Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2015.07.031

Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf essential oil of *Forsythia koreana* deciduous shrub

Xiao-Nan Yang, Imran Khan, Sun Chul Kang*

Department of Biotechnology, College of Engineering, Daegu University, Kyungsan, Kyungbuk 712-714, Republic of Korea

ARTICLE INFO

Article history:
Received 15 Jun 2015
Received in revised form 20 Jul 2015
Accepted 20 Jul 2015
Available online 29 July 2015

Keywords:
Antibacterial activity
Antioxidant activity
Cell material released
Essential oil
GC–MS
Potassium ion flux

ABSTRACT

Objective: To identify the chemical constituents of leaf essential oil of *Forsythia koreana* (*F. koreana*) and evaluate its effects on bacterial strains.

Methods: The essential oil of leaf of *F. koreana* was extracted by using hydrodistillation process and the volatile components investigated with the help of gas chromatography coupled with mass spectrometry. The antibacterial study was carried out with the help of agar disc diffusion method, MIC, MBC and viable count. The mode of action was determined with help of potassium ion flux, cellular material release and scanning electron microscopy. The antioxidant activity was determined with the help of 2, 3-diphenyl-2-picrylhydrazyl method, nitric oxide scavenging activity and superoxide anion radical scavenging assay.

Results: Total ten compounds were identified as *trans*-phytol (42.73%), *cis*-3-hexenol (12.95%), β-linalool (10.68%), *trans*-2-hexenal (8.86%), *trans*-2-hexenol (8.86%), myrcenol (3.86%), 4-vinylphenyl acetate (3.86%), (4Z)-4,6-heptadien-1-ol (3.18%), lemonol (2.73%) and benzeneacetaldehyde (2.27%) by gas chromatography coupled with mass spectrometry. The antibacterial study was demonstrated that leaf essential oil of *F. koreana* act against foodborne and other pathogenic bacteria. The mode of action revealed that this essential oil acted on the cytoplasmic membrane, resulting in loss of integrity and increased permeability. In addition, leaf essential oil of *F. koreana* was shown to be rich in linalool, which contributes to improved antioxidant activity.

Conclusions: These results show that leaf essential oil of *F. koreana* has great potential as a natural food preservative, antibacterial and antioxidant agent.

1. Introduction

Foodborne diseases include a series of illnesses and are a growing public health problem worldwide. For example, the Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis, and food poisoning [1,2]. *Listeria monocytogenes* (*L. monocytogenes*) is responsible for severe foodborne illness listeriosis, which is recognized as one of the emerging zoonotic diseases over the last two decades [3]. The Gram-negative bacteria *Escherichia coli* (*E. coli*) is present in the human intestine and causes urinary tract infection, choleocystitis, or septicemia [4]. Often, chemical preservatives such as synthetic antimicrobial agents are used in the food industry to prevent growth of foodborne and food-spoiling microbes. However, concerns about the safety of these chemicals have increased along with consumer demands for naturally processed foods. Currently, there is growing interest in natural antibacterial materials, such as extracts of herbs and spices, for the preservation of foods. Plant-derived essential oils of various species of edible and medicinal plants, herbs, and spices have long been used as natural agents for food preservation in the food industry due to the presence of antimicrobial compounds [5]. In general, plant-derived essential oils are considered as non-phytotoxic compounds and are potentially effective against microorganisms. In this context, identification and evaluation of natural products to control food pathogens and assure a safe, wholesome, and nutritious food supply has become an important international challenge.

*Corresponding author: Prof. Sun Chul Kang, Department of Biotechnology, College of Engineering, Daegu University, Kyungsan, Kyungbuk 712-714, Republic of Korea.
Tel: +82 53 850 6553
Fax: +82 53 850 6559
E-mail: sckang@daegu.ac.kr
Peer review under responsibility of Hainan Medical University.

1995-7645/Copyright © 2015 Hainan Medical College. Production and hosting by Elsevier (Singapore) Pte Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Plant-derived essential oils are composed of various chemical components, each of which has its own significant activity. Plant-derived essential oils can be obtained from various plants parts, including the flower, seeds, buds, leaves, twigs, woods, fruits, and roots. Activities of plant-derived essential oils can vary from one strain to another [6]. These essential oils have antimicrobial [7], antioxidant [8,9], antimycotic [10], antiviral [11], antiparasitic [12,13], antitoxicogenic [8,14] and insecticidal [15,16] properties. Each component present in essential oils is directly related to the properties mentioned above.

