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In vitro antibacterial activity and essential oil composition of Litsea petiolata Hook. f. leaves

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ARTICLE INFO	ABSTRACT
Article history: Received on: 16/03/2017 Accepted on: 20/07/2017 Available online: 30/09/2017	Essential oil of <i>Litsea petiolata</i> (Hook f) was extracted from their leaves collecting from Chiang Rai province, Northern Thailand. Hydrodistillation technique with Clevenger system was performed to extract <i>L. petiolata</i> essential oil. The essential oil composition was further analyzed by gas chromatographic-mass spectrometric technique. A total of 74 volatile constituents were identified. Representing 96.94% of the oil with 2-ketonesare
<i>Key words:</i> <i>Litsea petiolata</i> (Hook f), essential oil, antibacterial, composition, GC-MS.	the key compound group. The major identified volatile components were 2-undecanone (79.52%), 12-tridecen- 2-one (6.26%) and 2-nonanone (2.46%), respectively. The essential oil of <i>L. petiolata</i> (Hook f) leaves showed good antibacterial activity against tested Gram-positive and Gram-negative human pathogenic bacteria including <i>Bacillus cereus ATCC</i> 11778, <i>B. subtilis</i> TISTR 008, <i>Staphylococcus aureus ATCC</i> 25923, <i>S. epidermidis ATCC</i> 12228, <i>S. agalactiae</i> DMST 17129, <i>Escherichia coli</i> ATCC 25922, <i>Salmonella typhimurium ATCC</i> 13311, <i>Vibrio cholerae</i> DMST 2873 and <i>V. parahaemolyticus</i> DMST 21243. The zone of inhibition was obtained from 9.54±1.24 to 18.07±2.19 mm. The minimum inhibitory concentration (MIC) values were within the antibacterial activity range and varied between 31.25 and 500 µg/mL. The essential oil of <i>L. petiolata</i> (Hook f) leaves showed maximum antibacterial activity against <i>S. aureus ATCC</i> 25923 and <i>E. coli ATCC</i> 25922. This study indicates that <i>L. petiolata</i> (Hook f) leaf oil could be employed as a natural medicinal application in antibacterial drugs.

INTRODUCTION

Treatments of human health and welfare have been increased continuously according to numbers of infectious diseases. They are the major problems for immense morbidity and mortality in all parts of the world especially developing countries (Memish *et al.*, 2003). The usual infectious diseases including incidences of nosocomial and opportunistic infections have also expanded intensely. Moreover, new or drug resistant pathogens are increasing and developing day by day causing from number of infection diseases. Hospitalized patients with immunodeficiency may take high risk of severe and invasive infections. In order to improve the problem, new or novel antimicrobial agents are urgently needed.

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Plants, especially in developing countries, are considered as an important resource of various drugs to prevent diseases due to various biodiversites. They have been employed in classical remedies for curing of common illnesses by approximately 60-80% of the world's population (Zhang, 2004). Essential oils are aromatic viscous liquids produced from aromatic plants containing a group of secondary metabolites mainly terpenoids with aromatic properties (Stefanakis et al., 2013). They are used intensively as antimicrobial, antivirals, and antioxidants (Pandey et al., 2014). Essential oils are also considered to be safe ingredients for using as antibacterial additives (Tian et al., 2014). According to many reports on the antimicrobial and antioxidant capacity, essential oil has been selected as natural antimicrobial and antioxidant agents continuously (Hossain et al., 2014; Jallali et al., 2014). Litsea petiolata (Hook f) (family Lauraceae) is known in Thai as Tummung. It is a medium-sized trees intensively cultivated in the Southern and Northeastern parts of Thailand.

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Their leaves possess individually unique odor. No previous studies report the antibacterial properties of *L. petiolata* (Hook f) leaf oil while the related species such as *L. japonica* leaf extract presented anti-inflammatory and anti-oxidative activities (Yoon *et al.*, 2010). In addition to being flavoring foods, methanol extract of *L. petiolata* (Hook f) can also act as antioxidant agent reported by Suksamerkun *et al.* (2013).

In the present work, the essential oil of *L. petiolata* (Hook f) leaves from Thailand was extracted prior their chemical composition was investigated by gas-chromatographic-mass spectrometric analyses. Their antibacterial activities against tested pathogenic bacteria were also evaluated.

EXPERIMENTAL

Plant material

Fresh leaves of *L. petiolata* (Hook f) was collected in Chiang Rai province, Northern Thailand (99°87'10" E, 20°33'06" N) between 5:00 and 6:00 pm, in the first week on August 2016. Three year old *L. petiolata* was identified and deposited at the Mae Fah Luang University Botanical Garden, Chiang Rai, Thailand as voucher herbarium specimen of MFLU No. 10004.

