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Antimicrobial activity of *amurca* (olive oil lees) extract against selected foodborne pathogens

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Abstract

The antimicrobial activity of a methanolic extract of *amurca* (olive oil lees) was determined against both Gram-positive (*L. monocytogenes* and *S. aureus*) and Gram-negative (*E. coli* O157:H7 and *S. enteritidis*) foodborne pathogens at 10 °C or 37 °C using microdilution and disk diffusion methods, and its relative activity was compared to selected antibiotics. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of *amurca* extract ranged from 60 to 80 µl/ml at 37 °C after 24 h against all tested strains. At 10 °C, *amurca* was more inhibitory with MIC and MBC values of 40 and 60 µl/ml, respectively, after 7 d against tested strains. *Amurca* at 40 µl/ml reduced numbers of tested pathogens by 2.5 to 3.2 $\log_{10}$ CFU/ml at 10 °C after 7 d, but was not inhibitory at 37 °C after 24 h. Protein prepared from *amurca* was not antimicrobial. The relative antimicrobial activity (inhibition zone ratio) of 80 µl/ml *amurca* methanolic extract compared to chloramphenicol, erythromycin, gentamycin and tetracycline ranged from 0.36 to 1.0 against Gram-negative and from 0.45 to 2.0 against Gram-positive bacteria. In addition, *amurca* extract inhibited *E. coli* O157:H7 02-0628 and *S. aureus* 26127 which were resistant to tetracycline and chloramphenicol, respectively.

Keywords: *amurca*; foodborne pathogens; antimicrobial activity; olive oil; phenolic compounds.

Practical Application: *Amurca* extract showed potent antimicrobial activity against foodborne pathogens.

1 Introduction

Olive trees (*Olea europeaea* L) belong to the *Oleaceae* family which is comprised of 30 species, including jasmine (*Jasminum*), lilac (*Syringa*), and ash (*Fraxinus*) (Niaounakis & Halvadakis, 2006). Olive trees are ancient plants and fossils from the Tertiary period (1 million years ago) have been found in the Mediterranean basin. Moreover, artefacts such as stone mortars and mills used for olive processing go back to the year 5000 BC (Harwood & Aparicio, 2000). There are more than 850 million olive bearing trees worldwide, of which about 10 million occur in Jordan (Niaounakis & Halvadakis, 2006; Hashemite Kingdom of Jordan, 2007). The main products of cultivated olive trees are table olives and olive oil (Amici et al., 1991).

Olive oil can be produced either by traditional discontinuous pressing, or by a continuous centrifugation cycle (Kapellakis et al., 2008). Olive oil is the major product of the olive fruit milling process and generates, in addition to the solid waste or de-oiled cake, semi-solid and aqueous liquor by-products, including olive oil mill waste water and olive oil lees (*Amurca*) (Caputo et al., 2003; Niaounakis & Halvadakis, 2006).

Amurca is a dark, bitter-tasting, sticky, viscous liquid, which settles at the bottom of olive oil containers over time (Niaounakis & Halvadakis, 2006). It is abundant in Jordanian olive oil extracted under low pressure in traditional mills. Historically, *amurca* has been used by Mediterranean farmers as a herbicide, pesticide, fungicide and fertilizer. It has also been used as a dried fruit preservative according to ancient records from the area, and has found use as a polish for floors and has been used for oiling leather, wood, metals, and axles (Niaounakis & Halvadakis, 2006; Quiles et al., 2006; Smith & Secoy, 1975).

Amurca is composed of fat (49.4%), water (47.3%), carbohydrate (0.7%), protein (0.7%), ash (0.9%), and has a relatively high content of phenolic compounds (289 mg gallic acid equivalent/100g) including oleuropein, isopropyl-5-methyl phenol, sinapic acid, luteolin, gallic acid, kaempferol and 3-hydroxy phenol (Janakat & Hammad, 2013). These phenolic compounds showed strong inhibitory effects against several spoilage and pathogenic bacteria; for example, oleuropein inhibited production of enterotoxin B by *Staphylococcus aureus* (Tranter et al., 1993), inhibited growth of *Salmonella* Enteritidis (Tassou & Nychas, 1995), and germination of *B. cereus* T spores (Tassou et al., 1991).