Forsythia is a genus of flowering plants in the family Oleaceae as a deciduous shrub. The species *Forsythia koreana* (*F. koreana*) is widely cultivated in South Korea and blooms in the months of March to April with vast yields of flowers. Fruits of *F. koreana* have been investigated and are known to have anti-inflammatory, diuretic and dampheat-clearing effects [17]. However, there are no phytochemical and biological studies on leaves of *F. koreana* so far. Regardless of the considerable data on fruit oils of the *Forsythia* genus from different regions, there is a lack of information on the chemical compositions and bioactivities of other parts of *F. koreana* planted in South Korea. Therefore, the aim of the present investigation was to analyze the chemical composition and antibacterial activity of leaf essential oil of *F. koreana*.

2. Materials and methods

2.1. Chemicals and reagents

1,2-diphenyl-2-picrylhedrazyl (DPPH), sulfanilamide, naphthalene-diaminedihydrochloride, nitroferrycyanide, (III) dehydrate, xanthine oxidase, and Folin-Ciocalteau's phenol reagent were obtained from Sigma–Aldrich (St. Louis, MO, USA). Nitro-blue tetrazolium was purchased from fluka (Buchs, Switzerland). All other chemicals and solvents were of the highest commercial grade.

2.2. Plant materials

Leaves of *F. koreana* were collected from the campus of Daegu University, Kyungsan, Kyungbuk, South Korea. The plant was identified by its morphological features and database present in the library at the University. Euchre specimen (DU-FK968-L) was preserved in Daegu University for further reference.

2.3. Isolation of leaf essential oil

Fresh leaves of *F. koreana* were subjected to hydrodistillation for 4 h using a modified clevenger type apparatus. The resulting oil–water mixture was then extracted using dichloromethane. The organic layer was then separated, dried over anhydrous Na_2SO_4, filtered, and the solvent volatilized. The resulting yellowish oil was preserved in a sealed vial at 4 °C in the dark until further analysis.

2.4. Microbial strains (food spoiling and foodborne pathogens)

A panel of foodborne pathogenic bacteria, including *Salmonella enteritidis* (*S. enteritidis*) KCTC 12243, *E. coli* ATCC 8739, *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19118, *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 19166, *L. monocytogenes* ATCC 19116, and *L. monocytogenes* ATCC 10943 were used in this study. Bacterial cultures were revived on Luria–Bertani (LB) Agar plates and then transferred into LB broth medium maintained at 37 °C for overnight.

2.5. Gas chromatography–mass spectrometry (GC–MS) analysis

Quantitative and qualitative analyses of essential oil was performed using a GC–MS (Model QP 2010, Shimadzu, Japan) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). For GC–MS detection, an electron ionization system with ionization 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 temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 °C to 150 °C at a rate of 3 °C/min, held for 10 min, and finally raised to 250 °C at a rate of 10 °C/min. Diluted sample (1/100, v/v, in dichloromethane) of 1 μL was manually injected in split less mode. The relative percentages of oil constituents were expressed as percentage by peak area normalization.

Identification of components of essential oil was based on their retention indices, relative to a homologous series of *n*-alkane (C_8–C_{20}) on ZB-1 capillary column under the same operating conditions and computer matching with NIST MS libraries.

2.6. Assay for antibacterial potential

Standard agar disc diffusion method was used for antibacterial assay. Firstly, active cultures were diluted with LB broth to achieve an optical density of 10^7 CFU/mL for the test organisms at 600 nm by an UV/Vis Spectrophotometer (Optizen 2120 UV). Petri plates were prepared by pouring 20 mL of LB agar medium, which was allowed to solidify. Standardized inoculum (0.1 mL) containing 10^7 CFU/mL of bacterial suspension was poured onto an LB plate, uniformly spread, and then allowed to dry for 5 min. Whatman No.1 sterile filter paper discs (6 mm diameter) were impregnated with 5 μL/disc of essential oil or 5 μg/disc of positive compounds (streptomycin and tetracyclin) placed on inoculated LB agar. The plates were left at room temperature for 30 min to allow the oil to diffuse into the agar, after which the plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone against the tested bacteria.

2.7. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of essential oil was tested by the method described by Chandrasekaran and Venkatesalu (2004) [18]. The oil was dissolved in DMSO and incorporated into tubes containing LB broth medium to obtain a final concentration from 0 to 1% (v/v). Standardized suspension (10 μL) of each fresh tested organism (10^7 CFU/mL) was transferred to separate tubes, which were incubated at 37 °C in a shaking incubator for 24 h. The lowest concentration of the test samples, which did not show any visual growth of test organisms after macroscopic evaluation, was determined as MIC. Further,

concentrations showing complete inhibition of bacteria were identified. For this, 50 μL of each culture broth was transferred onto an agar plate, which incubated for a specified time and temperature as mentioned above. The complete absence of growth on the agar surface at the lowest concentration of the sample was defined as MBC. Streptomycin was used as a positive control.