Essential oil extraction

The extraction of *L. petiolata* (Hook f) leaf oil was obtained from 250 g fresh plants by hydro-distillation using Clevenger system, during 4 h. Excess water retained in crude essential oil was removed by using anhydrous sodium sulphate. Pure essential oil was collected in sealed vial. The essential oil was dissolved in dichloromethane (1/100 v/v) prior injecting to gas chromatographic-mass spectrometric tool.

Chromatography conditions

The volatile constituents of *L. petiolata* (Hook f) leaf oil was investigated using a Hewlett Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with an HP-5MS (5% phenylpolymethylsiloxane) capillary column (30 m \times 0.25 mm i.d., film thickness 0.25 µm; Agilent Technologies, USA) and interfaced to an HP model 5973 mass selective detector operating in electron impact mode with 70 eV. Injector and detector temperatures were set at 250 °C and 280 °C, respectively. One micro-liter of diluted essential oil was injected and their chemical composition was separated in the column held initially at 60 °C and then raised up to 240 °C with a rate of 3 °C/min. Helium, carrier gas, was used in this study with a flow rate of 1 mL/min.

The percentage peak area was calculated relative to total peak area of individual identified components. Identification of the volatile constituents was performed by comparing mass spectra with those of authentic reference compounds and retention indices toC₇-C₂₂*n*-alkanes, and using a comparison of the mass spectra of individual components with the reference mass spectra in the W8N08 and NIST08 databases, and literature (Adams, 2001).

Bacterial pathogens

Antibacterial activity tests of L. petiolata (Hook f) leaf oil were carried out against the Gram-positive bacteria including Bacillus cereus ATCC 11778, B. subtilis TISTR 008, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, S. epidermidis ATCC 12228 and S. agalactiae DMST 17129, and Gram-negative bacteria including Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Proteus mirabilis DMST 8212, Pseudomonas aeruginosa ATCC 27853, Salmonella typhi DMST 22842, S. typhimurium ATCC 13311, Shigella flexneri DMST 4423, Vibrio cholerae DMST 2873 and V. parahaemolyticus DMST 21243. These bacteria with ATCC and TISTR code were obtained from Department of Medical Science, Ministry of Health Bangkok and Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand while DMST code was obtained from Culture Collection for Medical Microorganisms, Department of Medical Sciences, Thailand.

Antibacterial activity

Screening of antibacterial activities of L. petiolata (Hook f) leaf oil was performed by the disk diffusion method (Mar and Pripdeevech, 2014). It was done using an 24 h culture at 37 °C in 10 mL tryptic soy broth medium (BIO BASIC INC., USA). All bacteria were cultured to nearly 105CFU/mL in solution of sterile saline. Culture suspension (300mL) were spread on a sterile plates containing Mueller-Hinton agar. The essential oil was dissolved in dichloromethane to obtain various concentrations; 1000, 500, 250, 125, 62.50, 31.25, 15.62 7.81 and 3.91 µg/mL. A sterilized 6-mm of paper disc (WhatmanTM, USA) moistened with 30 µL of sample was placed on the agar surface. A standard disc containing penicillin was used as reference control. All plates were sealed with sterile parafilm to prevent evaporation of the test samples. The plates were incubated at 37°C for 24 h. After the incubation period, the zone of inhibition and minimum inhibition concentration (MIC) was measured. Screening of antibacterial activity was experimented in triplicate, and mean value was also determined.

RESULTS AND DISCUSSION

The essential oil (10.89% w/w) yield was obtained from *L. petiolata* (Hook f) leaves. The chemical composition of *L. petiolata* (Hook f) leaf oil obtained was investigated by GC-MS which allowed identification of about 96.94% of oil constituents (Table 1). The major components from *L. petiolata* (Hook f) leaf oil were 2-undecanone (79.52%) , 12-tridecen-2-one (6.26%) , 2-nonanone (2.46%) and 2*E*,6*Z*-farnesol (1.67%) while *trans*-pulegol (0.78%) , 8-nonene-2-one (0.39%), abienol (0.34%) and 2*E*,4*E*-decadienol (0.22%) were considered as minor components. Pulpipat *et al.* (2011) also reported 2-undecanone, 2-nonanone and 2*E*,6*Z*-farnesol as the key volatile components of extract of *L. petiolata* (Hook f) leaves obtained by using supercritical fluid carbon dioxide extraction method.

