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Potential roles of essential oil and organic extracts of *Zizyphus jujuba* in inhibiting food-borne pathogens

Sharif M. Al-Reza [a,b], Atiqur Rahman [b], Jonghwi Lee [c], Sun Chul Kang [a,*]

[a] Department of Biotechnology, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea
[b] Department of Applied Chemistry and Chemical Technology, Islamic University, Kushtia 7003, Bangladesh
[c] Department of Chemical Engineering and Materials Science, Chung-Ang University, Dongjak-gu, Seoul 156-756, Republic of Korea

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ABSTRACT

This study was undertaken to examine the chemical compositions of essential oil and tested the efficacy of oil and organic extracts from seeds of *Zizyphus jujuba* against food-borne pathogens. The chemical compositions of the oil was analysed by Null. Twenty three compounds representing 91.59% of the total oil were identified. The oil (5 μl of 1:5 (v/v) dilution of oil with methanol) and extracts of hexane, chloroform, ethyl acetate and methanol (300 μg/disc) of *Z. jujuba* displayed a remarkable antibacterial activity against *Staphylococcus aureus* (ATCC 6538 and KCTC 1916), *Listeria monocytogenes* ATCC 19166, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* KCTC 2004, *Salmonella typhimurium* KCTC 2515 and *Escherichia coli* ATCC 8739. The scanning electron microscopic studies also demonstrated the effect of essential oil on the morphology of *Staph. aureus* ATCC 6538 at the MIC value, along with the potential effect on cell viabilities of the tested bacteria.

1. Introduction

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against food-borne pathogens (Deans & Ritchie, 1987). In recent years, two consumer-driven demands have arisen in the food industry. The first is the provision of fresh and natural food technology required minimal preparation while the second is the control of food safety (Knobloch, Pauli, & Iberi, 1989). Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food borne disease. Currently there is a growing interest in using natural antibacterial compounds, like essential oils and extracts of various species of edible and medicinal plants, herbs, and spices which have long been used as natural agents for food preservation in food and beverages due to the presence of antimicrobial compounds (Nychas, Tassou, & Skandamis, 2003). In general, plant-derived essential oils are considered as non-phytotoxic compounds and potentially effective against microorganisms. However, it has also been evident that when essential oils are inappropriately used, they can give rise to adverse effects to humans such as skin irritation, headache and nausea (Aromacaring, 2004). Caution is generally required if essential oils are to be taken internally or used on food commodities because of the possible cancer-causing effects of some of them (McGuffin, Hobbs, Upton, & Goldberg, 1997). In this context, the identification and evaluation of natural products for the control of these pathogens and to assure consumers a safe, wholesome, and nutritious food supply, can be considered an important international innovative challenge in food technology.

Furthermore, with an increasing of bacterial resistance to antibiotics, there is considerable interest in investigating the antimicrobial effects of essential oils and different extracts against a wide range of bacteria, to develop other classes of natural antimicrobials useful for food preservation. Among those, a Gram-positive bacterium *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mylotte, McDermott, & Spooner, 1987). Also, *Listeria monocytogenes*, responsible for the severe food-borne illness, listeriosis, has been recognised to be one of the emerging zoonotic diseases during the last two decades (Farber, 2000). The Gram-negative bacterium *Escherichia coli* is present in human intestines and causes urinary tract infection, coleocystitis or septicaemia (Singh, Chandra, Bose, & Luthra, 2000).

Zizyphus jujuba is a thorny rhamnaceous plant that is widely distributed in Europe and Southeastern Asia. Fruits of this plant are edible and different parts of *Z. jujuba* possess multiple medicinal properties such as antifertility, analgesic, and antidiabetes

* Corresponding author. Address: Department of Biotechnology, College of Engineering, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea. Tel.: +82 53 850 6553; fax: +82 53 850 6559.
E-mail address: sckang@daegu.ac.kr (S.C. Kang).

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(Ambasta, 1986; Erenmemisoglu et al., 1995). The local tribal people use the bark mixture of *Z. jujuba* to prevent pregnancy (Souleles & Shammas, 1998).

Therefore, the aims of the present study were (a) to examine the chemical composition of the essential oil from seeds of *Z. jujuba* by Null; and (b) to evaluate the antibacterial activity of essential oil from seeds of *Z. jujuba* and various organic extracts (hexane, chloroform, ethyl acetate and methanol) against a diverse range of food-borne pathogenic bacteria with emphasis for the possible future use of the essential oil and plant extracts as an alternative to chemical bactericides for food preservation.

