 ng/ml and t_{max} was 30 min; for the 0,21 mg drops the C_{max} was 0.88 ng/ml and the t_{max} was again 30 min. Finally, for the 0,9 mg drops the C_{max} was 6.53 ng/ml and the t_{max} 20 min. An *ex vivo* study was performed to measure the influence on the immunological system of the three different froms of drops. All preparations gave the same effects on TNF-α pro-inflammatory cytokines and the IL-8, IL-6, IL-12p70 and IL-10 chemokines. After 24 h from buccal administration a significant down-regulation of all these immune parameters was found. **References:** [1] Woelkart, K., Koidl, C., et al. (2005) J. Clin. Pharmacol. 45: 683 – 689. [2] Woelkart, K., Marth, E., et al. (2006) Int J Clin. Pharmacol. Ther. 44: 401 – 408.

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The effects of inhalation of essential oil odour from *Rosa damascena* Mill. on gerbils in two models of anxiety

Bradley BF[1], Starkey NJ[2], Brown SL[1], Lea RW[3]
[1]Department of Psychology, University of Central Lancashire, Preston. Lancashire. UK. PR1 2HE; [2]Department of Psychology University of Waikato.Private Bag 3105. Hamilton. New Zealand; [3]Department of Biological Sciences, University of Central Lancashire PR1 2HE

To investigate the anxiolytic effects of prolonged rose odour, mature gerbils (*Meriones unguiculatus*) were exposed to acute (24 hour), chronic (2 week) rose odour, or a no-rose condition. Their behaviour was assessed in the elevated plus maze and black white box. Results were compared with the effects of diazepam (1 mg/kg) i.p. The Jonckheere-Terpstra test was used, with the Mann Whitney U-test to examine significant group differences. In the elevated plus maze, spatiotemporal measures altered by diazepam, were unaffected by rose oil, whereas exploration, headdip frequency increased (acute $U = 100$, $p < 0.001$; chronic $U = 13$, $p < 0.001$). In the black white box rose oil had anxiolytic effects: latency to move from the white to the black compartment (acute $U = 182$, $p < 0.01$, chronic $U = 179$, $p < 0.05$), percentage time in the white compartment (acute $U = 168$, $p < 0.01$, chronic $U = 149$, $p < 0.01$) and exploration, rear-sniff frequency white (acute $U = 100$, $p < 0.001$; chronic $U = 99$, $p < 0.001$) increased. The percentage of time in the dark area decreased (acute $U = 160$, $p < 0.01$, chronic $U = 178$, $p < 0.05$). This anxiolytic profile strengthened after chronic exposure to rose odour, transitions between the compartments ($U = 167$, $p < 0.01$) and percentage of time moving around the arena ($U = 154$, $p < 0.001$) increased. The anxiolytic profile of rose oil odour in these models was unlike diazepam's benzodiazepine profile and perhaps more representative of serotonergic type anxiolytics. *Acknowledgements:* Thankyou to Dr Paul Pollard, Head of Department, The Department of Psychology, University of Central Lancashire for supporting this work.

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TRPV1 antagonist activity of the extract and compounds from the fruits of *Tetradium daniellii*

Rédei D[1], Vizler C[2], Pecze L[2], Oláh Z[2], Forgo P[3], Hohmann J[1]
[1]Department of Pharmacognosy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary; [2]Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Temesvári krt 62, H-6726 Szeged, Hungary; [3]Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary

Vanilloid receptor type 1 (TRPV1), which confers noxious heat and inflamatory pain signals in the peripheral nervous system, has recently been implicated as novel target in painkiller drug discovery [1]. *Evodia* species have been recognised as a source of TRPV1 ligands. Quinazolinocarboline alkaloids from *E. rutaecarpa*, evodiamine and rutaecarpine, are potent agonists of TRPV1 [2,3]. We noted that different extracts from *Tetradium daniellii* (Benn.) T.G.Hartley (syn. *Evodia hupehensis* Dode) affects Ca^{2+}-uptake *via* TRPV1. The cyclohexane extract from fresh fruits of *T. daniellii* inhibited capsaicin-induced TRPV1 in NIH3T3 cells ectopically expressing the receptor. Dose-dependent inhibition of TRPV1 was determined in bioactivity-guided fractionation of the extracts. The cyclohexane phase was consecutively subjected to 1) open column chromatography (OCC), 2) vacuum-liquid chromatography, 3) RP-OCC and 4) preparative TLC fractionation, which afforded two active constituents. The structures of the isolated compounds were determined by ^{1}H- and ^{13}C-NMR experiments as *cis*-9,*cis*-12-linoleic acid and (2*E*,4*E*)-*N*-isobutyldeca-2,4-dienamide (= pellitorine). Both compounds inhibited the capsaicin-evoked Ca^{2+}-uptake with an IC_{50} of 80 and 200 μg/ml, respectively. Our separation process allowed also identification of *N*-isobutyl-4,5-epoxy-2*E*-decadienamide and furocoumarins, which proved to be inactive. Our study provides additional evidence that polyunsaturated fatty acids and aliphatic alkylamides can inhibit channel opening of TRPV1. **References:** [1] Olah Z. et al. (2001) J. Biol. Chem. 276: 11021 – 110301. [2] Calixto JB. et al. (2005) Pharmacol. Therapeut. 106: 179 – 208. [3] Pearce L V. et al. (2004) Org. Biomol. Chem. 2: 2281 – 2286.

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Activity-guided isolation of antiproliferative compounds from *Achilleae collina*

Csupor-Löffler B[1], Hajdú Z[1], Réthy B[2], Zupkó I[2], Fakay G[2], Forgo P[3], Hohmann J[1]
[1]Institute of Pharmacognosy, University of Szeged, Szeged, Eötvös u. 6., 6720, Hungary [2]Institute of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Eötvös u. 6., 6720, Hungary, [3]Department of Organic Chemistry, University of Szeged, Szeged, Dóm tér 8., 6720, Hungary

Plants of the Asteraceae family are used in the medicine for a variety of indications. Many of them were investigated for their cytotoxic activity [1,2,3], but only limited data are available concerning the anticancer effects of the Central-European species. As a part of a comprehensive screening programme, we tested the Asteraceae species found in Hungary for antiproliferative effect by MTT assay. In the course of this work, the chloroform extract of *Achillea collina* L. herbs exhibited high antiproliferative activity on HeLa cell line. The present paper reports the identification of compounds responsible for the antiproliferative activity of the extract. The chloroform extract was fractionated using multiple chromatographic methods (VLC, CPC, PLC, gel filtration). Each separation step was controlled with antiproliferative assay on three human cell lines (HeLa, MCF-7, A431). As a result of our experiments, six active and four inactive compounds were isolated. The structures of the compounds were established by means of UV, NMR and mass spectroscopy, optical rotation data were also determined. Structurally, the active compounds can be classified as flavonoids (apigenin, luteolin, casticin, and centaureidin) and *seco*-pseudoguaianolides (paulitin and isopaulitin). Centaureidin, casticin and paulitin exhibited the most pronounced activity with IC_{50} 0.08 – 0.35 μM, 1.29 – 3.58 μM and 1.48 – 4.76 μM, respectively. Inactive compounds were also isolated, such as artemetin, millefin, deacetyl-matricarin and the psilostachyin C. The *seco*-pseudoguaianolide-type compounds are reported for the first time from this genus. **References:** [1] Heinrich, M. et al. (1998) Ann. Rev. Pharmacol. Toxicol. 38: 539 – 65. [2] Itharat, A. et al. (2004) J. Etnopharmacol. 90: 33 – 38. [3] Lee, C.C., Houghton, P. (2005) J. Etnopharmacol. 100: 237 – 243.

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Acute and Chronic Toxicity Studies of the Aqueous Extract of *Mezoneuron Benthamianum* Baill (Caesalpiniaceae)

Mbagwu HOC[1,2], Adeyemi OO[1]
[1]Department of Pharmacology, College of Medicine, University of Lagos, P.M.B.12003, Lagos, Nigeria; [2]Department of Pharmacolgy and Toxicology, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo, Nigeria

Mezoneuron benthamianum (MB) is used traditionally in Nigeria for the treatment of peptic ulcer, diarrhoea and pain. Acute and chronic toxicity studies of the aqueous extract of a mixture of the root and leaves of MB were carried out in rodents to evaluate the safety profile (1,2). In the acute studies, 7 groups of mice (10/group) were administered with 5 – 20 g/kg of MB orally and 250 – 2000 mg/kg intraperitoneally (ip).The treated animals were examined for toxic signs and compared with the controls. In the chronic study, MB was administered orally to 20 rats and 20 mice at a daily dose of 320 mg/kg. Control animals received 10 ml/kg/day of distilled water. During the treatment, animals were observed for signs of toxicity. In addition, feeding habit and mortalities among others were recorded. At the end of the treatment, haematological and biochemical parameters were determined; semen and spermatozoa morphology were assessed and the vital organs analysed histologically. In the acute studies, MB produced no toxicities or mortality with oral doses up to 20 g/kg. The LD_{50} for ip administration was 1021.31 mg/kg. The control animals for both oral and ip groups showed no ill effects. Except for increases in AST and ALT as well as mild inflammatory reactions in liver and testes in both species and a decrease in total protein in rats, other parameters showed no significant differences between control and treated animals in the chronic studies. Semen and spermatozoa morphology, however, showed very significant ($p < 0.05$) abnormalities when compared with control animals. These results show that the application of MB cannot be regarded as safe. **References:** [1] Thanabhorn, S., Jaijoy, K., et. al., (2006):Acute and subacute toxicity of the ethanolic extract from Lonicera japonica Thunb. J. Ethnopharm. 107: 370 – 373. [2] Yemitan, O.K. and Adeyemi O.O. (2004): Toxicity studies of the aqeous extract of Lecanidiscus cupanioides. Nig. J. Health and Biomed Sci. 3: 20 – 23.

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Biphasic Gastrointestinal Activity of the Aqueous Root Extract of *Talinum Triangulare* Jacq. Willd (Portulacaceae)

Adeyemi OO[1], Oyeniyi OP[1], Mbagwu HOC[1,2]
[1]Department of Pharmacology, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria; [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo, Nigeria

Talinum triangulare (TT) is a herbaceous perennial plant widely grown in tropical regions of the world. In Nigeria, it is cultivated in gardens and large farms and serves as one of the most important edible leaf vegetables (1). The root is used in the treatment of inflammation (2) and locally for other gastrointestinal upsets. In this study, we evaluated some gastrointestinal activities of the aqueous root extract of TT using three models: Intestinal propulsive movement (IPM), Castor-oil induced diarrhoea (COD) and Intestinal fluid accumulation (IFA). Pretreatment of rats with lower doses (50 – 200 mg/kg) caused a dose-dependent but non-significant increase in onset of diarrhoea and total diarrhoea score which were significantly ($p < 0.05$) less than that produced by liquid paraffin (10 ml/kg). The differences in other diarrhoeal parameters such as number of wet stools, total number of stools were neither significant nor dose-dependent. Higher doses (500 – 2000 mg/kg), however, produced a dose-dependent delay in onset of diarrhoea, frequency of stooling and total diarrhoea score. These parameters were significant only at 2000 mg/kg and were less than that of atropine (2 mg/kg). There were dose dependent and non-significant increases in IFA by the lower doses which were less than that of liquid paraffin. The higher doses significantly ($p < 0.05$) inhibited IFA. The inhibitions by the higher doses were significantly greater than that of atropine. Preliminary phytochemical screening revealed the presence of alkaloids, saponins, tannins, cardiac glycosides and phenols. These results show that TT produces biphasic gastrointestinal effects. Lower doses produce laxative, while higher doses produce anti-diarrhoeal, effects. **References:** [1] Nyananyo, B.L., and Olowokudejo, J.D. (1986): Taxanomic studies in the genus Talinum (Portulacaceae) in Nigeria. Wildenowie 15: 455 – 463. [2] ARCBS, (2004): Asean Regional Centre for Biodiversity Conservation: www.aseanbiodiversity.org/medicinal-plants.

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Studies on the comparison of the constituents from Korean and Chinese *Cynanchum* spp. and their effects on the hyperlipidemia in rats

Lee J[1], Yang G[1], Yoon YJ[1], Lee BH[1], Seong NS[2], Ham I[1], Choi HY[1]
[1]College of Oriental Medicine, Institute of Oriental Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul, Republic of Korea; [2]National Institute of Crop, 151 Seodun Dong Gwonseong-gu Suwon, Gyeongi-do, Republic of Korea

The constituents of *C. wilfordii* (CWK), *C. auriculatum* (CAK) in Korea and *C. auriculatum* (CAC) in China was compared by analysis of the TLC pattern. Their effects on the hyperlipidemia was investigated in rats induced by Triton WR-1339 and high cholesterol diet. The dried roots of CWK, CAK, CAC were extracted three times with 70% MeOH. The extract was suspended in water and then partitioned with chloroform and concentrated. The extracts (50 mg/kg, 200 mg/kg) were orally administrated every day for 7 days. In the hyperlipidemic rats induced by Triton WR-1339, CWK and CAK significantly decreased total cholesterol, triglyceride, otherwise significantly increased HDL. CWK and CAK significantly decreased AST and ALT. Also, in the hyperlipidemic rats induced by high cholesterol diet, CWK, CAK and CAC showed significant decreasing effects on total cholesterol, and especially CWK increased HDL and significantly decreased LDL. Korean CAK showed increasing effects on HDL. These results indicated that TLC analysis showed similar patterns between CWK, CAK and CAC, so the constituents of CWK and CAK is similar. CWK and CAK CAC were all effective in the hyperlipidemic rats induced by Triton WR-1339 and high cholesterol diet. *Acknowledgements:* This work was supported by the Second Stage of Brain Korea 21 project in Oriental Medical Science Center. **References:** [1] Kusama H. et al. (1988) Nippon Yakurigaku Zasshi 92(3):175 – 80.

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Studies on the comparison of the constituents from Bambusae caulis in Taeniam and their effects on the hyperlipidemia in rats

Tae J[1], Yang G[1], Lee J[1], Kim JB[1], Bang CS[1], Lee JJ[1], Ham I[1], Choi HY[1]
[1]College of Oriental Medicine, Institute of Oriental Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul, Republic of Korea

Bambusae Caulis in Taeniam is the green middle layer of stem of *Phyllostachys nigra* (Bambusaceae) after the bark had been removed and it is like dried green string shape. In the present study, the components of the *P. nigra* (PN), *P. bambusoides* (PB), Chinese Bambusae Caulis in Taeniam (BCT), P. pubescens (PP) and the bark of *P. nigra* (PNB) were compared and analyzed by TLC and HPLC as the Bambusae Caulis in Taeniam. The 70% methanol extracts of these were used for TLC and the 95% methanol extracts of these were used for HPLC. The effects of the 95% methanol extracts of PN, PB, PP, and BCT on rat with hyperlipidemia, induced by Triton WR-1339 injection, high cholesterol diet, and subacute alcohol stimulation were investigated. The level of total cholesterol, triglyceride, HDL, LDL, AST, ALT was measured. As a result, all extracts showed similar pattern in TLC analysis and PNB and PB showed similar pattern in HPLC analysis. The extracts (50, 200, 800 mg/kg) were orally administrated every day for 7days. In the hyperlipidemic rats induced by Triron WR-1339, all extracts decreased total cholesterol, triglyceride

and PN and BCT was superior to other exracts for inhibition of AST, ALT. Also in the hyperlipidemic rats induced by high cholesterol diet, all the extracts decreased total cholesterol, triglyceride, AST and ALT. And in the hyperlipidemic rats induced by alcohol, all the extracts dereased total cholesterol, triglyceride, LDL, AST and ALT. Therefore Bambusae Caulis in Taeniam may be used as a remedy for hyperlipidemia. *Acknowledgements: This work was supported by the Second Stage of Brain Korea 21 project in Oriental Medical Science Center.* **References:** [1] Rudel LL, et al. (2001) Curr. opin. lipidol 12: 121 – 7. [2] Min Zhanga, et al. (2005) Life Sciences 76: 2115 – 2124.

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Antitumor activity of xanthanolides from *Xanthium italicum* Moretti

Kovács A[1], Vasas A[1], Zupkó I[2], Réthy B[2], Forgo P[3], Hohmann J[1]
[1]Department of Pharmacognosy, University of Szeged, 6720 Szeged, Eötvös u. 6, Hungary; [2]Department of Pharmacodynamics and Biopharmacy, University of Szeged, 6720 Szeged, Eötvös u. 6, Hungary; [3]Department of Organic Chemistry, University of Szeged, Dóm tér 8, Hungary

The genus *Xanthium* (Asteraceae) is represented by limited number of species, which are distributed in all parts of the world. Some species were used in the traditional medicine for the treatment of basal cell carcinoma and different cancers and cold tumors.[1,2] In the course of our screening program for antiproliferative compounds in Asteraceae family found in Central and East Europe, extracts of *X. italicum* were evaluated. High cytotoxic activity was recorded for the lipophylic extract, thus this species was selected for bioassay-guided fractionation in order to identify the compounds responsible for the cytotoxic effect. *X. italicum*, a widespread common wead in South and Central Europe, was collected in various stage of vegetation. Different parts of the dried plant (radix, flower, stem and leaf) were extracted with methanol. The extracts were concentrated *in vacuo*, and then subjected to liquid-liquid partition with *n*-hexane and chloroform. The cytotoxic effects of the extracts were investigated on HeLa (cervix epithelial adenocarcinoma), A431 (skin epidermoid carcinoma) and MCF7 (breast epithelial adenocarcinoma) cell lines using the MTT assay. The most active *n*-hexane fraction of the leaves was separated by VLC, preparative TLC, HPLC and CPC with guidance of cytotoxic assay affording five compounds. The structures were established by means of NMR spectroscopy (^{1}H-NMR, JMOD, ^{1}H,^{1}H-COSY, NOESY, HSQC and HMBC) as xanthanolide-type sesquiterpene lactones. The cytotoxic assay on the three human cell lines demonstrated that the isolated xanthanolides possess remarkable cell growth inhibitory activity comparable to that of the positive control cisplatin. **References:** [1] Hartwell, J. (1968) J Nat Prod 31: 71 – 170. [2] Saxena, V.K., Mondal, S.K. (1994) Phytochemistry 35: 1080 – 1082

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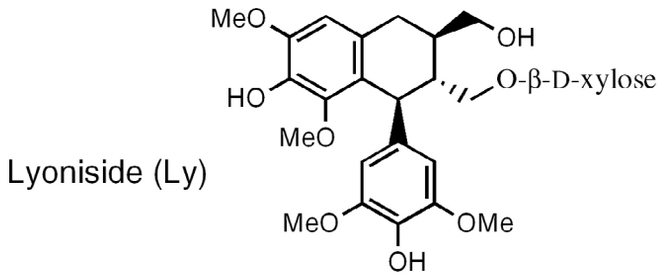
Prophylactic effect of the constituent of *Lyonia ovalifolia* against high fat diet-induced obesity rats

Kashima K[1], Yun YH[1], Fujikawa T[2], Ina H[1], Inoue H[1], Kunugi A[1]
[1]Tokyo University of Pharmacy & Life Science, 1432 – 1 Horinouchi, Hachioji, Tokyo 192 – 0392, Japan; [2]Mie University Graduate School of Medicine, 2 – 174 Edobashi, Tsu, Mie 514 – 8507, Japan

Lyoniside (Ly) is a phenyltetralin lignan isolated from *Lyonia ovalifolia* (Ericaceae) wood as a major component (1). In the course of our research on the pharmacological effects of Ly, we recently found a prophylactic effect against 35% high fat diet-induced obesity rats. In the test, rats were fed a diet containing 5% (normal diet, ND) or 35% (high fat diet, HFD) beef tallow with or without Ly (0.02% w/w and 0.06% w/w) for 3 months. Three month-administration of Ly (0.02%) demonstrated a 48.9% and 22.8% protective effect against an increase in the renal or testicular white adipose tissues (WATr and WATt, respectively) of rats. The protective effects were not dose-dependent. The administration of Ly (0.02%) decreased the circulating total cholesterol (T-CHO) and free fatty acid (FFA) levels, while the circulating level of triglyceride (TG) was unaffected, whereas 0.06% Ly maintained the effect on the circulating T-CHO levels and decreased the circulating TG levels. The circulating FFA levels were deteriorated oppositely. The circulating levels of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were unaffected by the 3-month administration of Ly. These results suggest that an improvement of blood lipid parameters by Ly markedly prevents the visceral fat accumulation in rats under HFD conditions. Furthermore, 12 known compounds were newly isolated from *Lyonia ovalifolia* wood.



Lyoniside (Ly)

Reference: 1. Yasue M. et al. (1960) *Yakugaku Zasshi*, 80: 1013.

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New 4,12-dideoxyphorbol esters from *Euphorbia pannonica* Host

Sulyok E[1], Rédei D[1], Dombi G[2], Hohmann J[1]
[1]Department of Pharmacognosy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary; [2]Department of Pharmaceutical Analysis, University of Szeged, Somogyi B. u. 4, H-6720 Szeged, Hungary

Ingenane, tigliane and daphnane diterpenes, called in common as phorboids, are typical constituents of the families Euphorbiaceae and Thymelaeaceae. Earlier studies on these compounds focused mainly on their powerful skin irritant and tumour promoting activities, which are the result of their protein kinase C activating potential. Further investigations have disclosed much wider pharmacological activities e.g. cytotoxic, antileukemic, antiviral, sedative and analgesic effects [1,2,3]. As part of our ongoing search for biologically active compounds from Hungarian Euphorbiaceae, we report herein the isolation and structure determination of two new diterpenes from *Euphorbia pannonica* Host. This plant is a perennial herb native to south, eastern and central regions of Europe. Phytochemical or biological investigations on *E. pannonica* have not been reported previously. The methanolic extract of the dried whole plants of *E. pannonica* was subjected to solvent partitioning to furnish dichloromethane and water-soluble fractions. The organic phase was fractionated by column chromatography on polyamide, then by vacuum liquid chromatography on silica gel. Selected fractions from these separations were further purified by CPC, preparative TLC and HPLC to yield two pure compounds. The structure elucidation was carried out by extensive NMR studies using advanced experiments (^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, HSQC and HMBC). The isolated compounds were identified as 4,12-dideoxyphorbol-20-benzoate-13-isobutyrate and 4,12-dideoxyphorbol-20-benzoate-13-isovalerianate. 4,12-dideoxyphorbols occur rarely, only the parent alcohol and its two esters were isolated earlier from *Excoecaria bicolor* [4]. **References:** [1] Fatope, M.O. et al. (1996) J Med Chem 39: 1005 – 1008. [2] El-Mekkawy, S. et al. (2000) Phytochemistry 53: 457 – 464. [3] Ma, Q.-G. et al. (1997) Phytochemistry 44: 663 – 666. [4] Karalai, C. et al. (1995) Phytother Res 9: 482 – 488.

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Cytotoxic Effects of Polyacetylenes from *E. Pallida* on Human Cancer Cell Lines and their Bioavailability through Caco-2 Cell Monolayers

Chicca A[1], Pellati F[2], Matthias A[3], Adinolfi B[1], Benvenuti S[2], Bone KM[3], Lehmann RP[3], Nieri P[1]

[1]Dep. Psychiatry, Neurobiology, Pharmacology and Biotechnology, University of Pisa, via Bonanno, 6, 56100, Pisa, Italy; [2]Dep. Pharmaceutical Sciences, University of Modena and Reggio Emilia, via G. Campi, 183, 41100, Modena, Italy; [3]MediHerb Research Laboratories, 3/85 Brandl Street, Eight Mile Plains, Queensland 4113, Australia

In previous studies, root hexanic extracts from the three medicinal *Echinacea* species (*E. purpurea, E. pallida* and *E. angustifolia*) exhibited a cytotoxic activity on human cancer cell lines and *E. pallida* was found to be the most cytotoxic one (1). Moreover, *E. pallida* root hexanic extract contains polyacetylenes, which are almost absent in the other two species of *Echinacea* where alkylamides are the main constituents (2,3). In the present study the cytotoxic effects of polyacetylenes single compounds isolated by a bio-guided assay fractionation from an *E. pallida* hexanic extract and their potential bioavailability were investigated. Antitumoral effects were assessed on human pancreatic MIA PaCa-2 and colonic COLO320 cancer cell lines. Cell viability was evaluated by the colorimetric WST-1 assay and apoptotic cell death was evaluated by an immunoenzimatic analysis of the cytosolic internucleosomal DNA enrichment and by the caspase 3/7 activity test. Bioavailability was studied using the Caco-2 cell monolayer, an *in vitro* model of intestinal permeability. All the isolated compounds exhibited a concentration- and time-dependent cytotoxicity (1 – 100 μM; 24 – 72 h) in both cell types and a greater potency on colonic cancer cells was observed. Apoptotic cell death was demonstrated to be involved in the cytotoxic effect of the most active polyacetylene, as revealed by both the assays used. Finally, polyacetylenes were found to cross the Caco-2 monolayer suggesting a likely bioavailability when taken orally. In conclusion, our data demonstrate, for the first time, an anticancer activity of constituents from *E. pallida* root hexanic extract and suggest their potential *in vivo* bioavailability. (1) Chicca A., Adinolfi B. et al., (2007) J. Ethnopharmacol. 110: 148 – 153 (2) Pellati F., Calò S. et al., (2006) Phytochemistry 67: 1359 – 1364 (3) Barnes J., Anderson L.A. et al.,(2005) J. Pharm. Pharmacol. 57: 929 – 954

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Diterpene Alkaloids from *Aconitum anthora* and *A. moldavicum*

Borcsa B[1], Forgo P[2], Veres K[1], Molnár A[3], Hohmann J[1]

[1]Department of Pharmacognosy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary, [2]Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary, [3]Department of Botany, Universtiy of Debrecen, Egyetem tér 1, H-4010 Debrecen, Hungary

Aconitum, *Delphinium* and *Consolida* species are known to produce various hydroxy-, alkoxy- and ester-substituted diterpene alkaloids. Many alkaloids exhibit high toxicity and a broad spectrum of pharmacological activities, including antinociceptive, anti-inflammatory, local anaesthetic and tyrosinase inhibitory activities [1,2]. In recent years it was demonstrated that some diterpene and norditerpene alkaloids exhibit antifeedant, insect repellent activity and cytotoxic effects against different human tumour cell lines with strong molecular selectivity [3]. As a part of our current studies on diterpene alkaloids of Ranunculaceae species *Aconitum anthora* L. and *A. moldavicum* L. were investigated. Seven alkaloids were isolated from the alkaloid-containing fractions of the methanolic extracts by means of multistep chromatographic purification, using CC, VLC, PLC, CPC and gelchromatography. The structures were determined with the aid of HR-ESIMS, ^{1}H-NMR, JMOD, ^{1}H,^{1}H-COSY, NOESY, HSQC and HMBC experiments. Isothalatisidine, 8,14-dimethoxyisothalatisidine and hetisinon were isolated from *A. anthora*. Two new compounds were identified from *A. moldavicum* besides ajacine and delcosine. With except of isothalatisidine [4] all alkaloids are reported for the first time from the investigated plant species. The accumulation of the diterpene alkaloids in the different organs of *A. anthora* was also examined using a newly developed TLC-densitometric method. It was found, that in the flowering period the accumulation of the alkaloids is highest in the generative organs. Taking into consideration the published antifungal, insecticide and antifeedant effects of diterpene alkaloids [3] it can be assumed that these compounds protect the reproductive organs of the plant against fungal infections and insect attacks. **References:** [1] Ameri, A. (1998) Prog. Neurobiol. 56: 211 – 223. [2] Shaheen, F. et al. (2005) Phytochemistry 66: 935 – 40. [3] González-Coloma, A. et al. (2004) J. Chem. Ecol. 30: 1393 – 1408. [4] Meriçli, A.H. et al. (2000) Pharmazie 55: 696 – 698.

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Interactions of Polyphenols with Immobilized Artificial Membrane and Human Serum Albumin Determined by High Performance Liquid Chromatography

Mornar A[1], Medić-Šarić M[1], Jasprica I[1], Krstulović A[2]

[1]Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry University of Zagreb, A. Kovaèića 1, 10 000 Zagreb, Croatia; [2]Department of Analytical Chemistry, Sanofi-Aventis, rue Georges Bizet 5, 91 160 Longjumeau, France

Within recent years, a promising new field of chromatography has opened up with immobilization of phospholipids and human serum albumin in columns and their use in prediction of drug candidates' pharmacokinetics. It is important to investigate early in the drug discovery both the oral absorption of new drug and the extent to which it binds to plasma proteins. In the last few decades plant polyphenols became very popular phytochemicals due to their presumed role in the prevention of various degenerative diseases, such as various types of cancer and cardiovascular diseases [1]. Until recently, relatively little information was available on their absorption and subsequent distribution and excretion in humans. The aim of our work was to investigate interactions of 30 polyphenols with immobilized artificial membrane (IAM) and human serum albumin (HSA) by high performance liquid chromatography in order to predict their oral absorption and plasma protein binding. The interaction of polyphenols with IAM was evaluated by using IAM.PC.DD 2 column. The solvent system (methanol-buffer) was used as a mobile phase with a varying content of organic modifier. The interaction of polyphenols with phospholipids was expressed as the logarithm of capacity factor, which was in the range from 1.345 to 3.581. The HSA binding values were derived from the gradient retention times using Chiral HSA column with buffer and 2-propanol as a mobile phase. The binding of polyphenols to HSA was in the range from 81.8% to 99.8%. Furthermore, the correlation between experimental and pharmacokinetic parameters predicted by different computer programs was considered in order to establish relationship between chromatographic behavior and predicted parameters. According to our research, chromatographic parameters proved to be useful for evaluation of lipophilicity, oral absorption and plasma protein binding properties of investigated polyphenols. **Reference:** 1. Havsteen, B. H. (2002) Pharmacol. Ther. 96: 67 – 202.

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A new screening strategy for CNS active plants by early *in vivo* characterization

Pedersen ME[1], Vestergaard HT[1], Hansen SL[1], Bah S[2], Stafford GI[3], van Staden J[3], Nielsen M[1], Jäger AK[1]

[1]University of Copenhagen, Faculty of Pharmacutical Sciences, 2 Universitetsparken, DK-2100 Copenhagen Ø, Denmark; [2]Traditional Healers Organisation, Mali; [3]University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

The aim of this study is to eliminate *in vivo* non-active plants early in the drug discovery process. Isolation of active compounds from

medicinal plants is a time and money consuming procedure. Thus, by early *in vivo* characterization of crude extracts from medicinal plants the risk of isolating *in vivo* non-active compounds would be reduced. Our screening strategy consists of three steps: (1) Initially the plants are screened using *in vitro* receptor binding assays (serotonin reuptake transport protein (SERT) and γ-amino butyric acid (GABA) binding assays). (2) The observed hits are subsequently tested in electrophysiological assays to obtain a functional characterization of the active compounds. (3) Finally the active plants and/or isolated compounds are tested *in vivo* in validated animal models (the modified forced swimming test (FST) to test for antidepressant activity; the pentylenetetrazole (PTZ) kindling model observe antiepileptic activity; the zero maze to test for anxiolytic properties). After the three-step screening strategy a bioassay-guided isolation of active compounds will be initiated. This approach minimizes the risk of isolating *in vivo* non-active compounds and thus focusing the search for new therapeutics. Crude extracts of 11 medicinal plants used in Mali for treatment of epilepsy and convulsions were screened for affinity to the benzodiazepine site (BDZ) on the $GABA_A$ receptor. Only one extract did not show binding to the BDZ site. But when the 11 extracts were tested in the functional assay, 4 extracts were considered non-active in the tested concentrations. The remaining 7 extracts showed activity in various degrees. Furthermore, the one extract without activity in the binding assay did show activity in the functional assay.

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Apoptosis induction and cell cycle alterations by extract of *Pistacia atlantica (Baneh)* in comparison to tamoxifen in human breast cancer T47D cells

Kamrani YY[1], Fouladdel S[1], Asgari T[2], Amin G[2], Esmaeelian B[3], Azizi E[1]
[1]Molecular Research Lab., Department of Pharmacology and Toxicology, and [2]Department of Pharmacognosy, Faculty of Pharmacy, Medical Sciences/ University of Tehran, [3]Department of Zoonotic Diseases, Shahriar veterinary network, Tehran, Iran

Identification of new anticancer drugs with synthetic or plant origin is one of the main topics of research in cancer research laboratories through out the world. Iran with its unique plant coverage has many varieties yet to be fully studied for their potential anticancer effects. We studied the anticancer effects of *Pistacia atlantica*, an Iranian species of the family of Anacardiaceae with the local name of *Baneh*, in human breast cancer T47D cells. The T47D cells were seeded in 96-well culture plates in the presence and absence of different concentrations of fruit-skin extract of *Baneh* to determine anticancer effects using the MTT assay. The apoptosis induction and cell cycle changes in T47D cells following exposure to *Baneh* extract were also determined by flow cytometric analysis using AnnexinV-FITC/PI and DAPI reagents, respectively. Extract of *Baneh* (IC_{50}= 1 mg/ml) showed strong growth inhibitory effects on proliferation of T47D cells when compared to RPMI control and Tamoxifen. *Baneh* extract showed significant increase of cells ($p = 0.005$) in G0/G1 and decrease of cells ($p = 0.005$) in G2/M phases of cell cycle in comparison to RPMI. Both *Baneh* extract ($p = 0.04$) and Tamoxifen ($p = 0.03$) showed significant apoptosis (PI-/AnnxV+) in T47D cells in comparison to RPMI. In addition, Tamoxifen ($p = 0.000$) showed significant cell necrosis (PI+/AnnxV-) in comparison to Baneh extract and RPMI. Our findings are the first data on potential anticancer effects of *Baneh* extract and its possible molecular mechanisms of action on human breast cancer T47D cell proliferation.

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Evaluation of the anti-herpes simplex virus activity of *Thymus longicaulis* L. (Lamiaceae)

Matta MK[1], Paltatzidou K[2], Triantafyllidou H[1], Lazari DM[1], Karioti A[2], Skaltsa H[2], Panagiotidis CA[1]
[1]Department of Pharmacognosy-Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece; [2]Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, 15771 Athens, Greece

The genus *Thymus* is one of the eight most important genera within the Lamiaceae family, with regard to its number of species. Thyme is a perennial herbaceous plant, indigenous to central and southern Europe, which is widely cultivated for its use as a tea, spice, and herbal medicine. Its leaf is listed both in the German and British Herbal Pharmacopoeias, and it has been used as a stomachic, carminative, diuretic, urinary disinfectant, and vermifuge. Herpes simplex viruses (HSV) are ubiquitous pathogens that cause a variety of diseases ranging in severity from mild to severe. Following a primary infection, HSV establishes a latent life-long infection within the nerve cells of the trigeminal ganglia, with periodic reactivations. Continuing our chemotaxonomic analyses of the Greek flora of the Lamiaceae family and the search for pharmacologically interesting compounds from these plants, here we evaluate the antiviral activity of the aerial parts of *T. longicaulis*. Specifically, we assess their virucidal activity against herpes simplex virus, type 1 (HSV-1), as well as their ability to inhibit the attachment and penetration steps of the HSV-1 infection cycle. Air-dried, powdered aerial parts of the above mentioned plant were extracted at room temperature with a series of solvents of increasing polarity, i.e. petroleum ether, CH_2Cl_2, MeOH, 1:1 mixture of MeOH-H_2O and H_2O. The dried extracts were dissolved either in DMSO or in sterile deionised and distilled water and they were tested for their above-described anti-HSV-1 activities, i.e. virucidal action and inhibition of virus entry. Significant anti-HSV activities were found in the dichloromethane and the methanolic extracts, which were subsequently subjected to bioguided chromatographic fractionation. The purity of five chromatographic fractions, possessing anti-HSV activities, was assessed by chromatography/spectroscopy and they were found to consist of pure compounds. We are in the process of analyzing the structures of these compounds, as well as further evaluating their mechanism of action at the molecular level.

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Evaluation of the activity of traditional Greek medicinal plants against herpes simplex virus

Matta MK, Sylignaki GI, Lazari DM, Panagiotidis CA
Department of Pharmacognosy-Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

Herpes simplex viruses (HSV) are important human pathogens causing a variety of diseases ranging in severity from mild to severe, and in certain cases, they can even become life threatening, especially in the case of highly susceptible adults. Following a primary infection HSV rests latent mainly in trigeminal ganglia and persists for the life-time of the host with periodic reactivations. The widespread use of nucleoside-based drugs, such as aciclovir (ACV), for the treatment of HSV infections has led to the emergence of drug-resistant HSV mutants. Therefore, the discovery of novel anti-HSV drugs, preferably targeting processes different than those affected by nucleoside analogues, i.e. virus DNA replication, deserves great efforts. Interestingly, it was found that traditional medicinal plants are promising sources for new anti-HSV drugs. The present study focuses on the evaluation of extracts derived from four Greek plants, i.e. *Artemisia absinthium*, *Echium italicum*, *Melissa officinalis* and *Onosma elengatissima*, either for their virucidal activity or their abilities to inhibit HSV-1 propagation. Air-dried, powdered aerial parts from the above mentioned plants were extracted at room tempera-

ture with a series of solvents of increasing polarity, i.e. petroleum ether, CH_2Cl_2, MeOH, 1:1 mixture of MeOH-H_2O and H_2O. The dried extracts were dissolved in DMSO or in sterile deionised and distilled water and tested both for cytotoxicity as well as for their antiviral action. MTT asays indicated that none of the above extracts, except the *E. italicum* aqueous extract, displayed significant cytotoxicity at concentrations up to 41 mcg/ml. Plaque reduction assays indicated that extracts from all four plants displayed anti-HSV activity. The CH_2Cl_2 and MeOH extracts were found to possess mainly virucidal activity whereas the more polar extracts displayed both virucidal activity and inhibition of the virus entry to the host cells *in vitro*. Specifically, the aqueous extract from *A. absinthium* displayed very potent inhibition of the HSV-1 attachment, without showing any virucidal or cytotoxic effects.

