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Chemical composition and antibacterial activities of essential oils of *Lavandula* × *hybrid* 'boysemberry ruffles', *L. pedunculata* 'princess' and *L.* × *hybrid* 'high five purple'

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Abstract: Essential oils of *Lavandula* plants are used in perfumery and therapeutic applications. They are mainly planted in Mediterranean area and several regions in Asia. According to its beneficial property, chemical composition and bioactivity of various species of *Lavandula* plants have been studied. Thus, this study aimed to extract the essential oils of three species from *Lavandula* plants grown in Thailand including *L.* × *hybrid* 'boysemberry ruffles', *L. pedunculata* 'princess' and *L.* × *hybrid* 'high five purple'. The yield of essential oils obtained among these samples were 0.11-0.24 %w/w. The chemical composition of all essential oils were investigated using gas chromatography-mass spectrometry. A total of 89 volatile compounds were identified. Four main compounds including 1,8-cineole, fenchone, camphor and α-pinene were detected in all samples with different contents. For the antibacterial activity assay, the experiment was performed by agar disc diffusion method against four Gram-negative bacteria (*Klebsiella pneumonia, Salmonella typhimurium, Escherichia coli* and *Pseudomonas aeruginosa*) and five Gram-positive bacteria (*Staphylococcus epidermidis, S. aureus, Bacillus subtilis, Enterococcus faecium* and *Streptococcus pyogens*). The result showed that the great antibacterial activity was obtained from the essential oil of *L. pedunculata* 'princess' compared to those found in essential oils of *L.* × *hybrid* 'boysemberry ruffles' and *L.* × *hybrid* 'high five purple', respectively. Different antibacterial activities among these samples could be correlated to major components such as 1,8-cineole, fenchone and camphor. It was indicated that essential oil of *L. pedunculata* 'princess' may be used as natural antibacterial agent in therapeutic applications.

Keywords: Antibacterial, chemical composition, essential oil, gas chromatography-mass spectrometry, *Lavandula*.

Introduction

Essential oils from plant species '*Lavandula'* have been receiving many interests due to their applications in many industries such as food, pharmaceutical, and cosmetic. In cosmetic industry, the oils can be used in the a production of perfumes, colognes, body wash and other cosmetics (Cavanagh and Wilkinson, 2005; Lesage-Meessen et al., 2015). The use of lavender essential oil might depend on their cultivar source due to the variety of composition. The volatiles constituents in the extracted oil are feasibly depend on many factors such as cultivar and extraction method. It has been found that linalool, linalyl acetate, 1,8-cineole, β-ocimene (both cis- and trans-), terpinen-4-ol and camphor are the main constituents of lavender oil. (Cavanagh and Wilkinson, 2002; Costa et al., 2012). *Lavandula* essential oil has the antimicrobial activity against various fungi and bacteria causing skin infection (Kunicka-Styczyńska et al., 2009). It was found that there is a relation between volatiles in the essential oil and antimicrobial strength. Linalool, a volatile components in lavender essential oil, has a strong antimicrobial strength (Guo et al., 2018). In other studies, it is reported that lavender oil is used for relieving symptoms of psoriasis, dermatitis, and eczema (Cavanagh and Wilkinson, 2002; Matos et al., 2009). Another factor that affect quality and yield of the oil is an extraction method. Hydrodistillation is chosen to extract essential oils in this study due to it is a popular method using less heat and retaining most of the volatiles in the sample (Costa et al., 2012). Therefore, the aims of this study are to extract essential oils from selected species, analyze volatile compounds using gas chromatography-mass spectrometry (GC-MS) and screen their antibacterial activities.

Materials and Methods

Plant materials

The sample *Lavandula* plants including *L. pedunculatas* 'princess', *L. x hybrid* 'high five purple' and *L. x hybrid* 'boysemberry ruffles' were collected from Angkhang Royal Agricultural Station, Chiang Mai, Thailand on April 2018.

Extraction of essential oils

Fresh arial part of each sample (250 g) was subjected to hydrodistillation. The essential oils were extracted for three h. The obtained essential oils were dried over anhydrous Na_2SO_4, and diluted with dichloromethane (1:100 v/v) prior subjecting to GC-MS injection port.

