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In vitro control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb.

Atiqur Rahman, Sun Chul Kang *

Department of Biotechnology, College of Engineering, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea

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ABSTRACT

The antibacterial potential of essential oil from flowers and ethanolic leaf extracts of *Lonicera japonica* Thunb. was evaluated for controlling the growth of a range of food-borne pathogens. Thirty-nine compounds representing 92.34% of the total oil were identified, of which *trans*-nerolidol (16.31%), caryophyllene oxide (11.15%), linalool (8.61%), *p*-cymene (7.43%), hexadecanoic acid (6.39%), eugenol (6.13%), geraniol (5.01%), *trans*-linalool oxide (3.75%), globulol (2.34%), pentadecanoic acid (2.25%), veridiflorol (1.83%), benzyl alcohol (1.63%) and phenylethyl alcohol (1.25%) were the major compounds. The oil and extracts revealed a remarkable antibacterial effect against *Listeria monocytogenes* ATCC 19116, *Bacillus subtilis* ATCC 6633, *B. cereus* SCK 11, *Staphylococcus aureus* (ATCC 6538 and KCTC 1916), *Salmonella enteritidis* KCTC 12021, *S. typhimurium* KCTC 2515, *Enterobacter aerogenes* KCTC 2190 and *Escherichia coli* ATCC 8739. However, no effect was observed for *Pseudomonas aeruginosa* KCTC 2004 and *E. coli* O157:H7 ATCC 43888. Our findings demonstrate that the oil and extracts derived from *L. japonica* might be a potential source of preservatives for use in the food or pharmaceutical industries.

1. Introduction

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food-borne diseases. The 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). *Listeria monocytogenes* is responsible for the severe food-borne illness, listeriosis, which 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). With the increase of bacterial resistance to antibiotics, there is considerable interest in investigating the antimicrobial effects of essential oils and different extracts against a range of bacteria, to develop other classes of natural antimicrobials useful for infection control or for the preservation of food. Thus essential oils and plant extracts are promising natural antimicrobial agents with potential applications in the food or pharmaceutical industries for the control of pathogenic bacteria.

The plant *Lonicera japonica* Thunb. (Caprifoliaceae), is a species of honeysuckle native to eastern Asia, including Japan, Korea, northern and eastern China, and Taiwan, which is a major invasive species in North America. *L. japonica* is traditionally used as a medicinal plant (Peng, Mei, Jiang, Zhou, & Sun, 2000). Pharmacological studies and clinical practice have demonstrated that *L. japonica* possesses many biological functions, including hepatoprotective, cytoprotective, antimicrobial, antioxidative, antiviral and anti-inflammatory (Chang et al., 1995). The constituents of this plant have been previously investigated and shown to contain iridoid glucosides (Kakuda, Imai, Yaoita, Machida, & Kikuchi, 2000) and polyphenolic compounds (Peng et al., 2000). The main polyphenolic components in *L. japonica* are hyperoside, chlorogenic acid, luteolin and caffeic acid (Kakuda et al., 2000; Peng et al., 2000). Some related investigations showed that hyperoside, chlorogenic acid and other flavones could be used to scavenge free radicals and have anti-inflammatory activity (Leung, Wu, Lin, & Lee, 2005). The major parts of this plant have medicinal properties; flower buds have anticancer and anti-inflammatory properties (Zhang, Yang, & Liu, 2008), leaf has antioxidant and tyrosinase inhibition properties (Byun, Jo, Lee, Jo, & Kim, 2004b) and stem has tyrosinase inhibition, xanthine oxidase inhibition, and nitrite-scavenging activities (Byun, Jo, Jeon, & Hong, 2004a). However, research on antibacterial properties of *L. japonica* is still scarce; only a few scientific articles reported some of these activities (Byun et al., 2004a; Shan, Cai, Brooks, & Corke, 2007). Despite its various medicinal properties, no report is available in the literature on chemical composition and antibacterial property of essential oil derived from flower parts of *L. japonica*. Hence, efforts have been made to investigate the antibacterial properties of the essential

* Corresponding author. Tel.: +82 53 850 6553; fax: +82 53 850 6559.
E-mail address: sckang@daegu.ac.kr (S.C. Kang).