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Antioxidant activity of selected Peruvian medicinal plants used in Calleria District

Svobodova B[1], Kokoska L[1], Kutilkova L[1], Polesny Z[1]
[1]Department of Crop Science and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic

The inceasing evidence that free radical-mediated damage to membranes, other lipid-containing structures, DNA and protein contributes to ageing and chronic diseases, such as cancer and coronary heart disease, has focused attention on natural antioxidants, which can play an important role in prevention of these diseases [1]. Therefore there is escalating interest in searching new antioxidants in medicinal and dietary plants [2]. The plants tested in this study were selected based on their traditional use in folk medicine among the Shipibo-Conibo ethnic group in Calleria District, Peru. The ethanol extracts of 14 Peruvian medicinal plants have been tested for their potential *in vitro* radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) in microplate assay [3]. Ascorbic acid was chosen as a standard pure compound next to ethanol extract of rosemary – a well documented plant with significant antioxidant activity [4]. All the crude extracts were found to have scavenging effects on DPPH in the range of 5.34 – 242 μg/ml. Among them, *Calycophyllum spruceanum* (Benth.) K. Schum., *Naucleopsis glabra* Spruce ex Pittier, *Triplaris* sp., *Phyllanthus ninuri* L., *Uncaria tomentosa* (Willd.) DC. and *Maytenus macrocarpa* (Ruiz & Pav.) Briq. possessed the strongest activity (EC_{50} 5.34; 5.45; 6.79; 6.91; 7.90 and 7.94 μg/ml resp.) comparable to *Rosmarinus officinalis* L. extract (EC_{50} 7.84 μg/ml) and ascorbic acid (EC_{50} 4.64 μg/ml). **References:** [1] de Beer, D. et al. (2005) Food Chem 90: 569 – 577. [2] Wiseman, H. (1996) J Nutr Biochem 7: 2 – 15. [3] Fukumoto, L.R. et al. (2000) J Ag Food Chem 48: 3597 – 3604. [4] Almela, L. et al. (2006) J Chromatogr A, 1120: 221 – 229.

P 532

Effect of two *Athamanta turbith* subspecies essential oils on some liver biochemical parameters in mice treated with carbon tetrachloride

Tomić A[a], Bozin B[b], Samojlik I[c], Milenković M[d], Mimica-Dukić N[e], Petrović S[a]
[a]Institute of Pharmacognosy, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade; [b]Department of Pharmacy; [c]Department of Pharmacology and Toxicology, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad; [d]Institute of Microbiology and Immunology, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade; [f]Chemistry Department, Faculty of Sciences, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

We investigated the effects of essential oils isolated from mature fruits of *Athamanta turbith* ssp. *hungarica* (Borbás) Tutin and *A. turbith* ssp. *haynaldii* (Borbás & Uechtr.) Tutin (Umbelliferae) on some liver biochemical parameters in mice intoxicated with CCl_4. Mice received single oral administration of water emulsion of essential oils (70, 140, 280 μl of essential oil/kg b.w.) three hours before CCl_4 was administrated (in single dose of 1 ml/kg b.w.). Twenty-four hours after intoxication, mice were sacrificed to obtain blood and liver. In liver homogenate activities of following enzymes were measured: peroxidase (Px), catalase (CAT), glutathione peroxidase (GSH-Px), lipid peroxidase (LPx) and xanthine oxidase (XOD), together with the content of reduced glutathione (GSH). In serum, the activities of aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) were estimated [1]. Statistical analysis was performed by ANOVA followed by Dunnett's 2-sided post hoc test. A mean difference was significant at the 0.05 level. Treatment with CCl_4 yielded a decrease in activities of CAT, XOD, GSH-Px, as well as of GSH content. Px, LPx, AST and ALT activities were increased. In applied doses, examined essential oils showed a certain hepatoprotective effect, since pretreatment with either of essential oils extenuated the effects caused by CCl_4. In mice treated only with essential oils, decrease in Px and CAT activity was detected, while values for other liver biochemical parameters did not vary significantly from the control group (not treated animals). Myristicin, the major compound in both essential oils, was previously reported to be a potent hepatoprotective agent [2]. **References:** [1] Popović, M. et al. (2002) Oxidation Comm. 20: 531 – 37. [2] Morita, T. et al. (2003) J. Agric. Food Chem. 51: 1560 – 65.

P 533

Realgar-derived Arsenic Compounds Induce Anti-proliferation and Apoptosis on Cultured HaCaT Keratinocytes

Lin ZX[1], Tse WP[1], Cheng CHK[2,3], Che CT[1]
[1]School of Chinese Medicine, Faculty of Science, The Chinese University of Hong Kong, Shatin, NT., Hong Kong SAR; [2]Department of Biochemistry and [3]Hong Kong Institute of Biotechnology, The Chinese University of Hong Kong, Shatin, NT., Hong Kong SAR

Our current research project focuses on the development of topical therapies from natural sources for psoriasis, a chronic immune-mediated inflammatory skin disease characterized by abnormal differentiation and hyperproliferation of keratinocytes. Previous studies conducted by our group have shown that the ethanolic extract of Realgar, a common Chinese medicinal substance, possesses potent inhibitory effect on the proliferation of the HaCaT cells, which are immortalized human epidermal keratinocytes. In the present study, three inorganic compounds derived from Realgar namely Arsenic (III) Oxide, Arsenic (V) Oxide and Arsenic Iodide were investigated for their anti-proliferative and apoptogenic effects on cultured HaCaT cells. All three arsenic compounds showed time and dose-dependent antiproliferative activities, with IC_{50}s being 2.4 μM, 16.0 μM and 6.8 μM respectively. Flow cytometric analysis revealed that these inorganic compounds elicited anti-proliferation of HaCaT cells through the induction of apoptosis based on evidence (i) DNA cleavage detected by TUNEL assay; (ii) the appearance of sub G1 peak through the analysis of cell cycle with propidium iodide (PI) staining; (iii) quantitative analysis of apoptosis by concomitant Annexin V-GFP and PI staining; and (iv) the expression of caspase 3 proteins. In a specificity test, all three Realgar-derived inorganic compounds exhibited less cytotoxicity to HS68 than that of HaCaT cells. In conclusion, we have unambiguously demonstrated that the arsenic compounds derived from Realgar exerted potent antiproliferative action on HaCaT cells via the induction of cellular apoptosis. The findings could be used for future development of new topical agents for psoriasis treatment. *Acknowledgement:* This project is funded by CUHK Direct Grant (Project Code: 2030363)

P 534

Anti-inflammatory effect of Mongolia and Vietnamese medicinal plants against LPS-induced NO release in the RAW 264.7 cell

Juan QH[1], Batmunkh T[2], Nga DT[1], Eun-Mi S[1], Joo YH[3], Burm-Jong L[1,2], Ah Koo K[1]

[1]Biohealth Products Research Center, [2]Department of Chemistry, [3]School of Biotechnology & Biomedical Science, Inje University, Gimhae 621 – 749, Republic of Korea

In order to study the anti-inflammatory activity of Vietnamese medicinal and Mongolian endemic plants, we collected the plants from the countries and prepared 50% ethanol extracts from 20 air-dried Mongolian plants and water and dichloromethane fractions from 50% ethanol extracts of 15 air-dried Vietnamese plants. Twenty extracts of Mongolian plants and forty-five fractions of Vietnamese plants were screened for their inhibitory effects on the nitric oxide (NO) production in the lipopolysaccharide (LPS)-stimulated RAW264.7 cells [1,2], a murine macrophage cell line. The results of the screening indicated: seven Vietnamese plant extracts and their fractions exhibited high inhibitory activity, which ranges from 45.2 ± 3.6% of the total extract of *Asarum glabrum* to 83.1 ± 0.6% of the dichloromethane fraction of it at the concentration of 50 μg/mL; eight Mongolia plants extracts exhibited moderate activity ranged from 20.7 ± 0.5% of *Salsola monoptera* to 34.9 ± 0.2% of *Jurinea mongolica* Maxim. Dexamethasone (DEX), the positive control, showed an inhibition of 46.4 ± 9.8% at the concentration of 10 μg/mL. These findings indicate that these Vietnamese and Mongolia traditional medical plants are beneficial for treatment against inflammatory conditions and may contain major constituent(s) with anti-inflammatory properties. *Acknowledgement:* This study was supported by a grant from the Ministry of Commerce, Industry and Energy (MOCIE) and the Korea Institute of Industrial Technology Evaluation & Planning (ITEP) through the Biohealth Products Research Center (BPRC) of Inje University. **References:** [1] John, M. et al. (1997) Annu. Rev. Immunol. 15: 323 – 50. [2] Sherman, M.P. et al. (1993) Biochem. Biophys. Res. Commun. 191: 1301 – 1308.

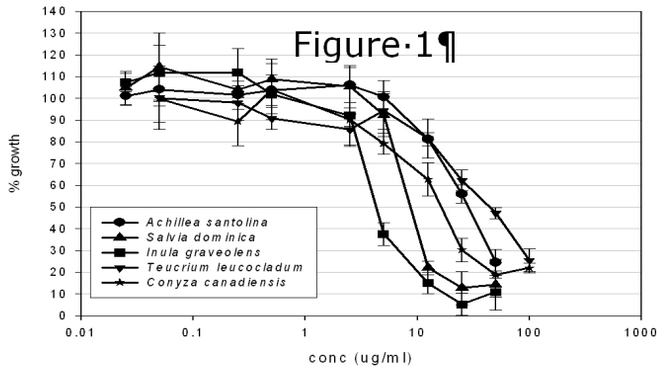
P 535

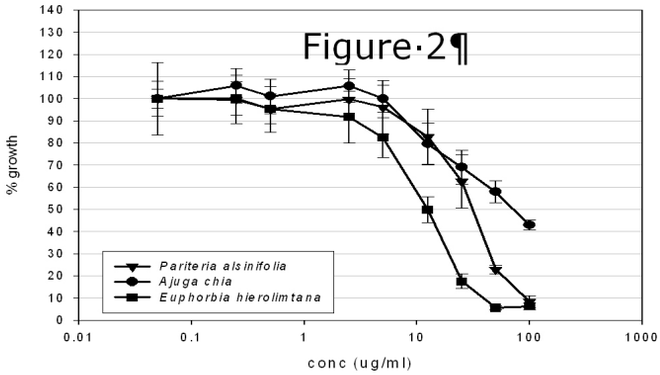
Comparison of antiproliferative activities of ethanolic plant extracts of the Jordanian flora using MCF7 and A549 cells

Abu Dahab R[1], Afifi-Yazar FU[1]

[1]Faculty of Pharmacy, University of Jordan, Queen Rania Street, 11942 Amman, Jordan

76 ethanolic extracts of medicinal herbs from the Jordanian flora, belonging to 67 species and 34 families, were evaluated for their antiproliferative activity on a breast cancer cell line (MCF7) and a lung adenocarcinoma cell line (A549). The cells were cultured in RPMI 1640 medium and incubated with the extracts for 72 hours. Sulphorhodamine B (SRB) assay was used to test for cytotoxicity [1]. From the tested crude extracts in MCF7 cells, *Inula graveolens, Salvia dominica*, *Conyza canadiensis* and *Achillea santolina* showed potent antiproliferative activities. The most active plant was *I. graveolens* with an IC_{50} of 3.83 μg/ml. Phytochemical screening indicated the presence of flavonoids and terpenoids in all active extracts. On the other hand in A549 cells, *Parieteria alsinifolia, Ajuga chia and Euphorbia hierosolyminata* showed more than 50% reduction in cell proliferation after 72 hours incubation with the cells. The most potent extract was *E. hierosolyminata* with an IC_{50} of 11.80 μg/ml. IC_{50} calculation for both cell lines with the active extracts are shown in Figure 1 (MCF7) and Figure 2 (A549). These results indicate the possible potential use of medicinal plants from the Jordanian flora as antineoplastic agents.





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P 536

Evaluating CYP3A4 inhibitory activity of Echinacea extracts using NMR and multivariate data analysis

Modarai M[1], Wilson N[1], Politi M[1], Suter A[2], Kortenkamp A[1], Heinrich M[1]

[1]The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX United Kingdom; [2]Bioforce AG, 9325 Roggwil, Switzerland

Previously we have shown that commercially available echinacea extracts vary widely in their inhibitory activity on CYP3A4 (IC_{50} values: 12.71 μg/ml -1812 μg/ml) in the supersome assay[1]. Modern NMR-spectroscopy and principal component analysis allows evaluation of differences between complex mixtures[2]. To correlate such differences to CYP3A4 inhibition we examined various Echinacea extracts. Six commercially available Echinacea extracts were chosen. Each extract was separated into ethanolic and water fractions, which were assayed for CYP3A4 inhibition. In tandem, 400 MHz ^{1}H- NMR spectra were obtained in deuterated ethanol or deuterium oxide with internal standards. Principal component analysis was conducted on the data. The inhibitory activity of the extracts resided mostly in the ethanolic fraction (with IC_{50} values ten folds lower than original extract e.g. Echinaforce IC_{50}: 22 μg/ml, vs. ethanolic fraction 2 μg/ml). ^{1}H NMR spectra of the ethanolic fractions showed a clear difference between the most and least active extracts. Greater inhibition was associated with the presence of peaks at 1 – 3 ppm and 6 – 8 ppm (visual inspection). Principal component analysis revealed good correlation between differences in ^{1}H spectra and IC_{50} values. Key contributors were identified in the regions: 0.875 ppm, 0.925 ppm, 1.275 ppm, 1.325 ppm. The ethanolic fraction of Echinaforce was further fractionated by SPE (C-18, water: ethanol 10% step gradient). Two potent fractions were identified (IC_{50}: 0.43 – 0.58 μg/ml). ^{1}H NMR analysis also revealed additional peaks

at ~7ppm, which were unique to these fractions. Amongst other compounds, we suspect that the ethanol soluble alkylamides, (potent CYP3A4 inhibitors) may be found in these fractions. *Acknowledgements:* Bioforce for funding this project. **References:** [1] Modarai, M. et al. (2007) Journal of Pharmacy and Pharmacology 59: 567 – 573 [2] Holmes, E. et al. (2006) Planta Med. 72: 771 – 785.

P 537

Prospects and Challenges of Cross Kingdom's Bioassay

Chang FR[1], Wu YC[1], Nozaki H[2], Chua NH[3], Dai JH[1], Lai WC[1], Hayashi K[2]
[1]Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan, [2]Department of Biochemistry, Faculty of Science, Okayama University of Science, 1 – 1 Ridai-cho, Okayama 700 – 0005, Japan,, [3]Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021

In year 2005, a brand-new cross kingdom's (from Plantae to Animalia) bioassay method was established [1], the transgenic Arabidopsis plant, *pER8-GFP*, harboring human estrogen receptor was taken as a powerful tool in searching and discovering natural estrogen-agonists/antagonists. The remarkable outcomes show that this assay tool could screen estrogen-agonist/antagonists from natural sources. To our best understanding, it is the first study in using the higher plants as an assay tool in screening the bioactive functions related to human beings. Since the assay model established, we have screened over 100 natural products' extracts (including well-known gynecologic traditional Chinese medicine) and fractions using bioactivity guided fractionation method and evaluated their potential for the uses of phytoestrogenic dietary supplements. Several natural pure compounds with estrogenic effect have been discovered. Meanwhile, the optimizing extracts with significant estrogenic activity of soybean and *Pueraria lobata* will be also reported. This new and special transgenic assay technology shows some new aspects and challenges in the further studies. *Acknowledgements:* 1. National Science Council, Taiwan **References:** [1] Chang F. R. et al. (2005) J. Nat. Prod. 68: 971 – 973

P 538

Antistress and antioxidant activity in mice after repeated treatment with St. John's wort extracts of different hyperforin content

Michalski C[1], Pomerenke A[1], Kroll U[2], Kelber O[2], Butterweck V[1]
[1]Department of Pharmaceutics, College of Pharmacy, University of Florida, POBox 100494, Gainesville, FL 32610, USA; [2]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany

Recent data from several reports indicate that free radicals are involved in the biochemical mechanisms underlying neuropsychiatric disorders in humans [1]. These reports suggest that there is a therapeutic benefit from antioxidant supplementation in manic-depressive patients [1,2]. In this study, we aimed to investigate the *in vitro* antioxidant capacity of chemically different St. John's wort (SJW) extracts using the ORAC assay and to determine *in vivo* the effect of subchronic treatment with the SJW extracts on antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in mouse brain homogenates as well as erythrocyte lysates. Temperature stress (50 °C) applied to SJW extracts significantly lowered the amount of hyperforin (extract A, no temperature stress, 3.26% hyperforin; extract B, 50 °C for 8 days, 1.37% hyperforin; extract C, 50 °C for 19 days, 0.14% hyperforin). The amount of hypericin only marginally changed under these conditions, no changes were observed for the flavonoids. The ORAC values for extract A, B and C ranged from 11.89 to 12.50 μmol of TE/g of dried weight; the results correlate with published data [3]. The decrease of hyperforin in the SJW extracts did not affect their *in vitro* antioxidant capacity which, therefore seems to be related to its phenolic compounds. Extract B and C increased enzyme activities in mouse brain homogenates (SOD, CAT, 41% and 44% vs. control) and erythrocytes lysates (GSH-Px, 56% and 61% vs. control) after 14 days of daily treatment comparable to fluoxetine and buspirone, indicating greater reduction of oxidative stress. **References:** [1] Herken, H. et al. (2007) Arch Med Res 38: 247 – 252. [2] Ozcan, M.E., (2004) Int Clin Psychopharmacol 19: 89 – 95. [3] Zheng, W. et al. (2001) J Agricul Food Chem 49: 5165 – 5170

P 539

Furocoumarins in phytomedicines: Is there a phototoxic risk?

Raquet N, Schmitz HJ, Schrenk D
Food Chemistry and Toxicology, University of Kaiserslautern, Erwin-Schroedinger-Strasse 52, D-67663 Kaiserslautern, Germany

Furocoumarins occur in plants used as food (e.g. grapefruit, parsley, parsnip) or in phytomedicines (*Ammi majus, Angelica archangelica*). In combination with UV light they can lead to phototoxic effects. Furthermore, in rats, a daily dose of 37.5 mg/kg b. w. of 8-methoxypsoralen (8-MOP) was toxic and carcinogenic. In humans [1,2], phototoxic effects were observed at 10 mg 8-MOP plus 10 mg 5-MOP (0.25 mg/kg b.w 8-MOP equivalents/kg b.w. in adults) or 14 mg 8-MOP (0.23 mg/kg b.w. in adults). The average daily furocoumarin intake per adult via food was estimated to be 1.45 mg in Germany [3] (24 μg/kg b.w.). Thus the daily intake of furocoumarins via food lies about 1000-fold lower than the phototoxic, genotoxic and carcinogenic doses in animals, and about 10-fold lower than the phototoxic 'threshold dose' in humans. With an assumed level of total furocoumarins in *Angelica* tincture of 1 mg/ml, and a daily dose of 1.5 g according to the German commission E monograph, a total daily intake of about 1.5 mg can be estimated. For an adult, this corresponds to an additional daily intake of 25 μg/kg b.w. being in the same range as the average intake via food. With respect to the phototoxic 'threshold dose', the intake via a phytomedicine as described would be in the range of 10%. In conclusion, intake of a phytomedicine containing *Angelica* root extract as described does not contribute in a relevant way to phototoxicity and is within the range of average intake via food. **References:** [1] Schlatter J (1991) Food Chem Tox 29: 523. [2] Brickl R et al. (1984) J Natl Cancer Inst Monogr 66: 63. [3] SKLM (2006) Toxicological Assessment of Furocoumarins in Foodstuffs, DFG, Bonn.

P 540

Antiproliferative and apoptotic effects of wheatgrass (*Triticum aestivum* L.) extracts on chronic myeloid leukemia (CML) cell line

Karadag A[1], Ozkan T[2], Altınok B[2], Aydos S[1], Sunguroglu A[1]
[1]Ankara University, Faculty of Medicine, Department of Medical Biology, Ankara, Turkey; [2]Ankara University, Institute of Biotechnology, Ankara, Turkey

Wheatgrass (*Triticum aestivum* L.) is a rich source of vitamins, antioxidants and minerals and it has higher phenolic and flavonoid content in the ethanol extracts than water extracts. Flavonoids in general have been shown to have anticarcinogenic, antimutagenic, properties. *C*hronic *M*yeloid *L*eukemia(CML) is a malignant hematopoietic stem cell disorder that is characterized by BCR-ABL fusion gene encodes cytoplasmic BCR-ABL oncoprotein with a constitutive tyrosine kinase activity that enhances the proliferation and anti-apoptotic capacity of affected cell clone. Antiproliferative and apoptotic effects of wheatgrass has not been studied yet. Here we report the effect of wheatgrass extract on CML cell viability, proliferation and apoptosis. 32Dp210 (BCR-ABL fusion gene (+) mouse CML cell line) and 32D (wild type mouse myeloid cell line) cells were grown in RPMI 1640 medium. Cells were incubated with wheatgrass ethanol extracts at final concentrations of 6.5% (w/v) and 13% (w/v) at 0,24,48,72 hours. Cell viability was detected by MTT and trypan blue assays. Apoptosis was determined morphologically and DNA laddering. Both of the concentrations were found to be statistically different ($p < 0.001$) in respect to their antiproliferative and apoptotic

effects than their controls. The results showed that the wheatgrass extract inhibited growth of 32Dp210 cells in a dose dependent manner compared to the control cell line(32D). At 6.5% (w/v) and 13% (w/v) concentrations of wheatgrass extracts induced apoptosis at 72, 24 hours respectively. In this study, it has been calculated that the death risk of 32Dp210 was found 6.2 times higher than 32D. It is concluded that wheatgrass extract inhibits proliferation of 32Dp210 cells through the induction of apoptosis.

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Variation in the chemical constituents and antioxydant activity in Stachys species

Háznagy-Radnai E[1], Czigle S[3], Veres K[1], Zupko I[2], Bezáková L[4], Janicsák G[5], Tóth E[5], Falkay G[2], Máthé I[1,5]

[1]Department of Pharmacognosy, [2]Department of Pharmacodynamics, University of Szeged, Eötvös Str. 6, Szeged H-6720, [3]Department of Pharmacognosy and Botany, [4]Department of Cell and Molecular Biology of Drugs, Comenius University, Odbojárov 10, SK-832 32, Bratislava 3, Slovakia, [5]Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót, H-2163 Vácrátót, Hungary

Chemical differences of volatile and non-volatile fractions of *Stachys officinalis, S. grandiflora, S. recta, S. annua, S. macrantha, S. sylvatica, S. germanica, S. byzantina, S. palustris* samples of different origin and time of harvest are studied together with their antioxidant activities. Plants were grown in Vácratót (Hungary) from seeds, obtained by botanical garden seed exchange programme. The steam distilled volatile oils, gained from the air-dried samples according to Ph.Hg VIII., were analysed with GC/FID (HP 5890 series II) and GC/MS (Finnigan GCQ) gas chromatographs [1]. The non-volatile (MeOH) fractions, after flesh column chromatograpy, were analysed by TLC/densitometry (Shimadzu CS-9301 PC) and HPLC (Shimadzu LC-10AS) for iridoids and for the derivatives of caffeic acid [2]. The total flavonoid, and polyphenol content (Ph.Eur.4.) and the enzyme-independent lipid-peroxidation using ox-brain homogenates [3] and in separate experiments anti-lipoxygenase activity, applying LOX obtained from rat lung were measured. In the volatile fractions of 27 – 73 identified components, the monoterpenoids (dominated by linalool) were in smaller concentration present than the sesquiterpenoids with β-caryophyllene in all samples. Acetyl harpagid, harpagid were ubiquitous in iridoid fractions, expect those from *S. annua*, it contained no iridoids. Volatile components varied mainly in their composition, while the iridoids in their quantity. Caffeic and rosmarinic acids were measured in low concentration. Correlation between effectiveness and the total phenolic content could only be justified. *S. sylvatic, S. recta* and *S. officinalis* extracts proved to be of more effective antioxidants than the control α-tocopherol succinat and ascorbic acid. *Acknowledgement: The authors are thankful for the financial support of OTKA T 43148 and APVVV-51 – 017905 Hungarian and Slovak Natonal Grants* **References:** [1] Radnai E., et al. (2003) Acta Horticulturae 597: 137 – 142. [2] E. Háznagy-Radnai, et al. (2005) J. Planar Chromat. (JPC) 18: 314 – 318. [3] E. Háznagy-Radnai, et al. (2006) Fitoterapia 77: 521 – 524.

P 542

Pharmacological evidence for the anti-inflammatory effect of STW 5 in colonic inflammation *in vivo*

Abdel-Aziz H[1], Wadie W[2], Khayyal MT[2], Kelber O[3], Okpanyi S[3], Weiser D[3]

[1]Al-Ahliyya Amman University, Faculty of Pharmacy and Medical Sciences, Al Salt Road, P.O.Box 183, 19328 Amman, Jordan; [2]Depts. of Pharmacology, Faculty of Pharmacy, Cairo University, Kasr-El-Aini Street, 11562 Cairo, Egypt; [3]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295 Darmstadt, Germany

STW 5 (Iberogast®) is a fixed combination consisting of 9 herbal components. Earlier evidence has shown it to be a multi-target preparation for gastrointestinal disorders, as functional dyspepsia and irritable bowel syndrome [1,2], which are in many cases triggered by a previous gastro-intestinal inflammation. Earlier *in vitro* studies point to an anti-inflammatory action of STW 5 [3,4]. The present study was undertaken to explore the anti-inflammatory activity of the drug *in vivo*. In an experimental model for inflammatory bowel disease, male rats were injected intra-colonically with 10 mg/kg trinitro benzene sulfonic acid (TNBS) in 50% ethanol under light ether anaesthesia. This induced the development of lesions, which were then examined macroscopically 4 days later. STW 5 was given orally in different dose levels (0.5 – 5 ml/kg) for 1 week before TNBS and for the 4 days to follow. It markedly reduced the area of lesions, colonic mass index, as well as prevented changes in myeloperoxidase and reduced glutathione. The effect was comparable to sulfasalazine, 300 mg/kg, which was used as reference drug in the same manner as STW 5. The beneficial effect of STW 5 was dose dependent. Furthermore, relevant pro-inflammatory cytokines, including TNFα, IL-1β, and ICAM-1, as well as prostaglandin E_2 and leukotriene B_4, were measured in both colonic tissue and blood in order to gain more insight into the mechanism of action of the drug. A positive correlation was observed between the therapeutic efficacy of STW 5 and the measured mediators. The findings point to a new evidence-based potential therapeutic usefulness of STW 5 (Iberogast®) potentially relevant also for its therapeutic effect in functional gastrointestinal diseases. **References:** [1] Holtmann G et al. (2004) Wien Med Wochenschr 154: 528 – 534. [2] Wagner H (2006) Phytomedicine 13: 122 – 129. [3] Schempp H (2004) Arzneim Forsch 54: 389 – 395. [4] Michael S et al. (2006) Gut 55 S V:A 206

P 543

Antimutagenic potential of different extracts of *Alkanna orientalis* (L.) Boiss evaluated by Ames Salmonella/microsomal test

Güvenalp Z[1], Özbek H[1], Demirezer LÖ[2], Özbek T[3], Güllüce M[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University, 25240, Erzurum, Turkey; [2]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey; [3]Department of Biology, Faculty of Art and Science, Atatürk University, 25240, Erzurum, Turkey

The Ames-*Salmonella*/microsome mutagenicity assay is also used to designate antimutagens and anticarcinogens that are removing the mutagen and carcinogen effects of many chemicals, including drugs and biocides [1]. The genus *Alkanna* (Boraginaceae) is represented by 31 species in the Flora of Turkey [2]. The present study was conducted to evaluate the antimutagenic activity of butanol extract, chloroform extract, and petrol ether extract obtained from the roots of *Alkanna orientalis.* Antimutagenic activities of the extracts were estimated by employing the plate incorporation Ames Salmonella histidine reversion assay by using the frame shift mutagen tester strain TA1535 and base pair substitution strain TA1537 against direct acting mutagens sodium azide (NaN_3), 4-Nitro-1-quinoline oksit (4-NPD). Butanol extract and petrol ether extract showed the antimutagenic activities related to dose-response in *Salmonella typhimurium* TA 1535 strain and TA 1537. While the butanol extract and petrol ether extract showed moderate antimutagenic activities in the TA1537 and TA1535 strains, respectively, as related to dose-response, no antimutagenic activity was observed with the chloroform extract by using each three doses and each bacterial strain. **References:** [1] Martelmans, K., Zeiger, E. (2000) Mutation Research 55: 29 – 60. [2] Huber-Morath A. (1978) In: Davis, P.H. (Ed.), Flora of Turkey and East Aegean Islands, Vol: 6. University Press, Edinburgh, pp. 414 – 434.

P 544

Antioxidant/prooxidant capacity of *Viscum album* L. extracts from different host trees affect their cytotoxic activity against AS30D tumour cell lines

Cebovic T[1], Spasic S[2], Popovic M[3]
[1]School of Medicine, Biochemistry Department, Hajduk Veljkova 3, 21000 Novi Sad, Serbia; [2]School of Pharmacy, Vojvode Stepe 450, 11000 Bolgrade, Serbia; [3]Faculty of Sciences, Trg D. Obradovica 5, 21000 Novi Sad, Serbia

Extracts from *Viscum album* L. have been reported to exert cytotoxic and immunomodulatory effects *in vitro* and *in vivo*. Interestingly, in dependence on the host tree on which mistletoe was grown, extracts exhibit different biological effects. Pursuing research on potentially useful pharmacological effects of this herbal drug, the possibility that observed antioxidant/prooxidant activity might depend significantly on the appropriate choice of its biological source was investigated. In this study we tested the hypothesis that an aqueous extracts from the European mistletoe growing on different host trees, exhibit cytotoxic activity against AS30D tumour cell line due to induction of oxidative stress in tumour cells. Some mistletoe extracts inhibited the tumour growth in a very high degree (0 – 40000cell/mm^3 in comparison to 120000 of the control). The activities of antioxidant enzymes showed the probable presence of oxidative stress in AS30D tumour cell line upon treatment with the mistletoe extracts. The amount of the reduced glutathione was significantly decreased in tumour cells upon treatment with the extracts (approx. 0,5nmolGSH/mL in comparison to 1,6 of the control), and the lipid peroxidation was strongly promoted (approx. 1,1 μmolMDA/mL in comparison to 0,03 of the control). It was also found that the higher cytotoxic activity of the examined extracts was followed by the induction of oxidative stress. Our data suggest that mistletoe extracts may be potentially useful in the reduction of the tumour development. Also, the biological source of this herbal drug needs to be more precisely defined, as observed activities were greatly dependent on plant material source.

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Antioxidant And Cytotoxic Activity of Several Red and White Wines Produced in Vojvodina (Serbia) Against EAC And AS30D Tumour Cell Lines

Cebovic T[1]
[1]School of Medicine, Biochemistry Department, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

The aim of this study was to test the hypothesis that red and white wines form Vojvodina could be used for their beneficial antioxidant effect due to the presence of the secondary biomolecules that proved to be good antioxidants. The effects of six red and white wines produced in Vojvodina, Serbia, were analysed. The results showed that the tested wine samples decreased the intensity of lipid peroxidation (78,8 – 193,2 in comparison to 229,2nmolMDA/mLliposomes of control). Furthermore, both red and white wine samples inhibited $OH^{\cdot}$ (0,52 – 1,1 in comparison to 1,1nmolMDA/mgdeoxyribose of control) and $DPPH^{\cdot}$ radical in a dosage dependent manner (24,4 – 86,2% of scavenged $DPPH^{\cdot}$ radicals). The examined wine samples, when applied at lower concentrations, inhibited the NO production in a statistically significant degree (0,9 – 23,5% inhibition). Thus, it can be hypothesized that the examined wines could be very strong and efficient antioxidants (red wines more than white wines). Cytotoxic activity of the examined wine samples against EAC and AS30D tumour cell lines was also analysed. Examined samples did not affect the viability of both EAC and AS30D tumour cell lines. But, interesting findings were made when tumour cells were implanted to NMRI mice. We have observed that pre-treatment with the wine samples (ethanol evaporated) significantly reduced tumour cell viability, and also decreased the cancer incidence (1000 – 1500cells/mm^3 in comparison to 120000 of control). Better results were obtained with the red wines. On the other side, post-treatment did not affect the tumour growth of both tumour types.

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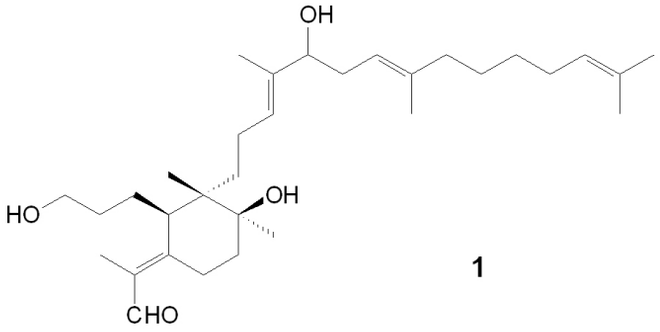
Cytotoxic compounds from *Iris tectorum*

Fang R[1], Houghton PJ[1], Hylands PJ[1]
[1]Pharmacognosy Laboratories, Pharmaceutical Sciences Division, Kings College London, London SE1 9NH, UK

Extracts of several Chinese plant species used traditionally to treat cancer were tested for cytotoxicity using the SRB assay [1] with three cancer cell lines (COR-L23, C32 and MCF-7) and a mammalian non-cancer cell line Hep G2. A chloroform extract of the rhizomes of *Iris tectorum* Maxim. gave the greatest cytotoxicity with 100 μg/ml giving less than 15% survival with all three cancer cell lines. The SRB assay was used for bioassay-guided fractionation of this extract and yielded two novel iridogermanal triterpenes IT4C and IT4D, two known iridogermanal triterpenes iridobelamal **1** and isoiridogermanal and two flavonoids, 7-OMe-aromadendrin and tectorigenin, whose structures were determined by advanced MS and NMR spectroscopy. The compounds were tested for cytotoxicity using the SRB assay against the four cell lines with iridobelamal **1** having the greatest activity (Table). Table: Cytotoxicity (IC_{50} μM ± SEM) of isolated compounds n = 3

Compound	Cell line			
COR-L23	MCF-7	C32		HepG2
IT4C	35 ± 1	18 ± 1	40 ± 2	29 ± 2
IT4D	19 ± 1	11 ± 1	25 ± 2	20 ± 1
Iridobelamal **1**	14 ± 0	11 ± 1	23 ± 1	18 ± 0
Isoiridogermanal	16 ± 2	11 ± 2	24 ± 1	22 ± 2
7-OMe-Aromadendrin	33 ± 1	21 ± 1	33 ± 3	25 ± 2
Tectorigenin	189 ± 6	105 ± 2	207 ± 14	149 ± 2



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P 547

Antioxidant activity of the essential oils of *Salvia officinalis* L

Cruz C[1], Miguel MG[1], Simões MTF[2], Figueiredo AC[2], Barroso JG[2], Pedro LG[2]
[1]Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8005 – 139 Faro, Potugal; [2]Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749 – 016 Lisbon, Portugal

Sage (*Salvia officinalis* L) is an important medicinal plant from the Mediterranean region. In the present work, the chemical composition and the antioxidant activity of *S. officinalis* oils were studied. The essential oils were isolated, from the dried aerial flowering parts of *S. officinalis* of commercial source, by hydrodistillation with different distillation times: 30 min, 1 h, 2 h and 3 h. The essential oils were analysed by GC and GC-MS. The antioxidant activity was assessed by three distinct methods: thiobarbituric acid reactive species (TBARS), reduction of the stable radical DPPH (2,2-diphenyl-1-picryl-hydrazyl) and the deoxyribose assay method for scavenging the hydroxyl radical. Each of the oils, obtained via the different hydrodistillation periods, was tested for antioxidant activity in the range of 100 – 22300 mg/L. The percentage of antioxidant activity was calculated by comparison with the negative control (methanol). α-Tocopherol was used as positive control for TBARS and DPPH

assays and manitol for scavenging the hydroxyl radical method. 1,8-Cineole, α-pinene and camphor were the dominant components of all the essential oils isolated by the different hydrodistillation periods. The scavenging ability of the oils for the DPPH radical ranged from 10 – 90%, the highest antioxidant capacity being obtained with the 3 h-oil at 22300 mg/L, with IC_{50}= 16912 mg/L. Likewise, the 3 h-oil showed the best antioxidant activity (79%) at 1000 mg/L, using the TBARS method, with IC_{50}= 217 mg/L. Generally, the oils' ability to scavenge OH radicals increased up to 1000 mg/L, decreasing at higher concentrations. The 1 h-oil showed the highest activity (73%). *Acknowledgements:* This study was partially funded by IFADAP under research contract AGRO 800.