2.8. Effect of essential oil on viable counts of tested bacteria

For viable counts, each tube containing a bacterial suspension (approximately 10^7 CFU/mL) in 2 mL of LB broth was inoculated with the MIC level of essential oil and incubated at 37 °C in a shaking incubator. Bacterial suspensions (10 μL) for viable counting were removed from tubes at 0, 20, 40, 60, 90, 120,150, 210, and 300 min time intervals and then diluted appropriately with sterile water. Each dilution (50 μL) was spread on the LB agar surface. The colonies were counted after 24 h of incubation at 37 °C. The controls without essential oil inoculation were treated under same experimental conditions as mentioned above.

2.9. Scanning electron microscopy (SEM)

Sample preparation for SEM was carried out according to Kockro method [19] with some modifications. Bacterial cells of *E. coli* ATCC 8739 and *L. monocytogenes* ATCC 10943 were treated with and without essential oil at the MIC level for 60 min, washed three times using 50 mM phosphate buffer solution (PBS, pH 7.3), and then centrifuged at 4000 rpm. After removing supernatant, centrifuged cells were suspended in new PBS. A thin smear of the suspension was then spread on a glass slide and fixed in 2.5% (v/v) glutaraldehyde (Electron Microscopy Science, Washington, USA) for 2 h. The specimen was dehydrated using sequential exposure per ethanol concentrations ranging from 30% to 100%. The ethanol was replaced by tertiary butyl alcohol. After dehydration, the specimen was dried with CO_2. The dried cells were coated with gold in a sputter coater (Hitachi, Japan). Samples were observed under a scanning electron microscope (Hitachi-S4300, Japan).

2.10. Assay of potassium ion flux

The concentration of free potassium ions in the bacterial suspension was measured after exposure to essential oils (at MIC value) in sterile peptone water (0.1%) for 0, 60, 120, 180, 240 and 300 min. At each pre-established interval, the extracellular potassium concentration was measured by following a photometric procedure using a Kalium/Potassium kit (Quantofix, GmbH, Wiesbaden, Germany). Control tubes without essential oils were tested. Results were expressed as the amount of extracellular free potassium (μM) ion at each interval of incubation.

2.11. Release of cellular material

Release of cellular materials at 260 nm was carried out in 2 mL aliquots of sterile peptone water (0.1% w/v) containing bacterial inoculum, after which essential oils (at MIC value) were added to the tubes. After 0, 60, 120, 180, 240, and 300 min of treatment, cells were centrifuged at 3500 rpm, and the absorbance of the obtained supernatant was determined at 260 nm by an Optizen UV/Vis Spectrophotometer. Control tubes without essential oils were tested. Results were expressed as the percentage of absorbing material of 260 nm min each interval with respect to time.

2.12. Antioxidant activity

Free radical scavenging activity was measured by stable DPPH according to Orhan and colleagues (2003) [20] with minor modifications. To test antioxidant activity, three concentrations of sample (25, 50, and 100 μg/mL) were dissolved in methanol. Each concentration (100 μL) was mixed with DPPH solution (900 μL of 0.004% w/v in methanol) and vortexed. Remaining amount of DPPH was determined based on decrease in absorbance at 517 nm. Inhibition of DPPH as a percentage was calculated by the formula:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction, which contains all reagents except the test sample, and A_{sample} is the absorbance of the test compounds.

2.13. Measurement of nitric oxide scavenging activity

Nitric oxide scavenging activity was determined by the Griess reaction (Griess *et al.*, 1879) [21]. Nitrite is one of the products of oxidative metabolism of NO. Sample (100 μL) was mixed with the same volume of Griess reagent (2% sulfanilamide in 4% phosphoric acid and 0.2% naphthyl ethylene diaminedihydrochloride in water) and incubated at room temperature for 10 min. The absorbance of the resulting chromophore was read at 540 nm. The activity was compared with sodium nitrite, which was used as a standard antioxidant.

2.14. Superoxide anion scavenging activity

Superoxide anion radical scavenging assay was carried out according to the method of Suh (Suh *et al.*, 2010) [22]. The reaction mixture consisted 0.25 mL of 0.8 mM xanthine in 0.1 mM potassium phosphate (pH 7.8), 0.15 mL of 0.5 mM nitro-blue tetrazolium in 0.1 mM potassium phosphate (pH 7.8), and 0.05 mL of sample solution. After incubation at 25 °C for 10 min, the reaction was started by adding 0.5 mU xanthine oxidase. The samples were kept at 25 °C for 20 min and then stopped. The results were calculated as the percentage of scavenging activity according to the following formula:

$$\%\text{scavenging} = [1-(S-S_b)/(C-C_b)] \times 100$$

where S, S_b, C, and C_b are the absorbance's of the sample treated with enzyme, sample without enzyme, control treated with enzyme, and control without enzyme, respectively.

2.15. Statistical analysis

The data were expressed as mean ± SD. Statistical significance was calculated between control and essential oil treated

group by using the Student's *t*-test. It was regarded as significant difference as $P < 0.05$.