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oil and leaf extracts. Leaves and flowers of this plant are using as herbal tea.

In this study, we examined the chemical composition of the essential oil from flower parts of *L. japonica* by GC–MS, and tested the antimicrobial efficacy of the oil and ethanolic leaf extracts. The choice of solvent for the extraction is based on the following criteria: it is generally accepted that ethanol is superior to methanol and acetone for extracting biologically-active components (e.g., flavonoids) from tea (Wang & Helliwell, 2001). Besides, ethanol is considered as safe (GRAS solvent).

2. Materials and methods

2.1. Plant material

The leaves and flowers of *L. japonica* were collected from Kyungsan city area of Republic of Korea in May 2007. The plant was identified on the basis of its morphological features and the database present in the library at the Department of Biotechnology, Daegu University, Republic of Korea; a voucher specimen has been deposited in the herbarium of the Department of Biotechnology, Daegu University, Republic of Korea.

2.2. Isolation of the essential oil

Plant materials were dried in the shade at room temperature for 10 days. The air-dried flower parts (300 g) of *L. japonica* 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 ethanolic extracts

The air-dried leaves of *L. japonica* (50 g) were extracted three times with 95% ethanol at room temperature. The volume of 95% ethanol used in each extraction was 500 ml. The extract was concentrated under reduced pressure by a vacuum rotary evaporator (EYELA N-1000, Japan) to yield an ethanol extract. The ethanol extract (7.2 g) was suspended in water and extracted successively with hexane, chloroform and ethyl acetate, to give hexane (2.12 g), chloroform (1.23 g), ethyl acetate (1.41 g) and residual ethanol subfractions (1.11 g), respectively. Solvents (analytical grade) for extraction were obtained from commercial sources.

2.4. Gas chromatography-mass spectrometry (GC–MS) analysis

The GC–MS analysis of the essential oil was performed using a Shimadzu (Kyoto, Japan) GC-MS (GC-17A) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 μm; Phenomenex, Torrance, CA). For GC-MS detection, electron ionisation 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 splitless mode.

The identity of the components of the essential oil was assigned by comparison of their retention indices (RI), relative to a series *n*-alkane on the ZB-1 capillary column and GC–MS spectra with the Wiley 6.0 MS data and literature data and whenever possible, by co-injection with authentic compounds (Adams, 2001). The relative amounts (RA) of individual components of the essential oil were expressed as percentages of the peak area relative to the total peak area.

2.5. Microorganisms

The following food spoilage and food-borne pathogens, obtained from the American Type Culture Collection (ATCC), were used in this study: *L. monocytogenes* ATCC 19166, *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 6538, *E. coli* ATCC 8739 and *E. coli* O157:H7 ATCC 43888. Food contaminating *Bacillus cereus* SCK 11 strain was obtained from Prof. Kun-Ho Seo, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea. Other pathogens such as *S. aureus* KCTC 1916, *Salmonella enteritidis* KCTC 12021, *S. typhimurium* KCTC 2515, *Enterobacter aerogenes* KCTC 2190 and *Pseudomonas aeruginosa* KCTC 2004 were obtained from the Korean Collection for Type Cultures (KCTC). 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 (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl, solidified with 1.5% agar when required) 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 oil and ethanolic extracts were dissolved in ethanol to a final concentration of 40 mg/ml and sterilised by filtration through 0.45 μm Millipore filters. The antibacterial test was then carried out by agar disc diffusion method (Murray, Baron, Pfaller, Tenover, & Yolke, 1995) using 100 μl of standardised inoculum suspension containing 10^7 CFU/ml of bacteria. To prepare standardised inoculum, bacteria were grown overnight in LB broth that was maintained at 37 °C with constant agitation until the density matched the turbidity of a 0.5 McFarland standard. Tube dilution with sterile saline was carried out to obtain inocula of 10^7 CFU/ml. Then sterile filter paper discs (6 mm diameter) were impregnated with 10 μl of diluted oil and extracts (corresponding to 400 μg/disc) separately, using capillary micro-pipette and placed on the inoculated LB agar. Negative controls were prepared by soaking the disc with ethanol only. Standard reference antibiotics, tetracycline and streptomycin (10 μg/disc, each from Sigma-Aldrich Co., St Louis, MO), 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. Each assay in this experiment was replicated three times.