The high concentration of phenolic compounds in olive oil contributes significantly toward its antioxidant and antimicrobial activity (Capasso et al., 1995; Kecel & Robinson, 2002; Markin et al., 2003; Pereira et al., 2006). The antimicrobial activity of oleuropein, hydroxytyrosol and tyrosol in virgin olive oil stems from their ability to interact with phosphatidylglycerol at the surface of the bacterial cell wall (Casas-Sanchez et al., 2007; Medina et al., 2006; Soler-Rivas et al., 2000).

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Several studies have found that virgin olive oil, olive fruit, olive oil mill waste water, and olive leaves possessed antimicrobial activity against a broad spectrum of microorganisms including Gram-negative and Gram-positive bacteria, fungi, viruses, and parasitic protozoan (Medina et al., 2006; Pereira et al., 2007; Ramos-Cormenzana et al., 1996; Soler-Rivas et al., 2000; Sudjana et al., 2009). However, no studies have examined the antimicrobial activity of *amurca*. Therefore, the objective of the current work was to evaluate aspects of the antimicrobial activity of *amurca* against a group of foodborne pathogens.

2 Materials and methods

2.1 Amurca samples

Amurca was obtained from a local olive oil mill in the province of Ajloun, Jordan. The samples were centrifuged at 3000 rpm (723 *xg*) for 20 min to sediment the *amurca* and allow its better separation from the remaining olive oil. The samples were kept at –18 °C until use.

2.2 Foodborne pathogens

Escherichia coli O157:H7 strains 02-0304 and 02-0628 which had become non-pathogenic (verotoxigenic negative) during storage were provided by Rafiq Ahmed, National Microbiology Laboratory, Public Health Agency, Canadian Science Centre for Human and Animal Health, Winnipeg, MB, Canada. *Listeria monocytogenes* strains 1 and 4 were isolated from processed meat and dairy products, respectively, in Jordan. *Salmonella* Enteritidis was provided by the Jordan Food and Drug administration, and *Staphylococcus aureus* strains 25923 and 26127 were from the Department of Nutrition and Food Technology, Jordan University of Science and Technology.

2.3 Amurca methanolic extract

Two hundred grams of *amurca* were soaked in 99% methanol at a ratio 1:3 (w/v) for 24 h at 4 °C. The mixture was homogenized using a household blender for one min at full speed, filtered through a double layer of cheese cloth, and centrifuged at 3000 rpm (723 *xg*) for 15 min. The supernatant was dried using a rotary evaporator (model 4001, Heidolph Laborota, Schwabach, Germany) at 40 °C and 120 rpm. The dried matter was re-suspended in a ratio of 1:2 with 2% (w/v) dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St Louis, MO, USA) and was sterilized by filtration with a disposable syringe filter unit (0.20 µm) (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and kept at –18 °C until used for antimicrobial assays.

2.4 Protein precipitation by ammonium sulphate

Protein in 100 ml of the *amurca* methanolic extract was precipitated by slowly adding ammonium sulphate until its concentration reached 100%. The mixture was centrifuged at 3000 rpm (723 *xg*) for 20 min at 4 °C. The supernatant was dialyzed against 0.05 M phosphate buffer, pH 7.2 for 24 h at 4 °C and the precipitate was re-suspended to its original volume using the same buffer. The dialyzed portion was sterilized by filtration with a disposable syringe filter unit (0.20 µm) and kept for antimicrobial assay (Janakat et al., 2004).

2.5 Determination of Amurca protein by the lowry method

A dilution series of a protein standard (0.3 µl/mL bovine serum albumin in distilled water) was prepared to yield concentrations of 0.03 to 0.18 µl/ml. Twenty microliters *amurca* samples were taken and protein concentration was determined using the Lowry method (Lowry et al., 1951).

2.6 Antimicrobial assay

(a) Preparation of culture. Test cultures (*E. coli* O157:H7 strains 02-0304 and 02-0628, *L. monocytogenes* strains 1 and 4, *S.* Enteritidis, and *S. aureus* strains 25923 and 26127) were kept at –40 °C in Brain Heart Infusion (BHI, Oxoid Ltd, Basingstoke, UK) broth containing 20% glycerol. Working cultures were prepared by transferring a single colony to Tryptone Soy Broth, TSB (Oxoid Ltd.) and incubated 37 °C for 24 h. Then 100 µl was transferred to 10 ml TSB prior to the experiment and incubated for 24 h at 37 °C.