2. Materials and methods

2.1. Plant material

The seeds of *Z. jujuba* were collected from the local area of Kyoungsan, Republic of Korea, in August 2008. Seeds were cleaned, dried and ground. Initially the seeds were identified by morphological features and an in-house data base by Prof. Man Kyu Huh. A voucher specimen number was deposited in the Herbarium of the College of Engineering, Department of Biotechnology, Daegu University, Republic of Korea.

2.2. Isolation of the essential oil

About 250 g ground seeds of *Z. jujuba* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4 °C until further analysis.

2.3. Preparation of organic extracts

The ground seeds (100 g) of *Z. jujuba* were extracted with 200 ml of each organic solvent (hexane, chloroform, ethyl acetate and methanol) separately for 7 days at room temperature and the solvents were evaporated by vacuum rotary evaporator (EYELA N-1000, Japan). The extraction process yielded in hexane (3.5 g), chloroform (5.2 g), ethyl acetate (6.1 g) and methanol (5.9 g) extracts. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, St. Louis, MO, USA).

2.4. Gas chromatography-mass spectrometry analysis

Quantitative and qualitative analysis of the essential oil was performed using a Null (Model QP 2010, Shimadzu, Japan) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 μm). For Null detection, an electron ionisation system with an ionisation energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 μl were manually injected in the split less mode. The relative percentage of the oil constituents was expressed as percentage by peak area normalisation.

Identification of components of the essential oil was based on their retention indices, relative to a homologous series of *n*-alkane (C_8–C_{20}) on the ZB-1 capillary column under the same operating conditions and computer matching with the Wiley 6.0 libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature data (Adams, 2001).

2.5. Microbial strains

A panel of food-borne pathogenic bacterial including four Gram-positive (*Staph. aureus* ATCC 6538, *Staph. aureus* KCTC 1916, *L. monocytogenes* ATCC 19166, *Bacillus subtilis* ATCC 6633) and five Gram-negative (*Pseudomonas aeruginosa* KCTC 2004, *Salmonella typhimurium* KCTC 2515, *E. coli* ATCC 8739, *Enterobacter aerogenes* KCTC 2190 and *S. enteritidis* KCTC 12021) were used in this study. All the strains were obtained from the Korea Food and Drug Administration (KFDA), Daegu, Republic of Korea. Active cultures for experimental use were prepared by transferring a loopful of cells from stock cultures to flasks and inoculated in Luria–Bertani (LB) broth medium at 37 °C for 24 h. Cultures of each bacterial strains were maintained on LB agar medium at 4 °C.

2.6. Antibacterial activity assay

The dried extracts were dissolved in the same solvent used for their extraction to a final concentration of 30 μg/μl and sterilised by filtration by 0.45 μm Millipore filters (Millipore Corp., Bedford, MA, USA). The antibacterial test was then carried out by an agar disc diffusion method (Murray, Baron, Pfaller, Tenover, & Yolke, 1995) using 100 μl of standardised inoculum suspension containing 10^7 CFU/ml of bacteria. The essential oil was diluted 1:5 (v/v) with methanol and aliquots of 5 μl were spotted onto the filter paper discs; while 10 μl of 30 μg/μl of each organic extract (300 μg/disc) was applied to the filter paper discs (6 mm diameter) and placed on the inoculated LB agar. Negative controls were prepared using the same solvents employed to dissolve the samples. Standard reference antibiotics, tetracycline and streptomycin (10 μg/disc, each from Sigma–Aldrich Co., St. Louis, MO, USA), were used as positive controls for the tested bacteria. The plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria. Where, 7–10 mm, weak inhibition; 11–14 mm, moderate inhibition; >15 mm, strong inhibition. Each assay in this experiment was replicated 3×.