Analysis of volatile compounds

The volatile components were also identified by GC-MS using a Hewlett–Packard 5973 MSD apparatus (Agilent 5973 network mass selective detector) from Agilent Technologies, USA with fused-silica capillary columns. The temperature program was started at 60°C and raised up to 220°C with a rate of 3°C/min. Helium was used as carrier gas with a flow rate of 1 mL/min. Injector and detector temperatures were set at 250 °C and 280°C, respectively. Electron impact ionization was employed with electron energy of 70 eV. The acquisition was performed in scanning mode (mass range m/z 35-300 u). Identification of all volatile compounds was performed by comparing their mass spectra to those obtained from NIST 05 and Wiley 7N libraries and literature (Angioni et al., 2006; Adams, 2017). Moreover, linear retention indices correlating with C_9-C_{18} n-alkanes were used to confirm identification.

Antibacterial activity assay

Antibacterial activity was performed using the paper disc diffusion method of Zaidan (2005). The bacteria strains including *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *S. pyrogens* DMST 17020, *Bacillus subtilis* TISTR 008, *Enterococcus faecium* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 13311 were obtained from the Department of Medical Science, Ministry of Health, Bangkok, Thailand. All strains were subcultured in Müller-Hinton Broth (YM, Difco, USA) at 37°C for 24 h. All pathogenic bacterial was further spread in Müller-Hinton agar. Different concentrations of essential oils were prepared by two-fold dilution method using hexane as solvent until the final concentration was obtained as follows: 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 mg/mL. Thirty microliters of each essential oil sample were dropped on a sterilized 6 mm diameter paper disc (Whatman™, USA), and the Petri dish plates were placed in culture plates prior incubating at 37°C for 24 h. Hexane and chloramphenicol antibiotic were used as negative and positive control, respectively. The result was reported as minimum inhibition concentration (MIC) and zone inhibition diameter. Each experiment was carried out in triplicate.

Result & Discussion

Essential oils from three *Lavandula* species, *L. pedunculatas* 'princess', *L. x hybrid* 'high five purple' and *L. x hybrid* 'boysemberry ruffles' were extracted by hydrodistillation method. All essential oils were clear with pale yellow and strong piquant smell. All essential oils yielded ranging between 0.11-0.24 %w/w. The results of volatile constituents in the essential oils from *Lavandula* species analyzed by GC-MS are listed in Table 1. A total of eighty-nine volatile constituents were detected in the essential oils extracted from the selected *Lavandula* species. Forty-five compounds were observed in the essential oil of *L. x hybrid* 'boysemberry ruffles' while the essential oils from *L. pedunculatas* 'princess' and *L. x hybrid* 'high five purple' contained 42 and 38 volatile compounds, respectively. It was also found that 1,8-cineole, fenchone, camphor and α-pinene were the main compounds in all *Lavandula* species with different contents. This might be the result of their genetic diversity. Camphor found to be the most abundant volatile in *L. pedunculata* 'princess' was similar to those found previously in the study of Gonçalves and Romano (2013). Camphor is known as antimicrobial (Zuccarini, 2009), thus the essential oil with the highest amount of camphor could be expected to provide the greatest antibacterial activity in this study. As show in Table 2. the essential oil of *L. pedunculatas* 'princess' showed the best result in the microbial inhibitory activities, considering from its ability to inhibit microbial growth by MIC and zone inhibition diameter. It was able to inhibit most of the bacterial pathogens used in this study. *S. aureus* was evaluated as the most sensitive bacteria with MIC and zone inhibition diameter of *L. pedunculatas* 'princess' essential oil being 0.39 mg/mL and 7. 37 mm, respectively. On the other hand, *K. pneumoniae* and *B. subtilis* were considered to be less sensitive when being tested with all essential oils. The greatest antibacterial activity of *L. pedunculatas* 'princess' might be due to the presence of major volatile compounds, such as 1,8-cineole, fenchone, camphor (Vakilian et al., 2011).

Conclusion

This study reports the essential oil composition and antibacterial activities of three different *Lavandula* species grown in Thailand. Each *Lavandula* species appeared to have unique volatile profiles and antibacterial activities. The essential oil of *L. pedunculata* 'princess' showed a broad spectrum of antibacterial activities, suggesting thatit may be used as antimicrobial agent in the food, cosmetic and therapeutic applications.