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Chromatographic separation, characterization and bioactivity guided assays of the aqueous root extract fractions of *Dalbergia saxatilis* HOOK. F. (Papilionaceae)

Yemitan OK, Adeyemi OO
Department of Pharmacology, College of Medicine of the University of Lagos, Idi-Araba, P.M.B. 12003 Lagos, Lagos, Nigeria

The aqueous root decoction of *Dalbergia saxatilis* (DS), is used to treat convulsive and anxiety disorders, pain and muscle tension in traditional African medicine. Separation of phytochemical constituents was conducted on DS using Sephadex G-50 packed chromatography into thirty 5 ml fractions. Preliminary chemical characterization was conducted on fractions employing the Agilent 8453 UV-Visible spectrophotometer between 200 and 600 nm; and phytochemicals present in fractions were confirmed by simple chemical tests on determination of phytochemicals in medicinal plants [1]. Based on the distribution of the phytochemicals, fractions 9 & 10; 11 & 12; 15 & 16; 17 & 18 (labelled as "A", "B", "C", "D", respectively), were pooled for biological tests. Bioactivity guided assays were done by investigating the CNS depressant effects of fractions on strychnine induced seizures [2], pentobarbitone hypnosis [2] and exploratory behavioural test [3] models. Results as depicted by the chemical tests method [1] showed chromatographic separation of DS into fractions of saponins (7 – 26), reducing sugar (5 – 21), soluble carbohydrates (5 – 27), tannins (5 – 19), phenols (5 – 19) and C-glycosides (15 – 18). Bioactivity guided assays showed that fraction "C" (200 mg/kg, p.o.), like phenobarbitone (40 mg/kg, i.p.) produced significant (P< 0.01) antiseizure effect; fraction "D" (200 mg/kg, p.o.) produced prolongation of pentobarbitone (40 mg/kg, i.p.) sleeping time, while fraction "A" (200 mg/kg, p.o.) produced suppression of exploratory behaviour, like chlorpromazine (4 mg/kg, i.m.). The unfractionated extract (200 mg/kg, p.o.), however, showed significantly (P< 0.05, ANOVA) higher effects than the fractions in these three models. The results suggest that the active CNS depressant phytochemicals may have been fractionated, but their effects are synergistic in the unfractionated extract. **References:** [1] Odebiyi and Sofowora (1978) Lloydia 41: 234. [2] Yemitan et al. (2001) Nig. J. Neurosci. 4: 33 – 40. [3] Dhara et al. (2002) Psychopharmacologia 44: 53 – 59.

P 549

The role of vitamin C on prevention of morphine addiction in rats

Rafati A[1], Dashti MH[2], Morshedi A[2]
[1]Dept. of physiology Shiraz medical university, Shiraz, Iran; [2]Herbal Medicine Research Center, Shahid Sadughi Medical University Yazd, Iran

Objectives: Today, addiction is one of the most important social problems. Morphine is an addictive drug which causes several alterations in human body. Both acute and chronic administration of morphine increase releasing of dopamine which leads to dependence and tolerance to it. One of the most important factors that prevent addicted people from abandonment is painful symptoms of withdrawal syndrome. So finding a method to decrease withdrawal symptoms can be a good protocol to defeat this challenge. Since vitamin C which is released from glutaminergic neurons is a modulator of central dopaminergic and glutaminergic transmissions, we decided to study the role of it on prevention and decreasing physical dependence of morphine addiction in rats. **Methods:** In this study we evaluated withdrawal symptoms (e.g. jumping, wet dog shaking) after naloxane injection in sham (normal saline i.p. injection), control (fixed doses of morphine i.p. injection) and test (500 mg/kg vitamin C i.p. injection before morphine daily) groups. **Results:** Our data showed that both the tendency and most of the withdrawal signs (jamping, standing and wet dog shaking) in the test animals were significantly less than the control animals ($p < 0.05$). **Conclusion:** Our findings support the use of vitamin C as a potent agent in treatment of addicts.

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Evaluating the Anesthetic Effect Of Clove Essence In Mice

Dashti MH, Morshedi A, Nooraldini SJ, Salami AS
Herbal Medicine Research Center, Shahid Sadughi Medical University Yazd, Iran. postal code:8915875918

Aims: Previous studies have reported the effect of clove (*Syzygium aromaticum*) extract as a local sedative in toothache, and as an anesthetic drug in aquatics such as trouts. In the present study we evaluated the anesthetic effect of clove essence in mice. **Methods:** In this study Syrian mice weighting 25 – 30 g were used. Different doses of clove essence (0.1 – 16 ml/kg) were injected intraperitoneally to 10 animals in each group. The anesthetic effect was evaluated immediately after the injection of Clove essence according to the expression of motion impairment or imbalance, akinesia, tail pinch unresponsiveness (as an index of complete anesthesia) and recovery to normal motion. **Results:** Our findings showed that clove essence in doses equal or less than 1 ml/kg did not produce complete anesthesia, but the administration of doses equal to or greater than 1.5 ml/kg in a dose dependant manner revealed a period of complete anesthesia lasting for 2 to 6 minutes. The dose – response curve for different anesthetizing doses was plotted and 1.8 ml/kg was determined as $ED_{50.}$ Mortality was observed by administrating 2.5 ml/kg Clove essence and by considering the mortality rate induced by different doses from initial to full lethal dose, LD_{50} for clove essence was 2.8 ml/kg. **Conclusion:** Although clove essence induces anesthesia in mice since its ED_{50} and LD_{50} are very close to each other this herb is not a very safe choice for anesthesia induction in mice.

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The Effect of Clove Essence on Tonic Pain In Mice

Morshedi A, Dashti MH, Salami AS
Herbal Medicine Research Center, Shahid Sadughi Medical University Yazd, Iran.postal code:8944157963

Objectives: Opioids are the most effective analgesics but they induce serious side effects such as dependence. So approaching to alternative antinociceptive agents has been the aim of many investigations. Previous studies have reported the effect of clove (*Syzygium aromaticum*) extract as a local sedative in toothache, and as an anesthetic drug in aquatics such as trout. In the present study we conducted to evaluate the effect of clove essence on tonic pain in mice. **Methods**: In this study the Formalin test was used for assessing animal's pain sensation. 28 male Syrian mice were randomly divided into 4 groups. Animals received normal saline (control group) and 3 different doses of clove essence 0.1, 0.2 and 0.4 mg/kg respectively (test groups) intraperitoneally 15 min before Formalin test. Pain scores were assessed during 1 hour post Formalin injection and the pain rates were statistically analyzed for each 5 min intervals. **Results:** Our findings showed that clove essence attenuates pain responses in a dose dependent manner. This analgesic effect in comparison with normal saline was significant for all administered doses ($P < 0.05$). The antinociception was more prominent in the

inflammatory phase than the acute phase of Formalin test ($P < 0.05$). **Conclusion**: According to our findings clove essence could attenuate the chronic pain induced by formalin injection in a dose dependant way.

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The analgesic effect of saffron extract in rats as compared with morphine sulfate

Vahidi AR, Bashardost N, Akhondi H
Medicinal herb center, Shahid Sadughi Medical University, Yazd, Iran. 8944157963

Objectives: In Iranian traditional medicine saffron (*Crocus sativus* L.*)* is known as a sedative, tranquilizer and antispasmodic agent. Crocetin with potent anti-inflammatory activities is a major ingredient of saffron. So in this study we conducted to evaluate the effect of saffron extract on inflammatory pain in rats. **Methods**: In this study 30 male Wistar rats weighing 200 – 250 grams selected randomly and divided into 5 groups. Animals in the first group received normal saline as control group, 3 groups received 3 different doses of saffron extract (0.5, 1 and 2 mg/kg respectively) and the last group received 1 mg/kg morphine sulfate. Aqueous extract of 2 g saffron was obtained at room temperature and was injected to rats intraperitoneally. Morphine sulfate (1 mg/kg) was used as positive control. The formalin test was used as a method of inflammatory pain inducing and pain scoring test. **Results**: Our findings showed that different doses of saffron extract (0.5, 1 and 2 mg/kg) reduce pain sensation during chronic phase of formalin test (1.68 ± 0.37, 1.51 ± 0.251and1.21 ± 0.41respectively) as compared to the control group (2.35 ± 0.34) in a dose dependent manner ($p < 0.05$). The analgesic effect in animals receiving 2 mg/kg saffron extract was more prominent and comparable to 1 mg/kg morphine sulfate. Mean pain score was 1.21 ± 0.41in test vs1.1 ± 0.25 in morphine injected group ($p > 0.05$). **Conclusion**: Our findings support the claim of traditional medicine indicating the saffron's antinociceptive activity.

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Anticholinergic effect of *Carum copticum* on intestinal motility of rats

Hejazian SH, Mahdavi M, Dashti MH
Herbal Medicine Research Center, School of Medicine; University of Medical Sciences, Yazd, Iran

Objective: It has been reported that *Carum copticum* Benth. Hook f., a plant in the Umbelliferae family, has some anticholinergic and antihistaminic activities on tracheal smooth muscle [1]. However, these effects of *C. copticum* are not yet identified with respect to mechanical activities of isolated ileum. The present study has been designed to find out the specific effects of *C. copticum* fruit extract on cholinergic activity of the rat ileum. **Methods**: In this study five pieces of isolated rat ileum were used for assessing the anticholinergic effects of alcoholic extracts of *C. copticum* fruit. using an organ bath and physiographic recording [2]. **Results:** Our findings showed that the maximum contractile response was obtained in the presence of 5×10^{-4} M acetyl choline. This contractile activity was reduced to 50 percent by pre-treating the specimens with 0.01 mg extract/ml bath. This concentration of *C. copticum* extract by itself showed a relaxant effect on ileum specimens as compared with control base line. This relaxant effect was reversed by pre treating the specimens with atropine sulphate (5×10^{-6} M) **Conclusion:** The results of this study indicated an anticholinergic property for the extract. **References:** [1] Boskabady MH, Shaikhi J, (2000), J Ethnopharmacol., 69: 217 – 27. [2] Kiely JM, Noh JH,(2005) J Surg Res,124(1):98 – 103

P 554

Mechanisms of action of antiosteoporotic effects of a special *Cimicifuga racemosa* (CR) extract: inhibition of the RANK-RANKL-osteoprotegerin triad

Seidlová-Wuttke D, Jarry H, Wuttke W
Department of Clinical and Experimental Endocrinology, University of Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany

Receptor activator of nuclear factor κB (RANK), its ligand RANKL and the RANKL decoy receptor osteoprotegerin (OPG) form a protein triad of importance for bone homeostasis maintenance. Estradiol-17ß (E2), testosterone (T) and the *Cimicifuga racemosa* extract BNO 1055 (CR BNO 1055) have antiosteoporotic effects following gonadectomy of female or male rats. Their mechanisms of action, however, remain unexplored. Rats were ovariectomized (ovx) or orchidectomized (orx) and substituted with E2, T and CR BNO 1055 via soy free food for 3 months. Cancellous bone mineral density of the tibia metaphysis was determined by quantitative computer tomography. Serum levels of RANKL, OPG, osteocalcin and RatLaps were determined by radioimmunoassays. In both, ovx and orx animals RANKL was suppressed by E2 and CR BNO 1055, while T suppressed RANKL levels in orx males only. OPG remained uneffected. In both sexes, osteocalcin was significantly reduced by E2. RatLaps were reduced by E2 and CR BNO 1055. E2 treated animals had the highest bone mineral density; this effect was partly shared by CR BNO 1055, but not by T. Thus, the bone sparing effect of E2 and CR BNO 1055 are partly mediated by inhibition of RANKL production. The E2 effects are most likely mediated via the estrogen receptor alpha. Since compounds in CR BNO 1055 do not bind to any type of estrogen receptors, the mechanism of RANKL inhibition by CR BNO 1055 remains unknown. The failure of T to prevent osteoporosis in orx animals despite reduced RANKL serum concentrations remains enigmatic.

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Antioxidant and radical scavenging effect of different parts of four Turkish *Juniperus* species

Çoban T[1], Konuklugil B[2]
[1]Department of Toxicology, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Türkiye; [2]Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Türkiye

The comparative antioxidant potential of *J. drupacea, J. oxycedrus, J. foetidissima and J. excelsa* leaf and fruit water extracts were studied. Their antioxidant activities were evaluated by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method and non-enzymatic rat hepatic microsomal lipid peroxidation method. Various antioxidant activities were compared with standard antioxidant vitamin E (α-tocopherol). Fruit and leaf extracts of *J. Durapacea* (IC_{50}; 24 μg/ml; IC_{50}; 35 μg/ml, respectively) and *J. foetidissima* (IC_{50}; 33.5 μg/ml; IC_{50}; 26 μg/ml, respectively) were found to be a good scavenger of DPPH radical when compared to vitamin E (IC_{50}; 13 μg/ml). *J.oxycedrus and J. excelsa fruit* extracts exhibited the weakest effect on DPPH radical with IC_{50} values of 115 and 104 μg/ml, respectively, compared with α-tocopherol. Almost all tested extracts exhibited very strong antioxidant properties when compared to vitamin E (α-tocopherol) with percent inhibition of 84 – 97% in the TBA assays at the 0.25 and 0.5 mg/ml concentrations. TBA assay results showed that *J. drupacea* leaf (95%) and fruit extracts have the strongest anti-lipid peroxidation activity (91 – 96%) at a dose of 0.25 – 0.5 mg/ml, respectively. *J. oxycedrus* leaf extract also exhibited strong anti-lipid peroxidation effect with percent inhibition of 88 – 97% while *J. oxycedrus* fruit extract showed moderate activity with percent inhibition of 48 – 65% in the TBA assay and DPPH methods. The results showed that *J. drupacea and J. foetidissima* extracts have higher antioxidant capacities than *J.oxycedrus* and *J. excelsa* fruit extracts.

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In vitro evaluation of the cytotoxicity of several eremophilane constituents of *Petasites hybridus* in rat hepatocytes

Bodensieck A[1], Gaunitz F[2], Gebhardt R[2], Bauer R[1]
[1]Karl-Franzens-University Graz, Inst. of Pharm. Sciences, Pharmacognosy, Universitaetsplatz 4, A-8010 Graz; [2]University of Leipzig, Med. Faculty, Inst. of Biochemistry, Johannisallee 30, D-04103 Leipzig

Several *Petasites hybridus* eremophilanes have been tested for cytotoxicity in primary rat hepatocytes by means of the MTT assay [1, 2, 3]. (8S)-8-Hydroxyeremophil-7(11)-en-12,8-olide, (8R,9β)-2-[(Angeloyl)oxy]-9-hydroxyeremophil-7(11)-en-12,8-olide and (8S)-2-[Methacroyl)oxy]eremophil-7(11)-en-12,8-olide were not cytotoxic up to a concentration of 0.5 mg/mL. No EC_{50} values were determinable for these less soluble compounds. (8S)-2-{[(Z)-3-(Methylsulfanyl)prop-2-enoyl]oxy}eremophil-7(11)-en-12,8-olide was not cytotoxic up to 1 mg/mL. (8R)-2-[Methacroyl)oxy]eremophil-7(11)-en-12,8-olide was the most cytotoxic constituent (EC_{50} approx. 0.3 mg/mL). The *non-steroid-like* 8α-conformers of both 8-H-isomeric couples of the 2-angeloyloxy and 2-methacroyloxy esters of eremophilanolide seemed to be more cytotoxic than the *steroid-like* 8β-H-conformers. The additionally measured cytotoxicity parameters mitochondrial dehydrogenase activity, ATP-content and LDH-leakage were considerably lower for the 8α-conformer of 2-[Methacroyl)oxy]eremophil-7(11)-en-12,8-olide than for the corresponding 8β-conformer [4]. This stereoselectivity points to a specific new cytotoxicity target. **References:** [1] Bodensieck, A et al. (2007) Helv Chim Acta: 90: 183 – 195. [2] Mosmann T J (1983) J Immunol Meth 65: 55 – 63. [3] Gebhardt R (1997) Toxicol Appl Pharmacol 144: 279 – 286. [4] Gaunitz F et al. (2003) Assay Drug Dev Technol 1: 469 – 477.

P 557

No evidence for modulation of endothelial nitric oxide synthase by the olive oil polyphenol hydroxytyrosol in human endothelial cells

Schmitt CA[1], Handler N[2], Heiss EH[1], Erker T[2], Dirsch VM[1]
[1]University of Vienna, Department of Pharmacognosy, Althanstraße 14, 1090 Vienna, Austria; [2]University of Vienna, Department of Medicinal Chemistry, Althanstraße 14, 1090 Vienna, Austria

Decreased nitric oxide (NO) availability in the vascular system is associated with atherosclerosis [1]. Upregulation of endothelial nitric oxide synthase (eNOS) expression and activity is considered as a strategy for the prevention of cardiovascular diseases. Olive oil is a cornerstone of the Mediterranean diet. Besides fat, it contains several phenolic compounds which are found especially in extra virgin olive oil. The polyphenolic fraction seems to contribute considerably to the beneficial effects associated with olive oil consumption. The polyphenol hydroxytyrosol (HT), which is present in olive oil and red wine, has shown antiatherogenic activity *in vitro* and *in vivo* [2],[3]. To elucidate underlying molecular mechanisms, we investigated possible effects of HT on eNOS using human endothelial cells (EA.hy926) [4]. Specifically, we addressed putative effects on eNOS promoter transactivation, eNOS enzyme activity and NO availability. Cells were treated with a broad range of HT concentrations (from 10 nM to 100 μM) and for different incubation times (15 min to 24 h). HT did not exert significant positive effects on eNOS in any of our assay systems. Neither did we find evidence for a possible synergism between the red wine polyphenol resveratrol and HT. We conclude that a direct modulation of eNOS is unlikely to account for the antiatherogenic properties of HT under non-inflammatory conditions of the endothelium. *Acknowledgements:* The authors would like to thank Dr. C.-J.S. Edgell (University of North Carolina) for EA.hy926 cells, Dr. P. Wohlfahrt (sanofi-aventis, Germany) for EA.hy926-he-NOS-Luc-cells and Drs Steffen Hering and Oskar Hoffmann for lab space to perform experiments with radioactive isotopes. **References:** [1] Förstermann U (2006) Biol Chem 387: 1521 – 33. [2] Carluccio MA et al. (2003) Arterioscler Thromb Vasc Biol 23: 622 – 9. [3] Gonzalez-Santiago et al. (2006) Atherosclerosis 188: 35 – 42. [4] Edgell C-JS, et al. (1983) Proc Natl Acad Sci USA 80: 3734 – 3737.

P 558

Treatment of the Kidney Stone by a Herbal Medicine Complex, STONREEZ: A Clinical Trial

Orafai H[1], Mohammadpor AH[2], Avari E[3], Molaqen H[3]
[1]Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences P.O.91775, Mashhad, I.R.Iran; [2]Department of Pharmaocology and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences P.O.91775, Mashhad, I.R.Iran; [3]Out patient Department Hashemi-Nejad Hospital, Mashhad University of Medical Sciences, Mashhad, I.R.Iran

A complex powder, *Stonreez*, containing 10 medicinal herbs, was introduced to 40 male volunteers suffering from stones in their kidneys between 25 – 45 years old who had been treated with customary antispasmodic drugs and showed failure to release their stones. They were taken as positive control group, and had been checked for non-existence of low blood pressure, and for stones in kidneys with both x-ray and ultrasound techniques before and after the treatment. They were taking 2 grams of the *Stonreez* during 2 – 15 days in 5 doses per day in the form of 150 ml infusion, until their stones were released. The formulation includes *Cerasus avium* fruits tail, *Lavandulla officinalis, Foeniculum vulgare, Cuminum ciminum, Cucumis sativus* and *Cucumis melo* seeds, *Althea officinalis* and *Aerva lanata* flowers, leaf and stem of *Thymus vulgaris* and forelock of *Zea Mays*. They were checked and standardized for the main markers, total phenolic compounds as tannic acid, linalyl acetate for *Lavandulla officinalis essential oil* and ashes for the others, before formulation. The collected stones released were 2 – 18 mm for the biggest diameter over all samples as measured by ruler and photographed. The statistical analysis showed that 92.5% of the volunteers had released their stones of 0.2 – 18 mm diameter. It is concluded that this formulation is a suitable medicament for kidney stone treatment without adverse effects which might be due to the combination of diuretics, disintegrants and spasmolitic effects (1 – 2). **References:** 1. Newall CA.,Anderson LA.,Philipson JD.,Herbal medicine, a guide for health-care professionals, London, The Pharmaceutical Press, 1996,90. 2. Wagner H.,Bladt S.,Zgainski EM., Plant drug analysis, New York, Springer 1984, p. 163 – 4,269 – 70.

P 559

Antioxidant and anti-HIV-1 integrase compounds from *Smilax corbularia* Kunth

Itharat A[1], Kejik R[2], Tewtrakul S[2], Watanaperomskul C[2]
[1]Applied Thai Traditional Medicine Center, Faculty of Medicine, Thammasart University, Rungsit Campus, Klongluan, Pathumtani, 12120 Thailand
[2]Department of Pharmacognosy and Pharmaceutical Botany Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkla 90110, Thailand

Smilax corbularia Kunth. (Hua-Khao-Yen-Neua) is commonly used to treat AIDS patients in Thai traditional medicine. The aim of this study was to investigate the antioxidant and anti-HIV-1 integrase activity of compounds of *S. corbularia* rhizome (1). The ethanolic extract and its compounds were used and isolated by bioassay-guided fractionation. They were tested for antioxidant activity by the DPPH assay (2) and for lipid peroxidation (3) and anti-HIV-1 integrase activity by a multiplate integration assay (MIA) (4). Two compounds isolated from the ethanolic extract were astilbin and engeletin. The ethanolic extract and its compounds exhibited high antioxidant activity in both assay. The ethanolic extract, astilbin and engeletin showed antioxidant activity with EC_{50} values of 2.5, 2.2 and 3.9 μg/ml, respectively in the DPPH assay and EC_{50} values of 46.9, 0.8 and 1.2 μg/ml, respectively in the lipid peroxidation assay. The IC_{50} values on HIV-1 integrase activity were 1.9, 30.2 and

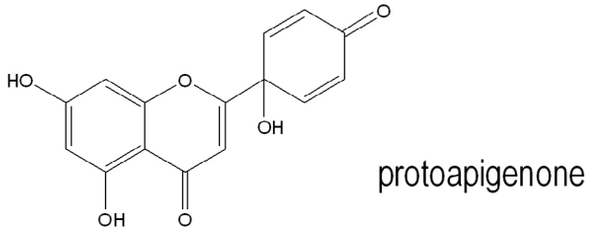
75.7 μg/ml, respectively. In conclusion, the extract of *Smilax corbularia* possessed high antioxidant activity as well as anti HIV-1 integrase activity. The compounds responsible for these activities are astilbin and engeletin. *Acknowledgement:* Prince of Songkla University for financial support. **References:** [1] Itharat, 1998, Specification and identification of plant called Hua-khao-yen. Thai Traditional Medicine Institute, Bangkok, p 42 [2] Yamasaki, K.(1994) Chem.Pharm.Bull.42,1663 – 1665 [3] Uchiyama, M.. et al. (1978) Anal. Biochem. 86, 271 – 278. [4] Tewtrakul, S. et al. (2002) Chem.Bull. 50, 630 – 635

P 560

First Total Synthesis of Protoapigenone and its Analogs as Potent Cytotoxic Agents

Lin AS[1], Nakagawa-Goto K[2], Chang FR[1], Yu D[2], Morris-Natschke SL[2], Wu CC[1], Chen SL[1], Lee KH[2], Wu YC[1]
[1]Graduate Institute of Natural Products, Kaohsiung Medical University, Kaoshiung 807, Taiwan; [2]Natural Products Research Laboratories, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599

A natural occurring flavonoid, protoapigenone, was isolated from the fern *Thelypteris torresiana* for the first time in 2005 [1]. This compound showed significant cytotoxic activity against five human cancer cell lines in the literature and had potential anti-tumor effects in an animal study [2]. In order to explore structure-activity relationships, identify additional active compounds, and investigate the mechanism of action, the first total synthesis of protoapigenone was achieved. By using hypervalent iodine reagent oxidation [3], 9 para-quinol analogs were also synthesized. All newly synthesized compounds and related intermediates were evaluated for *in vitro* cytotoxic activity. The result showed that these compounds possessed obvious cytotoxicity against five human cancer cell lines, HepG2, Hep3B, MDA-MB-231, MCF-7 and A549. Among them, 5,7-dimethoxy protoapigenone showed enhanced and notable activity against four cell lines (Hep3B, MDA-MB-231, MCF-7 and A549) with IC_{50} values of 0.17 – 0.40 μg/mL. 7-Methoxy protoapigenone, showed 2.1 – 13.7 fold greater cytotoxicity against all five tested cancer cell lines than parent compound. Furthermore, changing the A-ring from phenyl to naphthyl remarkably enhanced the activity. Analog 3-(1-hydroxy-4-oxocyclohexa-2,5-dienyl)-1*H*-benzo[*f*]-chromen-1-one strongly inhibited Hep3B cell growth with an IC_{50} value of 0.09 μg/mL and was more potent than the positive control doxorubicin against this cell line.



Acknowledgements: Grants CA 17625 from National Cancer Institute, National Science Council, Taiwan. **References:** [1] Lin A.-S. et al. (2005) Planta Med. 71: 867 – 870. [2] Unpublished data. [3] Wipf P. et al. (1994) J. Am. Chem. Soc. 116: 11678 – 11688.

P 561

Hepatoprotective activity from various parts of *Sonneratia caseolaris*

Charoenteeraboon J, Wetwitayaklung P, Limmatvapirat C, Phaechamud T
Faculty of Pharmacy, Silpakorn University, Nakhon-Pathom, Thailand, 73000

Sonneratia caseolaris L., family Sonneratiaceae, is well-known as mangrove tree which commonly found in mud lagoon. In Asia, its fruit and flower were used as vegetable and traditional medicines for cough and anti-parasites. The hepatoprotective activity of the methanolic extract from the different parts of *S. caseolaris*, stamen, sepal, meat of fruit, skin of fruit and persistent calyx, seed and pneumatophore, were investigated in HepG2 cells using MTT assay for cell viability determination. All crude extracts could protect the cell damage against chloroform toxicity. The hepatoprotective activities increased in the methanolic extracts obtained from various parts of *S. caseolaris* sepal, seed, skin of fruit and persistent calyx, pneumatophore, stamen, and meat of fruit, respectively, at a concentration of 50 μg/ml. The sepal extract could inhibit the chloroform toxicity in a concentration dependent manner and its activity was higher than that of silymarin, which was employed as the positive control. From this presented results it is suggested that *S. caseolaris* is a potential plant to further investigate for developing as hepatoprotective agent. *Acknowledgements:* Thailand Research Fund and Faculty of Pharmacy, Silpakorn University **References:** [1] Perry, L.M. 1980. Medicinal plants of east and southeast asia. MIT Press, Cambridge. [2] Mossman, T. J. (1983) Immunol. methods. 65: 55.

P 562

Effects of *Hypericum perforatum* oil extract on pentobarbital induced sleeping time

Cvejić J[1], Karanovic T[1], Simig M[1], Atanackovic M[1], Raskovic S[2], Jakovljevic V[2]
[1]Laboratory of Analysis of Natural and Pharmaceutical Products, Department of Pharmacy, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia;[2]Department of Pharmacology, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

Hypericum perforatum is a traditional herbal medicine which has been used for centuries as a topical remedy against ulceration and burns. It is well known for its vulnerary and epithelising properties and its oil extract can be used for treatment of wounds, bruises and other skin problems. Oil extracts can also be taken for stomach ache, colic, intestinal problems and as an expectorant for the congestion in the lungs. *Hypericum* could show pharmacologically significant interaction with drugs, if they are applied simultaneously. In this study, the effects of pretreatment with *H. perforatum* oil extracts on pentobarbital induced sleeping time were investigated. Crude oil extract (A) as well as extract made from a marketed formulation, namely a capsule with oil of *Hypericum* (B), standardised on 0,014% of hypericin, were used in our experiments. Oil extracts 0,04% were administrated intraperitoneally to white laboratory mice (both sexes, b.w. 20 – 30 g) 24, 18, 6, and 2 h before the administration of pentobarbital (40 mg/kg). Sleeping time, caused by pentobarbital, was measured as time between loss and regaining of the righting reflex. Compared with the control group (without any pretreatment), sleeping time in the group pretreated with extract A has been statistically and significantly prolonged, while pretreatment with extract B didn't show any statistically effect on induced sleeping time. The inductive time has been shorted in both groups, but not significantly. It is not yet clear why the extract made from marketed formulation did not show any significant effect on sleeping time. Future phytochemical investigations may help to elucidate this phenomenon.

P 563

Behavioral and chemical characterisation of MeOH extracts of two *Stachys* taxa

Kukić J[1], Savić M[2], Gavrilović I[2], Grayer R[3], Marin P[4], Tomić M[5], Petrović S[1]
[1]Institute of Pharmacognosy, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade, Serbia; [2]Institute of Pharmacology, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade, Serbia; [3]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK; [4]Faculty of Biology, Botanical Institute and Garden, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia; [5]Department of Biochemistry, Institute for Biological Research "Siniža Stanković", Despota Stefana 142, 11000 Belgrade, Serbia

In the quest for natural-based medicines, investigating the plant sources with putative psychotropic actions is a particularly challenging enterprise. Hereby, we report on the basic behavioral characterisation of the MeOH extracts of *Stachys plumosa* (SP) and *S. alpina* ssp. *dinarica* (SAD) (Lamiaceae). The behavioral activity of these extracts dosed in the range 50 – 400 mg/kg was examined in the adult male Wistar rats, with diazepam used as a positive control. The extracts were suspended with the aid of sonication in a solvent containing 85% distilled water, 14% propylene glycol, and 1% Tween 80, and were administered *i.p.*, 20 min before behavioral testing. The spontaneous locomotor activity, elevated plus maze and grip strength tests, predictive of sedative, anxiolytic and myorelaxant actions, respectively, were employed [1,2,3]. The analysis of variance was used in statistical evaluation. In the spontaneous locomotor activity test, there was a significant overall influence of treatment ($F(4,34)= 4.37$, $p = 0.006$); extract of SAD was devoid of measurable influences, whereas extract of SP exerted a significant hypolocomotor effect at the dose of 400 mg/kg ($p = 0.001$ for Dunnett's test). In the elevated plus maze, both extracts lacked any hints of anxiolytic activity. Similarly, neither of two extracts affected the grip strength of rats. In that regard, SP extract could be helpful in common sleep ailments, but not in anxiety or spastic disorders. HPLC analysis showed presence of verbascoside as the dominant compound in both investigated extracts. In SAD extract large amounts of 8-hydroxyflavone glycosides were detected, while their concentration in SP extract was very low. Chrysoeriol and apigenin glycosides were identified only in SP extract, comprising its major flavonoid fraction. **References:** [1] Savić, M.M. et al. (2006) Pharmacol Biochem Behav 84: 35 – 42. [2] Savić, M.M. et al. (2004) Pharmacol Biochem Behav 79: 279 – 90. [3] Gitler, D. et al. (2004) J Neurosci 24: 11368 – 80.

P 564

Phenolic compounds and antioxidant activity of *Achillea macrophylla* L. and *Achillea stricta* Schleicher from Valsesia (Italy)

Vitalini S[1], Fico G[1], Iorizzi M[2], Tomè F[1]
[1]Dipartimento di Biologia, Università degli studi di Milano, via Celoria 26, 20133, Italy; [2]Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territorio, Università degli Studi del Molise, Contrada Fonte Lappone, 86090, Pesche (Isernia) Italy

Valsesia is an area in the Western Italian Alps characterized by the presence of many wild and autochthonous plant species. Some of these plants are commonly used by the local population for pharmaceutical purposes and human nourishment as well as in veterinary medicine. In this work we present the results obtained by analysis of methanolic extracts and their fractions obtained from two species belonging to the *Achillea* genus (*A. macrophylla* L. and *A. stricta* Schleicher) collected in Valsesia. These species have shown significant antioxidant activity and the aim of this work was therefore the identification of constituents responsible for this activity. The MeOH extracts were separated by Sephadex LH-20 column chromatography; 22 and 19 fractions were obtained from *A. macrophylla* and *A. stricta* respectively. The fractions with different TLC profiles were tested for their antioxidant activity, evaluated as removal of the stable radical DPPH, total antioxidant capacity based upon the reduction of Cu^{++} to Cu^{+} and lipid peroxidation. Subsequently, the active fractions were purified using HPLC, and 4 active compounds could be isolated (Chlorogenic acid isomers, Rutin, Luteolin-7-O-glucoside, Apigenin-7-O-glucoside).

P 565

Radical scavenging ability of spanish red wine

Martin S, Gómez-Serranillos MP, Palomino OM, Naval MV, Ortega T, Carretero ME
Dpt. of Pharmacology. Faculty of Pharmacy. Universidad Complutense de Madrid. 28040-Madrid. Spain

Wine is a product from *Vitis vinifera* L. fruits that presents qualitative and quantitative differences in its phenolic composition which directly depends on the grape variety from which it is made. Red wine shows the most antioxidant, neuroprotective and cardioprotective effects, because of its high polyphenols content. Recent studies have shown that the dealcoholized fraction of wines can have important neuroprotective properties which depend on a wide number of factors such as climatic and cultivation process, winemaking technology, conditions of wine storage, etc. [1] In this study, the effect of one monovarietal young Spanish red wine (Merlot) on neuroprotection has been studied for the first time. This activity is assessed through its effect as free radical scavenger against different toxins ($FeSO_4$, H_2O_2 and $FeSO_4 + H_2O_2$) and by measuring the intracellular reactive oxygen species (ROS) generation through the fluorescence intensity of the ROS indicator 2',7'-dichlorofluorescein (DCF) [2]. First of all, the effect on cell survival was determined by 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium reduction assay (MTT) [3]. Astrocytes cultures have been used to test this neuroprotective effect as they are the most numerous cell types in the central nervous system and they support the physiology of associated neurons, interact with neurons and participate in the regulation of synaptic neurotransmission. They also play a crucial role in maintaining normal brain physiology during development and in adulthood. The results showed that Merlot wine improves cellular survival when exposed to the three different tested toxins at every assayed dose. In relation to the free radical scavenger activity, a decrease in the ROS generation is observed that is statistically significant ($p \leq 0.05$) at every assayed dose (6.8 ml/l; 10.2 ml/l and 13.6 ml/l) and every assayed toxin. The highest decrease is obtained with the dose of 13.6 ml/l of wine at 45 minutes and 1 hour with a percentage of ROS generation decrease of 76.4% and 71% at 1 h and 79.3% at 45 min, for $FeSO_4$, H_2O_2 and $FeSO_4 + H_2O_2$, respectively. *Statistical analyses:* Data were analyzed by analysis of variance (ANOVA), statistically significances were considered with $p \leq 0.05$. **References:** [1] Bertelli (2003). Drugs Exp Clin Res, 29: 169 – 70. [2] Fernández-Gómez et al. (2005). Neurobiol. of Disease, 20: 384 – 391. [3] Takahashi et al. (2002). Neurochem. Int., 40: 441 – 448.

P 566

Nigella Sativa L. Activity on Aminoacids Release in Mouse Brain Structures

El-Naggar T[1], Gómez-Serranillos MP[1], Palomino OM[1], Arce C[2], Carretero ME[1]
[1]Dpt. of Pharmacology. Faculty of Pharmacy. Universidad Complutense de Madrid. 28040-Madrid. Spain; [2]Dpt. of Biochemistry. Faculty of Pharmacy. Universidad Complutense de Madrid. 28040-Madrid. Spain

Nigella sativa L. (NS) is a vegetal species from the Mediterranean flora that has been traditionally used in folk medicine in the origin countries. NS methanolic extract has been proved to exert a potent Central Nervous System (CNS) (depressant action) and analgesic activity [1,2]. The aim of this work was to investigate the relationship between the observed depressant effects *in vivo* and the inhibitory amino acids levels in brain structures (hypothalamus (1), cortex (2), striatum (3)) in rats treated with NS. Seeds of NS were supplied

by the Medicinal and Aromatic Plants research Institute of Egypt (El Cairo, Egypt). Two groups of animals received the treatment with the methanolic extract of NS (2.5 g plant/kg) for 1 hour or 8 days, respectively; a third group served as a control. Then animals were sacrificed and the different brain structures were immediately extracted. The obtained structures were homogenized and centrifuged; the supernatant was collected and used to determine the amino acids content. A specific and stability-indicating HPLC method has been applied for the analysis of these amino acids. Samples were derivatized with dansyl-chloride and the amino acids were quantified by HPLC with ultraviolet-diode array detection. Data show that NS can modulate amino acid release in the studied brain structures (Table 1). The levels of GABA and Glycine in the NS-treated groups were higher than in the non-treated groups in a dose-dependent manner (Table 1). The use of HPLC on the qualitative and quantitative determination of the inhibitory amino acids is useful in following the pharmacological activity of central nervous system-active natural products.

	Control	Asp	Control	Glu	Control	Gly	Control	GABA
1	0.31 ± 0.01	0.40 ± 0.09*	1.31 ± 0.16	1.50 ± 0.21*	0.53 ± 0.09	0.35 ± 0.04*	1.27 ± 0.2	1.03 ± 0.17*
2	0.34 ± 0.05	0.34 ± 0.05	1.54 ± 0.29	1.48 ± 0.16	0.27 ± 0.07	0.44 ± 0.05*	0.59 ± 0.08	0.49 ± 0.07*
3	0.26 ± 0.04	0.28 ± 0.06	1.76 ± 0.04	1.69 ± 0.06	0.53 ± 0.11	0.55 ± 0.08	0.90 ± 0.12	0.67 ± 0.08*

Table 1. Amino acid content in every brain structure after 8 days of treatment with NS **References:** [1] El-Naggar T et al. (2003) J. Ethnopharmacol. 88: 63 – 8. [2] Kalus U et al. (2003) Phytother. Res. 17: 1209 – 14.