2.7. Minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of essential oil and organic extracts, standard NCCLS method was used (NCCLS, 2000). Active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks and inoculated in LB medium and incubated at 37 °C for 24 h. The cultures were diluted with LB broth to achieve an optical density of 10^7 CFU/ml for the test organisms at 600 nm by UV/Vis Spectrophotometer Optizen 2120UV & Optizen III (Shin, Bajpai, Kim, & Kang, 2007). Dilutions, to get the final concentration ranging from 0 to 1000 μg/ml of essential oil and extracts of hexane, chloroform, ethyl acetate and methanol in LB broth medium were prepared in a 96-well microplates. Finally, 20 μl inoculums of each bacteria strain (10^7 CFU/ml) was inoculated the microplates and the tests were performed at a volume of 200 μl. The plates were incubated at 37 °C for 24 h. The lowest concentration of the test samples, which did not show any visual growth of tested organisms after macroscopic evaluation, was determined as MIC, which was expressed in μg/ml.

2.8. Effect of essential oil on viable counts of bacteria

For viable counts, each of the tubes containing a bacterial suspension (approximately 10^7 CFU/ml) of *L. monocytogenes* ATCC 19166, *Staph. aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633 in LB broth medium was inoculated with the minimum inhibitory concentration of the essential oil in 10 ml LB broth, and kept at

37 °C (Bajpai, Rahman, & Kang, 2008). Samples for viable cell counts were taken out at 0, 20, 40, 60, 80 and 100 min time intervals. Enumeration of viable counts on LB plates was monitored as follows: after incubation, 1 ml of the resuspended culture was diluted into 9 ml buffer peptone water, thereby diluting it, and 1 ml of each dilution was spread on the surface of LB agar. The colonies were counted after 24 h of incubation at 37 °C. The controls were inoculated without essential oil for each bacterial strain with the same experimental condition as mentioned above.

2.9. Scanning electron microscopic (SEM) analysis

To determine the efficacy of essential oil and the morphological changes, SEM studies were performed on *Staph. aureus* ATCC 6538 treated with MIC of essential oil of *Z. jujuba*. Controls were prepared without essential oil. Further, to observe the morphological changes, the method of SEM was modified from Kockro method (Kockro et al., 2000). The bacterial samples were washed gently with 50 mM phosphate buffer solution (pH 7.3), fixed with 2.5 g/100 ml glutaraldehyde and 1 g/100 ml osmic acid solution. The specimen was dehydrated using sequential exposure per ethanol concentrations ranging from 30–100%. The ethanol was replaced by tertiary butyl alcohol. After dehydration, the specimen was dried with CO_2. Finally, the specimen was sputter-coated with gold in an ion coater for 2 min, followed by microscopic examinations (S-4300; Hitachi High Technologies America, Schaumburg, Illinois, USA).

2.10. Statistical analysis

The essential oil and different organic extracts were assayed for antibacterial activity. Each experiment was run in triplicate, and mean values were calculated. The statistical analysis was carried out employing one way ANOVA ($p < 0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

3. Results

3.1. Chemical composition of the essential oil

The hydrodistillation of the ground seeds of *Z. jujuba* yielded a pale yellow coloured oil (yield: ~0.8%, w/w). Upon GC/MS analysis, the essential oil was found to contain 23 different compounds, representing 91.59% of the total oil. The identified compounds are listed in Table 1 according to their elution order on a ZB-1 capillary column. The major compounds detected were eugenol (48.3%), isoeugenol (11.83%), caryophyllene (9.16%), eucalyptol (3.27%), caryophyllene oxide (3.14%), benzaldehyde (2.88%), veridiflorol (2.31%). α-Humulene (1.85%), tetradecanoic acid (1.77%), γ-muurolene (0.82%), trans-Z-α-bisabolene epoxide (0.78%), chavicol (0.68%) and δ-cadinene (0.65%) were also found to be the minor components of *Z. jujuba* oil in the present study.

Table 1
Chemical composition of essential oil from seeds of *Z. jujuba*.

RI[a]	Components	% RA[b]	Identification[c]
819	Ethylacetamide	0.28	RL MS
974	Hexanoicacid	0.56	RL MS
982	Benzaldehyde	2.88	RL MS
1059	Eucalyptol	3.27	RL MS
1073	Heptanoic acid	0.61	RL MS
1160	Benzyl acetate	0.47	RL MS
1173	Octanoic acid	0.32	RL MS
1189	Cinnamaldehyde	0.29	RL MS
1203	Chavicol	0.68	RL MS
1392	Eugenol	48.3	RL MS
1410	Isoeugenol	11.83	RL MS
1435	y-Murolene	0.82	RL MS
1469	5-Cadinene	0.65	RL MS
1494	Caryophyllene	9.16	RL MS
1530	Ledol	0.59	RL MS
1531	trans-Z-a-bisabolene epoxide	0.78	RL MS
1567	Caryophyllene oxide	3.14	RL MS
1568	Veridiflorol	2.31	RL MS
1570	Dodecanoic acid	0.45	RL MS
1579	a-Humulene	1.85	RL MS
1616	Turner one	0.23	RL MS
1769	Tetradecanoic add	1.77	RL MS
2183	Lin oleic acid	0.35	RL MS
	Total	91.59	