3. Results

3.1. Chemical composition of essential oil

The yield of leaf essential oil was about 1.2%, (v/w). Upon GC–MS analysis, 10 compounds were identified as *trans*-phytol (42.73%), *cis*-3-hexenol (12.95%), β-linalool (10.68%), *trans*-2-hexenal (8.86%), *trans*-2-hexenol (8.86%), myrcenol (3.86%), 4-vinylphenyl acetate (3.86%), (4Z)-4,6-heptadien-1-ol (3.18%), lemonol (2.73%), and benzeneacetaldehyde (2.27%) (Table 1).

3.2. Antibacterial activity

The *in vitro* antibacterial activity of leaf essential oil of *F. koreana* against foodborne pathogenic bacteria was assessed based on the presence or absence of inhibition zones (Table 2). Essential oil (5 µL/disc) exhibited potent antibacterial activity against all test pathogens, including *S. enteritidis* KCTC 12243, *E. coli* ATCC 8739, and *S. aureus* ATCC 6538 with inhibition zone diameters of 12.3, 8.0, 9.3 mm, respectively. Strains of *L. monocytogenes* (ATCC 19118, ATCC 19111, ATCC 19166, ATCC 19116, and ATCC 10943) showed inhibition zone diameters of 10.0, 6.9, 6.8, 6.2 and 9.7 mm, respectively.

3.3. MIC and MBC

As shown in Table 3, leaf essential oil showed greater susceptibility against Gram-positive *S. aureus* ATCC 6538 and one strain of *L. monocytogenes* (ATCC 10943) with MIC values of 0.2% and 0.1% (v/v), respectively. Leaf essential oil also exhibited moderate antibacterial activity with MIC values from 0.3% to 0.5% (v/v) against two Gram-negative bacteria, *S. enteritidis* KCTC 12243 and *E. coli* ATCC 8739, and the other four strains of Gram-positive *L. monocytogenes*.

Table 1

Composition of essential oil of *F. koreana* leaf.

Compound	MF	%[a]	Identification[b]
Aldehydes		11.13	
trans-2-Hexenal	$C_6H_{10}O$	8.86	MS, RI
Benzene acetaldehyde	C_8H_8O	2.27	MS, RI
Alcohols		24.99	
cis-3-Hexenol	$C_6H_{12}O$	12.95	MS, RI
trans-2-Hexenol	$C_6H_{12}O$	8.86	MS, RI
(4z)-4,6-Heptadien-1-ol	$C_7H_{12}O$	3.18	MS, RI
Oxygenated monoterpenes		17.27	
β-Linalool	$C_{10}H_{18}O$	10.68	MS, RI
Myrcenol	$C_{10}H_{18}O$	3.86	MS, RI
Lemonol	$C_{10}H_{18}O$	2.73	MS, RI
Oxygenated diterpenes		42.73	
trans-Phytol	$C_{20}H_{40}O$	42.73	MS, RI
Esters		3.86	
4-Vinylphenyl acetate	$C_{10}H_{10}O_2$	3.86	MS, RI
Total		99.98	

MF: Molecular formula; %[a]: Relative percentage (peak area relative to the total peak area); Identification[b]: MS comparison of mass spectra with NIST MS libraries and retention indexrelative to n-alkanes C_8–C_{20} on ZB-1 capillary column.

Table 2

Inhibition zone diameter produced by the essential oil of *F. koreana* leaf on the test pathogens.

Bacterial strains	Inhibition zone diameter (mm)		
	Essential oil of leaf (5 µ L/disc)	Strep to mycin (5 µg/disc)	Tetracylin (5 µg/disc)
S. enteritidis KCTC 12243	12.3 ± 0.5	–	–
E. coli ATCC 8739	8.0 ± 0.3	11.0 ± 0.8	–
S. aureus ATCC 6538	9.3 ± 0.4	6.8 ± 0.7	18.0 ± 0.7
L. monocytogenes ATCC 19118	10.0 ± 0.6	–	16.5 ± 0.7
L. monocytogenes ATCC 19111	6.9 ± 0.5	6.6 ± 0.3	17.2 ± 0.1
L. monocytogenes ATCC 19166	6.8 ± 0.5	6.4 ± 0.1	–
L. monocytogenes ATCC 19116	6.2 ± 0.1	–	16.8 ± 0.8
L. monocytogenes ATCC 10943	9.7 ± 0.4	20.6 ± 0.5	22.0 ± 0.1

Table 3

MIC and MBC of essential oil of *F. koreana* leaf against the test pathogens.