Table 1: Chemical composition of L. petiolata (Hook f) leaf oil with the percentage of content obtained by hydrodistillation

No.	Chemical composition of <i>L. petiolata</i> (Hook f) la Compound	RI	% area	No.	Compound	RI	% area
1	hexen-3-ol	779	0.05	38	epi-cubebol	1494	0.05
2	3Z-hexenol	850	0.09	39	β-bisabolene	1505	0.09
3	2Z-hexenol	861	0.04	40	2E,4E-dodecadienal	1517	0.09
4	tricyclene	917	0.04	41	citronellyl butanoate	1529	0.08
5	artemisia triene	924	0.04	42	furopelargone A	1537	0.05
6	benzaldehyde	963	0.06	43	1,10-decanediol	1548	0.08
7	sabinene	967	0.05	44	geranyl butanoate	1563	0.05
8	β-pinene	971	0.11	45	gleenol	1584	0.07
9	trans-meta-mentha-2,8-diene	979	0.04	46	globulol	1591	0.08
10	dehydroxy-trans-linalool oxide	993	0.04	47	epi-cedrol	1618	0.12
11	δ-2-carene	997	0.05	48	2-epi-αcedren-3-one	1625	0.08
12	α-phellandrene	1003	0.05	49	2E-hexenyl phenyl acetate	1636	0.04
13	α-terpinene	1014	0.06	50	Z-amyl cinnamaldehyde	1649	0.09
14	E-ethyl-2-methyl-2-pentenoate	1017	0.07	51	dihydro-eudesmol	1661	0.09
15	Z-β-ocimene	1033	0.04	52	E-citronellyl tiglate	1666	0.08
16	E-β-ocimene	1044	0.04	53	trans-methyl dihydro jasmonate	1681	0.05
17	isobutyl acetoacetate	1059	0.05	54	2E,6Z-farnesol	1706	1.67
18	8-nonene-2-one	1070	0.39	55	2E,6E-farnesoic acid	1818	0.04
19	2-nonanone	1081	2.46	56	7-hydroxy coumarin	1837	0.08
20	terpineol	1129	0.04	57	hexadecanol	1871	0.12
21	3Z-hexenyl isobutanoate	1143	0.12	58	5E,9Z-farnesyl acetone	1884	0.11
22	δ-terpineol	1162	0.11	59	5E,9E-farnesyl acetone	1905	0.16
23	2-decanone	1178	0.14	60	carissone	1926	0.08
24	trans-pulegol	1211	0.78	61	cembrene	1937	0.08
25	2-undecanone	1297	79.52	62	3E-cembrene A	1953	0.05
26	2E,4E-decadienol	1320	0.22	63	geranyl benzoate	1959	0.06
27	4-hydroxybenzaldehyde	1358	0.08	64	sclarene	1972	0.06
28	cyclosativene	1366	0.07	65	7-hydroxy-4-methyl coumarin	1978	0.09
29	linalool isobutanoate	1373	0.08	66	ethyl hexadecanoate	1993	0.13
30	2-dodecanone	1383	0.07	67	ethylene brassylate	2010	0.04
31	longifolene	1407	0.18	68	juvibione	2015	0.13
32	β-duprezianene	1422	0.08	69	canellal	2047	0.06
33	cis-a-ambrinol	1439	0.09	70	methyl linoleate	2106	0.14
34	cis-cadina-1 (6),4-diene	1461	0.06	71	methyl octadecanoate	2123	0.11
35	cis-muurola-4 (14) ,5-diene	1465	0.08	72	abienol	2147	0.34
36	12-tridecen-2-one	1479	6.26	73	incensole acetate	2184	0.19
37	2-tridecanone	1485	0.08	74	ethyl octadecanoate	2194	0.18
PI linear	temperature program retention index on DB-5 c	olumn			•		

RI, linear temperature program retention index on DB-5 column.

However, content of minor components in this study were varied when compared to those found in Pulpipat et al. (2011) due to different extraction method, various ecological and biological conditions, as well as genetic factor. The results for inhibition zone diameter and MIC assay of L. petiolata (Hook f) leaf oil and penicillin against tested bacterial pathogens are shown in Table 2. The MIC values of L. petiolata (Hook f) leaf oil were in the ranges of 31.25-500 µg/mL. The MIC values for Grampositive bacteria were 31.25-500 µg/mL. The L. petiolata (Hook f) leaf oil exhibited inhibitory effect against B. cereus ATCC 11778, B. subtilis TISTR 008 and S. epidermidis ATCC 12228 at MIC of 125 µg/mL. The MIC values of L. petiolata (Hook f) leaf oil against S. agalactiae DMST 17129 was higher than those of the other Gram-positive bacteria (500 µg/mL). The essential oil of L. petiolata (Hook f) leaves had bactericidal effect against some tested Gram-negative bacteria including E. coli ATCC 25922, S. typhimurium ATCC 13311, V. cholerae DMST 2873 and V. parahaemolyticus DMST 21243. Among the tested Gram-negative bacteria, the MIC values of V. cholerae DMST 2873 were higher than those of others (MIC= 250 µg/mL). In addition, S. aureus ATCC 25923 and E. coli ATCC 25922 showed more sensitivity to essential oil of L. petiolata (Hook f) leaves and the oil had antibacterial activities on both bacterial pathogens