RL comparison of retention index with bibliography.
[a] Retentionindices relative to *n*-alkanes C_g – C_{20} on ZB-l capillary column.
[b] Relative area (peak area relative to the total peak area).
[c] Identification: MS. comparison of mass spectra with MS libraries.

3.2. Antibacterial activity

The *in vitro* antibacterial activity of essential oil and various extracts (hexane, chloroform, ethyl acetate and methanol) of *Z. jujuba* against the employed bacteria was qualitatively assessed by the presence or absence of inhibition zones. According to the results given in Table 2, a total of nine food-borne pathogenic bacteria, including four Gram-positive and five Gram-negative bacteria were tested. The oil exhibited antibacterial activity against all four Gram-positive and three Gram-negative bacteria at the concentration of 5 μl of 1:5 (v/v) dilution with methanol. The oil exhibited a noticeable antibacterial effect against *Staph. aureus* (ATCC 6538 and KCTC 1916), *L. monocytogenes* ATCC 19166, *B. subtilis* ATCC 6633, *P. aeruginosa* KCTC 2004, *S. typhimurium* KCTC 2515 and *E. coli* ATCC 8739 with diameter of inhibition zones ranging from of 8.6~21.2 mm, as shown in Table 2. Various organic extracts of *Z. jujuba* also revealed a good antibacterial activity against all four Gram-positive and three Gram-negative bacteria (*P. aeruginosa* KCTC 2004, *S. typhimurium* KCTC 2515 and *E. coli* ATCC 8739), at the concentration of 300 μg/disc (Table 2). The methanol extract showed the strongest antibacterial effect against *S. aureus* KCTC 1916, *L. monocytogenes* ATCC 19166 and *B. subtilis* ATCC 6633 with their respective diameter zones of inhibition of 18.6, 17.2 and 16.8 mm, whereas the ethyl acetate extract showed the strongest effect against *Staph. aureus* ATCC 6538 (inhibition zone: 19.2 mm), compared to the standard drug streptomycin. On the other hand, hexane and chloroform extracts showed interesting antibacterial effect with inhibition zones in the range of 11.2–14.1 and 13.5–15.0 mm, respectively. In this study, in some cases, the oil and organic extracts (chloroform, ethyl acetate and methanol) exhibited higher antibacterial activity compared to streptomycin, while tetracycline showed higher activity in some other cases than the essential oil and solvent extracts. The blind control did not inhibit the growth of the tested bacteria. The various extracts (hexane, chloroform, ethyl acetate and methanol) from *Z. jujuba* exhibited a moderate inhibitory effect against *P. aeruginosa* KCTC 2004, *S. typhimurium* KCTC 2515 and *E. coli* ATCC 8739, with diameter zones of inhibition in the range 8.1–14.9 mm. No inhibitory effect was observed against *E. aerogenes* KCTC 2190 and *S. enteritidis* KCTC 12021 in all cases.

3.3. Minimum inhibitory concentration (MIC)

As shown in Table 3, the MIC values for the oil were found to be lower for *Staph. aureus* (ATCC 6538 and KCTC 1916), *L. monocytogenes* ATCC 19166 and *B. subtilis* ATCC 6633 (31.25–125 μg/ml) than that of *P. aeruginosa* KCTC 2004, *S. typhimurium* KCTC 2515 and *E. coli* ATCC 8739 (250–500 μg/ml). On the other hand, MIC values of various solvent extracts against the tested bacteria were found to

Table 2
Antibacterial activity of essential oil and various extracts of *Z. jujuba* against food-borne and spoilage bacteria.