(b) Microbroth dilution. Minimum Inhibitory (MIC) and Minimum Bactericidal (MBC) concentrations of *amurca* against tested organisms were determined as described by Al-Nabulsi et al. (2011) in Mueller Hinton (MH) broth (Himedia Ltd., Mambai, India). Ninety six well microtiter plates (Greiner Bio-One, CellStar™, Italy) were used to evaluate the effects of different concentrations of methanolic *amurca* extract on the viability of foodborne pathogens in the present study. Of the 300 µl well capacity, 250 µl was used as follows: 100 µl was from the tested culture diluted in MH broth to give a final inoculum of 6.0 $\log_{10}$ CFU/ml; 100 µl of MHB was added, then 5, 10, 15, or 20 µl of sterile methanolic *amurca* mixture (containing 33.3% (w/v) dry amurca extract) was added, and the volume was completed by the addition of 30, 35, 40 or 45 µl of sterile distilled water to give a final concentration 20, 40, 60, or 80 µl/ml of methanolic *amurca* extract, respectively. Negative control wells contained 200 µl MH broth plus 20, 15 or 10 µl of sterile *amurca* methanolic extract, and the volume was completed by the addition of 30, 35 or 40 µl of sterile distilled water. Positive control wells contained 100 µl culture mixed with 100 µl of MH broth and 50 µl of sterile distilled water. The purpose of the negative control was to enable the subtraction of its absorbance from the absorbance of other wells, since *amurca* had a dark color. Samples were incubated at 37 °C for 24 h or at 10 °C for 7 d and absorbance was read with a microplate reader at 620 nm (MR 5000, Dynatech, Mount Holly, NJ, USA). The MBC (the lowest concentration of antimicrobial required to kill 99.9% of the initial viable cells) was determined by plating 100 µl of the samples in each well on Tryptone Soy Agar, TSA (Oxoid).

(c) Agar well diffusion. Methods by Janakat et al. (2005) and Gupat et al. (2008) were used with some modification. Samples of 100 µl to yield a final concentration of 6 $\log_{10}$ CFU/ml of each test organism were spread on the surface of MH agar, MHA (Himedia Ltd.) and allowed to stand for 30 min at room temperature. Then sterile blank disks containing 25 µl sterile methanolic *amurca* extract (80 µl/ml) or sterile antibiotic disks

containing chloramphenicol (30 µg), erythromycin (15 µg), gentamycin (10 µg), or tetracycline (30 µg) were placed on the surface of MHA and incubated at 37 °C for 24 h. The inhibition zones produced were recorded to the nearest mm.

2.7 Statistical analysis

The data were analyzed using Statistical Analysis Software (SAS, version 7, 2000, USA). Factorial analysis of variance (ANOVA) was performed on all parameters. Means were calculated for all variables in the study and the least square means were used to determine significant differences. Findings with a p-value of < 0.05 were considered to be statically significant.

3 Results

3.1 Antimicrobial activity of amurca methanolic extract by microdilution at 10 °C or 37 °C

An obvious difference ($p < 0.05$) in the antimicrobial activity of different concentrations of *amurca* extract at 10 and 37 °C was observed against the foodborne pathogens (Tables 1, 2, 3). *Amurca* extract was more inhibitory at 10 °C where the MIC and MBC were 40 and 60 µl/ml, respectively, against all pathogens compared to 60 and 80 µl/ml except *L. monocytogenes* 4 and *S. aureus* 26127 which had MBC values of 60 µl/ml at 37 °C (Table 1). *Amurca* concentration at 40 and 60 µl/ml reduced numbers of tested pathogens by < 0.5 $\log_{10}$ CFU/ml and 2.5-9.8 $\log_{10}$ CFU/ml, respectively, compared to control at 37 °C after 24 h (Table 2); while at 10 °C, pathogen numbers were reduced by 2.5-3.2 $\log_{10}$ CFU/ml and 9.0-9.5 $\log_{10}$ CFU/ml at 40 and 60 µl/ml *amurca* extract, respectively. Moreover, all organisms tested were not detected at 60 and 80 µl/ml *amurca* extract at 10 and 37 °C, respectively (Table 3).