(MIC = $31.25 \ \mu g/mL$). The complex mixtures of various volatile components in the essential oil of L. petiolata (Hook f) leaves may afford multiple mechanisms for potential antibacterial activity. The major components of L. petiolata (Hook f) leaf oil were considered to play an important role in the antibacterial activity, while the minor volatile constituents were also detected to result in synergistic outcomes (Li et al., 2014). Previous reports on essential oils indicated that the most active essential oil compounds are terpene derivatives and organic molecules containing functional groups, while hydrocarbonsare the least active compounds (Burt, 2004; Kalemba and Kunicka, 2003). The major components of L. petiolata (Hook f) leaf oil were 2-ketones (88.92%) which was obtained from mainly 2-undecanone (79.52%). This component normally showed a moderate antibacterial activity (Reddy and Al-Rajab, 2016). Al-Shuneigat et al. (2015) reported that the anti-bacterial activity of Ruta graveolens essential oil was resulted from ketones. The antibacterial activity of L. petiolata (Hook f) leaf oil may be similarly correlated to its ketones content, while the minor compounds could be responsible for other synergistic properties such as 2E, 6Z-farnesol, Z-amyl cinnamaldehyde, longifolene, δ terpineol & dihydro-eudesmol also as reported by Burt (2004).

Table 2: Antibacterial activities of essential oil of L	petiolata (Hook f) leaf oil and pe	enicillin.
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Bacteria	Inhibition dia	MIC (µg/mL)		
	Essential oil	Penicillin	Essential oil	Penicillin
Gram-positive				
B. cereus ATCC 11778	11.76±2.81	15.08±0.91	125	3.91
B. subtilis TISTR 008	18.07±2.19	13.75±1.22	125	3.91
E. faecalis ATCC 29212	-	11.12±1.15	-	62.50
S. aureus ATCC 25923	10.14 ± 2.54	10.08±0.29	31.25	7.81
S. epidermidis ATCC 12228	10.25±1.67	12.25±2.28	125	15.62
S. agalactiae DMST 17129	9.68±1.39	15.23±2.38	500	15.62
Gram-negative				
E. coli ATCC 25922	9.54±1.24	10.41±1.56	31.25	7.81
K. pneumoniae ATCC 700603	-	9.15±2.54	-	500
P. mirabilis DMST 8212	-	8.42±3.12	-	500
P. aeruginosa ATCC 27853	-	7.75±1.20	-	125
S. typhi DMST 22842	-	11.12±1.15	-	1000
S.typhimurium ATCC 13311	9.81±2.17	9.55±1.10	125	7.81
S. flexneri DMST 4423	-	7.08 ± 2.17	-	500
V. cholerae DMST 2873	9.54±1.55	17.15±1.87	250	125
V. parahaemolyticus DMST 21243	9.98 ± 2.47	11.36±1.68	125	62.50
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-not detected

Normally, cell membrane is main target of essential oil components. It is extinguished resulting in ions leakage, membrane permeability, and obstruction of enzymes or proteins (Saad *et al.*, 2013; Hyldgaard *et al.*, 2012; Cox *et al.*, 2000).The broad spectrum antibacterial activity of *L. petiolata* (Hook f) leaf oil against tested pathogenic bacteria was detected in Grampositive bacteria. It was noted that Gram-negative bacteria may contain higher resistance to the *L. petiolata* (Hook f) leaf oil than those found in Gram-positive bacteria. This result may be related to the structure of cell wall in Gram-negative bacteria which is hydrophilic and constitutes mainly of a lipo-polysaccharide inhibiting the penetration of hydrophobic oil and avoiding the accumulation of *L. petiolata* (Hook f) leaf oilin the target cell membrane (Bajpai *et al.*, 2008).

Gram-positive bacteria were considered to be more sensitive to the *L. petiolata* (Hook f) leaf oil than Gram-negative bacteria (Amensour *et al.*, 2010). Essential oil of *L. petiolata* (Hook f) leaves was effective against most tested bacterial pathogens included in the study. However, the susceptibility of Gram-negative bacteria may be variable due to genetic factors. Thus, the antibacterial activity of *L. petiolata* (Hook f) leaf oil may be differed due to the existence of some targets (Boire *et al.*, 2013). Leaf oil of *L. petiolata* (Hook f) presented the great antibacterial potential toward *S. aureus* ATCC 25923 and *E. coli ATCC* 25922.

CONCLUSIONS

The *L. petiolata* (Hook f) leaf oil showed various degrees of antibacterial properties against tested pathogenic bacteria significant growth inhibiting effects on Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*). The potential of leaf oil of *L. petiolata* (Hook f) against these pathogens may suggest a scientific knowledge for the developing of this plant in the prevention and treatment of bacterial infections.

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