2.7. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of essential oil and ethanolic extracts was tested by two-fold serial dilution method (Chandrasekaran & Venkatesalu, 2004). The test samples of oil and ethanolic extracts were first dissolved in ethanol, and incorporated into LB broth medium to obtain a concentration of 2000 μg/ml and serially diluted to achieve 1000, 500, 250 125, 62.5 and 31.25 μg/ml. The final concentration of ethanol in the culture medium was maintained at 0.1% (v/v). A 10 μl standardised suspension of each tested organism (10^7 CFU/ml approximately) was transferred to each tube. The control tubes containing only bacterial suspension, were incubated at 37 °C for 24 h. The lowest concentration of the test samples, which did not show any growth of tested organism after macroscopic evaluation, was determined as the MIC.

2.8. Effect of essential oil on viable counts of bacteria

The essential oil which exhibited slightly higher antibacterial activity, as compared to ethanol extract, was chosen for viable counts of bacteria. Also, four bacteria were used on the basis of their sensitivity (the lowest MIC values) to the oil. For viable

counts, each of the tubes containing bacterial suspension (approximately 10^7 CFU/ml) of *L. monocytogenes* ATCC19166, *B. subtilis* ATCC 6633, *S. aureus* ATCC 6538 and *S. enteritidis* KCTC 12021 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, 30, 60, 90, 120 and 150 min time intervals. Enumeration of viable counts on LB plates was monitored as followings: 0.1 ml sample of each treatment was diluted into buffer peptone water, there by diluting it 10-fold, and 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 under the same experimental conditions as mentioned above.

2.9. Statistical analysis

The essential oil and ethanolic extracts were assayed for antibacterial activity. Each experiment was run in triplicate, and mean values were calculated. A Student's *t*-test was computed for the statistical significance of the results.

3. Results

3.1. Chemical composition of the essential oil

The hydrodistillation of the air-dried floral parts of *L. japonica* gave a dark yellowish oil with a yield of 0.43% (w/w). GC–MS analyses of the oil led to the identification of 39 different compounds, representing 92.34% of the total oil. The identified compounds are listed in Table 1. The oil contained a complex mixture mainly of oxygenated mono- and sesquiterpenes, and mono- and sesquiterpene hydrocarbons. The major compounds detected in the oil were *trans*-nerolidol (16.31%), caryophyllene oxide (11.15%), linalool (8.61%), *p*-cymene (7.43%), hexadecanoic acid (6.39%), eugenol (6.13%), geraniol (5.01%), *trans*-linalo oloxide (3.75%), globulol (2.34%), pentadecanoic acid (2.25%), veridiflorol (1.83%), benzyl alcohol (1.63%) and phenylethyl alcohol (1.25%) as shown in Table 1. Also, citronellyl acetate (0.97%), geranylacetone (0.92%), hexahydrofarnesyl acetone (0.87%), α-cadinol (0.85%), tetradecanoic acid (0.85%), dodecanal (0.83%), aromadendrene (0.82%), and 1,8-cineole (0.81%) were found to be the minor components of *L. japonica* oil (Table 1).

Table 1
Chemical composition of the essential oil of *Lonicera japonica* Thunb.

RI[a]	Components	% RA[b]
884	*cis*-4-Heptenal	0.66
961	1-Octen-3-ol	0.70
1003	*p*-Cymene	7.43
1005	1, 8-Cineole	0.81
1017	Acetophenone	0.74
1036	Benzyl alcohol	1.63
1061	*cis*-Linalool oxide (furanoid)	0.69
1076	Linalool	8.61
1085	*trans*-Linalool oxide (furanoid)	3.75
1136	Phenylethyl alcohol	1.25
1159	α-Terpineol (p-Menth-1-en-8-ol)	0.65
1163	Citronellyl acetate	0.97
1182	Decanal	0.46
1200	Dodecane	0.55
1238	Geraniol	5.01
1287	Undecanal	0.52
1368	Geranyl acetate	0.54
1372	*Cis*-Jasmone	0.58
1380	Eugenol	6.13
1397	Dodecanal	0.83
1414	β-Caryophyllene	0.65
1431	Geranylacetone	0.92
1438	Aromadendrene	0.82
1448	β-Ionone	0.70
1529	Elemol	0.52
1549	*trans*-Nerolidol	16.31
1550	Spathulenol	0.69
1566	Globulol	2.34
1567	Caryophyllene oxide	11.15
1568	Veridiflorol	1.83
1574	Epiglobulol	0.50
1626	α-Cadinol	0.85
1660	Junipher camphor	0.76
1697	*cis,trans*-Farnesol	0.68
1769	Tetradecanoic acid	0.85
1816	Hexahydrofarnesylacetone	0.87
1869	Pentadecanoic acid	2.25
1968	Hexadecanoic acid	6.39
2175	Octadecanoic acid	0.75
	Total	92.34