3.2 Antimicrobial activity of amurca methanolic extract by disk diffusion and its activity relative to selective antibiotics

The antimicrobial activity of *amurca* extracts determined by the dilution method were confirmed by disk diffusion. The *amurca* methanolic extract at 80 µl/ml gave inhibitory zones of 9 mm with all Gram-negative and Gram-positive bacteria except for *E. coli* O157:H7 02-0628 and *S. aureus* 26127, which showed inhibitory zones of 10 mm and 20 mm, respectively (Tables 4, 5).

Table 1. MIC and MBC (µl/ml) of selected foodborne pathogens strains at 37 °C after 24 h or at 10 °C after 7d.

Organism	10 °C		37 °C	
	MIC [a]	MBC [b]	MIC [a]	MBC [b]
E. coli O157:H7 02-0628	40	60	60	80
E. coli O157:H7 02-0304	40	60	60	80
L. monocytogenes 1	40	60	60	80
L. monocytogenes 4	40	60	60	60
S. Enteritidis	40	60	60	80
S. aureus 26127	40	60	60	60
S. aureus 25923	40	60	60	80

[a] The lowest concentration at which no visible growth occurred. [b] The lowest concentration cause 99.9% reduction of initial inoculation.

The relative antimicrobial activity of 80 µl/ml *amurca* methanolic extract to chloramphenicol, erythromycin, gentamycin and tetracycline (calculated as a ratio of inhibition zones, *amurca*/antibiotic) ranged from 0.36 to 1.0 against Gram-negative bacteria (Table 4) and 0.45 to 2.0 against Gram-positive bacteria (Table 5). It is notable that *E. coli* O157:H7 02-0628 and *S. aureus* 26127 were resistant to tetracycline and chloramphenicol, respectively, but the *amurca* extract at 80 µl/ml produced inhibitory zones of 10 and 20 mm, respectively (Tables 4, 5). The relative inhibitory activity of *amurca* in these instances was estimated to be 10- and 20-fold greater than the compared antibiotic.

3.3 Effect of protein precipitated from Amurca extract on growth of foodborne pathogens

When examined for inhibitory effects on pathogen growth, the proteins recovered after precipitation by ammonium sulfate from 40, 60 or 80 µl/ml *amurca* extract did not prove inhibitory against tested strains. In these tests, bacterial numbers increased about 4 $\log_{10}$ CFU/ml and reached 9.2 to 9.6 $\log_{10}$ CFU/ml by 24 h at 37 °C (Table 6).

4 Discussion

The *amurca* extract showed substantial antimicrobial activity against tested foodborne pathogens in the current study. The MIC values reported here were similar to those reported by Pereira et al. (2007) who found that extracted olive leaves inhibited the growth of *E. coli* and *S. aureus* with MIC values of 50 µl/ml and 25 µl/ml, respectively, at 37 °C. In another study, Pereira et al. (2006) reported that the MICs of a Portuguese table olive extract against *E. coli, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, ranged from 10 to 100 µl/ml at 37 °C. *B. cereus* numbers were reduced by 2.7 $\log_{10}$ CFU/ml in the presence of 5 µl/ml olive powder in BHI broth at 20 °C (Ferrer et al., 2009).

Since proteins recovered after precipitation from the a*murca* extract did not inhibit the growth of tested foodborne pathogens, it is possible that the potent antimicrobial activity of *amurca* reported in the present study may have been caused by the phenolic compounds present (Table 6). This would be consistent with previous reports showing that phenolic compounds in olive products (olive fruit, olive oil, olive leaves) and olive oil by-products (olive oil mill waste water, olive cake) exhibited antibacterial activity (Capasso et al., 1995; Kecel & Robinson, 2002; Markin et al., 2003; Pereira et al., 2006).

The antibacterial activity of phenolic compounds was believed due to their ability to inflict bacterial membrane damage and disrupt the cell wall peptidoglycan, which cause loss of structural integrity and leakage of intracellular cytoplasmic constituents such as protein, glutamate, potassium and phosphate (Caturla et al., 2005). Moreover, the hydroxyl group in phenolic compounds may bind the active sites of enzymes and change their substrate affinity. In addition, their lipid solubility and the degree of steric hindrance they cause may also contribute to their overall antimicrobial activity (Ceylan & Fung, 2004).

Table 2. Antimicrobial activity of *amurca* methanolic extract (40 to 80 µl/ml) against selected foodborne pathogens strains at 37 °C after 24h.