Bacterial strains	MIC (%, v/v)	MBC (%, v/v)	MIC of streptomycin (µg/mL)
S. enteritidis KCTC 12243	0.4	0.7	45
E. coli ATCC 8739	0.3	0.4	10
S. aureus ATCC 6538	0.2	0.5	30
L. monocytogenes ATCC 19118	0.4	0.6	35
L. monocytogenes ATCC 19111	0.4	0.6	50
L. monocytogenes ATCC 19166	0.5	0.8	50
L. monocytogenes ATCC 19116	0.4	0.6	50
L. monocytogenes ATCC 10943	0.1	0.3	10

3.4. Effect of essential oil on cell viability

Reducing effects of leaf essential oil of *F. koreana* on cell viabilities of *S. enteritidis* KCTC 12243, *E. coli* ATCC 8739, *S. aureus* ATCC 6538, and *L. monocytogenes* ATCC 10943 were observed during treatment for 300 min (Figure 1A). Leaf essential oil reduced the growth of test bacteria at minimum inhibitory concentrations, and 50% inhibition of cell viabilities was observed within 90 min of exposure in all test strains. After 150 min, 80% inhibition of Gram-positive bacteria (*S. aureus* ATCC 6538 and *L. monocytogenes* ATCC 10943) was observed, and exposure for 210 min to leaf essential oil resulted in complete inhibition of CFU numbers of these two strains. After 300 min, all test bacteria were completely inhibited by leaf essential oil.

3.5. SEM

SEM was carried out to visualize the effects of leaf essential oil of *F. koreana* on cellular morphologies of *E. coli* ATCC 8739 and *L. monocytogenes* ATCC 10943 (Figures 2 and 3).

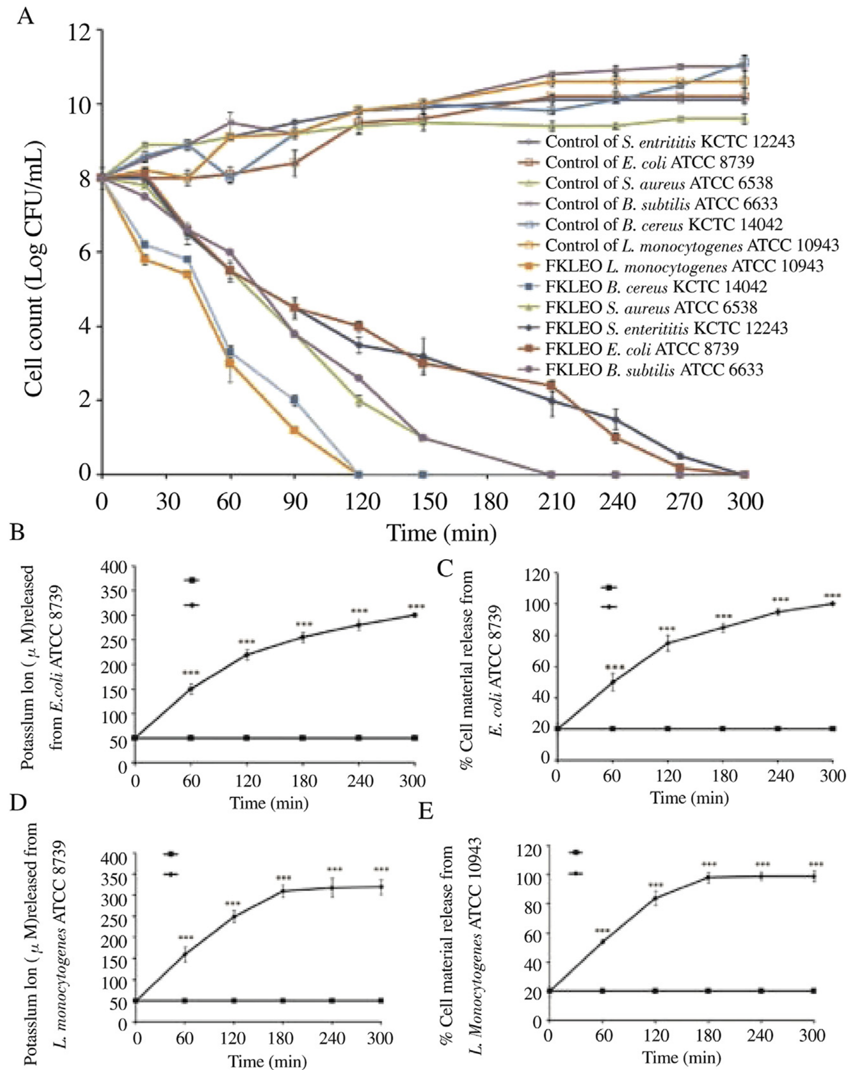

Figure 1. A. Effects of the essential oil of *F. koreana* leaf (MIC level) on cell viabilities of the test bacteria.
Control: cells without essential oil treatment; LEO: (leaf essential oil treatment).
B. Effect of the leaf essential oil of *F. koreana* on potassium release of *E. coli* ATCC 8739.
C. Effect of the leaf essential oil of *F. koreana* on cell material release of *E. coli* ATCC 8739.
D. Effect of the leaf essential oil of *F. koreana* on potassium release of *L. monocytogenes* ATCC 10943.
E. Effect of the leaf essential oil of *F. koreana* on cell material release of *L. monocytogenes* ATCC 10943.
Statistical significance was calculated between essential oil treated and control by using the Student's *t*-test. *Represents $P < 0.05$, **represents $P < 0.01$, ***represents $P < 0.001$.