[a] Retention index relative to *n*-alkanes on ZB-1 capillary column.
[b] Relative area (peak area relative to the total peak area).

3.2. In vitro antibacterial activity

According to the results given in Table 2, the oil and ethanol extract exhibited a potent inhibitory effect against *L. monocytogenes* ATCC 19166, *B. subtilis* ATCC 6633, *B. cereus* SCK 11, *S. aureus* (ATCC 6538 and KCTC 1916), *S. enteritidis* KCTC 12021, *S. typhimurium* KCTC 2515, *E. aerogenes* KCTC 2190 and *E. coli* ATCC 8739 with diameter of inhibition zones ranging from 10.3 to 20.3 mm. The oil and ethanolic extracts showed a significantly high antibacterial effect on *L. monocytogenes* ATCC 19166 and *B. subtilis* ATCC 6633. On the other hand, chloroform subfraction displayed the best antibacterial activity toward several strains, such as *L. monocytogenes* ATCC 19166, *B. subtilis* ATCC 6633, *B. cereus* SCK 11 and *S. aureus* ATCC 6538. The ethyl acetate subfraction showed good antibacterial effect against most of the bacteria tested (inhibition zones: 13.2–18.2 mm), whereas hexane subfraction inhibited moderately against some of the bacteria. However, the residual ethanol subfraction did not show any activity against all the bacterial strains tested (data not shown). Ethanol extract of *L. japonica* and its chloroform subfraction exhibited a moderate inhibitory effect against *E. aerogenes* KCTC 2190 and *E. coli* ATCC 8739 with diameter zones of inhibition in the range 10.3–12.4 mm. No inhibitory effect of either ethanolic extract or essential oil was observed against *E. coli* O157:H7 ATCC 43888 and *P. aeruginosa* KCTC 2004. The solvent did not inhibit the growth of any of the bacteria tested at the used concentration.

3.3. Minimum inhibitory concentration (MIC)

As shown in Table 3, the MIC values for the oil were found more susceptible to *L. monocytogenes* ATCC 19166, *B. subtilis* ATCC 6633, *S. aureus* ATCC 6538 and *S. enteritidis* KCTC 12021 (62.5–125 μg/ml) than those of *B. cereus* SCK 11, *S. aureus* KCTC 1916, *S. typhimurium* KCTC 2515, *E. aerogenes* KCTC 2190 and *E. coli* ATCC 8739 (250–500 μg/ml). On the other hand, MIC values of the ethanol extract and its derived subfractions of hexane, chloroform and ethyl acetate against the tested bacteria were found in the range 125–500 μg/ml (Table 3). Ethanol extract and its chloroform subfraction showed higher antibacterial activity by minimum inhibitory concentrations than did hexane and ethyl acetate subfractions. In this study, MIC values of essential oil were found to be more susceptible as compared to ethanolic extracts.

3.4. Effect of essential oil on viable counts of bacteria

Based on susceptibility, further, elaborative study carried out on *L. monocytogenes* ATCC19166, *B. subtilis* ATCC 6633, *S. aureus* ATCC

Table 2
Antibacterial activity of essential oil, ethanol extract and subfractions of ethanol extract of *Lonicera japonica* Thunb. against food-borne and spoilage bacteria.