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Chemical composition and antioxidant activity of *Salvia officinalis* L. cultivated in Georgia

Sagareishvili T, Bostoganashvili M, Malania M, Sikharulidze I
Iovel Kutateladze Institute of Pharmacochemistry, 36 P.Sarajishvili st., 0159, Tbilisi, Georgia

The chemical composition of the leaves of *Salvia officinalis* cultivated in Georgia has been investigated. The essential oil tops 1.1% consisting mainly of 1,8-cineole (17.5%), α-thujone (31.6%) and β-thujone (17.6%). Six flavonoids and five phenylpropanoids were found in the water and water-ethanolic extracts of the leaves. Rosmarinic acid, luteolin, luteolin-7-β-D-glucuronide, cinaroside (luteolin-7-β-D-glucoside), vicenin-2 (apigenin 6,8-di-C-D-glucoside) were isolated and the presence of choline was identified. Microelemental composition looks as follows: K (0.4%), Na (0.007%), Fe (0.053%), Cu (0.002%), Mn (0.018%), Zn (0.003%), and Ni (0.02%) [1 – 3]. Antioxidant activity of the extracts was evaluated *in-vitro* and *in-vivo* (Table) using TBARs method where malondialdehyde (MDA, a product of lipid peroxidation) is expressed as thiobarbituric acid reactive substances (TBARs) and spectrophotometrically detected in serum [4]. Data were processed statistically (SPSS 12.0). Antioxidant activity of *Salvia officinalis* leaves water extract

	MDA concentration (μmol/l; mean ± SD, n = 10)	
	in vitro	in vivo
Control	5.13 ± 0.16	9.4 ± 0.21
Salvia officinalis water extract	3.41 ± 0.09 *	6.8 ± 0.12 *

* – $p < 0.01$ vs. control

On the basis of chemical and biological investigations the biologically active alimentary additive "Salbin" has been elaborated. **References:** [1] Sagareishvili T. et al. (2000), Chem. Nat. Compounds, 36: 360 – 361. [2] Sagareishvili T. et al. (2006), Georgia Chem. J., 6: 557 – 559. [3] Sagareishvili T., Bostoganashili M. et al. (2006), Georgia Chem. J., 6: 560 – 561. [4] Turpeinen, A. et al. (1995), Lipids, 30: 485 – 492.

P 568

Changes in Antioxidant Activity, Total Phenolics and Ascorbic Acid Content during Fruit Ripening in Korean Cultivars of *Rubus coreanus*

Kim SH, Park Y, Han J, Chung HG
Korea Forest Research Institute, 44 – 3 Omokcheon, 441 – 350, Suwon, Republic of Korea

Rubus coreanus is a perennial shrub distributed in the southern part of Korea. Its unripe fruit has been used in traditional herbal medicine for the treatment of diabetes mellitus and sexual disinclination. The fruit of *R. coreanus* was also found to have antioxidant activity. While there are some data on the constituents and biological activities of *R. coreanus* fruits, there are no studies on the chemical composition and antioxidant activity of *R. coreanus* fruits during ripening. Therefore, changes in the overall antioxidant properties of *R. coreanus* fruit during ripening are studied. Correlations between total phenolic content and ascorbic acid were also examined. *R. coreanus* fruits of three clones (S-13, S-14, and S-16), grown in the Korea Forest Institute (Suwon) were utilized. The antioxidant activities of the fruit 5 days after fruit set of three clones (S 13, S 14, and S 16) were 59.9, 75.8, and 81.2% at the concentration of 125 μg/ml. Total phenolic content in fruit of 5 days after fruit set of three clones (S 13, S 14, and S 16) were 260.5, 254.3, and 235.5 μg/g, respectively. When fruits were 5 days old, vitamin C content were 572.4, 541.2, 574.4 μg/g for S 13, S 14 and S 16, respectively. Total phenolic content of *R. coreanus* fruits (S-13, S 14, and S 16) was correlated with antioxidant activity (R^2 = 0.87, 0.97, 0.94).

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Histomorphology of the metaphysis of the tibia of ovariectomized rats treated with estradiol-17ß or *Cimicifuga racemosa*

Wuttke W, Seidlová-Wuttke D
Dept. of Clinical and Experimental Endocrinology, University of Goettingen, Germany

Estradiol-17ß (E2) and the extract of *Cimicifuga racemosa* (CR) protect against development of osteoporosis following ovariectomy (ovx) of rats. This was previously shown utilizing quantitative computer tomography which measures bone mineral density, i.e. the degree of bone mineralisation. Bone stability, however is largely dependent on the microarchitecture particularly of the trabecular apparatuses of long bones. Therefore we investigated by means of quantitative histomorphometry the microarchitecture of the metaphysis of the tibia of ovx rats either treated with E2 (14 μg/day/animal) or with the special extract BNO 1055 (30 mg per day) over a period of 3 months given immediately after ovx (prophylactic approach) or starting 30 days after ovx (therapeutic approach). CT scans were taken prior to 45 and 90 days after ovx and animals were sacrificed after the last CT-scan, the tibiae harvested and embedded in epoxy resin, cut in 3 μm preparations and stained according to Goldner. By means of computer aided program the surface of the trabeculae within the endosteal area of the metaphysis of the tibia were evaluated. The qCT data indicated that sham treated animals lost more than 50% of their cancellous bone mineral density within 3 months after ovx. This loss was almost totally prevented by E2 and partially by the Cimicifuga racemosa extract in the prophylactic approach. This reflected also in the histomorphometric data: A massive loss of the number of trabeculae was observed in the sham treated ovx animals and this loss was largely prevented by E2 and the CR extract BNO 1055. The periosteal circumference at the level of the metaphysis of tibia was significantly enlarged in the ovx animals and the total cortical bone mass was also increased. This effect was also largely prevented by E2 and the CR extract BNO 1055. Osteocalcin is a surrogate parameter of osteoblast activity which can be measured in the serum. E2 caused lowest osteocalcin concentration at the end of the study while levels in BNO 1055 treated animals were significantly elevated compared to controls.

In the therapeutic approach trabecular rarification had occurred prior to treatment (~35%) but a further loss of cancellous density and of trabeculae was prevented by E2 and BNO 1055. Thus E2 and substance(s) present in BNO 1055 were able to maintain the structure of the bone although the underlying mechanisms appear to be different. E2 has more antiresorptive effects whereas CR BNO 1055 may stimulate osteoblast activity.

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Proerectile effect of 15*R*-16,17-seco-subincanadine E, an indole alkaloid isolated from *Aspidosperma ulei* stem bark

Campos AR[1], *Deocleciano Júnior OB*[2], *Uchoa DEA*[3], *Silveira ER*[3], *Rao VSN*[2]
[1]*Regional University of Cariri, Chemical Biology Departament, Rua Cel. Antonio Luiz, 1161, 63.105 – 000, Crato, CE, Brazil;* [2]*Federal University of Ceará, Physiology and Pharmacology Department, Rua Cel Nunes de Melo, 1127, 60.430 – 279, Fortaleza, CE, Brazil;* [3]*Federal University of Ceará, Organic and Inorganic Chemistry Department, Av. Mister Hull s/n, 60.455 – 760, Fortaleza, CE, Brazil*

The search of an effective, safe and easy to administer drug for use in erectile dysfunction, impotence and fertility has been a perennial pursuit of most societies from times immemorial to throughout the history [1]. Plants that belong to the genus *Aspidosperma* (Apocynaceae) are known to be very rich in indole alkaloids and have an ethnomedical history of use as traditional remedies for erectile dysfunction [2]. In the search for novel drugs effective against erectile dysfunction, this study examined whether the indole alkaloid 15*R*-16,17-seco-subincanadine E (SEC) isolated from *Aspidosperma ulei* Markgr. stem bark could manifest penile erection-related behavioural responses (penile erection, erection-like and genital grooming) in mice. In the method described by Rampin [3], intraperitoneal injection of SEC (12.5; 25 and 50 mg/kg) elicited all the three different behavioural responses in a manner similar to yohimbine (YOH 2 mg/kg), a known indole alkaloid. Seventy-five percent of mice treated with SEC ($p < 0.001$ *vs* control) showed penile erections, which were completely blocked by clonidine ($p < 0.001$ *vs* SEC), an alpha-2-adrenoceptor agonist and haloperidol ($p < 0.001$ *vs* SEC), a dopaminergic antagonist and as well as by L-NAME ($p < 0.05$ *vs* control), a nitric oxide synthase inhibitor. In conclusion, the data obtained in this study clearly demonstrate that SEC facilitates penile erection in mice possible through the activation of central dopamine and blockade of presynaptic alpha-2 adrenoceptors with a subsequent enhancement of nitric oxide release from the penile nerves and arteries. This study further supports the traditional use of extracts from *Aspidosperma* species in erectile dysfunction. *Acknowledgements:* CNPq, CAPES and FUNCAP, Brazil. **References:** [1] Shoeb Qureshi, S.S. et al. 2007. Phcog Mag. 3(9): 1 – 15. [2] Campos, A.R. et al. 2006. J Ethnopharmacol. 104: 240 – 244. [3] Rampin, O. et al. 2003. Life Sci. 72: 2329 – 2336.

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Salvia fruticosa tea drinking reduces the expression of sodium/glucose cotransporter 1 in enterocytes brush-border membrane of streptozotocin-induced diabetic rats

Azevedo MF[1], *Lima CF*[1], *Wilson JM*[2], *Koepsell H*[3], *Fernandes-Ferreira M*[1], *Almeida MJ*[1], *Pereira-Wilson C*[1]
[1]*Department/Center of Biology, University of Minho, Campus de Gualtar, 4710 – 057 Braga, Portugal;* [2]*Laboratory of Ecophysiology, CIIMAR – University of Porto, Rua dos Bragas 289, 4050 – 123 Porto, Portugal;* [3]*Institute of Anatomy and Cell Biology, University of Würzburg, Koellikerstrasse 6, 97070 Würzburg, Germany*

Considering the increasing prevalence of Type 2 *diabetes mellitus* and a lack of efficient treatment, there is a growing interest on the research of natural bioactive compounds. *Salvia fruticosa* (greek sage) is a medicinal plant to which antidiabetic properties have been attributed [1, 2] and previous results confirmed an effect on control of blood glucose in rats. The aim of this work was to study the antidiabetic effects of sage tea drinking at the levels of intestinal epithelium and insulin secretion in normal and streptozotocin (STZ)-induced diabetic rats. Sage tea was given *ad libitum*, instead of water, for 14 days to the treated animals. SGLT1 (Na^+/glucose cotransporter 1) and GLUT2 (facilitative glucose transporter 2) expression in enterocytes were evaluated by Western blotting. Effects on glucagon-like peptide 1 (GLP1) and islet regeneration (β cell insulin expression) were assessed by immunohistochemistry. Glucose and insulin were measured in the plasma. An increase in the expression of SGLT1 in brush-border membrane (BBM) ($P < 0.001$) and GLUT2 in crude homogenates ($P = 0.027$) was observed in diabetic animals. Sage tea only decreased SGLT1 expression by 63% ($P = 0.008$). Fasting plasma glucose levels stabilized in tea STZ-diabetic rats when compared to water controls. A slightly increase in plasma insulin of sage tea drinking diabetic rats was observed ($P > 0.05$), accompanied by an increase in the intensity of insulin signal from β cells compared with diabetic control rats ($P = 0.012$). GLP1 immunoreactive cells were fewer in number in diabetic compared with normal animals ($P = 0.002$), although GLP1 intensity was higher ($P = 0.035$). No effects of tea were detected. *Salvia fruticosa* tea seems to be beneficial on a diabetic condition, where an increased intestinal transport capacity exacerbates hyperglycaemia, mainly by reducing the SGLT1 expression in BBM. *Acknowledgements:* MFA and CFL are supported by FCT grants: SFRH/BD/12527/2003 and SFRH/BPD/26316/2006, respectively. This work was supported by FCT research grant POCI/AGR/62040/2004. **References:** [1] Alarcon-Aguilar, F.J. et al. (2002), Phytother. Res., 16: 383 – 6. [2] Perfumi M, et al. (1991), J Ethnopharmacol., 34: 135 – 140.

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Peroxisome Proliferator-Activated Receptor-γ (PPARγ) activity from *Cistus salvifolius* – Cistaceae

Arapogianni NE[1], *Halabalaki M*[1], *Hempel J*[2], *Wober J*[2], *Skaltsounis AL*[1], *Vollmer G*[2]
[1]*Laboratory of Pharmacognosy & Natural Products Chemistry, School of Pharmacy, Panepistimioupoli, Zografou, 155771, Athens, Greece;* [2]*Molekulare Zellphysiologie & Endokrinologie, Technische Universität Dresden, Zellescher Weg 20b, 01217 Dresden, Germany*

A number of medicinal/culinary herbs have been reported to improve glucose metabolism and to yield hypoglycemic effects in patients with diabetes. Since stimulation of insulin sensitivity appears to be a potential mechanism, PPARγ is a likely target molecule for small lipophilic compounds derived from metabolism and nutrition. As a member of the nuclear receptor superfamily, functionally PPARγ integrates the control of energy, lipid, and glucose homeostasis. The aim of this study was to investigate the activation of PPARγ by botanical products. *Cistus salvifolius* (Cistaceae), a wide spread evergreen shrub was selected as an example. It has revealed significant antihyperglycaemic activity in a preliminary screening of extracts from medicinal plants of Greek flora. The whole plant was extracted using organic solvents and water (cHex, CH_2Cl_2, MeOH, MeOH-H_2O, and H_2O). The MeOH-extract was re-extracted with EtOAc and BuOH to afford four additional sub-extracts. PPARγ specific activities were assessed in HEC-1B endometrial adenocarcinoma cells following co-transfection with a PPARγ expression and a PPRE containing J3-tk-Luc reporter plasmid. Extracts (doses 10 and 100 μg/ml) were tested in comparison to a solvent control and a positive control (10 μM troglitazone). Several extracts induced reporter gene activity with cHex-extract being most potent. For those fractions a clear dose response pattern (0,1 – 100 μg/ml) could be established. For this reason cHex-extract was fractionated using VLC and tested again. PPARγ activity thereby resided in several fractions. In summary, extraction and fractionation of *Cistus salvifolius* yields PPARγ stimulating activities with differing chemical nature. In conclusion, PPARγ represents a candidate molecule for the mediation of improvement of glucose metabolism by botanical/nutritional products.

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Inhibition of the Akt pathway by tetracyclic triterpenoids induces cell cycle arrest and triggers apoptosis in human prostate cancer cells

Estrada AC, Syrovets T, Büchele B, Schmidt T, Simmet T
Institute of Pharmacology of Natural Products & Clinical Pharmacology, University of Ulm, D-89081 Ulm, Germany

Akt is a family of kinases controling cell proliferation and apoptosis. Akt plays an important role in the progression and chemoresistance of human cancers. Akt1 and Akt2, but not the Akt3 isoform are expressed and constitutively active in the androgen-dependent LNCaP and androgen-independent PC-3 and DU 145 prostate cancer cell lines. Three structurally different synthetic Akt inhibitors exerted cytotoxic effects on prostate cancer cells indicating that the Akt pathway is indispensable for cancer cell proliferation. The oleogum resins from Boswellia species contain a complex mixture of triterpenoids that possess a number of biological activities including antitumor properties. In search for well-tolerated and stable Akt inhibitors, we have isolated the tetracyclic triterpenoids 3-oxo-tirucallic acid, α-acetyl-tirucallic acid and β-acetyl-tirucallic acid from the oleogum resin of Boswellia carterii and purified them by reversed phase HPLC to chemical homogeneity. The triterpenoids potently inhibited the activities of human recombinant Akt isoforms in *in vitro* kinase assays with IC_{50} for Akt1 of about 3 μM, 0.3 μM, 0.1 μM, respectively. Similarly, the triterpenoids inhibited with comparable efficacy the Akt activity immunoprecipitated from PC-3 cells, but did not affect the activity of immunoprecipitated IKK. The triterpenoids also inhibited the phosphorylation of cellular Akt, β-catenin and glycogen synthase kinase (GSK)-3β, whereas extracellular signal-regulated kinase (ERK)1/2 phosphorylation remained unaffected. In addition, the compounds down-regulated the expression of the crucial cell cycle regulators cyclin D1 and c-myc followed by hypophosphorylation of retinoblastoma protein, cell cycle arrest and apoptosis. Thus, the inhibition of Akt activity is sufficient to trigger apoptosis in prostate cancer cells. Tetracyclic triterpenoids inhibiting Akt might provide a novel approach for the treatment of chemoresistant human prostate cancer. *Acknowledgement: Supported by the Deutsche Krebshilfe*

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Topical comfrey root extract in painful osteoarthritis: results of a randomized, double-blind placebo-controlled clinical trial

Krug L, Staiger C
Merck Selbstmedikation GmbH, Rößlerstrasse 96, 64293 Darmstadt, Germany

The aim of this randomized, double-blind, placebo-controlled clinical trial (COPA) was to demonstrate the therapeutic efficacy of a comfrey root extract ointment (Extr. Rad. Symphyti) in the treatment of patients with painful osteoarthritis of the knee. The trial was performed in accordance with the Declaration of Helsinki/Hong Kong 1989/Somerset 1996 as well as ICH-GCP Guidelines. 220 patients (153 women, 67 men, mean age 57.9 years) received daily 6 g (3 x 2 g) of either an active ointment (Kytta-Salbe® f, containing comfrey root fluid extract 1:2, 35.0 g, extraction solvent ethanol 60% (v/v)) or a corresponding placebo, for 21 days. In the course of the study, the VAS sum score (primary target variable) decreased by 51.6 mm (54.7%) in the active therapy group and by 10.1 mm (10.7%) in the placebo group. The mean group difference of 41.5 mm or 44.0% points is significant ($p < 0.001$). The WOMAC sum score (secondary target variable) decreased by 60.4 mm (58.0%) in the active therapy group and by 14.7 mm (14.1%) in the placebo group. The mean group difference of 45.7 mm or 43.9% points is significant ($p < 0.001$). With regard to further exploratory target variables SF-36 (quality of life), angle measurement (knee mobility), CGI (clinical global impression) and global efficacy assessment by investigator and patient, a significant superiority was also demonstrated ($p < 0.001$, respectively) for the phytotherapeutic agent versus placebo. A total of 22 AEs occured in 22 patients (7 in the active therapy group, 15 in the placebo group). None did present as adverse drug reaction in the active therapy group. Conclusion: The comfrey root extract ointment reduced the symptoms of osteoarthritis of the knee significantly. The therapy lead to reduction of pain, improved knee mobility and better quality of life. Therefore, the present clinical trial has demonstrated that the topical application of a comfrey root extract is a sensible and safe treatment option for patients with painful osteoarthritis. **Reference:** Grube B, Grünwald J, Krug L, Staiger C (2007) Phytomedicine 14: 2 – 10.

P 575

Effects of cereal and nata de coco supplementation on serum lipids in human

Pakpeankitvatana V[1], Weratean K[2], Thongmung N[2], Komind S[2], Mesomya W[3]
[1]Faculty of Pharmacy, Mahidol University, Bangkok, Thailand; [2]Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [3]Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand

The purpose of the study was to evaluate cereals and nata de coco (*food* product produced by the bacterial *fermentation* of *coconut* water) supplementation on lipid status in 22 subjects with hyperlipidemia. The subjects consisted of 11 men and 11 women aged 32 – 75 yrs, and had serum total cholesterol (TC) level of ≥ 5.2 mmol/L, serum total triglyceride (TG) level of ≥ 1.7 mmol/L and LDL-C level of ≥ 3.4 mmol/L. The subjects were given 15 g of the supplement twice daily for 20 weeks. These daily 30 g supplement consisted of unpolished rice, hulled mung bean, sweet corn, and nata de coco and provided 122.6 kcal, 5.5 g of protein, 0.5 g of fat, 24.1 g of carbohydrate and 2.7 g of dietary fiber. After 20 weeks, the subjects were divided into 2 groups, according to their dietary compliance, group A: ≥ 90% compliance, and group B: < 90% compliance with the assigned supplement intake. There were 15 subjects in group A, and 7 subjects in group B. Results showed that in group A the mean TG levels at week 4, 8, and 16 and TC at week 16 were significantly different from week 0 ($p < 0.05$), but no significant differences were observed in LDL-C and HDL-C. In group B, no significant changes were observed. Thus, the results appeared to indicate that health food from cereals and nata de coco may reduce the serum TG and TC in hyperlipidemic patients.

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The effects of curcumin on social isolation-induced depression and lipid peroxidation in mice

Sumanont Y[1], Jarukamjorn K[1], Tiangtam N[1], Yongprapat K[1], Laha T[2]
[1]Faculty of Pharmaceutical Science, Khonkaen University, Khonkaen, 40002, Thailand; [2]Department of Parasitology, Faculty of Medicines, Khon Kaen University, Khon Kaen 40002, Thailand

Curcumin, an active component of *Curcuma longa*, has been reported to possess free radical scavenging and antiinflammatory activities [1]. This study investigated the antidepressant-like effect of curcumin in a model of depression, the forced swimming test (FST) in mice. Mice were induced depressive behavior using the paradigm of social isolation which was associated with significantly longer durations of immobility during forced swimming [2]. We also elucidated the protective effect of curcumin against social isolation-induced lipid peroxidation in brain. Mice were housed alone or in groups for a period of 6 weeks [3]. After isolation period, a group of the mice (n = 6) received sub-chronic treatment with curcumin (10 and 100 mg/kg/day, s.c.) and another group (n = 6) imipramine, a tricyclic antidepressant (20 mg/kg/day, p.o.) once daily for a period 7 days. Mice were then tested behaviorally by forced swimming. The results showed that treatment with imipramine significantly decreased the immobility time in the FST. A similar profile of action

was observed in the animals received curcumin (100 mg/kg/day). There was no alteration of locomotor activation as determined in open-field tests in both of imipramine and curcumin treatment. In addition, the lipid peroxidation assays showed that repeated treatment with curcumin (100 mg/kg/day) markedly decreased the thiobarbituric acid reactive substance levels, the end products of lipid peroxidation. These findings suggest that mice subjected to 6 weeks of social isolation stress produces depressive-like behavior and oxidative damage in the brain and that curcumin exerts an antidepressant effect and neuroprotective effect by reduction of lipid peroxidation. *Acknowledgements:* Khon Kaen University Research Grant for Young Researchers (2006). **References:** [1] Motterlini, R. et al. (2000) Free Radic Biol Med. 28: 1303 – 12. [2] Yates, G. et al. (1991) Physiol Behav. 49: 347 – 53. [3] Houng, NT. (2005) Biol Pharm Bull. 28: 1389 – 93

P 577

Influence of *Veratrum* alkaloids on the catecholamine release at the ductus deferens and the adrenal medulla of the rat

Kretschmer N[1], Jost S[2], Vierling W[2]

[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens University, Universitätsplatz 4/I, 8010 Graz, Austria; [2]Institute of Pharmacology and Toxicology of the Technical University Munich, Biedersteiner St. 29, 80802 Munich, Germany

An influence on catecholamine (CA) release can have positive effects in several diseases. For example, reduction of noradrenaline release can improve tachycardia or hypertension. An increase in CA release can be helpful in the case of bronchoconstriction and may ameliorate depressive disorders. We investigated the influence of different *Veratrum* alkaloids on CA release. In previous experiments, it could be shown that some of these alkaloids have a positive inotropic effect on the papillary muscle of the heart [1]. In our experiments, we used the ductus deferens and the adrenal medulla of the rat. By measuring isometric force of contraction induced by electrical stimulation and stimulation with noradrenaline, we determined the CA release at the ductus deferens in an indirect way. At the adrenal medulla we measured the CA release in a direct way via voltammetry using carbon fibre microelectrodes. Two of the tested cevератrum alkaloids, veratridine and cevadine, which are veracevine esters increased the CA release (EC_{50} values: about 0.25 and 0.15 μM, respectively) but germine-3-acetate and germitrine (germine esters) inhibited the release (EC_{50}: about 25 and 0.15 μM,). The investigated jerveratrum alkaloid veratramine also reduced the release (EC_{50}: about 15 μM). Moreover, a whole plant ethanol extract exerted a decreasing effect on the release of CA in the applied concentrations (EC_{50}: about 0.7 ml/l). From the results, it is concluded that there are remarkable differences between *Veratrum* alkaloids regarding their effect on CA release. In the whole plant extract, the CA release-inhibiting compounds seem to be dominant. **References:** [1] Honerjäger P. (1982) Rev. Physiol. Biochem. Pharmacol. 92: 1 – 74

P 578

Identification of phenolic compounds in six *Hypericum* species by LC/UV/MS TOF and their radical scavenging properties by post-column HPLC-DPPH derivatization method

Gođevac D[1], Zdunić G[2], Šavikin K[2], Novaković M[1], Milosavljević S[3], Petrović S[4]

[1]Institute of Chemistry, Technology and Metallurgy, Njegoževa 12, 11000 Belgrade, Serbia; [2]Institute for Medicinal Plant Research "Dr. Josif Panèić", T. Kožćužka 1, 11000 Belgrade, Serbia;[3]Faculty of Chemistry, Studentski trg 16, 11000 Belgrade, Serbia; [4]Institute of Pharmacognosy, Faculty of Pharmacy, V. Stepe 450, 11221 Belgrade, Serbia

Many of the *Hypericum* species have been used in traditional medicine throughout the world [1]. The aim of this study was to investigate chemical composition and free radical scavenging properties of six *Hypericum* species growing in Serbia. A comparative analysis of the phenolic components in the 70% EtOH extracts of *Hypericum acutum*, *H. androsaemum*, *H. barbatum*, *H. hirsutum*, *H. maculatum*, and *H. richeri* has been carried out using HPLC/DAD and high resolution TOF-MS. Quercetin, astilbin, I-3,II-8-biapigenin, orientin, 2''-*O*-acetylorientin, three caffeoylquinic acids, and eight flavonol-3-*O*-glycosides were identified in the extracts on the basis of their on-line UV spectra, accurate mass spectral data, and in comparison of retention times with those from the standards. Fingerprint analysis of the extracts revealed significant differences in the qualitative and quantitative chemical composition of the studied species. A post-column HPLC-DPPH derivatization method was used to identify the free radical scavenging compounds. The amount of DPPH quenched by antioxidants is measured by a UV detector as a decrease in absorption at 517 nm. According to HPLC-DPPH profiles of the extracts, all phenolic compounds except I-3,II-8-biapigenin exhibited radical scavenging properties. Free radical scavenging properties of the extracts, considering the integrated areas of all the negative peaks in a $DPPH^{\cdot}$ chromatogram were expressed as TEAC (Trolox equivalent antioxidant capacities) values. The most potent DPPH scavenger was *H. maculatum* with TEAC value of 0.37 mmol Trolox/g of extract, while *H. barbatum* showed the weakest capacity (0.19 mmol Trolox/g of extract). *Acknowledgements:* The authors acknowledge their gratitude to the Ministry of Science and Ecology of Serbia for the financial support. **Reference:** 1. Dall'Agnol, R. et al. (2003) Phytomedicine 10: 511 – 516.

P 579

Extracts and constituents of Edelweiss (*Leontopodium alpinum)* enhance cholinergic transmission: AChE inhibitory, ACh-increasing and memory improving properties

Schwaiger S[1], Hornick A[2], Rollinger JM[1], Phung Vo N[2], Danzl B[1], Prast H[2], Stuppner H[1]

[1]Institute of Pharmacy/Pharmacognosy, Josef-Möller-Haus, Innrain 52c, Leopold-Franzens University of Innsbruck, A-6020 Innsbruck, Austria; [2]Institute of Pharmacy/Pharmacology and Toxicology, Peter-Mayr-Str. 1, Leopold-Franzens University of Innsbruck, A-6020 Innsbruck, Austria

Extracts of the roots of Edelweiss (*Leontopodium alpinum* Cass.*)* were activity guided fractionated for their acetylcholinesterase (AChE) inhibitory activity in an *in vitro* enzyme test and in a TLC assay with bioactivity staining, both based on Ellman's method [1]. Since results of the applied methods were contradictory the ability of the crude extract and its sub-fractions to enhance acetylcholine (ACh) in rat brain was studied using the push-pull technique. The sub-fraction with the highest *in vivo* activity consisted of a mixture of four sesquiterpenes: modhephene, silphinene, isocomene and β-isocomene. The *in vivo*-efficacy of the single compounds did not correlate with the *in vitro* AChE inhibitory potency and is obliviously mediated by another mechanism of action. The most potent sesquiterpene, isocomene, was further investigated with behavioural tasks in mice for cognition-improving and cholinergic transmission-enhancing properties. The compound (42 nM; i.c.v.) improved the object recognition in scopolamine-impaired mice (50 nM; i.c.v.) and showed nootropic-like effects in the T-maze alternation task in normal and scopolamine-treated mice (50 nM; i.c.v.). Additionally, the sesquiterpene (42 nM; i.c.v.) reduced locomotor activity of untreated mice in the open field task while the activity induced by scopolamine (50 nM; i.c.v.) was abolished. The effects of isocomene *in vivo* including the amelioration of cholinergic deficit in the behavioural tasks are in accordance with those of cholinergic transmission-enhancing substances [2]. The mechanism of action seems not to be caused by AChE inhibition and remains to be elucidated. Taken together, isocomene and related constituents of *Leontopodium* deserve further interest because of the significant anti-amnesic and cholinergic transmission-related effects of yet unidentified mechanism of action. *Acknowledgements:* This work was supported by the Austrian Science Fund (P18379-B11). **References:** [1] Ellman, GL., et

al. (1961) Biochem Pharmac. 7: 88 – 95. [2] Prast, H., Rollinger J., Schwaiger S., Stuppner H. (2007) PCT Int. Appl. A1 20070118.

P 580

Deoxyelephantopin exhibits potent effects against breast tumor growth and metastasis *in vitro* and *in vivo*

Huang CC, Lo CP, Chiu CY, Hsieh MC, Shyur LF
Agricultural Biotechnology Research Center, Academia Sinica, Taipei 115, Taiwan, R.O.C

Elephantopus scaber L. (Compositae) is a popularly used herbal tea constituent or folk medicine for various medications such as anti-tumor, anti-inflammation, etc. In this study, a group of sesquiterpene lactones (SLs) were isolated from the *E. scaber* extracts using silica gel chromatography and structurally elucidated by various spectral analyses. A most abundant SL compound, namely deoxyelephantopin (DET), was further examined for its anti-tumor cell activity *in vitro* and *in vivo*. We observed that DET exhibited a strong anti-breast cancer cell proliferation effect, with IC_{50} value = 1.5 – 2.0 μg/ml, on either human (MCF-7) or murine (TS/A) cell line. DET also significantly induced cell-cycle arrest, apoptosis, and suppressed colony formation and cell migration in TS/A cells. We further investigated the preventive and therapeutic effects of DET on tumor growth and lung metastasis *in vivo* using TS/A tumor-bearing syngeneic BALB/c mouse model. We observed that DET exhibited profound effects on TS/A tumor formation. The TS/A tumor growth and size were found to be significantly suppressed in the DET-treated mice (10 mg/kgBW) with a T/C value of 64% that was superior or comparable to the effect of Taxol®, a well-known therapeutic drug for breast cancer. In the lung metastasis model, vehicle-treated animals had a survival rate of 50% and 0% at 25 and 34 days, respectively, whereas DET pretreated animals not only significantly prolonged the overall survival rate but also reduced the number of metastatic pulmonary foci. Molecular mechanism(s) underlying the anti-breast cancer activities and inhibition of tumor metastasis of DET are under investigation.

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Moringa oleifera Lam Root Bark Decoction (MORBD) in Urolithiasis Management – A pilot study

Saketh Ram T[1], Shridhar A[2], Rajasekaran R[1], Sampath Kumar K[2]
[1]*Indian Institute of History of Medicine(IIHM),3rdFloor, Osmania Medical College Building, Putlibowli, Hyderabad, 500095 Andhra Pradesh, India,* [2]*Srinivasa multi speciality hospital & Institute of Advanced research in Ayurveda [SMH-IARA], 5 – 120/2 Opp: Shah Theater, HMT Road, Chintal, Hyderabad, 500 054 Andhra Pradesh, India*

Aims: *Moringa oleifera* Lam (MO) is a widely used vegetable drug in the Indian subcontinent. Ayurveda, the traditional Indian Medical system [1], recommends MO root bark decoction (MORBD) for the treatment of urolithiasis. Alkaloids moringine and moringinine isolated from root bark [2]. Use of MORBD helps alleviate pain along with free passage of urinary calculi. Current studies also confirm safety [3], lithotriptic [4], anti-inflammatory [5], antispasmodic [6], diuretic [7]· hypnotic [8] activity of MO. Objective: To Evaluate the lithotriptic and anti-spasmodic activity of MORBD in humans. DESIGN: Randomized open labeled study. SUBJECTS: 30 Subjects of either sex, age group between 18 – 65 years diagnosed for urolithiasis (stone size < 10 mm) from SMH-IARA*In Patient Department and Out Patient Department. Subjects served as their own controls. INTERVENTION: Standardized MORBD, 40 ml, 12th hourly administered orally for 3 – 15 days. Pain measured on Visual Analogous Scale (VAS: 0 – 100), size of the calculi based on ultrasonography (USG). **Results:** Amongst the 30 (Male: 18, F: 12), size of calculus > 8 – 10 mm: 4 subjects (13.33%), size < 8 mm: 26 subjects (86.6%). 2 mm size reduction was observed in 9 (30%) subjects. Stones passed in 15 (50%) subjects. No change was observed in 6 (20%) patients. Mean pain on VAS before treatment 75.60 ± 23.31 and after treatment 23.40 ± 8.10. Significant ($p < 0.01$) reduction in pain was observed during the study. 50% of the subjects passed calculi within the treatment period (< 15days). CONCLUSION: This pilot study hints at the potential of MORBD as a drug of choice in urolithisis as an antispasmodic and lithotriptic agent. The observations need further evaluation in a larger collective. *Acknowledgments*: Dr.Ala Narayana, Director, Indian Institution of History of Medicine(IIHM),3rd Floor, Osmania Medical College Building, Putlibowli, Hyderabad, 500095 Andhra Pradesh, India **References:** [1] Sushruta, Acharya.Y.T,(1994 repriont) Sushruta Samhita, Chaukhamba Surabharati Prakashan, Varanasi, India. [2] Chopra R N et.al. (1996) Glossary of Indian Medicinal Plants, NISCOM, New Delhi, India, 4th reprint, P.170 [3] Mazumder UK et.al. (1999) Indian J Exp Biol. 1999 Jun;37(6): 612 – 4., [4] Karadi RV. et.al. (2006) J Ethnopharmacol. 2006 Apr 21; 105 (1 – 2):306 – 11. [5] Ndiaye M. et al .(2002) Dakar Med.; 47(2): 210 – 2. [6], [7] Caceres A.et al..(1992) J Ethnopharmacol. 1992Jun;36(3):233 – 7. [8] Ray K et.al. (2004) Indian J Exp Biol. 2004 Jun;42(6):632 – 5. **Srinivasa Multi Speciality Hospital & Institute of Advanced research in Ayurveda [SMH-IARA].*

P 582

Protection of Neurons against Amyloid β Protein (25 – 35)-induced toxicity by Korean mistletoe

Kim JY[1], Ju HS[1], Cho SO[1], Song KS[2], Seong YH[1]
[1]*Lab of Pharmacology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361 – 763, Republic of Korea,* [2]*College of Agriculture and Life-Sciences, Kyungpook National University, Daegu, 702 – 701, Republic of Korea,*

Semi-parasitic plants, mistletoes (Loranthaceae), have been traditionally used as a sedative, analgesic, anti-spasmodic, cardiotonic and anticancer agent. Amyloid β protein (Aβ) (25 – 35) is believed to play a central role in the pathophysiology of Alzheimer's disease (AD) [1]. In the present study, the protective effect of a methanol extract from whole plant of Korean mistletoe (KM; *Viscum album* L. var. *coloratum* Owhi containing *ca* 0.01% (w/w) of triterpenes) against Aβ (25 – 35)-induced neurotoxicity was examined in primary cultured rat cortical neurons. KM (10 to 50 μg/ml) prevented the Aβ (25 – 35) (10 μM)-induced neuronal cell death, as assessed by a 3-[4,5-dimethylthiazol-2-yl]-2,5-di-phenyl-tetrazolium bromide (MTT) assay and Hoechst 33342 staining. KM significantly inhibited Aβ (25 – 35)-induced elevation of the cytosolic Ca^{2+} concentration which was measured by a fluorescent dye, fluo-4 AM. KM also inhibited generation of reactive oxygen species induced by Aβ (25 – 35). The protective effect of KM against Aβ (25 – 35)-induced memory impairment in mice was examined using passive avoidance test [2]. Memory impairment model in mice was established via intracerebroventricular (i.c.v.) microinjection of Aβ (25 – 35) (8 nmol). Aβ (25 – 35)-induced memory impairment was markedly improved by chronic administration of KM (25 and 50 mg/kg, PO, 8 days). Data were expressed as mean ± SEM and statistical significance was assessed by one-way analysis of variance (ANOVA) with subsequent Tukey's tests. In conclusion, the protection against Aβ (25 – 35)-induced neurotoxicity in *in vitro* and *in vivo* by KM may explain its inhibitory action on the progression of AD, and provide the pharmacological basis of its clinical usage in treatment of neurodegeneration in AD. *Acknowledgements:* This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea. **References:** [1] Hsiao K. K., et al., (1995). Neuron, 15, 1203 – 1218 [2] Schwarzberg, H., et al., (1989). Neuropeptides, 13, 79 – 81

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Sanguisorbae radix and its active component, gallic acid, protect amyloid β protein (25 – 35)-induced neurotoxicity

Thuy Ha NT[1], Kim JY[1], Cho SO[1], Song KS[2], Seong YH[1]
[1]College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361 – 763, South Korea, [2]College of Agriculture and Life-Sciences, Kyungpook National University, Daegu, 702 – 701, South Korea

Sanguisorbae Radix from *Sanguisorba officinalis* L. (Rosacea) is widely used in Korea and China due to its various pharmacological activities [1],[2]. The present study investigated the effect of the methanol extract of SR and gallic acid, which was isolated as an active component from SR by an activity-guided purification, on amyloid β protein (25 – 35) (Aβ (25 – 35)), a synthetic 25 – 35 amyloid peptide, -induced neurotoxicity using primarily cultured rat cortical neurons. SR (10 to 50 μg/ml) and gallic acid (0.1 and 1 μM), inhibited Aβ (25 – 35) (10 μM)-induced neuronal cell death, as assessed by a 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and the number of apoptotic nuclei, evidenced by Hoechst 33342 staining. Pretreatment of SR and gallic acid inhibited 10 μM Aβ (25 – 35)-induced elevation of cytosolic calcium concentration ($[Ca^{2+}]_c$), which was measured by a fluorescent dye, fluo-4 AM. SR and gallic acid inhibited glutamate release into medium induced by 10 μM Aβ (25 – 35), which was measured by HPLC, and generation of reactive oxygen species. Data were expressed as mean ± SEM and statistical significance was assessed by one-way analysis of variance (ANOVA) with subsequent Tukey's tests. These results suggest that SR and gallic acid prevent Aβ (25 – 35)-induced neuronal cell damage by interfering with the increase of $[Ca^{2+}]_c$, and then by inhibiting glutamate release, generation of ROS. Furthermore, these effects of gallic acid may be associated with the neuroprotective effect of SR. *Acknowledgements:* This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea. **References:** [1] Cheng and Cao (1992) Phytochemistry 31, 1317 – 1320. [2] Shin et al. (2002) Immunotoxicol. 24, 455 – 468.