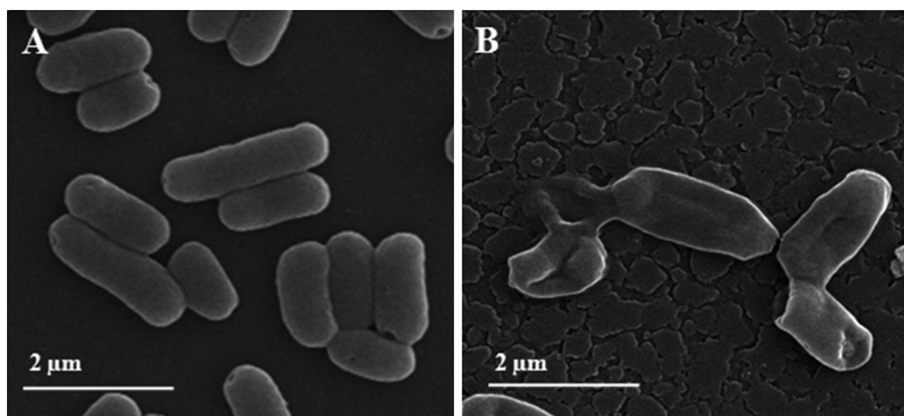

Figure 2. Effect of the essential oil of *F. koreana* leaf on morphological change of *E. coli* ATCC 8739.
A: bacteria without essential oil treatment (control) showing smooth and regular cell surface; B: bacteria treated with essential oil at the MIC level (3 000 μg/mL) after 60 min shows cell wall lysis.

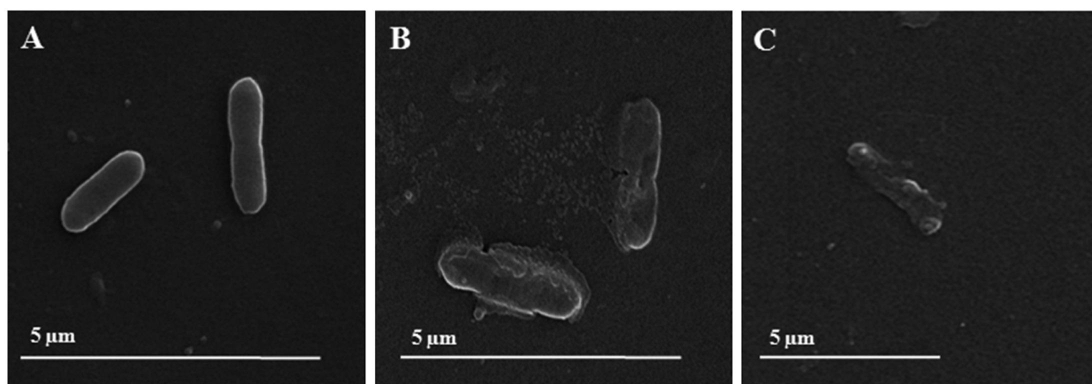

Figure 3. Effect of the essential oil of *F. koreana* leaf on morphological change of *L. monocytogenes* ATCC 10943.
A: bacteria without essential oil treatment (control) showing smooth and regular cell surface. B and C: bacteria treated with essential oil at the MIC level (1 000 μg/mL) after 60 min shows cell wall lysis.

Essential oil exhibited strong detrimental effects on morphology of *E. coli* ATCC 8739 (Figure 2B) and *L. monocytogenes* ATCC 10943 (Figure 3B and C). Control cells (without essential oil treatment) showed a regular, smooth surface as shown in Figures 2A and 3A. In contrast, cells inoculated with essential oil at MIC levels for 60 min showed changes in cell wall morphology, cell wall lysis (Figures 2B, 3B and C), and pore formation (Figure 3B). Release of cell materials also occurred in *L. monocytogenes* ATCC 10943 upon essential oil treatment (Figure 3B).

3.6. Release of potassium ions and 260-nm-absorbing material

Release of potassium ions and 260-nm-absorbing cell material from *E. coli* ATCC 8739 treated with leaf essential oil of *F. koreana* at the MIC level (3 000 μg/mL) is depicted in Figure 1B and C. Efflux of potassium ions from test bacteria occurred immediately after addition of essential oil, followed by a steady reduction along the evaluated intervals. The OD_{260nm} of filtrates of cells exposed to leaf essential oil revealed elevated release of cell materials according to time of exposure.