Microorganism	Zones of inhibition (mm)						
	Essential oil[a]	EtOH extract[b]	Subfractions of EtOH extract[c]			Antibiotics[d]	
			Hexane	$CHCl_3$	EtOAc	TC	SM
L. monocytogenes ATCC 19166	20.3 ± 1.1^{a}	16.2 ± 1.9^{a}	14.1 ± 0.7^{a}	18.2 ± 1.6^{a}	16.2 ± 1.5^{a}	19.2 ± 0.6^{bc}	14.2 ± 0.6^{de}
B. subtilis ATCC 6633	17.8 ± 0.7^{de}	15.4 ± 0.7^{a}	13.2 ± 0.8^{ab}	16.3 ± 1.2^{b}	13.2 ± 0.6^{b}	18.3 ± 0.5^{cd}	14.3 ± 0.6^{de}
B. cereus SCK 111	15.2 ± 1.1	14.0 ± 1.1^{ab}	12.2 ± 0.8^{b}	16.2 ± 1.3^{b}	13.2 ± 1.5^{b}	18.4 ± 0.7^{cd}	14.1 ± 0.6^{de}
S. aureus ATCC 6538	16.3 ± 0.9^{cd}	15.0 ± 1.2^{a}	13.1 ± 1.1^{ab}	15.1 ± 0.5^{bc}	14.2 ± 1.0^{b}	19.2 ± 0.7^{bc}	14.3 ± 0.7^{de}
S. aureus KCTC 1916	14.1 ± 1.1^{bc}	14.1 ± 1.2^{ab}	12.3 ± 0.7^{b}	14.1 ± 1.1^{cd}	14.1 ± 1.2^{b}	18.4 ± 0.5^{cd}	16.2 ± 1.2^{c}
S. enteritidis KCTC 12021	15.3 ± 1.2^{cd}	14.1 ± 1.4^{ab}	12.3 ± 1.1^{b}	14.0 ± 1.2^{cd}	14.2 ± 1.1^{b}	20.3 ± 1.2^{ab}	19.0 ± 0.5^{b}
S. typhimurium KCTC 2515	14.0 ± 1.5^{de}	14.2 ± 1.1^{ab}	10.1 ± 1.1^{c}	14.0 ± 1.2^{cd}	13.2 ± 1.6^{b}	21.3 ± 0.6^{a}	13.3 ± 0.6^{e}
E. aerogenes KCTC 2190	12.4 ± 1.2^{ef}	12.2 ± 1.7^{bc}	nd	12.2 ± 1.2^{de}	10.1 ± 0.7^{c}	17.3 ± 1.2^{d}	24.0 ± 0.7^{a}
E. coli ATCC 8739	12.1 ± 0.6^{f}	10.3 ± 1.4^{c}	nd	11.2 ± 1.2^{f}	nd	20.3 ± 0.5^{ab}	15.3 ± 1.1^{cd}
E. coli O157:H7 ATCC 43888	nd	nd	nd	nd	nd	20.1 ± 1.0^{ab}	14.0 ± 0.5^{de}
P. aeruginosa KCTC 2004	nd	nd	nd	nd	nd	21.3 ± 1.3^{a}	14.3 ± 0.6^{de}

Diameter of inhibition zones of essential oil including diameter of disc 6 mm (tested at a volume of 400 μg/disc).
EtOH extract (400 μg/disc).
Subfractions of EtOH extract (400 μg/disc).
Standard antibiotics: TC, tetracycline and SM, streptomycin (10 μg/disc).
nd, not detected. Values are given as mean ± S.D. (n = 3).
Values in the same column with different superscripts are significantly different (p < 0.05) by Duncan's test.

Table 3
Minimum inhibitory concentration of essential oil, ethanol extract and subfractions of ethanol extract of *Lonicera japonica* Thunb. against food-borne and spoilage bacteria.

Microorganism	Minimum inhibitory concentration (MIC)[a]				
	Essential oil	EtOH extract	Subfractions of EtOH extract		
			Hexane	$CHCl_3$	EtOAc
L. monocytogenes ATCC 19166	62.5	125	250	125	125
B. subtilis ATCC 6633	62.5	125	250	125	125
B. cereus SCK 111	250	250	500	250	250
S. aureus KCTC 6538	125	125	500	125	250
S. aureus ATCC 1916	250	125	500	125	250
S. enteritidis KCTC 12021	125	250	500	250	500
S. typhimurium KCTC 2515	250	250	500	250	500
E. aerogenes KCTC 2190	500	500	nd	500	500
E. coli ATCC 8739	500	500	nd	500	nd
E. coli O157:H7 ATCC 43888	nd	nd	nd	nd	nd
P. aeruginosa KCTC 2004	nd	nd	nd	nd	nd

nd, not detected.
[a] Minimum inhibitory concentration (values in μg/ml).