Organism	Initial number (log_{10} CFU/ml)	Control number, 24 h (log_{10} CFU/ml)	*Amurca* concentration (µl/ml)	Count after treatment, 24 h (log_{10} CFU/ml)	Reduction* (log_{10} CFU/ml)
E. coli O157:H7 02-0628	6.0 ± 0.05	9.5 ± 0. 20	40	9.5 ± 0.10	0.0
			60	6.1 ± 0.10	–3.4
			80	ND	–9.5
E. coli O157:H7 02-0304	5.8 ±0.17	9.3 ± 0.20	40	9.4 ± 0.30	+0.1
			60	6.8 ± 0.06	–2.5
			80	ND	–9.3
L. monocytogenes 1	5.3 ± 0.05	9.8 ± 0.20	40	9.7 ± 0.10	–0.1
			60	5.8 ± 0.10	–4.0
			80	ND	–9.8
L. monocytogenes 4	5.7 ± 0.17	9.0 ± 0.20	40	9.2 ± 0.30	+0.2
			60	ND	–9.0
			80	ND	–9.0
S. Enteritidis	5.9 ± 0.05	9.3 ± 0.20	40	9.0 ± 0.20	–0.3
			60	6.3 ± 0.30	–3.0
			80	ND	–9.3
S. aureus 26127	5.6 ± 0.16	9.8 ± 0.20	40	9.3 ± 0.20	–0.5
			60	ND	–9.8
			80	ND	–9.8
S. aureus 25923	5.6 ± 0.26	9.6 ± 0.20	40	9.3 ±0.20	–0.3
			60	6.7 ± 0.30	–2.9
			80	ND	–9.6

Values are expressed as mean ± SD (n = 3). ND: Bacterial cells were not detected by transferring 0.1 ml to 10 ml TSB and no growth was observed after 24 h at 37 °C (detection level was ≤ 1 CFU/ml). *Difference after 24 h. – magnitude of reduction compared to control, + increase in the viability compared to control.

Table 3. Antimicrobial activity of *amurca* methanolic extract (20 to 60 µl/ml) against selected foodborne pathogens strains at 10 °C by 7 d.

Organism	Initial number (log_{10} CFU/ml)	Control count, 7 d (log_{10} CFU/ml)	Amurca concentration (µl/ml)	Count after treatment, 7 d (log_{10} CFU/ml)	Reduction* (log_{10} CFU/ml)
E. coli O157:H7 02-0628	6.0 ± 0.05	9.3 ± 0.20	20	9.1 ± 0.10	–0.2
			40	6.5 ± 0.10	–2.8
			60	ND	–9.3
E. coli O157:H7 02-0304	5.8 ±0.17	9.1 ± 0.10	20	8.8 ± 0.20	–0.3
			40	6.6 ± 0.20	–2.5
			60	ND	–9.1
L. monocytogenes 1	5.3 ± 0.05	9.5 ± 0.20	20	9.2 ± 0.10	–0.3
			40	6.3 ± 0.20	–3.2
			60	ND	–9.5
L. monocytogenes 4	5.7 ± 0.17	9.0 ± 0.10	20	8.9 ± 0.20	–0.1
			40	6.2 ± 0.20	–2.8
			60	ND	–9.0
S. Enteritidis	5.9 ± 0.05	9.4 ± 0.40	20	9.1 ± 0.10	–0.3
			40	6.6 ± 0.20	–2.8
			60	ND	–9.4
S. aureus 26127	5.6 ± 0.16	9.3 ± 0.30	20	9.0 ± 0.10	–0.3
			40	6.3 ± 0.06	–3.0
			60	ND	–9.3
S. aureus 25923	5.6 ± 0.26	9.4 ± 0.20	20	8.8 ±0.20	–0.6
			40	6.3 ± 0.20	–3.1
			60	ND	–9.4

Values are expressed as mean ± SD (n = 3). ND: Bacterial cells were not detected by transferring 0.1 ml to 10 ml TSB and no growth was observed after 24 h at 37 °C (detection level was ≤ 1 CFU/ml). *Difference after 7 d. – magnitude of reduction compared to control, + increase in the viability compared to control.