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Anti-dementia effect of *Aralia cordata*: *in vitro* and *in vivo*

Cho SO[1], Ban JY[1], Song KS[2], Bae K[3], Seong YH[1]
[1]College of Veterinary Medicine and Research Institute of Herbal Medicine, Chungbuk National University, Cheongju, Chungbuk, 361 – 763, Republic of Korea; [2]College of Agriculture and Life-Sciences, Kyungpook National University, Daegu 702 – 701, Republic of Korea; [3]College of Pharmacy, Chungnam National University, Taejon 305 – 764, Republic of Korea

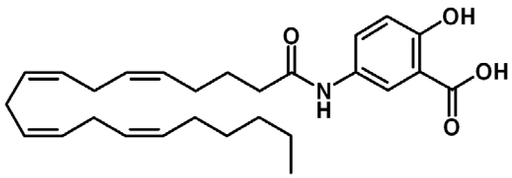
Alzheimer's disease (AD) is characterized by neuronal loss and extracellular senile plaque, whose major constituent is amyloid β protein (Aβ), a 39 – 43 amino acid peptide derived from amyloid precursor protein [1]. *Aralia cordata* Thunb. (Araliaceae) (AC) is a medicinal plant distributed in Korea, China and Japan. In a previous report, we demonstrated that the aerial part of AC contains diterpenes, triterpenes, saponins, sterols and cerebroside and its inhibitory activity against COX-1 and COX-2 [2]. The neuroprotective and antidementia effects of a 70% ethanol extract of aerial part of AC were examined using primarily cultured neurons and experimental animals in the present study. It was demonstrated that AC (1 – 10 μg/ml) inhibited Aβ (25 – 35)-induced neuronal apoptotic death, generation of reactive oxygen species and elevation of $[Ca^{2+}]_i$ in primarily cultured rat cortical neurons. Memory loss by Aβ (25 – 35) (8 nmol, i.c.v.), examined in passive avoidance test, was recovered by chronic treatment of AC (50 and 100 mg/kg, p.o., 8 days) in ICR mice. In the same system, brain and blood acetylcholinesterase activity induced by Aβ (25 – 35) was inhibited by AC supporting the *in vivo* results. In Morris water maze test [3], scopolamine (1 mg/kg, i.p.)-induced amnesia was prevented by AC administration (100 and 200 mg/kg, p.o., 5 days) in C57BL6 mice. Data were expressed as mean ± SEM and statistical significance was assessed by one-way analysis of variance (ANOVA) with subsequent Tukey's tests. From these results, it is concluded that the protection against Aβ (25 – 35)-induced neurotoxicity and dementia by AC may help to explain its inhibitory action on the progression of AD. *Acknowledgements:* BioGreen 21 Program (2006), Rural Development Administration, Republic of Korea. **References:** [1] Ivins, K. J. et al. (1999) J. Biol. Chem. 274: 2107 – 2112. [2] Lee, I. S. et al, (2006) Arch. Pharm. Res. 29(7):548 – 555. [3] Morris R., (1984) J. Neurosci. Methods, 11: 47 – 60.

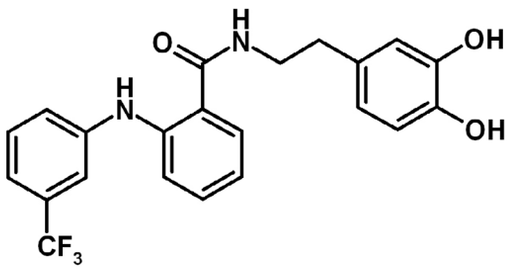
P 585

Potential drug metabolites as endocannabinoids and endovanilloids

Sinning C[1], De Petrocellis L[2], Di Marzo V[3], Imming P[1]
[1]Martin-Luther-Universität, Institut für Pharmazie, Wolfgang-Langenbeck-Str. 4, 06120 Halle, Germany; [2]Istituto di Cibernetica, CNR, Via Campi Flegrei 34, 80078 Pozzuoli, Italy; [3]Istituto di Chimica Biomolecolare, CNR, Via Campi Flegrei 34, 80078 Pozzuoli, Italy

Δ^9-Tetrahydrocannabinol is the most popular plant-derived cannabinoid [1]. Recently, endocannabinoids, e.g. arachidonoylethanolamide and arachidonoyldopamide were isolated [2]. They display activity at cannabinoid receptors (CB_1, CB_2) and the vanilloid receptor (TRPV1). We synthesised several fatty acid conjugates of drug substances as potential human metabolites. We tested the synthesised compounds against fatty acid amide hydrolase, CB_1, CB_2, TRPV1 and anandamide transporter *in vitro*. For instance, the arachidonic acid amide of mesalazine (**1**), an anti-inflammatory drug [3], was determined as a ligand at CB_1 (32% displacement at 1 μM) and as an inhibitor of anandamide reuptake (80% at 25 μM). In addition we replaced the arachidonic acid moiety in arachidonoyldopamide with NSAIDs as arachidonic acid mimetics. By this approach we found flufenamic acid dopamide (**2**) and mefenamic acid dopamide as potent agonists at TRPV1 displaying EC_{50} values in the nanomolar range of concentrations.





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Antioxidant Properties of some Extracts from *Adansonia digitata* L. Using Two Different Automated Cell-Free Assays

Hamed A[1,2], Shah T[1], Matthews H[1], Holmes A[1], Shahat AA[2], Fry J[1]
[1]School of Biomedical Sciences, University of Nottingham, NG7 2UH, UK; [2]Chemistry of Medicinal Plants Department, National Research Centre, Tahrir st., Cairo, Egypt

Naturally occurring antioxidants can help fight many diseases that include oxidant-degenerative reactions such as cancer [1]. Many

medicinal plant extracts and fractions have been proven as rich sources of antioxidant activities. In the present study the total alcoholic, ethyl acetate and water fractions of *Adansonia digitata* L. fruit pericarps were assessed for both radical scavenging and oxidant-reducing properties. The evaluation of radical scavenging and oxidant reducing activities were carried out using automated, 96-well, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging [2] and FRAP (Ferric-reduction antioxidant power) assays [3] respectively. Results were expressed as Trolox ® equivalents in both assays. In the DPPH assay, *Adansonia* total alcoholic extract and its two sub-fractions showed strong radical scavenging properies at low concentration (15 μg/ml) giving Trolox ® equivalents in the order: ethylacetate fraction = water fraction > total alcoholic extract. In the FRAP assay, the three extracts produced FRAP activity in the order: ethylacetate fraction > total alcoholic extract > water fraction at final reaction concentration of 62.5 μg/ml. The antioxidant activities shown in this study are consistent with a previous study [4] that revealed the occurrence of proanthocyanidins as major constituents in the total alcoholic extract. The results recommend extended study in cell-based assays to further elucidate the correlation of cell-free and cell-based systems in determination of the antioxidant potential of these plant extracts. *Acknowledgements:* Egyptian Government; Prof. F M Hammouda, National Research Centre (Cairo, Egypt). **References:** [1] Ames, B. N. et al. (1993) PNAS 90: 7915 – 22. [2] Nara, K. et al. (2006) Biosci. Biotechnol. Biochem.70: 1489 – 91. [3] Benzie I. F., Strain J. J. (1996) Anal. Biochem. 239: 70 – 6. [4] Shahat, A. A. (2006) Pharmaceutical Biol. 44: 445 – 50.

P 587

Heavy metal contents in samples of *Hypericum* and *Thymus* spec. collected from different mountain areas in Serbia

Djukic-Cosic D, Curcic M, Cmiljanovic M, Vasovic I, Matovic V
Instutute of Toxicological Chemistry, Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia

The beneficial effects of medicinal plants are well known from ancient times and consumption of different herbal extracts is recommended all over the world. Metallic elements are constitutive plant compounds with biological activity as essential or toxic agents in metabolism. Toxic effects of heavy metals on plants is very complex and can affect the content of pharmacologically active compounds in medicinal plants, and thereby, seriously impact the quality, safety and efficicacy of natural plant products. Therefore, among the other quality control analyses of the raw material of medicinal plants, determination of metals, especially toxic ones, is of special concern. The aim of this work was to determine contents of toxic metals like Cd and Pb in the herbs of *Hypericum* sp. and *Thymus* sp., collected during 2003, 2004, and 2005 from various localities of Serbian mountains: Golija, Zlatibor, and Rtanj. Cd and Pb contents in dried, homogenized and mineralized samples were determined by AAS (apparatus GBC 932AA). In almost all investigated samples Cd levels are higher than the ones proposed by WHO (0.3 mg Cd/kg dried plant materials). The contamination level of Pb can be classifed as normally low, because all samples contained less than 10 mg/kg Pb (proposed by WHO). The content of Cd in *Hypericum* sp. were higher than in *Thymus* sp. This work contributes to the investigations of toxic metals content in medicinal plants. Further investigations are needed in order to find out the correlation between metal pollution and content of pharmacologically active metabolites.

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Effect and mechanisms of action of the fixed combination STW 5 in secretion and motility of human colon *in vitro.*

Krüger D[1], Wagner S[1], Hann von Weyhern CW[2], Zeller F[3], Kelber O[4], Frieling T[5], Schemann M[1]
[1]Lehrstuhl für Humanbiologie, Wissenschaftszentrum Weihenstephan, Technische Universität München, Hochfeldweg 2, 85350 Freising-Weihenstephan, Germany, [2]Institut für Allgemeine Pathologie und Pathologische Anatomie, Klinikum r.d. Isar, Ismaninger Str. 22, 81675 München, Germany, [3]Chirurgische Abteilung, Klinikum Freising, Mainburger Str. 29, 85356 Freising, Germany, [4]Steigerwald Arzneimittelwerk GmbH, Havelstr; 5, 64295 Darmstadt; Klinikum Krefeld, Medizinische Klinik II, Lutherplatz 40, 47805 Krefeld, Germany

Clinical studies with the fixed combination phytomedicine STW 5 (Iberogast®) show a significant improvement of symptoms in irritable bowel syndrome [1]. Mechanisms of action may be effects on intestinal motility, as has been shown in studies in ileal preparations of guinea pig, mouse and rat [2],[3], as well as a stimulation of mucosal secretion [4]. We studied the mechanisms of action of the pro-secretory effect in mucosa/submucosa preparations from healthy human intestinal resectates (157 preparations from 47 patients) and in the human intestinal epithelial cell line T84. The effects on motility were studied in circular and longitudinal muscle of human colon (44 preparations from 11 patients). Serosal application of STW 5 (128 μg/ml-1024 μg/ml) lead to a dose-dependent increase of secretion in the human intestinal preparations as well as in the cell line. This action is sensitive to bumetanide (100 μM) and is reduced by the chloride channel blocker glibenclamide (400 μM), SITS (1mM), CFTR 172 inh (10 μM) and the adenylate cyclase inhibitor MDL 12330A (20 μM). Neuronal block with TTX (1 μM) reduces the secretory answer for 50%. Secretion induced by electrical field stimulation was not influenced. Studies in colonic preparation precontracted by carbachol (10 μM) showed a significant ($p < 0.05$) decrease of contraction amplitude and basal tone. Our results show that STW 5 (Iberogast®) increases chloride secretion by opening of calcium- and cAMP- dependent chloride channels and that it has a spasmolytic effect in human intestine. These effects may contribute to the symptome improvement in irritable bowel syndrome. **References:** [1] Madisch A et al. (2004) Aliment.Pharmacol.Ther. 19: 271 – 279. [2] Ammon et al. (2006) Phytomedicine 13 S V:67 – 74. [3] Heinle H et al. (2006) Phytomedicine 13 S V:75 – 79. [4] Krüger D et al. Neurogestroenterol Motil 18: 771

P 589

Cytotoxic activity of the traditional Thai medicinal plant preparation Benjakul and 4 isolated compounds

Tappayuthpijarn P[1], Itharat A[1], Sakpakdeejaroen I[1], Kumarpawa K[2]
[1]Applied Thai Traditional Medicine, [2]Department of Medical Science, Faculty of Medicine, Thammasart University, Rungsit campus, Klong Luang, Pathumthani, 12120, Thailand

Benjakul (BEN), a Thai traditional medicine preparation, is composed of five plants, *Piper chaba* fruit [PC], *Piper sarmentosum* root [PS], *Piper interruptum* stem [PI], *Plumbago indica* root [PL] *and Zingiber officinale* rhizome [ZO]. It is a balanced health preparation in Thai traditional medicine. From selective interviews of folk doctors in Southern Thailand, it was found that Benjakul was used as the adaptogen drug for cancer patients [1]. These plants and the preparation have been selected to study cytotoxic activity against three human cancer cell lines, large lung carcinoma (CORL23), prostate cancer cell lines (PC3) and liver cancer cell lines (HepG2) using the SRB assay [2]. From the Benjakul preparation also pure compounds were isolated and their cytotoxic activity was tested. From the EtOH extract four compounds were isolated [gingerol (Gin), shogaol (Sho), plumbagin (Plu) and piperine (Pip)]. The results shown below can support the usage of Benjakul to treat cancer patients. The four compounds isolated can be markers for standardization of the preparation. Table. IC_{50} values (μg/mL) against cell lines and antioxi-

dant activity [EC_{50} value (μg/ml)] of Benjakul and some of its ingredients

Plant extracts	PC	PS	PI	PL	ZO	BEN	Gin	Sho	Plu	Pip
CORL 23	15.8	32.9	18.4	3.4	7.9	19.8	26.2	2.1	0.5	10.5
PC 3	19.7	45.8	27.8	9.2	9.9	29.8	9.6	3.7	0.5	12.7
MCF-7	35.7	69.5	62.4	40.8	31.2	33.2	8.5	4.1	9.4	0.47

Acknowledgement: Faculty of Medicine, Thammasart University for the financial support **References:** [1] Itharat, A. et al. (1998) Wisdom of Southern Thai Traditional Doctors. Prince of Songkla University, Songkla, p. 126 – 129. [2] Skehan, P. et al. (1990) J. Natl. Cancer Inst., 82: 1107 – 1112.

P 590

Recent Developments in Risk Assessments of Herbal Medicinal Products: Unlimited limitation?

Schmidt M
Herbresearch Germany, Wartbergweg 15, D-86874 Mattsies

Recent risk assessments for compounds of toxicological concern in herbal preparations involved new approaches and tools for risk minimization such as safety factors, the Margin of Exposure (MOE) or Threshold for Toxicological Concern (TTC) method. These methods are now regularly applied to assess the toxicological properties of isolated single compounds, and to extrapolate the results to herbal preparations containing this constituent. Thus, dose limitations for herbal extracts are regularly proposed even when the toxicological properties of the single compound are not known from the extract preparation as such. The methods for risk minimization originate from environmental toxicology and were created to minimize contact to toxic impurities with no pharmacological benefit. Their use highlights a need for scientifically sound approaches to risk assessment for herbal medicinal preparations:

- In accordance with the recent EMEA concept paper [1], risk assessments must not be made on extrapolated suspicions which are not based on signals of adverse events.
- The safety factor, MOE and TTC methods are unsuitable for medicinal plants. They lead to random results which are obviously not in accordance with clinical experience.
- Metabolic factors and the scope of dietary exposure must be taken into consideration.
- Recently applied practises of risk assessment may lead to arbitrary and inadequate regulatory actions which may affect an in principle unlimited number of herbal preparations, regardless of the observation of adverse events.

References: [1] Concept paper on the development of a guideline on the assessment of genotoxic constituents in herbal substances/preparations (2006) EMEA/HMPC/413271/2006.

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Antidiarrhoeal Activity of the Ethyl Acetate Extract of *Baphia Nitida* (Papilionaceae)

Adeyemi OO, Akindele AJ
Department of Pharmacology, College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria

In our search for plants useful in the treatment of diarrhoea, we investigated the ethyl acetate extract of *Baphia nitida* (BN) using intestinal transit (1,2), enteropooling (3) and gastric emptying (4) tests. In the normal intestinal transit test, peristaltic index (PI %) for the control group (distilled water 10 ml/kg, p.o.) was obtained to be 83.64 ± 4.07. BN produced a non-significant increase in intestinal propulsion with PI values of 84.37 ± 1.72, 88.22 ± 2.71 and 91.80 ± 2.33 for doses of 100, 200 and 400 mg/kg (p.o.), respectively. The extract effected a significant ($P < 0.05$) dose dependent decrease in propulsion in the castor oil induced intestinal transit model. PI values were 56.85 ± 6.76, 36.84 ± 3.04 and 31.98 ± 2.60, respectively for BN at 100, 200 and 400 mg/kg vs. 89.33 ± 6.28 for control. The effect at 400 mg/kg was significantly lower than that of morphine, 10 mg/kg s.c. (20.29 ± 3.78), and was antagonized by isosorbide dinitrate, IDN (150 mg/kg, p.o.) but not by yohimbine (1 mg/kg, s.c.). This effect was not potentiated by atropine (1 mg/kg, s.c.). In the castor oil induced diarrhoea test, BN produced a significant increase in onset of diarrhoea (103.40 ± 8.74, 138.80 ± 17.04 and 174.8 ± 29.04 min., 100 to 400 mg/kg, vs. 47.60 ± 8.76 min. for control and 226.10 ± 12.57 min. for morphine). The severity of diarrhoea (diarrhoea score) was reduced (19.00 ± 2.26, 17.04 ± 1.89, 15.00 ± 2.05, 100 to 400 mg/kg, vs. 31.40 ± 2.11 for control and 7.7 ± 2.2 for morphine). This effect was not antagonized by IDN or yohimbine. The effect on severity was however potentiated by atropine. BN also reduced the number and weight of wet stools but did not have any significant effect on intestinal fluid accumulation and gastric emptying. Results obtained suggest that the ethyl acetate extract of *Baphia nitida* is endowed with antidiarrhoeal activity, which can be potentiated by muscarinic antagonists, due mainly to its inhibitory effect on gastrointestinal propulsion possibly mediated by interference with the nitric oxide pathway. **References:** [1] Hsu, W. H. (1982) Eur. J. Pharmacol. 83: 55 – 60. [2] Aye-Than, J.H. et al. (1989) J. Crude Drug Res. 27: 195 – 200. [3] Robert, A. et al. (1976) Prostaglandins 11: 809 – 814. [4] Droppleman, D.A. (1980) J. Pharmacol. Methods 4: 227 – 230.

P 592

Effects of oral administration of Silexan on behavioural parameters in rats and mice

Nöldner M, Luderer G
Preclinical Research, Dr. Willmar Schwabe GmbH & Co. KG, Willmar-Schwabe-Str. 4, 76227 Karlsruhe, Germany

Silexan is a new phytochemical preparation which is under development for the treatment of mild affective disorders. It contains essential oil obtained from the flowers of *Lavendula angustifolia*. Native preparations of lavender essential oil, are traditionally used in aromatherapy and in the flavouring industries. Since the 1980 s, a considerable number of studies have been carried out, and a wide range of biological activities were found for lavender oil or its main constituents linalool and linalyl acetate (1,2). For example, linalool shows anticonvulsant and antiphlogistic properties and modifies nicotine receptor function at neuromuscular junctions.(3,4,5,6,7). The aim of the present investigation was to determine potential central nervous effects of Silexan in animal models of depression, sleeping time and anxiety. For this purpose, the effect of oral administration of Silexan was investigated in rats and mice. Repeated oral administration of Silexan in a dose range between 3 and 30 mg/kg caused a statistically significant inhibition of the immobility time in the forced swimming test. Anxiolytic effects were tested in mice using the "light-dark-box" and the "elevated-plus-maze" models. Statistically significant effects were found in both models after single oral administration in doses of 3 to 30 mg/kg b.w. Influence on pentobarbital-induced sleeping time was investigated in mice after single and repeated oral administration of the test compound. The sleeping time was significant prolonged after oral administration of 3, 10 or 30 mg/kg Silexan. These results suggest that Silexan has anxiolytic and antidepressant properties and could modify the sleeping profile, which is frequently disturbed in affective disorders. **References:** [1] Cavanagh, H.M.A. et al. (2002) Phytotherapy Research 16: 301 – 308; [2] Heuberger, E. et al. (2004) Neuropharmacology 29 1925 – 1932; [3] Elisabetsky, E. et al. (1999) Phytomedicine 6 (2): 107 – 113; [4] Peana, A. et al. (2004) Eur. J. Pharmacol. 497: 279 – 284; [5] Re, L et al. (2000) Pharmacol. Res. 42 (2): 177 – 181; [6] Ghelardini, C. et al. (1999) Planta Med. 65: 700 – 703; [7] Ballabeni, V. et al. (2004) Phytomedicine 11: 596 – 601

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Effects of pomegranate extracts on rat uterine contraction

Promprom W[1], Kupittayanant S[1], Indrapichate K[1], Kupittayanant P[2]
[1]Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand; [2]Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand

Pomegranates (*Punica granatum* L.) have been widely used for their health benefits. They have been found to be effective in reducing heart disease risk factors [1] and may be effective against prostate cancer [2,3] and osteoarthritis [4]. However, the use of promegranates as uterotonic agents is not well understood. The aims of the study were to investigate the effects of pomegranate seed and peel extracts on rat uterine contraction. We particularly examined the effects of the extracts on phasic contraction arising spontaneously and investigated their underlying mechanisms. Pomegranate seed and peel were collected from local gardens and extracted using methanol. The extracts were then analyses by GC/MS. Rats were killed by asphyxiation with CO_2 and longitudinal uterine smooth muscles isolated. Isometric force was measured and the effects of the extracts studied. Several agents, previously reported to increase contraction in other smooth muscles, were found in both peel and seed extracts. When the extracts were applied on the uterus, they significantly increased uterine contraction. Their effects were dose dependent. The maximal effect of the seed and peel extract was observed at the dose of 2.5 mg/ml (2.0 – 2.6 mg/ml) and 0.7 mg/ml (0.3 – 0.9 mg/ml) respectively. Neither the seed extract nor the peel extract produced contraction in the absence of external calcium. Wortmannin, a specific inhibitor of myosin light chain kinase, significantly inhibited increases in contraction produced by both extracts. In conclusion, pomegranate seed and peel extracts have uterotonic effects. Increases in contraction produced by the extracts depend on external calcium and myosin light chain kinase. **References:** [1] Aviram, M. & Dornfeld, L. (2001) Atherosclerosis 158(1): 195 – 8. [2] Malik, A. et al. (2005) Proc Natl Acad Sci USA 102(41): 14813 – 8. [3] Adhami, V.M. & Mukhtar, H.B. (2006) Free Radic Res 40(10):1095 – 104. [4] Ahmed, S. et al. (2005) J Nutr 135(9): 2096 – 102.

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Reversible contraceptive efficacy of methanol extract of aerial parts of *Gynandropsis pentaphylla* in male albino rats

Gupta RS, Kachhawa JBS, Sharma A, Lahiri SM
Reproduction Physiology Section, Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur-302 004, India

In spite of the considerable development in contraceptive technology, search for male antifertility agents in plants continues to be a potential area of investigation. Many plants have been known to possess antifertility activity but limited attempts have been made to scientifically evaluate these claims. Hence the purpose of this study was to evaluate the antifertility and reproductive toxicity potential of *Gynandropsis pentaphylla* DC. (Capparidaceae) in male wistar rats. Oral administration of 100% methanol extract of aerial parts of *G. pentaphylla* (Capparidaceae) at the dose level of 20 mg/rat/day male albino rats for 60 days did not decrease body weight, while the testes and epididymides were significantly reduced, seminal vesicles and ventral prostate showed a significant reduction ($P \leq 0.001$). Treated animals showed a notable depression of spermatogenesis. As a result of 20 mg/rat/day extract feeding, the preleptotene spermatocytes, secondary spermatocytes, round spermatids and the mature Leydig cells decreased. At this dose level Leydig cell nuclear area and cytoplasmic area, as well as the cross sectional surface area of Sertoli cells, were significantly reduced ($P \leq 0.001$) when compared to controls. The reduced sperm count and motility resulted in 100% negative fertility. A significant fall in the total protein and sialic acid content in the testes, epididymides, seminal vesicle and ventral prostate, as well as in the glycogen content of testes was also observed. However a most exciting observation is that all the parameters were found in a non-significant manner after withdrawal of the drug. In conclusion methanol extract of *G. pentaphylla* have contraceptive activity in male rats with their reversible nature.

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Effects of ethanolic extract of *Mucuna pruriens* on sexual behaviour of normal male rats

Kupittayanant P[1], Munglue P[1], Saraphat W[1], Danoopat T[1], Kupittayanant S[2]
[1]Institute of Agricultural Production Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand; [2]Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand

The seeds of *Mucuna pruriens* (Ma Mui, MM) have been used in Thai medicine since ancient times for the treatment of male sexual disorders. The present study is aimed to investigate the effect of an ethanolic extract of MM on general mating behaviour, libido, and potency along with its adverse effects on sexually normal male rats. The suspension of the extract was administered orally at doses of 8, 48, 100, and 200 mg/kg, to different groups of male rats (n = 5) once a day for twenty-one days. The female rats involved in mating were made receptive by hormonal treatment. The general mating behaviour, libido and potency were determined. The adverse effects of the extract were also evaluated by observing the animals at least once daily for any overt sign of toxicity, food and water intake, stress and changes in behaviour. Oral administration of the extract significantly increased the mounting frequency, intromission frequency, and ejaculatory latency and caused significant reduction in the mounting latency and intromission latency. The most appreciable effect of the extract was observed at the dose of 200 mg/kg. The extract was also found to be devoid of any adverse effects. The results indicated that the ethanolic extract of MM produced a significant and sustained increase in the sexual activity of normal male rats, without any adverse effects. Thus, the resultant aphrodisiac effectivity of the extract lends support to the claims for its traditional usage in sexual disorders.

P 596

The efficacy of multi-drug therapy for the common cold with particular reference to Kan Jang fixed combination

Wikman G, Panossian A
Swedish Herbal Institute R&D, Prinsgatan 12 5tr, SE-413 05, Gothenburg, Sweden

The general, and very successful, approach of oriental medicines, such as Ayurveda and Unani in India, and Kampo in China and Japan, involves treatment with complex mixtures of a number of herbs. In some cases, the medicinal value of the preparation is entirely due to the combination of constituents and cannot be reproduced by one or two so-called active principles alone. Recent research has shown that in the treatment of viral diseases, fixed combinations of plant extracts often provide greater-than-expected medicinal benefits by virtue of multiple constituents that exhibit synergistic effects and also act upon different molecular targets. KanJang® oral solution, a fixed combination of standardised extracts of *Adhatoda vasica*, *Echinacea purpurea*, and *Eleutherococcus senticosus*, has been available in Scandinavia for around 20 years for the relief of symptoms associated with common cold, particularly coughing and irritability of the throat [1,2]. In recent randomised, double-blind controlled clinical studies, this preparation has been shown to have a significant effect on symptoms related to uncomplicated upper respiratory tract infections (i.e. coughing, quality of sleep, mucus discharge in the respiratory tract, and nasal congestion) in comparison with placebo or with a combination of *Echinacea purpurea* and *Eleutherococcus senticosus* alone. The significance of the results obtained in these studies is discussed with respect to the efficacy of KanJang in the treatment of acute respiratory infection and to the concept that

multi-drug therapy offers higher efficacy compared to mono-drug treatment of such infections. **References:** [1] Thom E, Wollan T (1997) A controlled clinical study of Kanjang mixture in the treatment of uncomplicated upper respiratory tract infections. Phytotherapy Res 2: 207 – 210. [2] Narimanian M, M. Badalyan, V.Panosyan, E. Gabrielyan, A.Panossian, G.Wikman, and H.Wagner (2005) Clinical evidence of high efficacy of fixed combinations of herbal extracts, with particular reference to Adhatoda vasica, in patients with non-complicated respiratory tract infections (bronchitis). Phytomedicine, 12, 539 – 547.

P 597

Chemical analyses of the essential oils of three *Fortunella* cultivars and a Greek traditional Kumquat Liqueur

Kontaratou V, Graikou K, Chinou I
Dept. of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, University of Athens, Athens GR-15771, Greece

Kumquats (*Fortunella sp.*) have long been used in traditional herbal medicine, for the treatment of colds and coughs [1] as well as in both the perfumery and food industry [2]. In this study, the oil content of kumquat peel, flower and leaf oils of three Fortunella species (*F. japonica, F. margarita* and *F. crassifolia*), cultivated in Greece was determined. From the oil from fruits of *F. margarita*, a traditional Greek Liqueur, has been produced. The volatiles from all species and the liqueur, were analysed by GC and GC/MS. The major compounds of all leaf oils were germacrene-D (9.0%-16.4%) and elemol (8.65%-13.17%), while in the flower oils limonene (27.75%-63.67%) was the most abundant constituent followed by valencene (8.69%) for *F. crassifolia* and germacrene-D (4.79%) for *F. japonica*. Limonene was also the abundant constituent in peel oils, followed by β-pinene (6.31%) for *F. crassifolia* and myrcene for both *F. japonica* and *F. margarita* (5.34%, 7.79%, respectively). Significant differences among oil components from kumquat leaves, flowers and peels were observed. The analysis of the volatiles of the fruit liqueur resulted that linalool and limonene were the most abundant constituents (40.2%, 10.3% respectively). All oils and major compounds showed an interesting antimicrobial broad spectrum of activities as they have been assayed against nine bacteria and fungal strains and three foodborne pathogenic bacteria (MICs 0.05 – 1.5 mg/ml) *Acknowledgements:* University of Athens (Special Account for Research Grant (70/4/8807) as well as PAVET project (70/9075) and Korres NaturalProduct S.A. **References:** [1] Yun, S. B. (1993) Research of Korean Food History; Shinkwang Publishing: Seoul, Korea, p 274. [2] Sawamura, M. (2000) J. Agric. Food Chem., 4: 131 – 4164.

P 598

Evaluation of Indian food spices for their beneficial hypoglycemic activity

Seema A[1], Akbar M[2], Shamshad A[3], Mohammad I[1]
[1]Regional Research Institute of Unani Medicine, (Central Council for Research in Unani Medicine, the University of Kashmir Campus, Srinagar, J&K 190006 India; [2]Department of Biochemistry, the University of Kashmir, Srinagar, J&K 190006, India; [3]Central Council for Research in Unani Medicine, New Delhi, India

Aqueous homogenates (10% w/v) of components of Indian dietary spices *Allium cepa* (onion), *Allium sativum* (garlic), *Curcuma longa* (turmeric, 1% w/v) and *Zingiber officinale* (ginger) when administered with 0.5 ml/kg body weight in alloxan induced hyperglycemic male Wistar rats showed significant antihyperglycemic activity 30 minutes after glucose loading in rats under OGTT. The activity continued even after 2 hours. The fall of glycemia was in the range of 15 – 35% as compared to controls with minimum and maximum being with *Curcuma longa* and *Allium cepa* treated animals, respectively. Combinational (2, 3 and 4 plant extracts) studies did not show any significant additive effects.

P 599

Biological activity of *Zanthoxylum limonella* (Dennst.) Alston. essential oil and its formulation

Chaiyong S[1], Jatisatienr C[1], Dheeranupatana S[1], Jatisatienr A[1]
[1]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

The biological properties of seven plants i.e. *Boesenbergia pandurata* (Roxb.) Schltro., *Curcuma longa* L., *Cymbopogon citratus* (De ex Nees) Stapf., *Cymbopogon nardus* (L.) Rendle., *Illicium verum* Hook.f., *Zingiber cassumunar* Roxb. and *Zanthoxylum limonella* (Dennst.) Alston. were investigated by determination of the LC_{50} of their essential oils in the brine shrimp (*Artemia salina* Leach.) lethality test (BST) (Meyer *et al.*,1982). The plant which gave the best efficiency was selected, formulated with different solvents and tested in the BST again. The insecticidal efficiency of the best formulation was confirmed by the leaf disk choice test and the topical application method with common cutworm. The results showed that *Z. limonella* gave the highest activity against brine shrimps with an LC_{50} value of 0.93 ppm, followed by *C. longa*, *Z. cassumunar*, *I. verum*, *C. nardus*, *C. cittratus* and *B. pandurata* with LC_{50} values of 2.20, 3.42, 4.59, 7.68, 9.99 and 27.36 ppm, respectively. Five formulations of *Z. limonella* essential oil consisted of 1% essential oil along with different solvents and other supplement substances in the following ratios i.e. formula 1: 95% ethanol, Tween 80 and water (20:5:74), formula 2: pine oil, 95% ethanol, Tween 80 and water (1:20:5:73), formula 3: wintergreen oil, 95% ethanol, Tween 80 and water (1:20:5:73), formula 4: sesame oil, 95% ethanol, Tween 80 and water (1:20:5:73) and formula 5: sunflower oil, 95% ethanol, Tween 80 and water (1:20:5:73). The results of biological testing of the five *Z. limonella* essential oil formulations showed that formula 2 had the highest activity against brine shrimps after 24 hrs with an LC_{50} value of 0.75 ppm, followed by formula 1, 3, 4 and 5 with LC_{50} values of 2.98, 3.32, 9.50 and 12.10, respectively. Finally, the results from the leaf disk choice test on the common cutworm showed that formula 2 displayed its antifeedant activity at a concentration of 0.1 and 0.5%. For the topical application method, 10% of formula 2 gave 23, 23, 30, 33 and 33% mortality 3, 6, 12, 24 and 48 hours after application, respectively. *Acknowledgments:* We thank the Chiang Mai University (CMU) for financial support. We are grateful to Assoc.Prof.Dr.S. Wechapikul, Faculty of Pharmacy and Assoc.Prof.Dr.D. Phudsuk, Faculty of Science, CMU for their excellent assistance and suggestion. **References:** [1] Meyer, et al. (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45: 31 – 34.

P 600

St. John's Wort (*Hypericum perforatum* L.,Clusiaceae) polysaccharides affect the signal transduction of human skin keratinocytes and fibroblasts

Deters AM
Institute for Pharmaceutical Biology and Phytochemistry, Westfalian Wilhelms University of Münster, Hittorfstr. 56, 48149 Münster, Germany

The traditional dermatological use of *Hypericum perforatum* L., Clusiaceae, extracts is currently undergoing a renaissance and a potential use in treatment of topic dermatitis is discussed. Since the use of *H. perforatum* extracts for topical treatment of skin diseases like atopic dermatitis has been discussed, the intention of the present study was to determine the most active polysaccharide fraction of the crude extract influencing keratinocytes and even fibroblasts. The crude polysaccharide fraction was fractionated by anion exchange chromatography (AEC) with regard to the polymer acidity followed by treatment of skin cells with 10 μg/ml of each obtained polysaccharide. qRT-PCR studies elucidated the influence of polysaccharides on the expression of proliferation (EGF-R, FGF2-R, InsR, STAT6, FGF-7) and differentiation (PKC-α, SMAD 3) related genes. The crude polysaccharide of St. John wort water extract induced the differentiation as measured by involucrin and keratin K1 and K10 content, while no effects on proliferation were observed. In the case of the

AEC, fractions the two most acidic polysaccharides revealed a very strong effect on keratinocyte differentiation examined morphologically as well as by determination of involucrin. Gene expression analysis demonstrated that the PKC-α gene was the preferred up-regulated gene in keratinocytes. The effect on primary fibroblasts was different: Mitochondrial activity as well as proliferation was enhanced. Examination of proliferation specific genes showed that the EGF-R, STAT6 and FGF-7genes were up-regulated as compared to the untreated control cells. Apoptotic and necrotic cells were not observed after treatment of skin cells with St. John wort polysaccharides. In conclusion it could be shown that the acidic polysaccharides exert the greatest influence on keratinocyte differentiation and that their influence on epidermal keratinocytes differs from their effect on dermal fibroblasts.