Microorganism	Zones of inhibition (mm)						
	Essential oil	Various extracts				Antibiotics	
		Hexane	$CHC1_3$	EtOAc	MeOH	TC	SM
Staph. aureus ATCC 6538	21.2 ± 0.6^{a}	14.1 ± 1.2^{a}	15.0 ± 0.6^{a}	19.2 ± 1.2*	19.1 ± l.l^{a}	17.3 ± 0.6^{a}	14.1 ± 0.6^{a}
Staph. aureus KCTC 1916	19.2 ± 1.5^{b}	13.6 ± 0.6^{a}	14.1 ± l.l^{a}	18.1 ± 1.0^{b}	18.6 ± 1.0^{a}	18.1 ± 0.6^{a}	14.0 ± l.l^{a}
L. monocytogenes ATCC 19166	17.3 ± 0.9^{c}	13.1 ± 1.2^{a}	14.8 ± 0.6^{a}	16.2 ± 0.6^{b}	17.2 ± 0.6^{b}	17.4 ± 0.7^{a}	13.6 ± 0.6^{a}
B. subrilis ATCC 6633	16.1 ± 1.2^{c}	11.2 ± 1.1^{b}	13.5 ± 1.0^{ab}	14.2 ± 1.1_{c}	16.8 ± 0.6^{b}	17.8 ± 0.5^{a}	13.3 ± 0.6^{a}
P. aeruginosa KCTC 2004	12.3 ± l.l^{d}	10.6 ± 0.6^{b}	11.0 ± 1.2^{b}	13.2 ± l.l^{cd}	14.9 ± 1.2^{c}	16.9 ± l.l^{a}	14.0 ± l.l^{a}
S. typhimurium KCTC 2515	8.6 ± 0.6^{e}	8.1 ± 1.1^{c}	10.3 ± l.l^{bc}	12.3 ± 0.6^{d}	13.8 ± l.l^{cd}	16.6 ± 0.6^{ab}	13.0 ± 0.6^{a}
E. coli ATCC 8739	9.2 ± 1.2	ni	9.2 ± 0.6^{c}	12.1 ± 0.7^{d}	12.8 ± 0.6^{d}	15.3 ± 0.6_{bc}	12.8 ± 1.2^{ab}
E. aerogenes KCTC 2190	ni	ni	ni	ni	ni	14.1 ± 1.1^{c}	11.1 ± 0.6^{b}
S. enteritidis KCTC 12021	ni	ni	ni	ni	ni	12.3 ± 1.2_{d}	10.5 ± l.l^{b}

Diameter of inhibition zones of essential oil including diameter of disc 6 mm (tested at a volume of 5 uldisc). Various extracts (300 μg disc).
Standard antibiotics: TC, tetracycline and SM, streptomycin (10 mug disc), ni. no inhibition. Values are given as mean ± S.D. (n = 3).
Values in the same column with different superscripts are significantly different ($p < 0.05$).

Table 3
Minimum inhibitory concentration of essential oil and various extracts of *Z. jujuba* against food-borne and spoilage bacteria.

Microorganism	Minimum inhibitory concentration (MIC)				
	Essential oil	Various extracts			
		Hexane	$CHC1_3$	EtOAc	MeOH
Staph. aureus ATCC 6538	31.25	250	125	62.5	62.5
Staph. aureus KCTC 1916	62.5	500	250	250	125
L. monocytogenes ATCC 19166	62.5	250	125	125	62.5
B. subtilis ATCC 6633	125	250	250	125	125
P. aeruginosa KCTC 2004	500	1000	500	250	250
S. typhimurium KCTC 2515	250	1000	500	500	500
E.coli ATCC 8739	500	ni	1000	500	500
E. aerogenes KCTC 2190	ni	ni	ni	ni	ni
S. enteritidis KCTC 12021	ni	ni	ni	ni	ni

Minimum inhibitory concentration (values in μg/ml), ni. no inhibition.

be in the range of 62.5–1000 μg/ml (Table 3). Methanol and ethyl acetate extracts showed higher antibacterial activity by minimum inhibitory concentrations as compared to hexane and chloroform extracts. In this study, the Gram-positive bacteria were found to be more susceptible to the essential oil and various solvent extracts than the Gram-negative bacteria.

3.4. Effect of essential oil on viable counts of bacteria

Based on the susceptibility, further, elaborative studies carried out on *Staph. aureus* ATCC 6538, *L. monocytogenes* ATCC 19166 and *B. subtilis* ATCC 6633, displayed different sensitivities of the essential oil. The effects of the essential oil on the growth of all the tested bacterial strains demonstrated the reduced viability of the tested bacteria at MIC concentration of the essential oil. At 20 and 40 min exposure, fold inhibition of *Staph. aureus* ATCC 6538 was observed at MIC concentration of the essential oil. Also the steep decline in CFU numbers was observed at 60 and 80 min exposure against *L. monocytogenes* ATCC 19166 and *B. subtilis* ATCC 6633. The exposure time of essential oil for complete inhibition of cell viability of all the tested bacterial strains were found to be 80–100 min (Fig. 1).