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Table 1. Volatile constituents of essential oils from three *Lavandula* species analysed by GC-MS.

No.	Compound	RI*	RI**	% relative peak area		
				L. x hybrid **'boysemberry ruffles'**	***L. x hybrid*** **'high five purple'**	***L. pedunculatas*** **'princess'**
1	tricyclene	918	921	0.11±0.18		0.31±0.27
2	α-pinene	928	932	2.31±0.07	0.02±0.00	4.75±0.18
3	α-fenchene	940	945		1.60±0.07	
4	camphene	941	946	1.97±0.08		4.78±0.14
5	sabinene	962	969	0.37±0.13	0.10±0.0.5	0.13±0.07
6	β-pinene	970	974	0.95±0.08	0.47±0.11	0.37±0.11
7	3-octanone	976	979			0.02±0.00
8	myrcene	984	988	0.33±0.13	0.34±0.10	0.32±0.04
9	3-octanol	985	988	0.06±0.10		0.11±0.03
10	δ-2-carene	998	1001	1.45±0.11		
11	α-phellandrene	999	1002			0.11±0.05
12	δ-3-carene	1002	1008			1.87±0.07
13	α-terpinene	1010	1014		0.02±0.00	0.13±0.04
14	p-cymene	1015	1020		0.33±0.13	0.09±0.03
15	o-cymene	1018	1022			0.47±0.10
16	limonene	1020	1024			9.73±0.11
17	1,8-cineole	1022	1026	28.81±0.14	12.29±0.15	9.73±0.10
18	Z-β-ocimene	1029	1032		1.05±0.10	0.70±0.07
19	β-ocimene	1041	1044	0.23±0.13		0.02±0.00
20	pulegone	1048	1053			0.02±0.00
21	γ-terpinene	1050	1054	0.08±0.04		0.19±0.02
22	cis-linalool oxide	1060	1067	0.18±0.02		
23	cis-vertocitral C	1070	1076	0.07±0.02		
24	camphenilone	1072	1078	0.09±0.04		0.13±0.05
25	fenchone	1079	1083	5.50±0.13	21.91±0.14	23.73±0.13
26	isoterpinolene	1080	1085		2.09±0.13	
29	trans-vertocitral C	1096	1105	0.02±0.00		
30	camphor	1138	1141	33.62±0.17	46.65±2.13	30.30±1.24
31	isoborneol	1148	1155			0.56±0.17
32	δ-terpineol	1156	1162	1.52±0.11	0.58±0.14	
33	lavandulol	1161	1164		0.50±0.04	
34	terpinen-4-ol	1170	1174	0.87±0.08		
35	santalone	1173	1177			0.71±0.13
36	p-methyl acetophenone	1173	1179		0.08±0.10	
37	α-terpineol	1180	1186	1.21±0.13	0.74±0.10	0.47±0.13
38	trans-isocarveol	1183	1187	0.61±0.14		
39	myrtenal	1190	1195		0.17±0.10	
40	verbenone	1199	1204	0.20±0.17	0.12±0.16	0.18±0.14
41	endo-fenchyl acetate	1214	1218		0.20±0.11	
42	carvone	1234	1239	0.09±0.02		0.18±0.08
43	iso-isopulegyl acetate	1275	1283		0.66±0.13	2.84±0.13
44	bornyl acetate	1283	1287	5.28±0.28		
45	lavandulyl acetate	1284	1288		0.02±0.00	
46	geranyl formate	1295	1298	1.06±0.14		
47	cis-pinocarvyl acetate	1305	1311	0.22±0.13		1.79±0.12
48	neo-verbanol acetate	1315	1319	0.13±0.10		
49	E-cis-jasmonol	1324	1328		1.07±0.14	