P 601

Reed mace (*Typha latifolia* L., Typhaceae) seed polysaccharides exert differing effects on human skin fibroblasts and keratinocytes

Gescher K, Deters AM
Institute for Pharmaceutical Biology and Phytochemistry, Westfalian Wilhelms University of Muenster, Hittorfstr. 56, 48149 Muenster, Germany

In North America *Typha latifolia* L., Typhaceae, is used for more than 4000 years for treatment of skin disorders, burns and diarrhoea. The whole plant exhibits high carbohydrate content so it was interesting to investigate effects of the seed and seed hair polysaccharide on the main skin cells. Polysaccharides were obtained by an ethanolic precipitation of a water-extract. The dialyzed and lyophilized crude polysaccharide was fractionated by AEC in one neutral and three acidic polysaccharides. Human primary keratinocytes and fibroblasts as well as HaCaT-keratinocytes were treated with 10 μg/ml crude polysaccharide and AEC-fractions to determine their influence on cell viability (MTT-test), necrosis (determination of LDH) and proliferation (BrdU-assay). The differentiation behaviour of primary keratinocytes was determined by the keratin and involucrin expression. At least qRT-PCR was carried out to investigate the expression of proliferation and differentiation specific genes. The crude polysaccharide containing little amounts of polyphenols reduced proliferation of keratinocytes and fibroblasts significantly. The AEC fractions affected the cell physiology in distinct ways relating to the treated cells: A 10 μg/ml concentration of neutral and slight acid fractions stimulated the keratinocyte but not the fibroblasts proliferation. The results were supported by the differential gene expression analysis by the reason that genes for EGFR, FGFR2, InsR und STAT6 were up regulated. The keratin and involucrin content as markers of keratinocyte differentiation arises significantly in primary keratinocytes treated with polysaccharide fractions and to a lesser extend after incubation with the raw polysaccharide. These results also coincide with the expressions of differentiation specific gene SMAD 3. The results show that the activity of polysaccharides depends on their composition, the effect is down to the used cell system. The observed modified cell physiology attributes to the observed changes in gene expression after treatment of cells with plant polysaccharides.

P 602

Biological effect of a glycolic extract of the stem bark of *Stryphodendron adstringens* (Mart.) Coville on cicatrisation of cutaneous wounds in mice

Figueira ELZ[1], Luz Neto N[2], Coelho VNP[1], Fungheto SS[1], Bernardes VV[1], Soare JS[2]
[1]Centro Universitário Unieuro – UNIEURO, Av. das Nações, Trecho 0, Conjunto 5, CEP 70200 – 000 – Brasília-DF, Brazil; [2]União Educacional do Planalto Central – UNIPLAC, Área Especial n° 02 – Setor Leste – Brasília-DF, Brazil

The stem bark of *Stryphnodendron adstringens* (Mart.) Coville is extensively applied in traditional medicine in Brazil as an important cicatrisation and antibacterial agent. The healing effect of a propyleneglycolic *S. adstringens* (Mart.) Coville stem bark extract, containing 12.7% tannins, was evaluated applying 50 μL twice a day (12.8 mg of tannins/day) in cutaneous wounds of female and male mice (*Mus musculus*) after 7 and 14 days of treatment, and these results were compared with 70 mg (twice a day) of commercial drugs Nebacetin® (neomycin sulphate 0.7 mg/day and bacitracin 35 I.U./day) and Iruxol® (chloramphenicol 1.4 mg/day and colagenase 0.084 U/day), the drugs most often prescribed as healing agent in Brazil. Epithelial cell proliferation in the area of re-epithelialisation of the wounds was evaluated by histological analysis. The epidermal growth was increased by the glycolic extract of stem bark of *S. adstringens* (Mart.) Coville and by Iruxol® and Nebacetin® treatment. However, the highest increase in epidermal growth was achieved by the propyleneglycolic stem bark extract of *S. adstringens*, followed by Iruxol® treatment. These results confirm the ethnopharmacological value of *S. adstringens* and suggest new studies to describe the action mechanisms of tannins in wound healing.

P 603

Cytotoxic activities of 4 natural products – carnosic acid, xanthohumol, caffeic acid phenethyl ester and (-) eburnamonine

Nga DT[1], Juan QH[1], Kyung KE[2], Batmunkh T[2], Yeon SJ[3], Hoon KY[3], Sup KM[3], Sun LG[3], Burm-Jong L[1,2], Ah KK[1]
[1]Biohealth Products Research Center, [2]Department of Chemistry, Inje University, Gimhae 621 – 749, [3]Hanmi Pharmaceutical Co, Research center, Gyeonggi-Do 445 – 813, Republic of Korea

In a cytotoxic or anti-proliferation assay, 20 compounds derived from natural products have been screened for cytotoxic effects against A549 cell line using MTT assay. Among them, carnosic acid, xanthohumol, caffeic acid phenethyl ester and (-) eburnamonine showed activies at concentrations of 50 μM and 10 μM. These four active compounds were subjected to cytotoxicity studies in two other cell lines (A431 and SK-BR3), using SRB assay. While carnosic acid, xanthohumol and (-) eburnamonine still showed cytotoxic activity, caffeic acid phenethyl ester showed no activity in A431 cell line and low activity against SK-BR3 cell line (IC_{50}= 258 μM). Xanthohumol possessed the strongest cytotoxic effects against all 3 cell lines with IC_{50} values of 24.16 μM, 16.14 μM and 1.68 μM in A549, A431 and SK-BR3 cells, respectively. Because A431 and SK-BR3 cells are known to over-express tyrosine kinase receptors, we have studied the four compounds for KDR and Her2 tyrosine kinase receptor inhibitory activity by using fluorescence polarization. The results showed that except caffeic acid phenethyl ester, which showed low inhibitory activity on KDR at a high concentration of 10 μM, the other compounds were inactive. In conclusion, carnosic acid, xanthohumol and (-) eburnamonine showed cytotoxic activity in A549, A431 and SK-BR3 cell lines but their activities are not mediated though the inhibition of KDR or Her2 receptors. The cytotoxic activity of caffeic acid phenethyl ester is just limited to A549 cell line, not for the other two cell lines, which suggests a specific activity of this compound. *Acknowledgement:* This study was supported by a grant from the Ministry of Commerce, Industry and Energy (MOCIE) and the Korea Institute of Industrial Technology Evaluation & Planning (ITEP) through the Biohealth Products Research Center (BPRC) of Inje University. **References:** [1] Lee F. Allen et al., (2003) Semin Oncol 30 (suppl 16) 65 – 78. [2] Daniel S. Krause et al., (2005) N Engl J Med 353: 172 – 87. [3] Stephen R Wedge et al., (2005) Cancer Res 65 (10): 4389 – 400.

P 604

Study on anti-inflammatory effects of silymarin on UV irradiated guinea pig skin

Esmaeelian B[1], Kamrani YY[1], Naderi MM[2], Rafeie SM[2], Amanlou M[1], Azizi E[3]
[1]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Medical Sciences/University of Tehran, P.O.Box: 14155 – 6451, Tehran, Iran; [2]Department of small animal internal medicine, faculty of veterinary, science & research of Tehran Azad university, Tehran, Iran; [3]Molecular Research Lab., Department of Pharmacology and Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Medical Sciences/University of Tehran, Tehran, Iran

Epidemiological, clinical and laboratory studies have implicated solar ultraviolet (UV) radiation as a tumor initiator, tumor promoter and complete carcinogen, and excessive UV exposure can lead to the development of various skin disorders including melanoma and non-melanoma skin cancers [1]. Considering skin damages due to UV irradiations of the sun and need of UV-protective products, we investigated the efficacy of silymarin to prevent the side effects of these rays. For this survey 120 albino guinea pigs, in the same age and sex were selected and randomly divided into four groups with thirty animals in each group. The skin of the lumbar region of each animal was shaved. Experimental group 1 received 9 mg silymarin in 20 µl acetone topically while control group 1 received only 20 µl acetone topically. Experimental group 2 received 50 mg silymarin orally and finally the control group 2 received nothing [2]. The light type was UV-B, 220V, 30W, with the dose of 180mj/cm^2. The results of clinical and pathological observations showed that silymarin in topical and oral use resulted in a significant decrease (79% and 67% respectively) in pathological damage incidences of skin due to UV irradiation. Oral and topical use of silymarin significantly reduced the side effects of UV irradiation and it can be used in topical ointments as well. Acknowledgements: 1. Tehran University of Medical Sciences and Pharmaceutical Sciences Research Center 2. Dr. HR.A.Ashtiani **References:** [1] Manjeshwar S. et al. (2006) J. Photochem. Photobiol. Sci 5: 243 – 253. [2] Farrukh A. et al. (2002) J. Skin Pharmacol. Applied Skin Physiol. 15: 297 – 306.

P 605

The response of immunoreactive nerve fibres in osteoarthritis to orally administered *Zingiber officinale* extract

Ganabadi S[1], Sukardi S[2], Fakurazi S[2], Yaakub H[3]
[1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, [2]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, [3]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Malaysia

Zingiber officinale, commonly known as ginger is a well known natural remedy that has long been used by people in many parts of the world as food additive and traditional medicine. Knowing the anti-inflammatory action of *Z. officinale*, it has become the most popular herbal medication for rheumatic diseases. The current work was undertaken to evaluate the effect of this extract on the efferent and afferent components of the peripheral nervous system, since this system had been shown to contribute to the inflammatory process which in turn will cause a severe joint damage in arthritis. Chemical lesioning of these fibres showed a decrease in the inflammatory response in arthritis [2], and oral administration of SP antagonist or dorsal rhizotomy markedly reduces the severity of inflammation in animal models [1]. This improved the cure from arthritis. Therefore, the respond of PGP 9.5-, SP-, CGRP- and NPY-immunoreactivity in experimentally-induced osteoarthritis with supplementation of *Z. officinale* extract was studied. Changes after the treatment were statistically evaluated by innervations of the synovial membrane. There was significant improvement in PGP 9.5-, SP-, CGRP- and NPY- immunoreactivity in the synovial membrane of treated animals compared with that for controls. Results of this study suggest oral administration of *Z. officinale* extracts can be a good alternative treatment for osteoarthritis. **References:** [1] Kane, D. et al. (2005) Ann. Rheum. Dis. 64(2): 325 – 327. [2] Nissalo, S. et al. Ann. N.Y. Acad. Sci. 966(1): 384 – 399.

P 606

Epigallocatechin gallate protects curcumin against oxidative degradation and mechanistic studies

Lee CY[1,2], Chien YW[1,2]
[1]InnovaTherapeutics Research Center, [2]Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, 100 Shih-chuan 1st Rd., Kaohsiung 807, Taiwan

Curcumin (CM) and Epigallocatechin gallate (EGCG), both are natural antioxidants and known to produce beneficial pharmacological activity [1]. CM has been reported to have a low stability toward oxidants; its oxidative status could be repaired by EGCG [2]. EGCG has shown a stronger DPPH-scavenging potency ($IC_{0.200}$) than CM (2.9 vs. 13.6 µM) [3]. To study the mechanism for the stabilizing effect of EGCG on CM, CM (10 µM) was incubated in an egg phosphatidylcholine (EPC) solution (0.9 mg/mL, pH 6.5), with or without EGCG (20 µM), at 37 °C for 72 hours and 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH) was added to provide free radical species for degradation of CM. With the addition of EGCG, the degradation of CM was reduced by more than 2 folds (from 88.4% to 40.4%). In the presence of AAPH (10mM), CM was observed to decline to baseline within 2 hours; while EGCG was found to slow down the degradation of CM by 3 times. While EGCG had shown to prolong the decline of CM to 6 hours, itself was declined to the baseline in 2 hours. On the other hand, CM was found to produce no protection on EGCG against the AAPH. With or without the co-existence of CM, EGCG was found to degrade at a rate of 78.7%/hr, while EGCG was degraded at a much slow rate of 8.3%/hr in the absence of AAPH. Furthermore, EGCG, CM and their combination were found effective in delaying the AAPH-induced lipid peroxidation of EPC, as assayed by thiobarbituric acid-reactive substances, for 2, 2, and 4 hours, respectively, which roughly corresponded to the length of time required to deplete CM and EGCG. Taken together, EGCG could provide a protective action to CM against the oxidative degradation of free radical, and this protection appears to be attributed to the radical-scavenging power of EGCG. *Acknowledgement*: 1. Supported by Development fund for Innovative Therapeutics. 2. National Science Council in Taiwan. **References:** [1] Aggarwal et al. (2006) Biochem. Pharmacol. 71: 1397 – 1421. [2] Jovanovic et al. (2001) J. Am. Chem. Soc. 123: 3064 – 3068. [3] Lee & Chien (2006). Taiwan Pharm. Soc. Conference-2006.

7. Other related topics

P 607

The effect of *Bacillus subtilis* FZB24® on flowers quantity and quality of saffron (*Crocus sativus* L.)

Sharaf-Eldin MA[1,5], Elkholy S[2,5], Fernández JA[3], Junge H[4], Cheetham RD[5], Guardiola JL[6], Weathers PJ[5]
[1]Medicinal and Aromatic Plants Dept., National Research Centre (NRC), 33 Elbehoth St., Dokki, Cairo-12311, Egypt; [2]Plant Transformation and Biopharmaceuticals lab, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Centre (ARC), 9 Gamaa St., Giza-12619, Egypt; [3]School of Agronomy (ETSIA) & Group of Biotechnology (IDR), University of Castilla-La Mancha, E-02071, Albacete, Spain; [4]ABiTEP GmbH, Glienicker Weg 185, D-12489, Berlin, German; [5]Department of Biology and Biotechnology, Worcester Polytechnic Institute (WPI), 100 Institute Rd., Worcester, MA 01609, USA; [6]School of Agricultural Engineering, Polytechnic University of Valencia, 46022, Valencia, Spain

The effect of *Bacillus subtilis* FZB24® on saffron (*Crocus sativus* L.) was studied at Worcester Polytechnic Institute (WPI), USA, using saffron corms provided by Universidad de Castilla-La Mancha

(UCLM), Spain and the powder form of *B. subtilis* FZB24® provided by FZB Biotechnik GmbH, Germany [1,2]. Saffron corms were soaked in water for 15 min before sowing (T1 = C.: untreated control), corms of treatment two (T2) were soaked in *B. subtilis* FZB24 solution at 2 g/1000 ml water for 15 min, while corms of T3, T4 and T5 were drenched with FZB24 at 2 g/10 L after 6, 10 or 14 wks of sowing date, respectively. The following parameters were evaluated starting from sowing date: days to sprout, number of sprouts and leaf length after 10 wks, days to flower, number of flowers/corm, FW of stigma yield [g]/1st flower and total stigma FW [g]/corm. Chemical analysis of stigmas showed that *B. subtilis* FZB24 altered the crocin, picrocrocin, crocetin and safranal contents of saffron compared to the untreated control. In contrast, there were no significant differences in vegetative growth. These results will prove useful in the implementation and improvement of saffron production technology. *Acknowledgements:* Support for this research provided in part by the ICSC-World Laboratory Scholarship, Switzerland to Dr. M.A. Sharaf-Eldin. **References:** [1] Kilian, M. et al. (2000) Pflanzenschutz-Nachrichten Bayer 1: 72 – 93. [2] Krebs, B. et al. (1998) JPDP 105: 181 – 197.

P 608

Safety monitoring of Thai Herbal National Essential Drug Lists

Oppamayun Y[1], Suwannakaesawong W[1], Kaewpaneukransri W[1], Sripirom P[1]

The ADR Monitoring Center, Food and Drug Administration, Ministry of Public Health, Nondthaburi 11000, Thailand

Background The Thai government has set up a national plan for promoting and monitoring of medicinal plants grown in Thailand in order to achieve self-reliance on drug supplies. In 1999, the Thai Herbal National Essential Drug List (HNEDL) contained five medicinal plants, i.e. Curcuma longa, Andrographis, Zingiber montanum, Clinacanthus nutans and Senna alata. The National ADR (adverse drug reactions) Monitoring Center launched the project to monitor ADRs associated with these medicinal plants in October 2000. Aims ADRs which occurred from these medicines should be reported, studied and analysed. Method The surveillance based on spontaneous reporting had been used to collect patients data on usage and to detect ADRs which occurred from medicinal plant products during October 2000-September 2003. Results A total of 1077 reports were gathered from 141 hospitals around the country. The most frequently used medicinal plant was Curcuma longa, with the indication of flatulence. The assessment of ADRs was evaluated. 222 (35%) reports of Curcuma longa ADRs were collected. Of these, 40% concerned gastrointestinal system disorders such as abdominal pain, diarrhea, flatulence, anorexia, nausea, vomiting and feeling of hunger. 31% concerned the whole body or general disorders including headache, fever, chest pain, dizziness etc. Others were dyspnoea and yellow eyes. Causality assessments varied from probable scale to unlike scale. Conclusion Nowadays, Thailand has issued an advanced HNEDL and new medicinal plants have been added to the HNEDL 2004. The good surveillance system of medicinal plant products will enable consumers to safely use them. However, there are a lot of implications and challenges for pharmacovigilance of medicinal plant products. There is still a need for public enlightenment and education in this field. Currently, the development of schemes linking both practitioners and consumers to optimize active and spontaneous reporting of ADRs is in progress.

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Autecology, ethnomedicinal and phytochemical studies of *Nepeta binaludensis* Jamzad, a highly endangered medicinal plant of Iran

Nadjafi F[1], Koocheki A[2], Rezvani Moghaddam P[3], Honermeier B[4], Asili J[5]

[1]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran; [2], [3]Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran [4]Institut fuer Pflanzenbau und Pflanzenzuechtung, Justus Liebig University, Gießen, Germany [5]Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Nepeta binaludensis Jamzad is a rare medicinal plant of Iran which is highly endangered by severe harvesting and unsustainable herbal collection. A research was conducted to study the ecobiological background, ethnomedicinal use and chemical properties of this species in the wild habitats during the years 2003 and 2004, to understand their conservation biology as well as to predict their behavior under systematic cultivation. Results indicated that this species grows in north-facing slopes at altitudes of 2300 to 2700 m, mean annual rainfall ranging from 350 – 370 mm and mean annual temperature of 6 – 7 °C. This plant grows on light soils with a neutral pH and poor in mineral content. Height of individual plants varies from 46 to 52 cm with a crown diameter of 39 to 42 cm, a plant density of nearly 4 $pl.m^{-2}$ and a dry matter of 23 to 72 $g.m^{-2.}$ Plant density, biomass, plant height, crown diameter and also soil coverage of this species decreased by increasing slope inclination. There was no relationship between essential oil content, slope and altitude. The whole growing period of this species is about 153 days being equivalent to 1978.9 GDD. Results showed that the aerial parts of *N. Binaludensis* are used mostly by local people to treat digestive disorders, nervous disorders and depression. Essential oils of the aerial parts of plants collected from two regions, Dowlat Abad and Freizi, were slightly yellow and the yields were 0.5%(v/w) in both regions. Eighteen components representing 95.2% and 97.5% of the total oils of these regions were identified, respectively. The major constituent of the oxygenated monoterpene-rich oils was 1,8 Cineole (77.8% and 73.2% respectively). **References:** Franz C: Plant Research and Development 1993; 37: 101 – 111. Pushpangadan, P: On conservation biology, domestication and commercial cultivation of wild medicinal and aromatic plants. In: Eds. Raych and Huri: Recent Advances in Medicinal, Aromatic and Spice Crops. New Delhi: Today and, Tomorrows Printers & Publishers; 1992. 2: 431 – 436. Rustaiyan A and Nadji K: Flav Fragr J 1999; 14: 35 – 37.

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Molecular identification of the traditional Tibetan medicinal plant *Gentianopsis paludosa* (Gentianaceae) using diagnostic PCR and PCR-RFLP based on nrDNA ITS regions

Xue CY, Li DZ

Laboratory of Plant Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan China

Gentianopsis paludosa (Munro ex J. D. Hook) Ma (Gentianaceae) is a widespread species and commonly used in Tibetan folk medicine as clearing away the heat-evils and removing toxic substances [1]. There are 10 species of Gentianaceae recorded as herbal drugs in the Tibetan Medicines [2]. The other species are often marketed as *G. paludosa*, and thus, the therapeutic effects of *G. paludosa* are not achieved. Methods to distinguish *G. paludosa* from the 9 other species of Gentianaceae are limited by the current morphological and chemical methods [3]. In this investigation, two molecular methods for authentication were applied based on the sequences of nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) regions. For diagnostic PCR, a pair of species-specific primers was designed and used for the rapid identification of *G. paludosa*. For PCR-RFLP, we identified a distinctive site which can be recognized by the restriction endonuclease Dra β in the nrDNA ITS1 region of *G. paludosa*. PCR-RFLP analysis was established to differentiate *G. paludosa* from the other species of Gentianaceae. These methods provide effective

and accurate identification of *G. paludosa*. *Acknowledgment:* Support for this research was provided by the Natural Science Foundation of China (NSFC) (Project 30200018) and the Natural Science Foundation of Yunnan (NSFY) (Project 2006C0050M). **References:** [1] Yang YC, (1991) Tibetan Medicines. p. 111. [2] Wang HD, Tan CY et al., (2006) J. Etnnopharmacol.105: 114 – 117. [3] Tai-Wai Lau D, Shaw PC, (2001) Planta Med. 67: 456 – 60.

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Biochemical studies on the volatile oils of *Laurus nobilis* L. plants grown in Egypt

Ibrahim ME[1], El-Sawi SA[2]
[1]Cultivation and Production of Medicinal and Aromatic Plants Dept &
[2]Pharmacognosy Dept. National Research Centre-Dokki, (12622), Giza, Egypt

As a part of an intensive screening program to introduce new species of medicinal and aromatic plants to Egyptian cultivation and industry, the plant Bay laurel (*Laurus nobilis* L.- Lauraceae) [1] was analysed. The chemical composition of the fresh essential oil isolated by hydrodistillation was investigated by GC and GC-MS [2].The fresh oil was obtained in 0.5 – 0.8% (v/w). It consisted mainly of (50.38%) 1,8 cineole. Additionally other oxygenated monoterpenes were identified. The principal compound were α-terpinenyl acetate (19.97%) and terpineol 4- (6.48%), accounted for 26.45% of the oil. Additional oil constituents found in concentrations above 1% include α-terpinene, eudsmol γ, α-terpineol and 3-carene. Experiments were carried out to test qualitative and quantitative differences on the oil stored under cold storage conditions (4 ^{0}C) for one year. Minor variations in the content of the oil were obtained using cold storage conditions. Increases of 1,8 cineole, α-terpinene, terpinolene and α-terpineol and decreases of terpinen-4-ol, α-terpinenyl acetate and γ-eudesmol content were observed. The antimicrobial activities of the two oils were tested using the inverted petriplate method. The volatile oils showed prominent antimicrobial activities against fungi, Gram positive and Gram negative bacteria at a very low concentration (10 µl). **References:** [1] Mabey R., Mclntyre M., Michael P., Duff G. and Stevens J. (1988). "The New Age Herbalist". Collier Books Macmillan Publishing Company New York. p. 76. [2] Adams, R. P.(1989) Identification of Essential oils by Ion Trap Mass Spectroscopy. Academic Press, New York.

P 612

Sumac: An underutilized plant in rural communities of Khorasan

Tabrizi L
Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box: 91775 – 1163, Mashhad, Iran

Sumac (*Rhus coriaria* L.) belonging to the Anacardiaceae family is a small tree or shrub. It grows widely in Mediterranean countries, North Africa, South Europ, Afghanistan and Iran (1). *R. coriaria* is a medicinal plant popularly known to people in Iran due to its multiple applications such as pharmaceutical, condimental and industrial properties. Its main medicinal effect is related to tannins. Also it has multiple biological effects including antibacterial, antimicrobial and antioxidant (1,2). Dehbar county in Khorasan province is one of the main natural habitats of *R. coriaria* in Iran, where in autumn fruits are collected wildly from its natural habitat by rural people. This species is critical to the livelihood of many rural people of the area and has the potential to alleviate poverty being a source of income generation for local communities. Fruits being accepted as 'wild organic product' are collected by local communities on the basis of cooperative systems. In such cases, natural resource authorities of the area allow the rural people to collect the fruits and share the benefits. This has lead to proper protection of this plant in the vicinity of villages. Morphological characteristics and yield differ between northern and southern slopes where in northern slopes, shrubs have an average plant height of 157 cm, crown area of 232 cm, plant density of 14375 plant/ha and fruit yield of 1507 kg/ha compared to southern slopes with an average plant height of 112 cm, crown area of 203 cm, plant density of 14500 plant/ha and fruit yield of 789 kg/ha. Better understanding of habitat and plant criteria based on autecological studies and also means of propagation provide insights to better utilization. **References:** 1. Ozcan, M. et al. 2004. Bulg. J. Plant Physiol. 30:74 – 84. 2. Lauk, L. et al. 1998. Phytotherapy Res.12:s152-s153.

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The effect of Cu^{2+} on the accumulation of daidzein, genistein and coumestrol in the tuberous roots of White Kwao Krua [*Pueraria candolle*i Grah. *var. mirifica* (Airy Shaw et Suvatabandhu) Niyomdham]

Manakasem Y[1], Chalardkid P[1], Chanrat P[1]
[1]School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand

The White Kwao Krua [*Pueraria candollei* Grah. *var. mirifica* (Airy Shaw et Suvatabandhu) Niyomdham] is a famous medicinal plant of Thailand. Its tuberous roots accumulate estrogen like substances such as daidzein, genistein and coumestrol. The purpose of this study was to investigate the form and concentration of Cu^{2+} that can maximise daidzein, genistein and coumestrol in the tuberous roots of the White Kwao Krua. Two experiments were conducted on 1 and 3 year old plants of White Kwao Krua during 2001 – 2004 at Suranaree University of Technology. Experiment 1 was a 3^3 factorial in RCBD with 4 replications. The forms of Cu^{2+} compounds used were $CuCl_2$, $CuSO_4$ and Cu-EDTA. The concentrations of Cu^{2+} were set at 0, 100, 300 and 500 ppm. The extraction and the analysis of daidzein and genistein were done according to the method of Murphy [1] and Frank et al. [2] by a HPLC technique. Experiment 2 was RCBD with 4 treatments and 4 replications. The treatment were $CuCl_2$, $MnCl_2$ and $FeCl_2$ at 1,000 ppm each, and distilled water was used as control. The amount of coumestrol was examined using the method of Khanna et al. [3] by TLC technique. The Cu^{2+} at 300 ppm showed the highest amount of daidzein (44.69 ppm) and genistein (28.45 ppm). All treatments with Cu^{2+} compounds gave more daidzein and genistein than the control. $CuCl_2$ at 1,000 ppm resulted in the highest amount of coumestrol. $CuCl_2$, $MnCl_2$ and $FeCl_2$ at 1,000 ppm can stimulate coumestrol accumulation. *Acknowledgements:* Suranaree University of Technology and the Thailand Research Fund (TRF). **References:** [1] Murphy, P. A. (1981) J. Chromatogr. 211: 166 – 169. [2] Frank et al., (1994) J. Agri. Food Chem. 42: 1905 – 1913. [3] Khanna et al., (1999) Transactions of the Illinois State Academic of Science. 92: 167 – 179.

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Self-mating effect on growth traits and silymarin production for some selected lines among milk thistle (*Silybum marianum* L.) varieties

Ibrahim MM[1], Ottai MES[1], El-Mergawi RA[2]
[1]Genetics & Cytology Dept.,National Research Center, Postal Code 12622,. Dokki, Cairo, Egypt; [2]Botany Dept., National Research Center., Postal Code 12622, Dokki, Cairo, Egypt

Ten selected lines for each purple and white head flower varieties of milk thistle, *Silybum marianum* were assessed for five growth traits and silymarin production among three generations: open parents, selfing progenies and selfing offspring. Highly significant variations existed between lines, varieties and generations as well as their interactions in all tested traits. The line characters for each variety were subjected to analysis of variance only for open parents opposite to selfing offspring, and seemed highly significant variablities. The selfing offspring generation produced higher mean value in all purple variety traits except no. of flower heads. Contrarily, the parent generation produced higher values in all white variety traits

except fruit yield (FY). Lines 34 and 9 for purple and white varieties respectively were the best lines in both open and selfing generations. Coefficient of variation, genotypic and phenotypic coefficients of variation as well as broad sense heritabilities and genetic advance for most of studied growth traits were improved in the selfing offspring generation to indicate that milk thistle traits were governed with additive gene effects. Fruit yield trait had the highest parent offspring regression and narrow sense heritability in both varieties. On the other hand lines 34, 22 and 28 in the purple as well as 9, 2 and 13 in the white variety were the highest, medium and lowest fruit yield, respectively and subjected for fruit content of silymarin using HPLC. Concentration and total yield of six detected silymarin compounds showed wide variations between lines, varieties and generations and ranged from 11.92 to 62.85 mg/g and between 329.8 to 2121.3 mg/plant, respectively. Selfing improved the silymarin contents in the purple lines, but reduced the content of white lines. Interesting to notice is that silymarin production has the same pattern of fruit yield trait, so selection should be based on this trait to produce new improved high yielding silymarin genotypes.

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Biodiversity of endemic plants of the Western Balkan area as a source of new medicines

Redžić S

Dep.of Pharm.Botany, Fac. of Science and Fac. of Pharmacy University of Sarajevo, 33 – 35 Zmaja od Bosne, 71 000 Sarajevo, Bosnia and Herzegovina

The biodiversity of W Balkan includes 7,000 vascular plants. Ethnobotanical research have confirmed so far use of 700 species in human and veterinarian phytotherapy and nutrition [1,2]. Aim of this research was to determine a level of diversity of medicinal wild flora, as well as, based on its phylogenetic-biochemical relationships, to evaluate diversity of the possible medicinal flora and its potential in terms of the occurrance of new chemical compounds and their use in modern phytotherapy. In order to achieve all planned objectives, the following methodology has been applied: field research on different profiles, including ethnobotanical interviews, followed at the end by a comparative taxonomic-biochemical evaluation. Among plants that could be potentially significant in terms of the pharmacology 225 endemic species of W Balkan were identified. The most significant new resources belong to the following genera: *Picea*, *Pinus, Juniperus, Drypis, Silene, Aquilegia, Helleborus, Alyssum, Cardamine, Potentilla, Astragalus, Genista, Oxytropis, Euphorbia, Rhamnus, Viola, Athamanta, Eryngium, Pancicia, Peucedanum, Seseli, Primula, Gentiana, Asperula, Vincetoxicum, Halacsya, Moltkia, Acinos, Micromeria, Salvia, Satureja, Stachys, Teucrium, Thymus, Euphrasia, Pedicularis, Scrophularia, Veronica, Plantago, Lonicera, Viburnum, Knautia, Scabiosa, Campanula, Edraianthus, Symphyandra, Achillea, Amphoricarpos, Centaurea, Crepis, Leucanthemum, Senecio, Fritillaria, Lilium, Scilla, Dioscorea, Crocus, Iris* and *Arum*. Phylogenetic relationships with known species indicates that afore mentioned gen pool hides new metabolites, such as alkaloids, heterosides, saponins, essential oils and other secondary metabolites. **References:** 1. Redzic, S. (2006) Proc.1st IFOAM Intern. Conf. Organic Wild Production, 117 – 141. 2. Redzic, SJ. (2006) Ecol. Food & Nutr. 45, 3, 189 – 232.

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Mas cotek (*Ficus deltoidea*): A new potential medicinal plant in Malaysia

Musa Y[1], Mohd Lip J[2]

[1]Rice and Industrial Crops Research Centre, MARDI Telong, 16310 Bachok, Kelantan, Malaysia; [2]Technical Services Centre, MARDI Serdang, 50774 Kuala Lumpur, Malaysia

Mas cotek (*Ficus deltoidea*) is gaining popularity among the local herbal practitioners. It is traditionally used for the postpartum treatment and as health tonic. Mas cotek is an epiphyte and found growing mainly on the branches of higher plants (1). Efforts to develop the technology for the commercial planting were established. These include identification of potential accessions, requirement for planting medium, water and other agronomic practices on the incidence of pests and diseases were also conducted. Studies on its bioactive contents in the effort to develop an effective and safe standardized products based on mas cotek were also conducted. The collection effort has successfully collected 31 morphologically different accessions (2). Assessment made showed that emas cotek requires well drained soils such as bris for optimum growth. Among the things that need special considerations is the use of soil ameliorant and water management. Planting of emas cotek under 50% shade in the open field (monoculture) system produced yields two times higher than the unshaded plants. Besides the field planting, emas cotek can also be commercially produced under containerized systems using polybags. Some of these accessions (MFD 2, MFD 4, MFD 6 and MFD 9) showed good growth and were capable of producing more than 1,200 kg/ha dry yield six months after planting (3). Biochemical studies conducted show that mas cotek leaves contain 0.04% of moretenol. This compound has the potential to be used as a chemical marker or biomarker due to a significant amount of moretenol in the leaves. An analytical method for analyzing the moretenol content by GC-MS was developed. *Acknowledgements: Ministry of Science, Technology and Innovation, Malaysia (MOSTI) for funding this project through IRPA Fund No. EA 1126* **References:** [1] Musa, Y. (2005). Bul. Teknol. Tanaman 2: 35 – 48. [2] Musa, Y. et al. (2005) Bul. Teknol. Tanaman 1: 29 – 36, [3] Musa, Y (2006) J. Trop. Agric. Fd. Sc. 34: 229 – 235

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Clonal propagation of *Crataegus monogyna* Jacq. (Lindm.)

Wawrosch C[1], Prinz S[1], Soleiman Y[1], Kopp B[1]

[1]Department of Pharmacognosy, University of Vienna, A-1090 Vienna, Austria

Hawthorn (*Crataegus* spp.) is an important medicinal plant which exhibits clinically proven improvements of cardiac functions [1]. Five hawthorn species plus hybrids, including *Crataegus monogyna* Jacq. (Lindm.), are allowed for the drug "Crataegi folium cum flore" (Hawthorn leaf and flower), and a minimum content of 1.5% flavonoids is claimed [2]. Highly fluctuating quality due to wild collection of the plant material from different species and genotypes resulted in increasing interest in controlled field culture of defined clones [3]. In vitro-propagation could provide an opportunity for the production of genetically homogenous seedlings. Winter buds were collected from a mature *C. monogyna* tree in spring and were surface-sterilized prior to inoculation on MS medium [4] with 10 μM zeatin. Nodal explants were excised from regenerated shoots and inoculated on MS media supplemented with factorial combinations of the plant growth regulators 6-benzylaminopurine and indole-3-butyric acid. Micropropagated shoots were subsequently potted into soil after dipping the base in Seradix® B3 rooting powder, acclimatized in a mist chamber, and then transferred to a test plot after further hardening in the greenhouse. The flavonoid patterns of single plants of an 8 years old clone were analyzed by HPLC. The contents of the major flavonoids were uniform within the trees. The flavonoid pattern of the in vitro-multiplied individuals showed a good concurrence with the mother plant, too. Our results indicate that micropropagation of *Crataegus monogyna* through nodal culture can be a potential alternative to the slow conventional propagation through cuttings. In respect of flavonoid content, genetically homogeneous plantlets for further field culture can be produced, thus making possible a standardization of the final herbal medicinal product at an early stage of the production chain. **References:** [1] ESCOP Monographs (2003). Thieme. Stuttgart. [2] Pharmacopoeia Europaea, 5th edn. (2005). [3] Sonnenschein, M., Plescher, A. (2005) Pharm. Unserer Zeit 34: 42 – 47. [4] Murashige, T., Skoog, F. (1962) Physiol. Plant. 15: 473 – 497.

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Dendrobium huoshanense C. Z. Tang et S. J. Cheng: micropropagation and anticataract acitivity

Luo JP[1], Wawrosch C[1], Kopp B[1]
[1]Department of Pharmacognosy, University of Vienna, A-1090 Vienna, Austria

Dendrobium huoshanense C.Z. Tang et. S. J. Cheng, an endangered orchid species used in traditional Chinese medicine for curing cataract, throat inflammation, fever and chronic superficial gastritis [1], was mass-propagated through protocorm-like bodies (PLBs) induced from stem segments of seedlings on Murashige and Skoog (MS) medium [2] supplemented with indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), α-naphthaleneacetic acid (NAA), 6-benzylamino purine (BAP) and kinetin (Kin) at different concentrations alone or in their combinations. The highest frequency (92.8 ± 4.9%) of PLB formation with an average of 23 PLBs per explant occurred on MS medium containing 9.84 μM IBA and 4.44 μM BAP. PLBs proliferated fast when cultured in liquid 1/2MS medium without growth regulators. Kin at 20 μM was the most effective to stimulate shoot development. Over 600 shoots were obtained from one gram PLBs within 3 months of culture. Further rooting was executed on MS medium containing 1.0 μM IBA. Total soluble polysaccharides extracted from micropropagated plantlets showed the same fractions as those from the wild plants using DEAE-Cellulose anion-exchange chromatography. These polysaccharides, when administrated to rats treated with streptozotocin (STZ, 45 mg kg^{-1}) at 100 – 200 mg $kg^{-1}day^{-1}$, displayed a significantly inhibitory effect on development of diabetic cataract. Compared to the groups treated only with STZ, polysaccharides at 200 mg $kg^{-1}day^{-1}$ caused significant decreases in blood sugar level (50.5 ± 2.6%) and advanced glycation end productions (40.2 ± 5.1%). The results suggest that in vitro propagation systems developed here would provide sterile, consistent plantlets for investigation of bioactivity and germplasm conservation of *D. huoshanense*. *Acknowledgements:* The support from the Austrian Exchange Service is gratefully acknowledged. **References:** [1] Bao, XS. et al. (2001) Medical Dendrobii in China. Fudan University Press. Shanghai. [2] Murashige, T., Skoog, F. (1962) Physiol Plant, 15: 473 – 479

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Molecular authentication of the traditional Chinese medicinal plant *Euphorbia humifusa* and *E. maculata*

Xue HG, Zhou SD, Deng XY, He XJ
College of Life Science, Sichuan University, 24 South Section 1 Yihuan Road Chengdu, Sichuan, 610065, People's Republic of China

Botanical supplements for health enhancement are being increasing used all over the world [1]. The integrity of herbal supplements is of great importance because adulteration or contamination of a botanical product can have a direct effect on the efficacy or even safety of the product by introducing unknown phytochemicals [2]. Here we provide a convenient, on-line method for the quick molecular identification of *Euphorbia humifusa* and *E. maculata*. These species are listed as source plants of Chinese medicine 'Dijincao' in the Chinese Pharmacopoeia to treat dysentery and colitis for thousands of years [3]. The method is based on an oligonucleotide barcode: a diagnostic combination of several oligonucleotides (probes) specifically allocated within the internal transcribed spacer 1 and 2 (ITS1 and ITS2) sequences of the rDNA repeat [4, 5]. The barcode was developed on the basis of 128 sequences of 56 vouchered specimens which displayed in total 72 ITS1 and ITS2 haplotypes. Oligonucleotide sequences which are constant in all known ITS1 and ITS2 of *Euphorbia* but different in closely related genera, were used to define genus-specific probes. The library of species-specific probes identifies 75 species. Verification of the DNA-barcode was done by a blind test on 28 unknown isolates of *Euphorbia*, collected in Central and Southwest China. The obtained results were in total agreement with NCBI BLAST of vouchered records and morphological analysis. We conclude that oligonucleotide barcode is a powerful tool for the routine identification of "Dijincao" species and should be useful as a complement molecular tool to traditional methods. *Acknowledgements:* This research was supported by the National Natural Science Foundation of China (grant no. 30670146) and National Infrastructure of Natural Resources for Science and Technology (grant no. 2005DKA21403). **References:** [1] Zhao, Z. et al. (2006) Planta Med. 72: 865 – 874. [2] Lum, MR. et al. (2005) Planta Med. 71: 841 – 846. [3] The state Pharmacopoeia Commission of the PRC. (2000) Pharmacopoeia of the People's Republic of China. Beijing: Chemical Industry Press 1: 84. [4] Baldwin, BG. et al. (1995) Annals of the Missouri Botanical Garden 82: 247 – 77. [5] Druzhinina, IS. (2005) Fungal Genetics and Biology 42: 813 – 828.