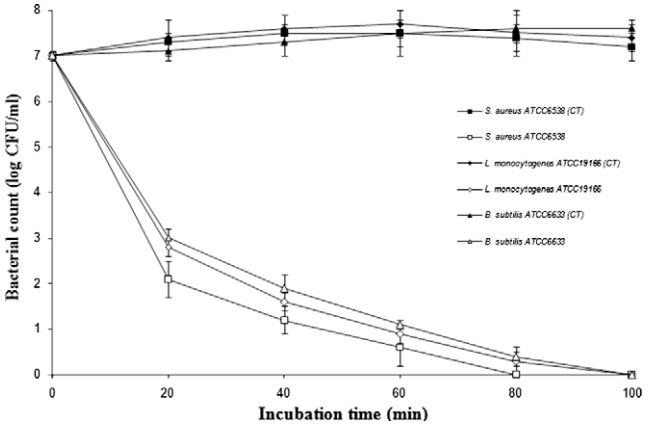

Fig. 1. Effect of *Zizyphus jujuba* essential oil (MIC concentration) on viability of the tested bacteria. CT: control without treatment.

3.5. Scanning electron microscopic (SEM) analysis

Scanning electron microscopic (SEM) analysis showed strong detrimental effect of essential oil on the morphology of *Staph. aureus* ATCC 6538. The control cell in the absence of the oil showed a regular, smooth surface as shown in Fig. 2 (A1). In contrast, cells inoculated with the essential oil at MIC value (31.25 μg/ml) revealed severe detrimental effects on the morphology of cell membranes, showing pore formation and lysis of the membranes integrity, as shown in Fig. 2 (A2 and A3).

4. Discussion

Plant-derived essential oils due to their antimicrobial content possess potential significance as naturally occurring agents for food preservation. Many volatile compounds naturally occurring in various essential oils possess strong antibacterial activities, thereby considered as natural antibacterial agents to inhibit the growth of food-borne pathogens (Cowan, 1999). The renewal of

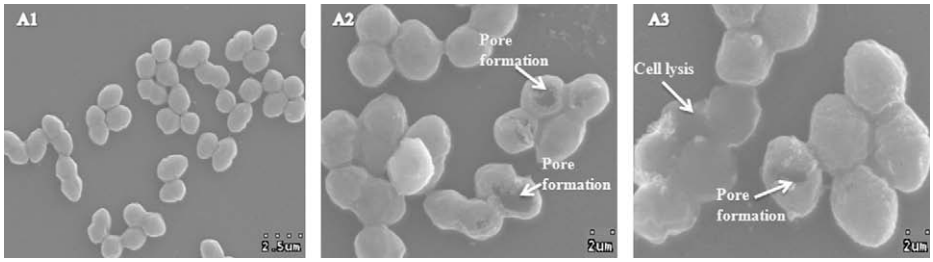

Fig. 2. Effect of essential oil on morphological changes of *Staphylococcus aureus* ATCC 6538. A1: Bacteria without essential oil (control); A2 and A3: Bacteria treated with essential oil at MIC values (31.25 μg/ml) showing pore formation and cell lysis.

interest in food science and technology, and increasing consumer demand for effective natural products means that quantitative data on plant based essential oils is required. The use of essential oils may improve food safety and overall microbial quality. If essential oils were to be more widely applied as antibacterials in foods, the organoleptic impact would be important. Foods generally associated with herbs, spices or seasonings would be the least affected by this phenomenon and information on the flavour impact of oregano essential oil in meat and fish supports this. The flavour, odour and colour of minced beef containing 1% v/w oregano oil improved during storage under modified atmosphere packaging and vacuum at 5 °C and were almost undetectable after cooking (Skandamis & Nychas, 2001). The addition of thyme oil up to 0.9% (v/w) in a coating for cooked shrimps had no ill effects on the flavour or appearance (Outtara, Sabato, and Lacroix, 2001). Individual essential oil components, many of them being approved food flavourings, also impart a certain flavour to foods. In addition, it is recommended to apply essential oils or their compounds as part of a hurdle system and to use it as an antimicrobial component along with other preservation techniques, e.g. in combination with reduced temperature and reduced pH or using a synergistic combination of essential oils and their compounds (Ultee, Slump, Stechini, & Smid, 2000). The antimicrobial activities of extracts obtained from spices, herbs, and other aromatic plants or parts thereof using organic solvents or steam distillation have been recognised for many years. Plants and plants extracts have been used since antiquity in folk medicine and food preservation, providing a range of compounds possessing pharmacological activity (Deans & Svoboda, 1990). Various publications have documented the antibacterial activity of essential oil constituents and plant extracts (Bajpai et al., 2008; Bhattacharjee, Chatterjee, Chatterjee, & Chandra, 2006; Ji-Hyun, 2004).