Although *amurca* contains 9 times more phenolic compounds than olive oil (Janakat et al., 2013), its inhibitory effects in the present study were less than previously reported for olive oil. For example, the MIC and MBC of olive oil extract were 0.4 µl/ml and 0.93 µl/ml, respectively, against *L. monocytogenes* at 37 °C (Bubonja-Sonje et al., 2011). Virgin olive oil also reduced *L. monocytogenes* and *S.* Enteritidis numbers by approximately 3 log CFU/g after 30 min in mayonnaise and salads (Medina et al., 2007). These differences in antimicrobial action may have been the result of variation in the phenolic profiles of the olive oil and *amurca* as well as differences in the concentration and activity of specific compounds in these products. The most bactericidal phenolic compounds in olive oil were found to be the dialdehydic form of decarboxymethyl

Table 4. Relative antimicrobial activity[1] of 80 µl/ml *amurca* methanolic extract against selected Gram-negative pathogenic strains.

Foodborne Pathogen	Antibiotic[2]	Inhibition Zone (mm)	Inhibition Zone (mm) by *amurca*	Relative Antimicrobial Activity
E. coli O157:H7 02-0628	Chloramphenicol	10	10	1.00
	Erythromycin	12	10	0.83
	Gentamycin	17	10	0.59
	Tetracycline	0	10	CD[3]
E. coli O157:H7 02-0304	Chloramphenicol	18	9	0.50
	Erythromycin	10	9	0.93
	Gentamycin	16	9	0.56
	Tetracycline	14	9	0.64
S. Enteritidis	Chloramphenicol	24	9	0.36
	Erythromycin	11	9	0.82
	Gentamycin	19	9	0.47
	Tetracycline	14	9	0.64

[1] Relative antimicrobial activity = (mean diameter of inhibition zone of *amurca*/mean diameter of inhibition zone of reference antibiotic). [2] Chloramphenicol 30 µg, erythromycin 15 µg, gentamycin 10 µg, tetracycline 30 µg. [3] CD, calculated differently. Relative inhibition was estimated as 10-fold greater for *amurca*.

Table 5. Relative antimicrobial activity[1] of 80 µl/ml *amurca* methanolic extract against selected Gram-positive pathogenic strains.

Bacterial strain	Antibiotic[2]	Inhibition zone (mm)	inhibition zone (mm) by *amurca*	Relative antimicrobial activity
L. monocytogenes 1	Chloramphenicol	20	9	0.45
	Erythromycin	11	9	0.82
	Gentamycin	16	9	0.56
	Tetracycline	12	9	0.75
L. monocytogenes 4	Chloramphenicol	20	9	0.45
	Erythromycin	11	9	0.82
	Gentamycin	15	9	0.60
	Tetracycline	12	9	0.75
S. aureus 26127	Chloramphenicol	0	20	CD[3]
	Erythromycin	13	20	1.54
	Gentamycin	20	20	1.00
	Tetracycline	10	20	2.00
S. aureus 25923	Chloramphenicol	21	9	0.43
	Erythromycin	11	9	0.82
	Gentamycin	18	9	0.50
	Tetracycline	14	9	0.64

[1] Relative antimicrobial activity = (mean diameter of inhibition zone of *amurca*/mean diameter of inhibition zone of reference antibiotic). [2] Chloramphenicol 30 µg, erythromycin 15 µg, gentamycin 10 µg, tetracycline 30 µg. [3] CD, calculated differently. Relative inhibition was estimated as 20-fold greater for *amurca*.

Table 6. The $\log_{10}$ number of selected foodborne pathogens in the presence of 40 to 80 µl/ml of ammonium sulfate-precipitated protein from *amura* methanolic extract at 37 °C.

Organism	Initial number ($\log_{10}$ CFU/ml)	Bacterial numbers ($\log_{10}$ CFU/ml) at *amurca* concentration (µl/ml) in ASPP after 24 h at 37 °C			
		0	40	60	80
E. coli O157:H7 02-0628	5.6±0.31[b]	9.6±0.10[a]	9.4±0.30[a]	9.5±0.22[a]	9.4±0.23[a]
E. coli O157:H7 02-0304	5.8±0.20[b]	9.4±0.16[a]	9.4±0.10[a]	9.2±0.20[a]	9.3±0.16[a]
L. monocytogenes 1	5.3±0.17[b]	9.1±0.20[a]	9.3±0.10[a]	9.5±0.10[a]	9. 2±0.31[a]
L. monocytogenes 4	5.7±0.11[b]	9.3±0.24[a]	9.2±0.10[a]	9.3±0.31[a]	9.4±0.20[a]
S. Enteritidis	5.9±0.19[b]	9.2±0.21[a]	9.5±0.20 [a]	9.2±0.15[a]	9.2±0.10[a]
S. aureus 26127	5.6±0.20[b]	9.1±0.16[a]	9.1±0.15[a]	9.2±0.17[a]	9.3±0.11[a]
S. aureus 25923	5.6±0.20[b]	9.2±0.21[a]	9.3±0.11[a]	9.5±0.20[a]	9. 2±0.10[a]