As shown in Figure 1D and E, release of potassium ions and 260-nm-absorbing cell material from *L. monocytogenes* ATCC 1093 treated with leaf essential oil of *F. koreana* at the MIC level (1 000 μg/mL) occurred immediately after addition of essential oil, followed by a steady reduction along the evaluated intervals. The OD_{260nm} of filtrates of cells exposed to leaf essential oil revealed elevated release of cell materials according to time of exposure.

3.7. Antioxidant activity

The DPPH, nitric oxide, and superoxide anion scavenging activities of leaf essential oil of *F. koreana* are shown in Table 3. The IC_{50} (50% free radical inhibitory concentration) values of leaf essential oil were (660 ± 20), (1 280 ± 70), and (1 080 ± 40) μg/mL, respectively. Leaf essential oil showed improved antioxidant activity as possessed lower IC_{50} value on the three free radicals.

4. Discussion

Much attention has been given to plant-derived essential oils, as they possess various pharmaceutical, antibacterial, antioxidant, antiviral, antiinsecticidal, antimycotic, and antitoxinogenic activities [8,10,11,13]. The volatile constituents of *F. koreana* leaves are a mix of oxygenated terpenes (β-linalool, myrcenol, lemonol, and *trans*-phytol) and oxygenated derivatives, including aldehydes (*trans*-2-hexenal and benzeneacetaldehyde), alcohols [*cis*-3-hexenol, *trans*-2-hexenol, and (4Z)-4, 6-heptadien-1-ol], and esters (4-vinylphenyl acetate).

Historically, many plant oils have been used as topical antiseptics. The use of essential oils may also improve food safety and quality. In the present study, the results of MIC determination showed that leaf essential oil of *F. koreana* exhibits potent activities against food spoilage and foodborne pathogenic bacteria, such as *S. enteritidis* KCTC 12243, *E. coli* ATCC 8739, *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19118, ATCC 19111, ATCC 19166, ATCC 19116, and ATCC 10943 with IC_{50} values ranging from 0.1 to 0.5% (v/v). The antibacterial activity of leaf essential oil could be attributed to components such as β-linalool, myrcenol, and lemonol, which have been found to be the major constituents of several essential oils or exhibit potential antimicrobial activity [23,24]. Other components are also critical and may have a synergistic effect or potentiating influence. SEM study of *E. coli* ATCC 8739 and *L. monocytogenes* ATCC 10943 determined that essential oil acted on cell membranes, resulting in loss of integrity and increased permeability. Increased leakage of potassium ions indicates that the cell membrane structure was damaged by essential oil as compared to the control group.

Release of potassium ions and 260-nm-absorbing material from *E. coli* ATCC 8739 and *L. monocytogenes* ATCC 10943 was stimulated by exposure of cells to leaf essential oil of *F. koreana*. The results indicate that essential oil acted on the cytoplasmic membrane, resulting in increased permeability. The cytoplasmic membrane acts as a permeability barrier to the passage of ions such as H^+, K^+, Na^+, and Ca^{2+} [8]. Increased leakage of K^+ indicates that the membrane structure was damaged by the essential oil. Further, SEM analysis of these two bacterial cells also demonstrated the destructive effect of essential oil on cell membranes as compared to the control group. Choi and associates (2000) [9] examined the DPPH scavenging activities of 21 authentic compounds, including linalool (DPPH scavenging activity of linalool was 50.3 mg trolox equiv/mL). In short, leaf essential oil of *F. koreana* caused physical destruction *via* loss of cytoplasmic integrity. Further, the high content of linalool could be responsible for the increased antioxidant activity of leaf essential oil against

the tested pathogens, which supports the notion that leaf essential oil of *F. koreana* is a promising food preservative.

In summary, leaf essential oil of *F. koreana* is rich in oxygenated diterpenes, oxygenated monoterpenes, and alcohols and displays significant antibacterial activity against foodborne and pathogenic bacteria such as *S. enteritidis* KCTC 12243, *E. coli* ATCC 8739, *S. aureus* ATCC 6538, and several strains of *L. monocytogenes* ATCC 19118, *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 19166, *L. monocytogenes* ATCC 19116, and *L. monocytogenes* ATCC 10943. Therefore, leaf essential oil of *F. koreana* might be used as an anticorrosive additive to improve food quality and control growth of food-spoiling microbial pathogens. Future research is necessary to understand the involved mechanisms, especially against other foodborne pathogens and as a food preservative. Furthermore, essential oils will be studied using human tumor cell lines to explore their role as a therapeutic agent.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol Rev* 2012; **36**: 815-836.

[2] Krakauer T, Stiles BG. The staphylococcal enterotoxin (SE) family SEB and siblings. *Virulence* 2013; **4**: 759-773.