In this study, the essential oil and methanol extract exhibited remarkable activity against some of the representative food-borne and spoilage pathogenic bacteria such as *Staph. aureus* KCTC 1916, *L. monocytogenes* ATCC 19166, *B. subtilis* ATCC 6633, *P. aeruginosa* KCTC 2004, *S. typhimurium* KCTC 2515 and *E. coli* ATCC 8739. This activity could be attributed to the presence of oxygenated mono- and sesquiterpene hydrocarbons such as eucalyptol, caryophyllene, caryophyllene oxide, and these finding are in agreement with the previous reports (Larsen & Knochel, 1997). However, the antibacterial effect of various solvent extracts was comparable to the standard antibiotics. *Z. jujuba* mediated oil also contained high percentage of eugenol, isoeugenol, caryophyllene, eucalyptol, caryophyllene oxide, benzaldehyde and veridiflorol as earlier reported the major components of the various essential oils, which have enormous potential to inhibit microbial pathogens (El-Sakhawy, El-Tantawy, Ross, & El-Sohly, 1998; Larsen & Knochel, 1997; Tewtrakul, Yuenyongsawad, Kummee, & Atsawajaruwan, 2005). Those claims are further supported by our findings; indicating high contents of eugenol, isoeugenol, caryophyllene, eucalyptol, caryophyllene oxide, benzaldehyde and veridiflorol; comprising 80.89% of the oil (Table 1). On the other hand, the components in lower amounts such as α-humulene, tetradecanoic acid, γ-muurolene, trans-Z-α-bisabolene epoxide, chavicol and δ-cadinene also contributed to antimicrobial activity of the oil (El-Sakhawy et al., 1998; Melliou, Stratis, & Chinou, 2007; Shafia, Rosammaa, Jamilb, & Reddy, 2002). It is also possible that the minor components might be involved in some type of synergism with the other active compounds (Marino, Bersani, & Comi, 2001).

Also, the results from viable count assay revealed that exposure of the MIC concentration of the oil had a severe effect on the cell viability of the tested bacteria. *Staph. aureus* ATCC 6538 was found to be most sensitive to the oil. The oil also exerted its maximum bacterial activity against *L. monocytogenes* ATCC19166 and *B. subtilis* ATCC 6633, as evidenced by the reduction in CFU numbers observed at 60 and 80 min exposure and complete inhibition of cell viability at 100 min exposure of essential oil. Based on the MIC values, further, an elaborative study carried out on *Staph. aureus* ATCC 6538 to visualise the effect of essential oil on the morphology of the bacteria cell. SEM analyses were performed and demonstrated to the alter cell morphology as compared to the control group (Fig. 2). Such morphological abnormalities mainly occurred due to the disruption of membrane structure as evidenced by the previous findings (Koyama, Yamaguchi, Tanaka, & Motoyashiya, 1997).

In this study, the Gram-positive bacteria were found to be more susceptible to the essential oil and various solvent extracts than Gram-negative bacteria. This is probably due to the cell membrane of Gram-positive bacteria, which can interact directly with hydrophobic compounds of essential oils, whereas the external cell wall around the cell membrane of Gram-negative bacteria is hydrophilic and blocks the penetration of hydrophobic oil and avoids the accumulation of essential oils in target cell membrane (Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007).

This result may indicate that essential oil and various extracts from seeds of *Z. jujuba* can be used as natural preservatives in food against the well-known causal agents of food-borne diseases and food spoilage. Therefore, essential oils and plant extracts are being considered as potential alternatives to synthetic bactericides or as leading compounds for new classes of natural bactericides.

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