P 620

Genetic improvement of *Centella asiatica* L. for high yields of asiaticoside

Dalave SC, Apparao BJ
Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, At/Po. Loni, Tal. Rahata, Dist. Ahmednagar, Maharashtra, India, PIN – 413 713

Centella asiatica (L) Urban (Family: Apiaceae) is a valuable medicinal plant, widely used in healing of wounds and as a brain tonic. Asiaticoside, a trisaccharide triterpene, is the most active compound in this plant that has been associated with the healing of wounds and duodenal ulcers. In the present investigation, attempts were made to improve the genotype of *C. asiatica* for higher yield of asiaticoside, employing the techniques of polyploidy breeding. Young developing shoots of *C. asiatica* were treated with saturated paradichlorobenzene (*p*DB) for varying time intervals. It was found that treatment with this antimitotic agent for 6 hours was effective in inducing autotetraploidy in this plant. The chromosomes counts have confirmed the ploidy of the plants. The autotetraploids were grown in the field for about 7 generations and compared with the control plants for morphometric traits and asiaticoside contents. The autotetraploids of *C. asiatica* showed vigorous growth, broad leaves, statistically significant increase in overall size of the plant and increase in fresh and dry weights. HPTLC analysis of dried leaf powder of control and autotetraploid plants, after 7^{th} generation, revealed that the autotetraploids produced double the amount of asiaticoside as compared to the control plants.

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Biotransformation of digitoxigenin – a plant secondary metabolite – by the fungus *Cochliobolus lunatus*

Pádua RM[1], Oliveira AB[2], Souza Filho JD[3], Takahashi JA[3], Vieira GJ[3], Abreu e Silva M[4], Braga FC[2]
[1]Friedrich-Alexander-Universität, Lehrstuhl für Pharmazeutische Biologie, Staudtstr. 5, D-91058 Erlangen, Germany; [2]Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270 – 010 Belo Horizonte – MG, Brazil; [3]Departamento de Química, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270 – 010 Belo Horizonte – MG, Brazil; [4]Faculdade de Farmácia, Universidade Federal de Ouro Preto, Rua Costa Sena, 171, 35400 – 000 Ouro Preto – MG, Brazil

Cardiac glycosides are plant secondary metabolites used to treat congestive heart failure. They also exhibit a wide spectrum of biological activities, including anti-carcinogenic, acaricidal, antifilarial, molluscicidal and antibacterial properties. Biotransformation of cardenolides has been investigated either as a strategy to obtain new derivatives or to convert the A-type cardenolides into the corresponding C-type compounds, which have clinical relevance [1]. The fungus *Cochliobolus lunatus* and its conidial anamorphous form *Curvularia lanata* are known for their capacity of hydroxylating Δ^{4-5} steroids at position 11β, 14α and 7α [2]. The biotransformation of digitoxigenin **1**, the aglycone of digitoxin, by *C. lunatus* was investigated. The biotransformation reaction was carried out in a 4-day process, resulting in the isolation of four products. Their structures

were elucidated by 1D and 2D NMR data as 1β-hydroxydigitoxigenin **2**, 7β-hydroxydigitoxigenin **3**, 8β-hydroxydigitoxigenin **4** and digitoxigenone **5** (Fig. 1). The observed hydroxylation sites are distinct from those previously described for Δ^{4-5} steroids in reactions with *C. lunatus*. The production of **4** by a biotransformation reaction is clear evidence that *C. lunatus* hydroxylases involved in the reaction are not affected by the steric hindrance of the 14β-OH group [3]. Therefore, it is feasible to obtain a product with two vicinal hydroxyls at 8β and 14β-positions. Hydroxylation of **1** at 1β-position is of special interest, since some glycosides of 1β-hydroxydigitoxigenin have been reported to exhibit potent *in vitro* activity against ovarian adenocarcinoma and lung carcinoma [4]. *Acknowledgments:* We are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the master fellowship to R.M.P. **References:** [1] Kreis, W. et al. (1992) J. Biotechnology 26: 257 – 273. [2] Holland, HL. et al. (1998) Steroids 63: 484 – 495. [3] Pádua, RM. et al. (2007) J. Braz. Chem. Soc. [Submitted]. [4] Baek, NI. et al. (1994) Planta Med. 60: 26 – 29.

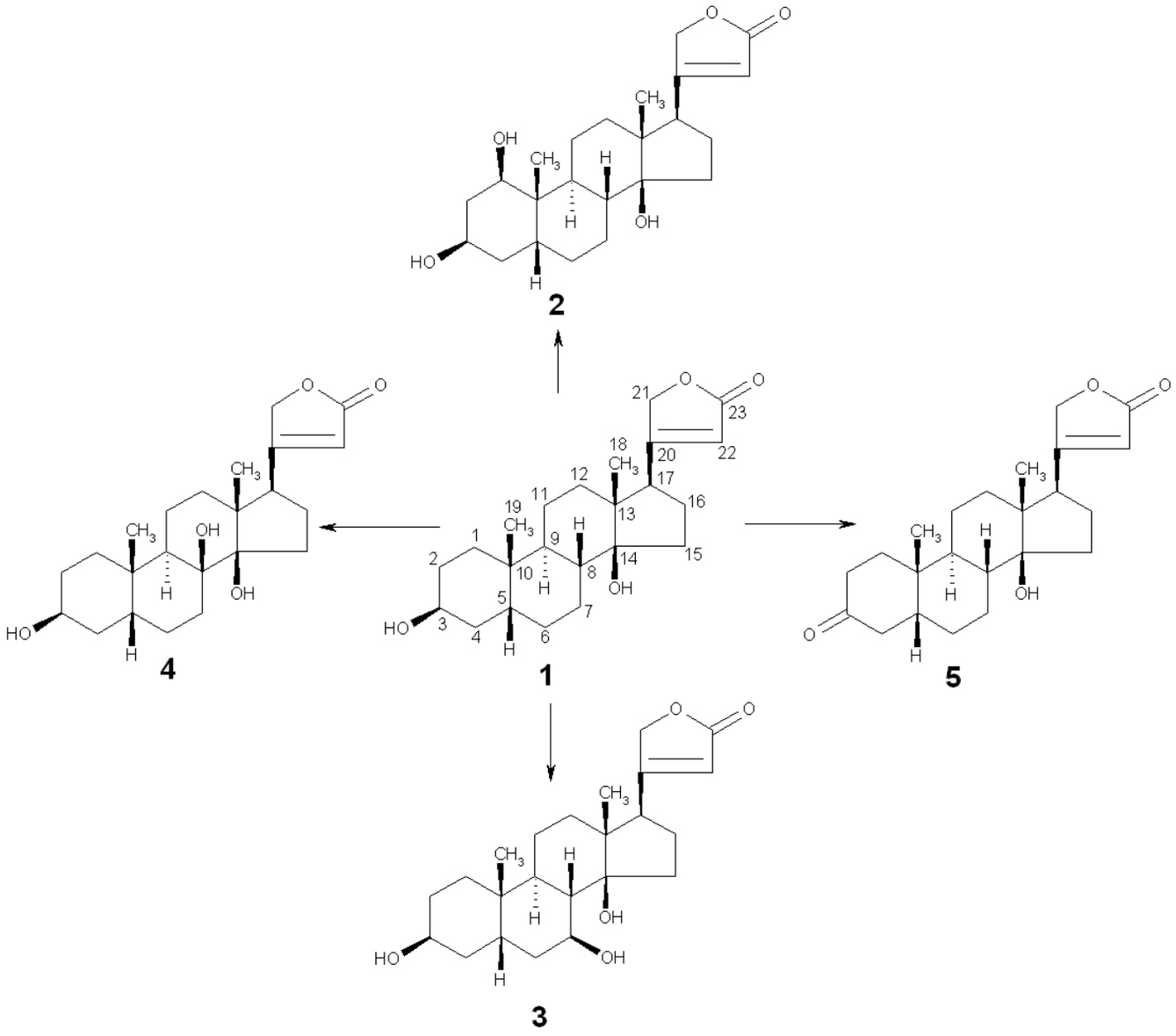

Fig. 1: Chemical structures of biotransformation products from digitoxigenin (1) by *C. lunatus*

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Formation of lignans in plant cell culture of *Schisandra chinensis*

Bøezinová L[1], Vlažínová H[2], Havel L[2], Bohatcová I[2], Chvátalová K[1], Slanina J[1]

[1]Department of Biochemistry, Faculty of Medicine, Masaryk University, Komenského nám. 2, CZ-66243 Brno, Czech Republic; [2]Department of Plant Biology, Faculty of Agronomy, Mendel University of Agriculture and Forestry Brno, Zemìdìlská 1, CZ-613 00 Brno, Czech Republic

The fruit of *Schisandra chinensis*, a woody liana, has been used for centuries in traditional Chinese medicine. The fruit is prescribed for the treatment of hepatitis in China. The active principles are lignans with unusual structures derived from dibenzo[a,c]cyclooctadiene [1]. These lignans have been shown to possess a broad range of biological activities. Recently, it has been found that lignan γ-schizandin strongly inhibits P-glycoprotein, the overexpression of which is the most frequent cause for multidrug resistance of cancer cells [2]. *S. chinensis* cultures derived from immature zygotic embryos were developed on Murashige and Skoog and VW5 medium. Embryogenic cultures were established on the medium containing thidiazuron, 2,4-dichlorophenoxyacetic acid and benzylaminopurine [3]. An analytical method based on HPLC-UV was used for the determination of six lignans in plant cell cultures. Our previous

results showed that the concentration of lignans in plant cell cultures was mostly lower than that in fruit and leaves. We also detected the lignans in liquid culture media, but the amount of lignans inside the cells was substantially higher. In order to release the lignans stored within the cells, we explored the cultivation of five cell lines with the neutral polymeric resin, Amberlite XAD-2. We found that the addition of Amberlite XAD-2 not only notably enlarged portions of extracellular lignans (from 2 – 29% to 28 – 99.9%), but also greatly enhanced the production of lignan deoxyschizandrin (14 – 200-fold as compared with the control), whereas the formation of other lignans was increased only moderately (less than 5-fold). Importantly, Amberlite XAD-2 did not reduce the growth of cell cultures (80 - 177% of the control). Deoxyschizandrin adsorbed on the polymeric resin can be easily recovered and purified without destruction of the cells. *This work was supported by Grant Agency of Czech Republic (No. 522/07/0995 and 301/03/H005)* **References:** [1] Slanina, J., Glatz Z. (2004) J. Chromatogr. B 812: 215 – 229. [2] Qiangrong, P. et al. (2005) Biochem. Biophys. Res. Commun. 335: 406 – 411. [3] Smížková, A. et al. (2005) Biol. Plant. 49: 451 – 454.

P 623

Effect of jasmonate on ginsenoside biosynthesis in adventitious root culture of *Panax ginseng*

Marsik P, Langhansova L, Vanek T
Laboratory of Plant Biotechnologies, Joint Laboratory of the Institute of Experimental Botany Academy of Sciences of the Czech Republic v.v.i. and Research Institute of Crop Production, Rozvojova 263, 166 00 Prague 6 – Lysolaje, Czech Republic

Ginsenosides are considered as principal biological active compounds of *Panax* species, which have a strong reputation since ancient times for being tonic, regenerating, and rejuvenating. Natural sources of wild growing plants have been overexploited and are therefore very limited. Hence the new ways of the production of *Panax* bioactive constituents are explored [1, 2]. In this study the effect of jasmonic acid (JA) on production of main ginsenosides (Rb1, Rb2, Rc, Rd, Rg1, Re and Rf) by the root culture of *Panax ginseng* was evaluated. Non-transformed adventitious root culture was established from *in vitro* regenerated plantlets cultivated on liquid medium with addition of plant regulators (IBA) [3]. JA (10 mg/L) was added to culture 64, 28, 21, 14 and 7 days before harvest. Compared with untreated control (15.88 mg/g of dry weight), total ginsenoside content increased as much as 33% in cultures treated with JA (23.67 mg/g), whereas growth was slightly suppressed. The increase was mainly caused by panaxadiol ginsenosides (Rb1, Rb2, Rc, Rd) (16.12 mg/g in comparison with 7.56 mg/g in control), whereas content of panaxatriol glycosides (Rg1, Re and Rf) was almost the same in control and treated roots (about 8.0 mg/g). The time of the application of JA before harvest had no significant effect on total production as well as panaxadiol and panaxatriol ginsenosides ratio. *Acknowledgements: This work was supported by KJB 400550705 research project and COST 1P0OC926.001 project.* **References:** [1] Yu, K.W., Gao, W.Y., Hahn, E.J., Paek, K.Y. (2002) Biochem Eng J 11: 211 – 215. [2] Langhansova L, Marsik P, Vanek T (2005) Biol Plantarum 49: 463 – 465. [3] Choi, S.M., Son, S.H., Yun, S.R., Kwon, O.W., Seon, J.H., Paek, K.Y. (2000) Plant Cell Tiss Org 62: 187 – 193.

P 624

Micropropagation of the medicinal plant *Dioon merolae* (Zamiaceae, Cycadales), an endangered cycad species.)

Cabrera Hilerio-Sandra L[1], Saucedo Gutiérrez S, Sandoval Zapotitla E[2], Guzmán Márquez JM[3], Mata Rosas M[5], Cruz Sosa F[1], Chávez Ávila VM[2], Litz RE[4]
[1]*Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco No. 186, Col. Vicentina, C. P. 09340 México, D.F;* [2]*Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, C. P. 04510 México D.F;* [3]*Facultad de Ciencias, Universidad Nacional Autónoma de México, C. P. 04510 México D.F;* [4]*TREC, University of Florida, USA,* [5]*Instituto de Ecología, A. C., Xalapa, Veracruz. México*

Cycads play an important role in Mexican Pharmacopeia as remedies for snake bites, ulcers and burns. This species are endangered and their propagation by alternative means offer available plant material for their study, conservation and use. Organogenic cultures were induced from zygotic embryo and megagametophyte explants of the Chiapas State (México) endangered cycad species, *Dioon merolae* De Luca Sabato & Vazquez Torres. Cycads are classified in an ancient order of gymnosperms. Plant growth medium consisted of B5 macronutrients, Murashige and Skoog micronutrients and organics, 60 g l^{-1} sucrose, 4 g l^{-1} GELRITE, and supplemented with 2,4-Dichlorophenoxyacetic acid (2,4-D) (0, 0.45, 2.26, 4.52, 9.05 μM) and kinetin (0, 2.32, 4.60, 9.30, 13.90 μM) arranged as a 5 x 5 factorial in a randomized block design. Callus became friable and yellow after 2 – 4 weeks; initiation occurred on a wide range of medium formulations from megagatophyte explants. In comparison, callus initiation from explanted zygotic embryos occurred on fewer medium formulations, and adventitious shoot induction occurred from callus on formulations with K (2.32 μM) and of 2,4-D (0.45 and 2.26 μM), embryos tended to have a morphogenic response, germinating and simultaneously developing shoots. This technique has a great potential for preservation of the highly endangered cycads.

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An investigation on in vitro propagation of *Colchicum luteum* and its evaluation of colchicine for the economic benefit of rural people in West Bengal

Gangopadhyay M[1], Bhattacharya R[1], Chakraborty D[2], Bhattacharya S[3], Mitra A[2], Bhattacharya S[1]
[1]*Medicinal plant Laboratory, Department of Botany, Bose Institute, 93/1, APC Road, Kolkata-700009 India;* [2]*Natural product Biotechnology group, AGFE, Indian Institute of Technology, Kharagpur- 721302 India;* [3]*Department of Biotechnology and Instrumentation, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252 India*

India is bestowed with a rich wealth of medicinal plants due to its diverse agro climatic and regional topography but indiscriminate collection from the wild has brought much of this biodiversity to the brink of extinction. The present research was aimed to introduce a commercially viable rare medicinal plant, Colchicum luteum from the semi-temperate, high altitude region of our country to the tropical agroclimatic zone of lower Indian Gangetic plains in view of producing the raw material of the species at higher rate than that obtained by natural propagation. Colchicine, the commercially important secondary metabolite of the plant is used in conventional crop breeding, in preparing anti-gout drugs and in anti-cancer research. A high multiplication rate of C. luteum (12 shoots on average/explant) was achieved in vitro by culturing small segments of bulbs in MS medium[1] supplemented stepwise with 8.88 μm BAP for shooting and IAA (5.71 μm) or IBA (4.92 μm) for rooting and bulblet formation. The hardened plants survived ex-vitro by 99%. Colchicine of 1 year old bulbs was estimated by TLC and HPLC followed by UV-vis spectral analysis. Genetic fidelity of the in vitro-raised plants was checked using a genetic marker. The finding of our study ensures feasibility of cultivation of this important plant in the proposed agro climatic region and may provide scope to generate a source of income, especially for the underprivileged section of the rural com-

munity. **References:** [1] Murashige, T., Skoog, F. (1962) Physiol. Plant. 15 (3): 473 – 97

P 626

Clonal micropropagation of *Hippophae rhamnoides* ssp. *carpathica*

Vantu S[1]
[1]Al. I. Cuza University, Faculty of Biology, B-dul Carol 11, 700506 Iasi, Romania

Sea buckthorn is an unique and valuable plant species by its content regarding the bio-active substances(1, 2). The technologies of vegetative multiplication offer opportunities to conserve and propagate superior genotypes. This study was designed to develop a micropropagation system for cloning elite sea buckthorn. This includes procedures and media for initiation, multiplication and rooting of sea buckthorn cuttings in vitro (3). Two varieties of Hippophae rhamnoides ssp. carpathica have been introduced to tissue culture and successfully propagated. A protocol for the micropropagation was developed starting with shoot tip (4, 5). Shoot apices with 1 or 2 leaf primordia were cultured on MS medium, supplemented with BAP and IAA. BAP was the most suitable growth regulator with the optimal concentration of 0,10 – 0,25 mg/l for initiation and 0,4 – 1 mg/l for multiplication. The average rate of multiplication was 3 – 4 shoots/explant per month. The capacity for shoot differentiation depended on concentration of cytokinin in the shoot-induction medium. Rooting of these shoots was achieved on MS medium without growth regulators. After 45 days, the regenerated plants were transplanted into pots in a greenhouse and these were subsequently planted in the experimental field. The two varieties of Hippophae rhamnoides ssp. carpathica regenerated "in vitro" displayed an vigorous growth capacity to the natural environment. **References:** [1] Dris, R. (2005) Crops-Growth, Quality and Biotechnology, Chanhan, A, Ramteke, R.S, Dris, R. (Eds), WFL Publisher [2] Li, T.S.C., Schroeder, W.R. (1996) Horticultural Technology 6: 370 – 378 [3] Montpetit, D., Lalonde, M. (1988) Plant Cell, Tissue Organ Culture, 15: 189 – 200 [4] Yao Ying, M (1995) Agricultural Science in Finland, 4(5/6): 503 – 512 [5] Vantu, S. (2006) The Proceedings of the 4 th Conference on the Medicinal and Arormatic Plants of Southeast European countries, ALMA MATER Publishing House: 273 – 276

P 627

Influence of extracellular calcium on flavonoid accumulation by cell cultures of *Hypericum androsaemum*

Paranhos A
Faculty of Pharmacy, University of Coimbra, Rua do Norte, 3000 – 295 Coimbra, Portugal

Hypericum androsaemum L., commonly known in Portugal as tutsan, is a plant species used in folk medicine for its diuretic and hepatoprotective properties [1]. The diverse flavonoids and phenolic acids present in the plant are supposed to be responsible for such biological activities. Suspension cultures of *H. androsaemum* were established from hypocotyl-derived callus using MS medium supplemented with 2,4-D (1 mg/L) and BA (0.5 mg/L). At different time points of culture growth, the total flavonoid and total hydroxycinnamic acid contents of cells were evaluted according to [2] and [3], respectively. The accumulation kinetics of these compounds were similar, with maximum levels occurring on the 14th day of the growth cycle. Culture of cells for 14 days in nutrient media containing high concentrations of $CaCl_2$ (15 or 18 mM) induced a 2-fold increase in the accumulation of flavonoids and a slight rise in the levels of hydroxycinnamic acids. Furthermore, an enhancement of flavonoid contents was also observed 72 h after adding 15 mM $CaCl_2$ or 5 μM calcium ionophore A23187 to 11-day-old control cultures. Conversely, identical treatments using either the calcium chelator EGTA (3 mM) or the calcium channel blocker verapamil (100 μM) decreased markedly the flavonoid levels registered on day 14. These results suggest that extracellular calcium plays an important role in flavonoid metabolism of *H. androsaemum* cell cultures. *Acknowledgements:* Center of Pharmaceutical Studies **References:** [1] Novais, M. et al. (2004) J. Ethnopharmacol. 93: 183 – 195. [2] Lamaison, J., Carnat, A. (1990) Pharm. Acta Helv. 65: 315 – 320. [3] Lamaison, J. et al. (1991) Pharm. Acta Helv. 66: 185 – 188.

P 628

5β-steroid reductase (At5β-StR) from the cardenolide-free plant *Arabidopsis thaliana*. Molecular cloning, heterologous expression and biochemical characterisation

Müller-Uri F, Herl V, Fischer G, Rühl C, Kreis W
FAU Erlangen-Nuernberg, Institute of Biology, Chair of Pharmaceutical Biology, Staudtstr. 5, 91058 Erlangen, Germany

As progesterone 5β-reductase activity has so far only being detected in cardenolide-producing plants like *Digitalis* and *Isoplexis* species, the gene is supposed to play a key role in cardenolide biosynthesis. During cardenolide formation the progesterone 5β-reductase catalyses the conversion of progesterone to 5β-pregnane-3,20-dione, representing the first stereo-specific step during the biosynthesis of these secondary metabolites [1]. GenBank searches (Blast) and alignments (ClustalW) using the gene and protein sequences from *Digitalis lanata* and *Isoplexis canariensis* [2 – 4] directed our attention to a putative protein described for *Arabidopsis thaliana*). Sequence identity of the three proteins and genes turned out to be 69% and 70%, respectively. Using specific primers we isolated a cDNA clone from *A. thaliana* leaves encoding a putative progesterone 5β-reductase (At5β-StR). The open reading frame of At5β-StR was 1167 nucleotides corresponding to 388 amino acids. Over-expression of a His-tagged fusion At5β-StR protein (pQAt5β-StR) was achieved in *E. coli* using the pQE expression vector system (Qiagen, Hilden, Germany). The pQAt5β-StR was purified under native conditions on a Ni-nitrilotriacetic acid (Ni-NTA) matrix, its molecular mass of about 45kDa was determined by SDS-PAGE. The purified pQAt5β-StR was enzymatically active, catalysing the reduction of progesterone into 5β-pregnane-3,20-dione. Other steroid compounds were also accepted as substrates like cortexone and 4-androstene-3,17-dione. NADPH is the only co-substrate accepted and cannot be replaced by NADH. The biochemical characterisation of pQAt5β-StR will be presented. As *A. thaliana* lacks cardenolides but contains other steroid compounds like brassinosteroids [5], our experimental results raise the question whether At5β-StR is involved in metabolic pathways others than that for cardenolide-biosynthesis. **References:** [1] Gärtner, D. E., Seitz, H. U. (1990) FEBS Lett. 271, 239 – 242. [2] Kreis, W., Hensel, A., Stuhlemmer, U. (1998) Planta Med. 64, 491 – 499. [3] Herl, V., Fischer, G., Müller-Uri, F., Kreis, W. (2006) Phytochemistry 67, 225 – 231. [4] Herl, V., Fischer, G., Bötsch, R., Müller-Uri, F., Kreis, W. (2006) Planta Med. 72, 1163 – 1165. [5] Bishop, G.J. Koncz, C. (2002) The Plant Cell 14 Suppl., 97 – 110.

P 629

The bifidogenic effect of *Agave sisalana* leaf juice

Rada V[1]*, Killer J*[1]*, Kokoska L*[2]*, Tomankova E*[1]*, Smehilova M*[1]
[1]Czech University of Life Sciences, Department of Microbiology, Nutrition and Dietetics, Prague 6, 165 21 Czech Republic; [2]Czech University of Life Sciences, Institute of Tropics and Subtropics, Prague 6, 165 21 Czech Republic

Twenty bifidobacterial strains were tested for the ability to utilize saccharides from agave leaf (*Agave sisalana* Perrine ex Engelm.). Overnight bacterial cultures grown in Wilkins-Chalgren broth (Oxoid, UK) were inoculated to the medium containing tryptone (10 g/l), nutrient broth No. 2 (10 g), yeast extract (5 g), tween 80 (1 ml), L-cysteine hydrochloride (0,5 g), distilled water (700 ml) and agave leaf juice (300 ml). All cultures were incubated at 37 °C for 72 h under anaerobic conditions. Then, the optical density at 620 nm was determined. Wilkins-Chalgren broth was used as a control medium. Contents of carbohydrates were determined before and

after incubation in all bifidobacterial cultures. The following methods were used: phenol-sulfuric acid method for total saccharides, fructan assay procedure kit (Megazyme, Ireland) for oligofructans and reflectoquant tests (Merck, Germany) for fructose and glucose. Agave oligofructans were important source of carbon and energy for all bifidobacteria tested. In most strains, oligosaccharides were utilized in preference to monosaccharides. All strains were able to grow in the medium with agave leaf juice. Most of strains grew significantly better in the medium with agave leaf juice compared to control – Wilkins-Chalgren broth. *Acknowledgement:* This study was supported by grant MSM No. 6046070901 of Ministry of Education, Youth and Sports of Czech Republic

P 630

Phytosterol, tocopherol and squalene content of selected nuts, seeds, legumes and cereals

Ryan E[1], Galvin K[1], O'Connor TP[1], O'Brien NM[1]
[1]Department of Food and Nutritional Sciences, University College Cork

Phytosterols, tocopherols and squalene, bioactive components present naturally in plant foods, all have potential health benefits. There are limited or old data pertaining to the presence or quantities of these compounds in certain foods. Therefore, the objective of the present study was to determine the levels of phytosterols, tocopherols and squalene in selected nuts, seeds, legumes and cereals. In short, the method comprised acid hydrolysis followed by lipid extraction and alkaline saponification, prior to analysis by HPLC. Total phytosterol content (β-sitosterol, campesterol and stigmasterol) ranged from 33.3 mg/100 g pumpkin seeds to 271 mg/100 g pistachio nuts. Generally, β-sitosterol was the predominant sterol in all foods. Total tocopherol content (α-tocopherol and β + γ-tocopherol) ranged from 0.1 mg/100 g rye to 16.1 mg/100 g peas. Squalene was identified in all foods employed in this study and was particularly abundant in Brazil nuts, pumpkin seeds and quinoa. In conclusion, the present study indicates that nuts, seeds, legumes and also cereals are important natural sources of phytosterols. Recent studies suggest that the present natural intake of phytosterols positively alters cholesterol metabolism and that relatively modest increases in this intake could improve public health. In addition, they contain appreciable amounts of tocopherols and squalene. *Acknowledgement*: Department of Agriculture and Food under the Food Institutional Research Measure (FIRM) as administered by the National Development Plan 2000 – 2006

P 631

The obtention of an oil/water nanoemulsion containing the *Calceolaria chelidonioides* flowers ethanol extract and its skin hydration power

Falcão DQ[1,3], Costa ER[2], Kuster RM[1], Vian L[3], Nielloud F[3], Menezes FS[2,4]
[1]Núcleo de Pesquisas de Produtos Naturais – CCS – UFRJ, 21941 – 590, Brazil; [2]Departamento de Produtos Naturais e Alimentos, Faculdade de Farmácia – CCS – UFRJ, 21941 – 590, Brazil; [3]Faculté de Pharmacie, Université Montpellier I, 34030,, France; [4]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland

Nanoemulsions are biphasic systems with droplet size in the nanometric scale (20 – 200nm). Oil/water nanoemulsions are promising colloidal drug carrier systems for diverse therapeutic applications as topical, intravenous, oral and ocular [1]. They are non-equilibrium systems, a high energy input generally from mechanical devices or from the chemical potential of the components, is required for their formation [2]. In this work we prepared an oil/water nanoemulsion with the ethanolic extract obtained from flowers of *Calceolaria chelidonioides* 5% with droplet size of 45,46 ± 3,64nm and high stability using a classical macroemulsion protocol by mixing two formulation concepts, the H.L.B. and the phase diagram, to choose the best non-ionic surfactant match. Calceolaria chelidonioides is an original Brazilian plant belonging to the Scrophulariaceae family. This plant has been studied in our group and we were able to identify its antimicrobial and antioxidant properties [3]. We evaluate this nanoemulsion skin hydration power in vivo with female volunteers between (20 – 40 years old). The measurement of the skin humidity was carried out by a Corneometer® CM820 using the capacitance method, before and 10, 20, 30, 45 minutes after the administration. Two moisturizing comercial products well known by its properties, Nivea Soft® from Nivea® and Ictyane® from Ducray® were used as control, and the formulated nanoemulsion without the plant active was evaluated. The C. chelidonioides' nanoemulsion showed some interesting skin hydration actitvity higher than Nivea Soft® cream, especially after 45 minutes, showing an increase of 25% in skin hydration. However, the most important hydration power was obtained with the nanoemulsion base which showed 30% of increase after 45 minutes. *Acknowledgements:* CAPES, CNPq. **References:** [1] Yilmaz, E. et al. (2006) Int. J. Pharm. 307: 232 – 238. [2] Solans C. et al. (2005) Curr. Opin. Coll. Interf. Sci. 10: 102 – 110. [3] Falcão, D.Q. et al. (2006) Braz. J. Pharmacognosy 16: 73 – 76.

P 632

Agro-chemical and insecticidal studies on *Iberis amara* plant grown in Egypt

Hendawy SF[1], Kamel AM[1], Sharaf-Eldin MA[1]
[1]Medicinal and Aromatic Plants Dept., National Research Centre (NRC), 33 Elbehoth St., Dokki, Cairo-12311, Egypt

The effect of different types of sulphur on growth and chemical constituents of *Iberis amara* grown in Egypt were studied in two successive seasons under Egyptian environmental conditions at the NRC Experimental Farm Station, Al-Giza. The insecticidal activity of different extracts of *I. amara* was investigated on cabbage aphids. Potassium sulphate increased plant height comparing to both ammonium sulphate and sulphur with a record of 74 cm. Ammonium sulphate at a rate of 476 kg/ha recorded the highest number of suckers (48 suckers/plant). Sulphur at rate of 357 kg/ha recorded highest number of branches (35 branches/plant). The highest fresh weight of herbs and flowers reached 340 g and 278 g when plants fertilized with 476 kg/ha ammonium sulphate and 357 kg/ha potassium sulphate, respectively. Ammonium sulphate at rate of 476 kg/ha leads to the highest contents of carbohydrate and fixed oils which reached 13.95%, 13.62% and 14.0%, 12.85% in first and second seasons, respectively. The highest content of glucosinolates and phenolic compounds was recorded when *I. amara* plants were treated with 476 kg/ha ammonium sulphate while cucurbitacins content reached the maximum by the treatment of 357 kg/ha ammonium sulphate. Insecticidal activity of different extracts showed that the higher concentration of ethanol extract induced the highest mortality for cabbage aphid as it induced 66.66% mortality after 96 hr followed by water extract after autolysis and chloroform extracts which induced 53.33% mortality at the high concentration. In contrast, hexane extract exerted the lowest effect on this insect.

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The importance of tannins, catechins and caffeine in the forming of tea aroma

Dmowski P[1], Šmiechowska M[1], Rój A[1]
[1]Gdynia Maritime University, Department of Commodity and Cargo Sciences, Morska Str. 83, 81 – 225 Gdynia, Poland

Tea is one of the eldest beverages known to mankind. Its quality is determined, among others, by aroma and taste properties. Tea is an aqueous infusion of leaves of *C. sinensis*, for which numerous biological activities have been reported, including antimutagenic, antibacterial, antioxidant and cancer preventing properties. It is commonly assumed that catechins and caffeine are responsible for these health benefits of tea. Recent research indicates that tannins express antioxidant properties and they may be used in the prophylactics of civilisation diseases [1]. It is also commonly assumed that both

catechins and tannins are responsible for aroma of tea. The aim of this work is estimation of the content of catechins, like: (+)-C, (-)-EC, (-)-EGC, (-)-ECG, (-)-EGCG, caffeine and tannins in tea imported to Poland from various countries of origin. Catechins and caffeine identification from chromatograms of examined teas' extracts (1 g of tea in 100mL boiling water) was performed by comparison of retention times with respective standards purchased from Sigma Aldrich [2].The determination of tannins was performed utilising a method based on formation of insoluble tannins salts with copper (II) cation. The tea sensory quality was estimated and scored by professional tea tasters. Two grams of tea sample were infused with 100mL freshly boiled water for 5 min. the grading system was based on a total score of 5, of which 20% was for the infusion colour, 35% for the tea aroma and 45% for the taste [3]. Total quality scores of nineteen tested tea samples (respectively – 8 samples of leave and fannings tea) ranged from 2.7 to 3.8 with a mean value of 3.34 for leave tea and 2.99 for fannings. Linear correlation analysis showed that concentrations of caffeine and tannins were positively and significantly correlated with total quality score while correlations between the concentrations of catechins and the total quality score were not statistically significant. *Acknowledgement: This paper was financed from Republic of Poland scientific funds in the years 2005 – 2007, as a research project, within grant no. 2 P06T 031 29.* **References:** [1] Chung, K-T. et al. (1998) Crit. Rev. Food Sci. Nutr. 38: 421 – 464. [2] Wang H., et al. (2000) Food Chem. 68: 115 – 121. [3] Liang H., et al. (2003) Food Chem. 80: 283 – 290. [4] Owuor P.O,. et al. (1999) Food Chem. 66: 147 – 152.

P 634

Microbiological quality of tea imported to Poland from Asia and South America

Dmowski P[1], Šmiechowska M[1], Steinka I[1]
[1]Gdynia Maritime University, Department of Commodity and Cargo Sciences, Morska Str. 83, 81 – 225 Gdynia, Poland

The quality of tea and its microbiological cleanness are influenced by the following factors, such as: the kind of soil, weather conditions, conditions of the storage and transportation. Koga et al. reported that on tea plantations, at the sour reaction of acid soil (pH< 6) along with the decrease pH follows the set-back of the development of microorganisms. Also it was observed that along with the decrease of the contents of water in soil from 52.5% the quantity of microorganisms falls by half. Additionally, too long process of withering of tea leaves, their excessive exsiccation, the prolonged process of the classification and irregular passage of the packing and transport of the raw material can contribute to the development of microbes [1,2,3,4]. Samples of black tea from Argentina (12), China (12) and Indonesia (15) were selected for this study. The mesophilic total count was determined by Total Plate Count. The samples were incubated in the plates at $30^0C \pm 1^0C$ for 48 hours. Count of yeast and moulds was determined in the media – Dichlorane Glycerol Agar (DG18). The samples were incubated in the plates at $25^0C \pm 1^0C$ for 5 days. Results show that Total Plate Count contents in examined tea samples was highly diversified. It ranged from 1.54 log cfu/g to 5.59 log cfu/g. The highest quantities of microbiological contaminates were obtained from Argentinian teas – average 4.14 ± 0.90 log cfu/g, whereas the lowest in Chinese teas – average 3.63 ± 1.2 log cfu/g. This analysis did not show a significant influence of region of origin. It was found that yeasts and moulds in samples of tea were variable and dependent on the country of origin (ranged from 1.40 log cfu/g to 5.13 log cfu/g). The highest quantities of yeasts and moulds were determined in Argentinian teas – average 3.81 ± 1.03 log cfu/g. This analysis showed a significant influence of country of origin. *This paper was financed from Republic of Poland scientific funds in the years 2005 – 2007, as a research project, within grant no. 2 P06T 031 29.* **References:** [1] Chou, C. et al. (1999) International Journal of Food Microbiology 48: 125 – 130. [2] Koga, K. et al. (2003) Journal of Bioscience and Bioengineering 5: 429 – 434. [3] Sanaka, S. et al. (2000) Journal of Bioscience and Bioengineering 1: 81 – 85. [4] Yam, T.S. et al. (1997) FEMS Microbiology Letters 152: 169 – 174.