No.	Compound	RI*	RI**	% relative peak area		
				L. x hybrid 'boysemberry ruffles'	*L. x hybrid* 'high five purple'	*L. pedunculatas* 'princess'
50	trans-carvyl acetate	1335	1339	3.16±0.28		
51	α-ylangene	1370	1373	0.19±0.14		
52	α-duprezianene	1383	1387	0.85±0.13		
53	β-cubebene	1383	1387			0.02±0.00
54	iso-longifolene	1384	1389		0.13±0.07	0.05±0.02
55	sesquithujene	1400	1405		0.05±0.02	
56	acora-3,7(14)-diene	1401	1407	0.20±0.13		
57	α-barbatene	1403	1407	0.02±0.00		
58	α-gurjunene	1406	1409	0.22±0.11	0.02±0.00	
59	α-cedrene	1407	1410			0.15±0.05
60	aromadendrene	1433	1439	0.08±0.02		
61	Z-β-farnesene	1435	1440			0.02±0.00
62	γ-muurolene	1472	1478	0.15±0.04		0.02±0.00
63	amorpha-4,7(11)-diene	1474	1479		0.05±0.02	0.10±0.07
64	β-vetispirene	1491	1493	0.18±0.04		
65	valencene	1494	1496	0.06±0.02		
66	epizonarene	1496	1501			0.05±0.02
67	α-chamigrene	1498	1503			0.07±0.02
68	premnaspirodiene	1499	1505		1.36±0.13	
69	Z-α-bisabolene	1499	1506	0.11±0.04		
70	δ-amorphene	1507	1511		0.09±0.02	
71	γ-cadinene	1508	1513	0.32±0.04		
72	sesquicineole	1509	1518		0.02±0.00	
73	7-epi-α-selinene	1517	1521	0.05±0.02		
74	10-epi-cubebol	1525	1533	0.11±0.04		
75	E-β-ionol acetate	1531	1537		0.07±0.02	
76	α-calacorene	1538	1542			0.19±0.13
77	trans-dauca-4(11),7-diene	1547	1556		0.22±0.11	
78	E-nerolidol	1552	1562		0.10±0.03	
79	longipinanol	1560	1567			0.40±0.07
80	zierone	1565	1574	0.37±0.13		
81	santalenone	1567	1576		0.02±0.00	0.05±0.02
82	β-copaen-4α-ol	1581	1590	0.93±0.07	0.51±0.07	0.02±0.00
83	khusimone	1591	1604	0.18±0.14		
84	β-humulene epoxide	1595	1614		0.06±0.02	
85	epi-cedrol	1605	1618		0.37±0.15	
86	β-acorenol	1619	1637		0.12±0.14	
87	α-muurolol	1629	1644			0.65±0.08
88	himachalol	1648	1652	0.17±0.08		
89	α-bisabolol oxide B	1649	1658		0.02±0.00	
%yield (w/w)				0.24±0.13	0.11±0.32	0.22±0.18
Total of volatile components				45	38	42

RI*; calculated retention indices, RI**; retention indices from literature Adams (2017).

Table 2. Antibacterial activities of essential oils from three *Lavandula* species and chloramphenicol expressed as inhibition zone diameter (mm) and MIC.

Bacterial strain	**Essential oil** MIC (mg/mL) (diameter±SD)			**Chloramphenicol** MIC (µg/mL) (diameter±SD)
	***L. pedunculatas* 'princess'**	***L. x hybrid* 'boysemberry ruffles'**	***L. x hybrid* 'high five purple'**	
Klebsiella pneumoniae	12.50 (7.67±0.06)	6.25 (7.40±0.17)	12.50 (8.17±0.21)	500.00 (9.30±2.50)
Salmonella typhimurium	1.56 (7.23±0.15)	6.25 (7.30±0.10)		500.00 (8.25±2.10)
Escherichia coli	0.78 (8.37±0.15)	12.50 (7.27±0.15)	12.50 (7.57±0.15)	7.81 (10.80±1.11)
Pseudomonas aeruginosa	0.78 (7.33±0.11)	12.50 (7.67±0.32)		
Staphylococcus epidermidis	3.12 (7.37±0.15)	12.50 (7.83±0.15)	12.50 (7.33±0.23)	15.62 (9.60±2.20)
Staphylococcus aureus	0.39 (7.37±0.06)			15.62 (12.40±1.40)
Bacillus subtilis	12.50 (7.53±0.21)			125.00 (13.10±1.10)
Enterococcus faecium		12.50 (7.37±0.21)		500.00 (7.21±0.18)
Staphylococcus pyogens	3.12 (7.33±0.15)	1.56 (7.10±0.10)		15.62 (9.60±2.20)