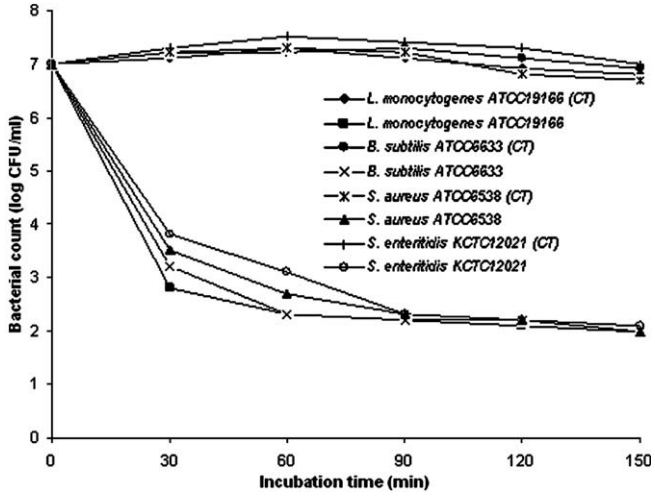

Fig. 1. Effect of *Lonicera japonica* Thunb. essential oil (MIC concentration) on viability of the tested bacteria. CT: control without treatment.

6538 and *S. enteritidis* KCTC 12021, displayed different sensitivities of the essential oil. The results showed that essential oil at MIC concentration strongly inhibited the growth of all the tested bacterial strains. At 60 min exposure, steep decline in CFU numbers was observed against *S. aureus* ATCC 6538 and *S. enteritidis* KCTC 12021. Exposure of 90 min of the essential oil MIC revealed potential effect of antibacterial activity as remarkable decrease in CFU numbers against all the bacterial strains tested (Fig. 1).

4. Discussion

Since ancient times aromatic plant extracts have been in use for many purposes, such as food, drugs and perfumery. Historically, many plant oils and extracts have been reported to have antimicrobial properties. Also, the renewal of interest in food industry and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Essential oils, which are odorous and volatile products of plant secondary metabolism, have a wide application in food flavouring and preservation industries. In recent years, several researchers have reported that the oxygenated mono- or sesquiterpenes, and mono- or sesquiterpene hydrocarbons are the major components of essential oils which exhibit potential antibacterial activities (Cakir, Kordali, Zengin, Izumi, & Hirata, 2004).

Also, the results of the antibacterial screening showed that essential oil, ethanol extract and its derived subfractions of hexane,

chloroform and ethyl acetate have potential activity against some representative food-borne pathogens, except for *Pseudomonas* and *E. coli* O157. The antibacterial activity *L. japonica* essential oil could be contributed to the presence of some major components, such as *trans*-nerolidol, caryophyllene oxide, linalool, *p*-cymene, hexadecanoic acid, eugenol, geraniol, *trans*-linalo oloxide, globulol, pentadecanoic acid, veridiflorol, benzyl alcohol and phenylethyl alcohol (El-Sakhawy, El-Tantawy, Ross, & El-Sohly, 1998; Lago et al., 2004; Melliou, Stratis, & Chinou, 2007). On the other hand, the components in lower amounts such as citronellyl acetate, geranylacetone, hexahydrofarnesyl acetone, 1,8-cineole, α-cadinol, tetradecanoic acid, dodecanal, and aromadendrene may also contribute to antimicrobial activity of the oil (Melliou et al., 2007; Vardar-Ünlü, Silici, & Ünlü, 2008). It is also possible that the minor components might be involved in some type of synergism with the other active compounds. Further, the antibacterial activity of ethanolic extracts could be attributed to the presence of some bioactive phenolic compounds (chlorogenic acid, luteolin, and protocatechuic acid) in *L. japonica* leaves and these finding are in agreement with a previous report (Chang & Hsu, 1992). The antibacterial activity of EtOAc fraction could also be attributed to the presence of phytochemicals, such as biflavonoids (3'-*O*-methyl loniflavone and loniflavone), luteolin and chrysin in *L. japonica* leaves, as shown by Kumar et al. (2005). It has been shown that phenolic compounds were abundant in leaves, while oxygenated sesquiterpenes, alcohols and phenolics were the main constituents in the flowers.