P 635

Effects of irrigation regimes and plant density on yield and essential oil production in hyssop (*Hyssopus officinalis*)

Khazaie HR[1], Nadjafi F[2], Motahari S[1]
[1]Ferdowsi University of Mashhad. Faculty of Agriculture. Department of Agronomy. Mashhad. Iran; [2]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran

A research project was conducted at the Research Station, College of Agriculture, Ferdowsi University of Mashhad, during 2003 and 2004 to study the effects of irrigation regimes and plant density on yield and essential oil production in hyssop. A split plot experiment based on a complete randomized block design with three replications was used. The experimental treatments comprised all combinations of three irrigation regimes, i.e. 7, 14 and 21 days interval, allocated in the main plots and three plant densities, i.e. 5, 7 and 10 plants/m^2 allocated in sub plots. Results indicated that irrigation regimes have significant effects on crop yield and essential oil yield in the first year but the effects were not significant in the second year of the study. The highest yield and essential oil content was observed at 7 days irrigation interval. There was no significant difference between different plant densities in both years of the study. Essential oil percentage was not affected by irrigation regimes and plant densities in both years. **References:** [1] Parta, D, D.M. Anwar, S. Saudan, A. Parsad. and D.V. Singh.1999. Aromatic and medicinal plants of salt and moistures stress conditions. Proceeding of a symposium held in India, March. 1997. pp.347 – 350. [2] Dinda, K. and L.E Cracker.1998. Growers Guide to Medicinal Plants. HSMP Press. Pub. Amherst. [3] Hornock, L 1992. Cultivation and Processing of Medicinal Plants. Academ. Pub. Budapest.

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cDNA cloning of prenyl diphosphate phosphatase from *Croton stellatopilosus* Ohba

Nualkaew N[1,2], Guennewich N[3], Springob K[3], De-Eknamkul W[2], Zenk MH[4], Kutchan TM[3]
[1]Khon-Kaen University, Khon-Kaen, 40002, Thailand; [2]Chulalongkorn University, Bangkok, 10330, Thailand; [3]Leibniz Institute of Plant Biochemistry, Halle/Saale D-06120, Germany/Donald Danforth Plant Science Center, St. Louis, MO 63132, USA; [4]Biocentre, Martin-Luther University, Halle/Saale D-06120, Germany/Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

Geranylgeraniol (GGOH) is an acyclic diterpene that posesses apoptotic activity to cancer cells [1]. It has been proposed to be the main intermediate of the biosynthetic pathway of plaunotol, an antipeptic ulcer drug from *Croton stellatopilosus* [2]. Our enzymological studies showed that GGOH is formed from the dephosphorylation of geranylgeranyl pyrophosphate (GGPP), through sequential monodephosphorylation [3], by the action of GGPP phosphatase enzyme [4]. As part of our interest in manipulating the gene of GGPP phosphatase for the production of GGOH in *Escherichia coli* system, we began with cloning of cDNA encoding prenyl diphosphate phosphatase from *C. stellatopilosus*. The degenerated primers were designed from the alignment of amino acid sequences of prenyl diphosphate phosphatase in database. The full-length gene was obtained by RACE-PCR. The cDNA contained an open reading frame encoding 888 amino acids with a calculated molecular mass of 33.6 kDa. The phosphatase motif [5] was included in the deduced amino acid sequence consisting of KX_6RP, PSGH, and SRX_5HX_3D. Its amino acid sequence showed 71% identity to phosphatidic acid phosphatase from *Vigna unguiculata*. The topology prediction of the enzyme indicated that it was a transmembrane protein with 6 transmembrane regions. The recombinant prenyl diphosphate phosphatase and its 4 designed truncated genes were expressed in *Escherichia coli* BL21(DE3)RIL.

Detection of their phosphatase activities by using [1-^{3}H]GGPP and farnesyl pyrophosphate ([1-^{3}H]FPP) as substrates showed that their enzymatic products of [1-^{3}H]GGOH and [1-^{3}H]FOH, respectively, were formed in the assay mixture. The results suggested the potential of GGOH production by the recombinant *E. coli* although the expression of the recombinant gene was still in low level. *Acknowledgements:* The Thailand Research Fund (TRF), The German Academic Exchange (DAAD), Leibniz Institute of Plant Biochemistry **References:** [1] Nakaya, M. et al. (1997) Biochem Biophys Res Comm 234: 641 – 645. [2] Tansakul, P., and De-Eknamkul, W. (1998) Phytochemistry 47: 1241 – 1246. [3] Nualkaew, N. et al. (2005) Tetrahedron Lett 46: 8727 – 8731. [4] Nualkaew, N. et al. (2006) Phytochemistry 67: 1613 – 1620. [5] Stukey, J. and Carman, G.M. (1997) Protein Sci 6: 469 – 472.

P 637

Impact of induced autotetraploidy on endogenous levels of alkaloids In *Withania somnifera* Dunal

Auti SC[1], Dalave SC[2], Apparao BJ[2]
[1]H.P.T. College of Arts and R.Y.K. College of Science, Nashik, Maharashtra, India; [2]Padmshri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, At/Post-Loni, Maharashtra, India, PIN 413 713

Withania somnifera (L.) Dunal (family: Solanaceae), is an important medicinal plant. Roots and leaves of this plant are a valuable source of alkaloids like withaferin, and withanolides. The present investigation was undertaken with the objective of inducing autotetraploidy in this plant employing colchicine and assessing its impact on total alkaloid yield. Ten days old seedlings of *Withania* were treated with different concentrations of colchicine (0.05, 0.1, 0.2, 0.3 and 0.4%) for varying time intervals (1, 2, 3, 4, and 5 hrs). Later they were transferred to a polyhouse where they were allowed to grow along with their control diploid plants till the plants attained maturity. The total amount of alkaloids was estimated from the control and autotetraploid plants using gravimetric analysis. It was observed that the plants subjected to treatment with 0.3% colchicine for 2 hours showed doubled chromosome number (2N = 4x = 96) and exhibited morphological variations, viz., increase in height of the plant, larger leaves and increased number of berries per plant as compared to control plants. The autotetraploid plants yielded 1.5 times more alkaloids as compared to diploid plants.

P 638

Morphological, structural and biochemical modification induced by air pollutants in some *Plantago* species

Gostin I[1], Olteanu Z[1], Oprica L[1]
"Al. I. Cuza" Iasi University, Faculty of Biology, Bdul Carol I no. 11, 700506 Iasi, Romania

Some morphological, structural and biochemical parameters of three species of the genus *Plantago*, which may be considered as biomarkers, were investigated in order to establish what modifications occur under the influence of pollutants. The material was represented by aerial organs of *Plantago media*, *P. lanceolata* and *P. major* collected from sites with different pollution degrees of the Ceahlau mountain. Peroxidases are enzymes possessing the capacity of catalyzing several biochemical reactions, besides their important function in the organism, e.g. in plant defense reactions [1]. The peroxidase activity determinated was 0,01419 U/ml for *P. media* and 0,1029 U/ml for *P. lanceolata*. These small values evidence that these plants are more resistant to pollution agents, a fact shown in the reduced peroxidase activity. Additionally the superoxide-dismutase activity was investigated. The increase of SOD activity in *P. lanceolata* and *P. media* harvested from heavily polluted sites shows a fast reaction of the plants to oxidative stress, and these plants can be considered, from this point of view, as being resistant to pollution [2]. The leaf structure of the investigated species show some dark deposits in the assimilatory cells, especially in the spongy parenchyma. This is an argument which demonstrates the role of the stomata (located especially in the lower epidermis) in penetration of the leaf lamina by the air polutants. Solid deposits are present on both sides, in the upper and lower epidermis. **References:** [1] T. Kawano (2003) Plant Cell Rep., 21: 829 – 837; [2] R. Bernardi, C. Nali (2001) J. Phytopathol. 149: 477 – 480.

P 639

The effect of mineral vs. bio fertilizer on growth, yield and essential oil contents of fennel (*Foeniculum vulgare* Mill)

Sharaf-Eldin MA[1], Mahfouz SA[1]
[1]Medicinal and Aromatic Plants Dept., National Research Centre (NRC), 33 Elbehoth St., Dokki, Cairo-12311, Egypt

The use of biofertilizers, in preference to chemical fertilizers, offers economic and ecological benefits. Therefore, this research work was carried out at the National Research Centre (NRC) Experimental Station (30 °05'N, 31 °22'E), located at about 22 m altitude, in the north of Egypt, Al-Giza Governorate during two successive seasons. The effect of inoculation with *Azospirillum, Azotobacter*, *Bacillus megatherium*, the mixture of them, 50% of NPK (according to the recommended dosage by the Egyptian Ministry of Agriculture and Land Reclamation) or 100% of NPK (control), was investigated. Among these six treatments the inoculantion with *B. megatherium* reluted in the highest plant height. There were no significant differences among all treatments registered for plant fresh weight, number of branches/plant, plant dry weight and yield of fennel fruits/plant. Both fungal and bacterial inoculants altered the uptake of NPK and the contents of carbohydrates [1]. The essential oil characterization by gas liquid chromatography revealed that the level of anethol was significantly enhanced by the treatments with bio fertilizers [2]. **References:** [1] Stamford, N. et al. (2007) Bioresource Technology 98: 1311 – 1318. [2] Kapoor, R. et al. (2004) Bioresource Technology 93: 307 – 311.

P 640

Influence of light irradiance on total phenols content, antioxidant capacity, growth and photosynthetic pigments of yarrow grown in fields and controlled environment

Giorgi A, Licheri GL, Mingozzi M, Cocucci M
Di.Pro.Ve.- Dipartimento di Produzione Vegetale- Università degli Studi di Milano, Via Celoria, 2 – 20133 Milano, Italy

Secondary metabolites biosynthesis in plant tissues is influenced by several environmental factor such as light, temperature, water availability etc. The aim of the present work was to study the effect of light irradiance on total phenols accumulation and antioxidant capacity in yarrow (*Achillea millefolium* L. spp *collina*) plants grown in two experimental fields (Dazio and Bormio) located in Valtellina in central Italian alps. 2-years old plants were grown in full sun or covered by a shadow net which reduced light irradiance by 70%. Phytochemical analyses were performed on methanolic extracts obtained from fresh leaf and inflorescence collected at full blooming. In both fields, a significant decrease in phenol accumulation and antioxidant capacity was observed in leaf samples harvested from shady plants compared to those in full sun, whereas there were no differences in inflorescence extracts. Regardless of light irradiance and organ, plants grown in Dazio had higher phenol levels and antioxidant capacity than those in Bormio and this was inversely correlated with plant growth. Photosynthetic pigments analysis showed higher content of chlorophyll a and b in shady plants compared to full sun ones. To verify the effect of light irradiance on phytochemical parameters in controlled environment we established *in vitro* culture of yarrow. Experiments conducted using photomixotrophic and photoautotrophic *in vitro* plants exposed to high and low light irradiance caused a similar response on phenol accumulation, antioxidant capacity and photosynthetic pigments supporting our field results.

P 641

Endophytic paclitaxel synthesizers – search and investigation

Staniek A[1], Woerdenbag HJ[1], Czepnik M[2], Szamalek M[2], Baer-Dubowska W[2], Krawczyk K[3], Budzianowski J[3], Zwart JW[4], Kayser O[1]
[1]Pharmaceutical Biology Dpt., University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, the Netherlands; [2]Biochemistry Dpt., Poznan University of Medical Sciences, ul. Swiecickiego 4, 60 – 781, Poznan, Poland; [3]Pharmaceutical Botany Dpt., Poznan University of Medical Sciences, ul. Sw. Marii Magdaleny 14, 60 – 861, Poznan Poland, [4]Botanische Tuin De Kruidhof, Schoolstraat 29 B, 9285 NE, Buitenpost, the Netherlands

While the 1990 s brought quite an abundance of reports on paclitaxel-producing endophytes, with the discovery of *Taxomyces andreanae* in 1993 being merely a starting point [1], no conclusive follow-up data on fungal metabolite profile or the genetic background of the biosynthetic pathway leading to paclitaxel is available as yet. Pursuing the idea of a microbial paclitaxel source providing for an inexhaustible supply of this antineoplastic blockbuster, two objectives were established in course of the hereby presented on-going research. The main emphasis was put on the in-depth study of the aforementioned endophyte, with the experimental procedure undertaken on alternative levels. Metabolic level, with detection of paclitaxel and other taxanes being the main goal, and genomic – aiming at revealing the microbial route leading to paclitaxel, encompassing genes encoding for the consecutive biosynthetic enzymes. As no decisive evidence supporting paclitaxel production in *Taxomyces andreanae* under our experimental conditions has been obtained, a thorough analysis of endophytic extracts aiming at the verification of their presumed antiproliferation activity was taken up. As investigated upon a human ovarian cancer cell line, the fungal extracts conferred a substantial antineoplastic influence. The natural products responsible for the observed proliferation arrest are, as yet, unknown. The second aspect of our pursuit was the study of a relictual conifer, *Wollemia nobilis* – an ancient tree reported to be host to a myriad of endophytic fungi, including *Pestalotiopsis guepinii* – a presumed paclitaxel producer [2]. The initiated screening for the endophytes of the pine in question resulted in obtaining several microbial entities. Further experimental steps, aiming at the isolation of taxol-producing specimens are underway. **References:** [1] Stierle, A. et al. (1993) Science 260: 214 – 216. [2] Strobel, G. et al. (1997) Aust. J. Bot. 45: 1037 – 1082

P 642

Plant-based ethnic remedies for hypertension from Malaysia

Ibrahim H[1], Yusoff MM[2]
[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; [2]Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 25000 Kuantan, Pahang, Malaysia

Hypertension is among the most common medical problem currently encountered in many ethnic communities. It is referred to as the "silent killer" and if left untreated, carries with it a substantial morbidity and mortality. Malaysia has rich and biologically diversified natural resources with over 15,000 flowering plants and 2,000 medicinal plants. An inventory of selected ethnic communities in East and West Malaysia was undertaken in an effort to identify potentially antihypertensive plant species used within the traditional pharmacopoeia of the communities. Respondents were randomly selected and interviews were conducted with community elders and persons knowledgeable in traditional medicine. Specific questionnaires were used and whenever possible plants documented in the survey were processed for voucher specimens. The data revealed that there are variations and differences in the method of preparation and utilization of plants as remedies for hypertension. While there has been a notable decrease in the practice of using plant-based remedies by the ethnic communities; interest in developing product based on such knowledge is on the upsurge around the world. Twenty species common to the ethnic communities studied are selected for discussion in this presentation. *Acknowledgements:* University of Malaya, Universiti Malaysia Pahang. **References:** [1] Goh S.H., Chua C.H., Mok J.S.L. and Soepadmo E.. (1995) Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases. Pelanduk Publications. Kuala Lumpur.

P 643

Contemporary phytotherapy in Australia: an ethnobotanical survey

Wohlmuth H[1], Brooks LO[2]
[1]Department of Natural and Complementary Medicine, Southern Cross University, PO Box 157, Lismore NSW 2480, Australia; [2]Research Methodology Unit, Southern Cross University, PO Box 157, Lismore NSW 2480, Australia

In Australia, Western herbal medicines are prescribed primarily by qualified herbalists and naturopaths. This survey aimed to examine plant use and prescribing patterns in contemporary phytotherapy in Australia. 68 responses from practitioners were included in the analysis. In total 322 species had been prescribed during the preceding 12 months; 309 of these were angiosperms and among these 286 were dicotyledons. The Asteraceae and Lamiaceae s.l. contributed the most species (33 and 30, respectively). The most widely prescribed species were *Glycyrrhiza glabra* (prescribed by 100% of respondents), *Vitex agnus-castus* (100%), *Actaea racemosa* (98.6%), *Hypericum perforatum* (98.6%) and *Zingiber officinale* (98.6%). The most frequently prescribed species were *Withania somnifera* (reported among the four most frequently prescribed herbs by 50%), *Glycyrrhiza glabra* (37%), *Echinacea* spp. (34%), and *Silybum marianum* (24%). Regression analysis was carried out using the total number of species in each family as the predictor and the number of species from the family used medicinally as the dependent variable [1]. Relative to their size, the Lamiaceae, Apiaceae and Asteraceae emerged as the most productive medicinal families (highest positive residuals). These results are discussed in a phytochemical context. On average, 74% of all prescriptions were in the form of liquids, 21% were in solid dose form for oral use and only 5% were for topical use. Practitioners reported combining several herbs in a prescription in nine out of ten cases; 75% of all prescriptions combined 4 – 6 herbs. **References:** [1] Moerman, DE (1991) J Ethnopharmacol 31: 1 – 42.

P 644

Genetic diversity of Iranian accessions of *Satureja hortensis* L. using RAPD markers

Hadian J[1], Fakhr-Tabatabaei SM[1], Naghavi MR[2]
[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Tehran, Karaj 31587, Iran [2]Department of Plant Breeding, Faculty of Agriculture, University of Tehran, Karaj 31587, Iran

In this study RAPD markers were used to determine the diversity level among 28 Iranian *Satureja hortensis* L. accessions. Sixteen decamer random primers were used for PCR reactions, among which 12 showed reliable polymorphic patterns. These primers which produced 181 were polymorphic. Cluster analysis of the genotypes was performed based on data from polymorphic RAPD bands, using Jaccard's similarity coefficient and UPGMA clustering method. The highest and lowest similarities detected between genotypes were 0.96 and 0.20, respectively. At a similarity of 60%, the genotypes were divided into three sub-clusters. Cophenetic correlation coefficient between similarity matrix and cophenetic matrix of dendrogram was relatively high (r = 0.9) showing the goodness of fit of the dendrogram. RAPD markers proved to be a useful tool for studying the genetic diversity of *S. hortensis*.

P 645

Biosorption of copper, cobalt and nickel (II) by marine brown algae *Sargassum* sp. in packed column

Esmaeili A[1], Soufi S[2], Rustaiyan A[2]
[1]Department of Chemical Engineering, North Tehran Branch, Islamic Azad University, P.O.Box 19585/936, Tehran, Iran; [2]Department of Marin Sciences and Technologiesy, Islamic Azad University, North Tehran Branch, P.O.Box 19585/936, Tehran, Iran

Heavy metal pollution represents an important environmental problem due to its toxic effects and accumulation throughout the food chain and hence in the human body. Biosorption is a potential alternative to traditional processes of metal ions removal. Biosorption utilizes the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediated or purely physico-chemical pathways of uptake. Biosorption has been studied in various types of biomass including marine algae, bacteria and fungi. Marine algae, a renewable natural biomass have attracted the attention of many investigators as organisms to be tested and used as new supports to concentrate and adsorb metal ions [1]. Modelling of biosorption isotherm data is important for predicting and comparing biosorption performance. Two, three and even four-parameter isotherm models are available for modelling adsorption data [2]. The objective of the present work was to asses the potential of *Sargassum* sp. for the biosorption of copper, cobalt and nickel. The experiments were conducted to study the effect of important design parameters such as pH, retention time, initial concentration and mass of biosorbent. At pH:4 the maximum uptake of Cu^{2+} and at pH:7 the maximum uptake of Co^{2+} and Ni^{2+} were obtained. Kinetic studies showed that about 80 – 90% of the total metal ions biosorption occur within 40 min. The results showed that biosorption stages follow from second – order kinetic model. By increasing the initial concentration, uptake increased. The 3.5 g of biosorbent shown higher uptake. Due to its outstanding copper, cobalt and nickel uptake capacity *Sargassum* sp. proved to be an excellent biomaterial for accumulating and recovering these ions from industrial solutions. Equilibrium data follow from Langmuir and Freundlich isotherms well. The high correlation coefficient showed the suitabvility of this method. Table 1. First-order and second -order kinetic models constants

kinetic models		Cu	Ni	Co
		-0.022	-0.036	-0.013
First-order	K_1 $(min)^{-1}$	-0.008	-0.013	-0.008
		-0.013	-0.017	-0.005
	R^2	1	1	1
		0.001	0.001	0.001
second -order	K_2 (g/mg.min)	0.003	0.005	0.002
		0.002	0.004	0.004
	R^2	1	1	1

Table 2. Isotherm constants of two-parameter models

Two-parameter models		Cu	Ni	Co
	q_{max} (mg/g)	0.50	5.00	3.12
Langmuir	b (L/mg)	0.15	0.01	0.03
	R^2	0.84	0.97	0.98
	K_f (L/g)	0.001	0.04	0.14
Freundlich	n	0.34	1.03	1.45
	R^2	0.90	0.94	0.94

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P 646

A molecular approach for the discrimination of medicinal Astragali radix used in TCM

Heubl G[1], Woelkart K[2], Wenzig EM[2], Heydel B[2], Bauer R[2]
[1]Fakultät für Biologie, Organismische Biologie, Systematische Botanik, Ludwig-Maximilians Universität München, Menzingerstr. 67, D-80638 München, Germany; [2]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl Franzens-University Graz, Universitätsplatz 4, A-8010 Graz, Austria

The genus *Astragalus* L. (Fabaceae) is represented in the Flora of China with approximately 360 species, many of them occurring as local endemics [1]. Regarding the morphological variability within the genus, species delimitation is a general problem [2]. Although the main source of the popular chinese drug huangqi (radix Astragali) is derived from *Astragalus mongholicus* BUNGE var. *dahurica* (DC.) PODLECH (Syn.: *A. membranaceus* (FISCH.) BUNGE) and *A. mongholicus* BUNGE var. *mongholicus* (Syn.: *A. membranaceus* (FISCH.) BUNGE var. *mongholicus* (BUNGE) P.K.HSIAO) other taxa such as *A. hoantchy, A. lehmannianus, A. tongolensis, A. chrysopterus, A. aksuensis, A. tribulifolius, A. floridulus, A. dshimensis, A. tribulifolius, A. lepsensis, A. ernestii, A. laxmanniii* or *A. penduliflorus* are commonly used as adulterants [2,3]. Thus the correct identification of huangqi is indispensible for phytochemical investigations and therapeutical application in TCM. Benefiting from molecular techniques, DNA markers have become popular for identification and authentication of plant species. The use of DNA sequencing (nuclear ITS and 5S- rDNA; plastidal *trn*L-F) combined with PCR-RFLP have shown that this approach is suitable for the identification at the species level. The utility of DNA-based markers has been applied successfully for authentication of selected Chinese medicinal plants cultivated in Bavaria, a project conducted by the Bavarian State Research Center for Agriculture (LfL) in Freising-Weihenstephan. Concerning phytochemistry, HPLC analyses of many samples from *Astragalus mongholicus* revealed no significant differences in the content of the main active component astragaloside IV (AGS-IV). **References:** [1] Xu, Lang-Ran & D. Podlech (2005) Astragalus. In: Flora of China. Vol. 10 (Fabaceae – in preparation). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis (www.eFloras.org) [2] Valant-Vetschera and C. Zyka (2005) Proc. WOCMAP III, Vol. 1: 41 – 48. [3] Pui Yin Yip and Hoi Shan Kwan (2006) J. Ethnopharmacol. 106: 222 – 229. [4] Ma, X. Q., Shi Q., Duan, J.A,. Dong. T. X and K.W. Tsi (2002) J. Agric. Food Chem. 50: 4861 – 4866

P 647

The value of medicinal plants in Ecuador

Gachet MS[1], Bauer R[1], Muñoz R[2]
[1]Institute of Pharmaceutical Sciences, Pharmacognosy, Karl-Franzens-University Graz, Universitaetsplaz 4/I, 8010 Graz, Austria; [2]Laboratorio de Química Orgánica e Investigaciones Aplicadas, Escuela Politécnica Nacional, Ladron de Guevara E11 – 253, POBOX: 17 – 01 – 2759, Quito, Ecuador

Approximately 80% of the world population uses traditional medicine, principally plant-based, to treat diseases [1]. In developing countries, the use of these plants is a valuable resource, and therefore, traditional medicine provides an important alternative to primary health systems [1]. Ecuador is a country which has very high biodiversity and also ancient cultural traditions in the use of plants as a source of medicine. Ethnobotanical studies and associated investigations of natural products provide data which not only helps to preserve these traditions but, contribute in the conservation and protection of these fragile ecosystems. As many phytochemical investigations have based the selection of their target of study from ethnobotanical experience, this review will mention some examples of constituents found in native Ecuadorian species. Flavonol glycosides from *Croton menthodorus* Breth. (Euphorbiaceae) have been proposed to alleviate symptoms of morphine withdrawal [2]. Minquartynoic acid from *Minquartia guianensis* Aubl. (Olacaceae) showed strong anti-protozoal activity [3]. Benzophenanthridine alkaloids

from *Bocconia integrifolia* Bonpl. (Papaveraceae) presented antitumoral [4] activity. Acetylated flavonoid glycosides from *Scoparia dulcis* L. (Scrophulariaceae) enhanced the activity of nerve-growth-factor mediated neurite (PC12D) cells [5] among many others. *Acknowledgements:* This research is part of a dissertation funded by the Austrian Exchange Service (ÖAD). **References:** [1] Macias, M., et al. (2005) J. Ethnopharmacol. 97: 337 – 50. [2] Capasso, A. et al. (1998) Pharm. Biol. 36: 310 – 4. [3] Rasmussen, H., et al. (2000) J. Nat. Prod. 63: 1295 – 6. [4] Oechslin, S., et al. (1991) J. Nat. Prod. 54: 519 – 24. [5] Li, Y., et al. (2004) J. Nat. Prod. 67: 725 – 7.

P 648

Hexa-, hepta- and nonaprenylhydroquinone isolated from marine sponges *Sarcotragus muscarum* and *Ircinia fasciculata* induce apoptosis in Hct116 cells

Watjen W[1], Rohrig R[1], Putz A[2], Konuklugil B[3], Proksch P[2]
[1]Heinrich-Heine-Universität, Institute of Toxicology, P.O. Box 101007, 40001 Düsseldorf, Germany; [2]Heinrich-Heine-Universität, Institute of Pharmaceutical Biology, Universitätsstr. 1, 40225 Düsseldorf, Germany; [3]University of Ankara, Faculty of Pharmacy, 06100- Tandoğan, Ankara, Turkey

Marine organisms have proved to be a source of potent pharmacologically active compounds [1 – 3]. Three polyprenyl-1,4-hydroquinone derivates (hexaprenyl-1,4-hydroquinone **1**, heptaprenyl-1,4-hydroquinone **2** and nonaprenyl-1,4-hydroquinone **3** were isolated from the Zoobenthos-inhabiting sponges *Sarcotragus muscarum* (Porifera: Demospongiae) and *Ircinia fasciculata* (Pallas) from the Aegean Sea. We analysed cytotoxic effects of these substances against three tumour cell lines: Hexaprenylhydroquinone exerted highest toxicity in all cell lines (H4IIE, HepG2 and Hct116 cells, respectively; H4IIE IC_{50} approx. 2.5 μM (**1**) compared to 25 μM (**2**, **3**), MTT assay, 24 h incubation). The mode of cell death was further analysed in Hct116 cells showing an apoptotic mode of cell death by these compounds (caspase-3 activation, nuclear fragmentation). Further experiments showed that the compounds exerted prominent antioxidative activity comparable to that of Trolox in the TEAC assay. In conclusion, the prenylated hydroquinones isolated from the marine sponges *S. muscarum* and *I. fasciculata* showed prominent cytotoxic activities against various cancer cell lines, exhibiting an apoptotic cell death in Hct116 cells. This may lead to the generation of new lead substances in cancer therapy. *Acknowledgements This research was supported by a grant (SBAG104S109) from TÜBİTAK and JÜLİCH* **References:** [1] Carte, B.K. (1996) Biosciences 271 – 286. [2] Halvorson, H.O. (1998) New Engl. J. Higher ed. Econ. Dev.13,28 – 42. [3] Blunt, J.W., et al. (2003) Nat. Prod. Res. 20,1 – 48.

P 649

The effect of cultivation density on dark-leaved willow (*Salix myrsinifolia*) growth and phenolics

Julkunen-Tiitto R, Sorsa S
Faculty of Biosciences, Natural Product Research Laboratories, University of Joensuu, P.O.Box 111, FIN-80101 Joensuu, FINLAND

Willow leaves, buds and bark contain condensed tannins, flavonoids, phenolic acids and phenolic glycosides [1]. *Salix myrsinifolia* leaves are very rich in glucosidic derivatives of salicyl alcohol, such as salicortins and tremulacins which have been used as analgetic, anti-inflammatory and antipyretic components in traditional herbal preparations. Phenolics have been shown to vary widely based on different environmental factors [2,3]. We studied the effect of planting density on dark-leaved willow shoot growth and leaf chemistry. Willow clonal cultures (8 clones) were established from cuttings on the field covered by black plastic mulch. The phytomass of shoots was measured after the second growing season, and phenolics were methanol extracted and HPLC-analyzed from dried and milled leaves. The increase in cutting density significantly decreased the plant size and relative phytomass. There was no difference in phenolic concentration in leaves grown in any density levels. Different clones showed a high variation in growth and phenolic content, and the concentration of some phenolics (such as hyperin, chlorogenic acid and (+)-catechin) positively correlated with the size of willow plants. The trade-off between dark-leaved shoot growth and phenolic constituent accumulation was not found. **References:** [1] Julkunen-Tiitto R, Sorsa S. (2002) J. Chem Ecol. 27 (4): 779 – 789. [2] Hansen AH et al. (2006) Oecologia 147: 1 – 11. [3] Nyman T, Julkunen-Tiitto R. (2005) Phytochemistry 66: 2836 – 2843.

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Survey of plants used by traditional birth attendants (TBAS) in Southwest Nigeria

Osunderu O[1], Neighmogha T[2]
[1]Nigeria Natural Medicine Development Agency, (Fed Ministry of Science and Technology),P O Box 17331, Ikeja, Lagos, Nigeria; [2]National Institute of Medical Research, PMB 2013, Yaba, Lagos, Nigeria

Medicinal plants are the primary source of medicines used by Traditional Birth Attendants in Nigeria. Several medicinal plants of global importance originate in the country. 85% of the population make use of traditional medicine (2, 3). The National Demographic and Health Surveys Report (NDS, 1999) indicate that only 37% of births take place in conventional health centers or hospitals. The purpose of the study is to document the common plants used by Traditional Birth Attendants in Southwest Nigeria. This is to assist the government formulate policies that will to document, regulate and promote the practice. 300 questionnaires were distributed to TBAs in the South Western geographical zones of Nigeria comprising Oyo, Ondo and Lagos States. 68% of the respondents were women while 32% were men. 41% do not refer cases but adhere to strict traditional methods by making use of plants. The later group may not want to refer their cases because of the unhealthy working conditions that exist between them and the orthodox doctors. (4). The type of method or medicinal plant used is related to the symptoms observed (1). This also reveals one of the deficiencies of traditional birth attendants as the method of diagnosis is not adequate in most cases although they enjoy increasing popularity. The herbs used by the practitioners include: *Carica papaya, Bilighia safida, Afromomum melegueta*. **References:** [1] Carpenter et al. (1995): World Health Forum: 198 – 199. [2] WHO traditional medicine strategy 2002 – 2005 Geneva, World Health Organization, 2002 (WHO/EDM/TRM 2002. (1). [3] WHO report on traditional medicine, my documents/WHO traditional medicine.htm 15/06/2006. [4] Wirth DP (1995): J. Social Science Medicine; 249 – 60.

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Effect of ultrasound on the kinetics of extraction, phenolic and flavonoid compounds from *Plantago major* L. leaves

Lazić M[1], Stanisavljević I[1], Stojièević S[1], Veliković D[2], Veljković V[1]
[1]Faculty of Technology, Bulevar oslobođenja 124, 16000 Leskovac, Serbia; [2]Zdravlje-Actavis, Vlajkova 199, 16000 Leskovac, Serbia

The kinetics of ultrasound-assisted (UE, 40 kHz) and classical solvent (CE, maceration) extraction of extractive substances (ES) from dry *Plantago major* L. leaves using 70% ethanol (plant-to-solvent ratios 1:10 g/ml) at room temperature, as well as the total contents of phenolic and flavonoid compounds of dry extracts, were studied. The extractive substances (ES) yield obtained by Soxhlet extraction (9 hours, 12 extraction cycles) was taken to represent the ES content in the plant material. Total contents of phenolic and flavonoid compounds were determined by the Folin-Ciocalteu [1] and aluminium chloride colorimetric methods [2], respectively. The extraction procedure occurred in two main stages: the first, washing or dissolution of the ES near the particle surface and the second, slow extraction or diffusion from the solid particles to the liquid extract. The second stage was described mathematically using the empirical model of Ponomaryov and the theory of unsteady-state diffusion

through plant material [3]. Ultrasound positively affected the ES yield and the kinetics of extraction. The extract obtained by CE from contained the higher total contents of phenolic and flavonoid compounds than the that obtained by UE, which is believed to be the result of their degradation by interaction with highly reactive hydroxyl radicals formed during sonication of the aqueous solvent [4]. *Acknowledgements:* Ministry of science and environmental protection of the Republic of Serbia, projects OI 142073b. **References:** [1] Singleton V. L., Rossi J. A., (1965) Amer. J. Enol. Viticult. 16 (3): 144 – 158. [2] Chang C. et al. (2002) J. Food Drug Anal. 10 (3): 178 – 182. [3] Velièković D. et al. (2001) Ultrason. Sonochem. 13 (2): 150 – 156. [4] Paniwnyk L. et al. (2001) Ultrason. Sonochem. 8 (3): 299 – 301.

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Water as extraction solvent for willow bark and artichoke leaves

Spriano D, Meier B
University of Applied Sciences, Gruental, CH-8820 Waedenswil, Switzerland

Pure water is rarely used in extraction technology. However, water extracts will be monographed in Ph Eur for willow bark and artichoke leaves [1,2]. In both cases, some special phytochemical aspects have to be taken into consideration to establish a convenient and reproducible extraction protocol. In willow bark, hydrolysis of the salicin derivatives like salicortin and tremulacin is improved compared to ethanol/water mixtures. Approximately 53% of the total salicin will be detected as salicin after an extraction with demineralised water at 25 °C. The rate can be increased up to 70% by adjusting the pH to 7.2 during the process. The hydrolysis of the salicin esters shows a similar kinetic compared to pure salicortin [3]. Chlorogenic acid has been selected as lead compound in Ph Eur monographs of artichoke leaves and extracts [2] by the working group 13B. Hardly any chlorogenic acid will be extracted by using cold water. The situation changes drastically with hot water. The best yield will result at 70 – 80 °C by maceration during one hour. The fingerprint is equivalent to the fingerprint of the herbal drug. At higher temperatures (90 °C and boiling water) a reduction of the chlorogenic acid is observed. A further peak elutes just before the chlorogenic acid. This peak is supposed to be an isomerization product of the chlorogenic acid. Furthermore, the fingerprint is changed by an additional peak between chlorogenic acid and flavonoids. It can be concluded that aqueous extracts are a useful and, in some cases, an equivalent complement to ethanolic extracts. Temperature and pH influence thereby the spectrum of the extracted substances. *Acknowledgments:* We thank SWISSMEDIC, Department of Pharmacopoeia, for the financial support. **References:** [1] EDQM (2006) Draft monograph, Willow bark dry extract. Pharmeuropa 18.1, 158 – 159. [2] EDQM (2007) Draft monograph, Artichoke leaf dry extract. Pharmeuropa 19.1, 56 – 57. [3] Meier B. (1988) Salicylate in einheimischen Arzneipflanzen, Schweizer Apotheker-Zeitung 126, 725 – 733.

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Method of gentle extraction and subsequent concentration at low temperature of natural compounds in the extract from plants

Norddahl B[1], Christensen K[1]
[1]Institute for Chemical Engineering, Bio- and Environmental Technology, University of Southern Denmark. Campusvej 55, 5230 Odense M, Denmark

A unit performing a simple and effective way to extract active phytochemicals from the plant specimens has been developed. The unit is mobile enabling operation near the place of collection of plant specimens reducing waste of potential valuable phytochemicals. The design is based on counter current liquid extraction following a pretreatment, where the plant material is harvested and macerated to an extent increasing the extraction of relevant components. The pretreated plant material is fed to an inclined conveyor, counter currently with the extracting solution. The technology, known as solid/liquid extraction is developed on the basis similar to sugar extraction from sugar beets, albeit in a much more compact form. The equipment has been tested on extraction of ethereal oils from dried, stored oregano and extraction of natural compounds from freshly harvested *Artemesia* with 96% EtOH as the solvent. Preliminary results from a continuous oregano extraction show efficiency between 55% and 85% of a more ideal laboratory batch extraction of a marker compound like carvacrol, which is most abundant in the ethereal oil. The operation can be repeated with another liquid in order to extract compounds not soluble in the previous liquid and thus enabling optimal extractions of all compounds. Practically all known extracting liquids can be used by this equipment. Extracts from the extraction operation can subsequently be concentrated using a membrane distillation unit operating with hydrophobic microporous membranes for polar solvents and pervaporation membranes for unpolar solutes, where solutes with a low vapour pressure are retained, while components with higher vapour pressures permeate the membrane including water and most other solvents. The operation is performed at relatively low temperature enabling concentration of extracted solutes without deterioration due to influence of high temperatures.

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Editor-in-Chief
Prof. Dr. Luc Pieters, University of Antwerp, Department of Pharmaceutical Sciences, Universiteitsplein 1, B-2610 Antwerp, Belgium, e-mail: luc.pieters@ua.ac.be, phone: +32 3 820 27 15, fax: +32 3 820 27 09

Editorial Offices
Dr. Claudia Schärer, Institute of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4053 Basel, e-mail: claudia.schaerer@unibas.ch

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