[3] Cossart P, Lebreton A. A trip in the “New Microbiology” with the bacterial pathogen *Listeria monocytogenes*. *FEBS Lett* 2014; **588**: 2437-2445.

[4] Ulett GC, Totsika M, Schaale K, Carey AJ, Sweet MJ, Schembri MA. Uropathogenic *Escherichia coli* virulence and innate immune responses during urinary tract infection. *Curr Opin Microbiol* 2013; **16**: 100-107.

[5] Dahiya P, Purkayastha S. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian J Pharm Sci* 2012; **74**: 443-450.

[6] Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. *Curr Med Chem* 2003; **10**: 813-829.

[7] Parimala M, Shoba FG. *In vitro* antimicrobial activity and HPTLC analysis of hydroalcoholic seed extract of *Nymphaea nouchali* Burm. F. *BMC Complement Altern Med* 2014; **14**: 314-361.

[8] Doughari JH, Ndakidemi PA, Human IS, Benade S. Antioxidant, antimicrobial and antiverotoxic potentials of extracts of *Curtisia dentata*. *J Ethnopharmacol* 2012; **141**: 1041-1050.

[9] Choi HS, Song HS, Ukeda H, Sawamura M. Radical-scavenging activities of citrus essential oils and their components: detection using 1, 1-diphenyl-2-picrylhydrazyl. *J Agric Food Chem* 2000; **48**: 4156-4164.

[10] Cannas S, Molicotti P, Ruggeri M, Cubeddu M, Sanguinetti M, Marongiu B, et al. Antimycotic activity of *Myrtus communis L.* towards *Candida* spp. from isolates. *J Infect Dev Countr* 2013; **7**: 295-298.

[11] Rothan HA, Zulqarnain M, Ammar YA, Tan EC, Rahman NA, Yusof R. Screening of antiviral activities in medicinal plants extracts against dengue virus using dengue NS2B-NS3 protease assay. *Trop Biomed* 2014; **31**: 286-296.

[12] Pandey R, Kalra A, Tandon S, Mehrotra N, Singh HN, Kumar S. Essential oil compounds as potent source of nematicidal compounds. *J Phytopathol* 2000; **148**: 501-502.

[13] Sukphan P, Sritularak B, Mekboonsonglarp W, Lipipun V, Likhitwitayawuid K. Chemical constituents of *Dendrobium venustum* and their antimalarial and anti-herpetic properties. *Nat Prod Commun* 2014; **9**: 825-827.

[14] Ultee A, Smid EJ. Influence of carvacrol on growth and toxin production by *Bacillus cereus*. *Int J Food Microbiol* 2001; **64**: 373-378.

[15] Ali MS, Ravikumar S, Beula JM, Anuradha V, Yogananth N. Insecticidal compounds from *Rhizophoraceae* mangrove plants for the management of dengue vector *Aedesa egypti*. *J Vector Borne Dis* 2014; **51**: 106-114.

[16] Konstantopoulou I, Vassilopoulou L, Mavragani-Tsipidou P, Scouras ZG. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. *Experientia* 1992; **48**: 616-619.

[17] Kim NY, Kang TH, Song EK, Pae HO, Chung HT, Kim YC. Inhibitory effects of butanol fraction of the aqueous extract of *F. koreana* on the nitric oxide production by murine macrophage-like RAW 264.7 cells. *J Ethnopharmacol* 2000; **73**: 323-327.

[18] Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *J Ethnopharmacol* 2004; **91**: 105-108.

[19] Kockro RA, Hampl JA, Jansen B, Peters G, Scheihing M, Giacomelli R. Use of scanning electron microscopy to investigate the prophylactic efficacy of rifampin-impregnated CSF shunt catheters. *J Med Microbiol* 2000; **49**: 441-450.

[20] Orhan I, Aydin A, Colkesen A, Sener B, Isimer AI. Free radical scavenging activities of some edible fruit seeds. *Pharm Biol* 2003; **41**: 163-165.

[21] Griess P. Bemerkungen zu der abhandlung der HH. Weselsky und Benedikt ‘Uber einige azoverbindungen’. *Ber Deut Chem Ges* 1879; **12**: 426-428.

[22] Suh HJ, Kim SR, Lee KS, Park S, Kang SC. Antioxidant activity of various solvent extracts from *Allomyrina dichotoma* (Arthropoda: Insecta) larvae. *J Photochem Photobiol* 2010; **99**: 67-73.

[23] Inouyea S, Takizawab T, Yamaguchia H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *J Antimicrob Chemother* 2001; **47**: 565-573.

[24] Chiang HM, Chiu HH, Lai YM, Chen CY, Chiang HL. Carbonyl species characteristics during the evaporation of essential oils. *Atmos Environ* 2010; **44**: 2240-2247.