Also, the results from the viable count assay revealed that exposure of the MIC of the oil had a severe effect on the cell viability of the tested bacteria. *L. monocytogenes* ATCC19166 and *B. subtilis* ATCC 6633 were found to be most sensitive to the oil. The oil also exerted its maximum bacterial activity against *S. aureus* ATCC 6538 and *S. enteritidis* KCTC 12021, as evident by the significant reduction in microbial counts at 60 and 90 min exposure to the essential oil. Our research group also found similar potential inhibitory effect of *Nandina domestica* Thunb. essential oil against *L. monocytogenes*, *B. subtilis*, and *S. aureus* (Bajpai et al., 2008).

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 aromas from the oil were almost undetectable after cooking (Skandamis & Nychas, 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.

Phenolic compounds such as chlorogenic acid, luteolin, and protocatechuic acid, 3'-O-methyl loniflavone and loniflavone, have been found in alcoholic extract and ethyl acetate fraction of *L. japonica* leaves (Chang & Hsu, 1992; Kumar et al., 2005), and recent studies have suggested that they may possess multiple therapeutic functions for various human diseases including liver cancer (Yip, Chan, Pang, Tam, & Wong, 2006). In recent years phenolic compounds have gained increasing interest because they exhibit beneficial health effects due to their potential antioxidant and pharmaceutical properties (Cai, Luo, Sun, & Corke, 2004). Thus ethanolic leaf extracts of *L. japonica* with high antibacterial activity selected in this study could be a potential source for inhibitory substances against some food-borne pathogens and they may be candidates for using in foods or food-processing systems.

In this study, the Gram-positive bacteria were found to be more susceptible to the essential oil and various solvent extractions than Gram-negative bacteria. Deans, Noble, Hiltunen, Wuryani, and Penzes (1995) observed that the susceptibility of Gram-positive and Gram-negative bacteria to plant volatile oils had little influence on growth inhibition. However, some oils appeared more specific, exerting a greater inhibitory activity against Gram-positive bacteria. It is often reported that Gram-negative bacteria are more resistant to essential oils. The hydrophilic cell wall structure of Gram-negative bacteria is constituted essentially of a lipopolysaccharide that blocks the penetration of hydrophobic oil and avoids the accumulation of essential oils in target cell membrane (Bezic, Skocibusic, Dinkic, & Radonic, 2003). This is the reason that Gram-positive bacteria were found to be more sensitive to the essential oil, ethanol extract and various ethanol-derived subfractions of *L. japonica* than those of Gram-negative bacteria. Among the Gram-negative bacteria, the oil and ethanolic extracts were not active against *Pseudomonas* and *E. coli* O157. The bacterium *Pseudomonas* has shown resistance to most essential oils and virtually all known antimicrobials and antibiotics, due to a very restrictive outer membrane barrier, highly resistant even to synthetic drugs (Bezic et al., 2003). It has been observed that the antibacterial properties of plant essential oils against *E. coli* O157:H7 are dose-dependent (Burt, 2004). Using the paper disc diffusion method, Ozcan, Sagdic, and Ozcan (2003) observed that thyme essential oil at low concentration had no antibacterial activity against *E. coli* O157:H7. However, this is not true for all oils. The reason for this behaviour has not been yet adequately elucidated.

In conclusion, the results of our study suggest the possibility of using the oil or organic extracts of *L. japonica* as natural antimicrobials in the food industry because they possess strong antibacterial activities. The use of natural antibacterial agents will be suitable for applications in the food industry because this plant is not known to be toxic, because it has been consumed by mankind for centuries without showing any signs of toxicity. A further study is in the pipeline to evaluate the bio-active compounds present in various organic extracts of *L. japonica*. Also, further research is needed in order to obtain information regarding the practical effectiveness of essential oil or extracts to prevent the growth of food-borne and spoilage microbes under specific application conditions.

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