Values are expressed as mean ± SD (n = 3). *P*-values were calculated by Students *t*-test. Means with different superscripts [a,b,] differ significantly $P < 0.05$. ASPP indicates ammonium sulfate-precipitated protein.

oleuropein and ligstroside aglycones, hydroxytyrosol and tyrosol (Medina et al., 2006). The MICs of oleuropein and hydroxytyrosol against *S. aureus* strain 25923 and *Salmonella* Typhi strain 6539 were 0.24 to 7.85 µg/ml and 62.5 to 500 µg/ml, respectively (Bisignano et al., 1999).

In general, Gram-positive bacteria are more sensitive to olive oil than Gram-negatives (Medina et al., 2007). In the present study, *L. monocytogenes* 4 and *S. aureus* 26127 had lower *amurca* MBCs at 37 °C than the other organisms. Casas-Sanchez et al. (2007) also reported that Gram-negative bacteria

were more resistant than Gram-positive bacteria towards oleuropein. This may be attributed to the protection from *amurca* exposure afforded the Gram-negatives by their outer cell (wall) membrane, which is absent from Gram-positive bacteria.

The antimicrobial activity of olive products (olive fruit, olive oil, olive leaves) and olive oil by-products against foodborne pathogens at refrigerated temperatures have not been previously reported, except that Ferrer et al. (2009) found that 1.5 to 5 µl/ml olive powder inhibited growth of *B. cereus* at 7 °C. The inhibitory effects of *amurca* were improved by lower storage temperature as reflected by the lower MICs and MBCs at 10 °C compared to 37 °C, but complete bacterial inactivation took longer at 10 °C. It is likely that the lower temperature delayed the hydrolytic and oxidative degradation of phenolic compounds in the *amurca* extract. It has been reported that the total phenolic content of olive oil was increased in cold pressed olives (Parenti et al., 2008). Also, oil that was obtained from olives stored at 5 to 10 °C experienced lower hydrolytic, oxidative degradation and off-odour development (Clodoveo et al., 2007; Inarejos-García et al., 2010). Therefore, *amurca* extract should be considered a potential natural antimicrobial for enhancing the keeping quality of foods that will be stored for extended periods at refrigerated temperatures.

The increased occurrence of foodborne pathogens resistant towards antibiotics puts pressure on authorities that regulate their clinical and agricultural use, but serves as motivation for researchers to find effective natural antimicrobials to use as food preservatives. The effectiveness of natural antimicrobials, such as plant-derived antimicrobials have been demonstrated against foodborne pathogens (Holley & Patel, 2005; Tajkarimi et al., 2010). The results of the present study indicated that *amurca* extract was inhibitory to some antibiotic resistant bacteria which suggests *amurca* extract might be used as an alternative hurdle to address the more frequent occurrence of antibiotic resistant bacteria in the environment, thus reducing the risk associated with foodborne pathogen infection.

5 Conclusions

The results showed that *amurca* extract has potent antimicrobial activity against Gram-positive (*L. monocytogenes* and *S. aureus*) and Gram-negative (*E. coli* O157:H7 and *S.* Enteritidis) foodborne pathogens. Further, *amurca* extract was inhibitory to two antibiotic resistant pathogenic bacteria. The greater antimicrobial activity of *amurca* extract at 10 °C than 37 °C may prove to be of value for maintaining the safe shelf-life of perishable foods. This antibacterial activity may be explained by the high content of phenolic compounds in the *amurca* extract. Further investigation will be conducted to evaluate the effectiveness of *amurca* extract in inhibiting the foodborne pathogens in model food systems and examine the sensory qualities of foods treated with the *amurca* extract.

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