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Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oil from the Leaves of *Lindera fragrans* Oliv.

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Abstract: The chemical composition of the essential oil obtained by hydrodistillation from the leaves of *Lindera fragrans* Oliv. was determined by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS). Sixty two compounds accounting 76.45% of the essential oil were identified. The main constituents found to be spathulenol (27.63%), ledol (6.81%), *β*-caryophyllene (4.01%), (+)-*cis*-limonene oxide (3.69%), *α*-cadinol (3.24%). The disc diffusion method on antimicrobial activities revealed that it has remarkable inhibition effect against *Escherichia coli* (CP009072.1), *Staphylococcus aureus* (CP009361.1), *Pseudomonas aeruginosa* (CP015117.1) and *Candida albicans* (FJ159643.1). Antioxidant capacity of the essential oil was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and *β*- carotene bleaching assay, and it did not show effective antioxidant activity.

Keywords: *Lindera fragrans* Oliv.; essential oil; antimicrobial; antioxidant; GC-MS; spathulenol.

1. Plant Source

The leaves of the *Lindera fragrans* Oliv. (2 kg) were collected from Jiange County (32° 17' 15.80" N and 105° 31' 29.16" E; Alt. 540 m), Sichuan Province, China, in April 2016. The species was identified by Associate Prof. Liang-Ke Song (School of Life Science and Engineering, Southwest Jiaotong University, China), and a voucher specimen (SWJTU-201604) was deposited at the Herbarium of School of Life Science and Engineering, Southwest Jiaotong University, China.

2. Previous Studies

The genus *Lindera* (Lauraceae) is an important natural source for tradional medicinal and perfume applications, and it grows in the temperate zone of Asia and North America. The genus

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consists of approximately 100 species in all around of the world [1]. *Lindera fragrans* Oliv., widely distributed in Sichuan and Yunnan Province of China, is used in folk medicine for treatment of gastric pain and gastric ulcer. The leaves of *L. fragrans* are also used as mosquito repellent in Sichuan Province [2].

Secondary metabolites of several species of the genus such as, *Lindera setchuenensis* Gamble [3], *Lindera nacusua* (D. Don) Merr. [4], *Lindera erythrocarpa* [5], *Lindera aggregate* [6], *Lindera glauca* (Sieb. et Zucc.) [7, 8], *Lindera neesiana* Kurz [9], and *Lindera chunii* Merr. [10] were reported. While the leaf essential oil of *L. strychnifolia* can inhibit the proliferation of HepG2 cell [11, 12], essential oil of *L. umbellata* and its component linalool induced apoptosis and differentiation of HL-60 cell [13]. However, no related report was found about the chemical composition and bioactivities of the essential oil of *L. fragrans*, which inspired us to investigate chemical composition and antimicrobial activities of the essential oil *L. fragrans*.

3. Present Study

The essential oil was obtained by hydrodistillation from the leaves of the species and yield of the process were determined as 0.85% (v/w) on the basis of the oil volume and the dry weight of the plant material used (Method employed for GC and GC-MS were given in supporting information S1). The analyses reveal the presence of 101 peaks but 62 compounds could be identified, which accounted for 76.45% of the total peak area. The main compounds were determined as spathulenol (27.63%), ledol (6.81%), *β*-caryophyllene (4.01%), (+)-*cis*-limonene oxide (3.69%), *α*-cadinol (3.24%), *β*-eudesmol (2.55%), (-)-humulene epoxide II (2.32%), caryophyllene oxide (2.27%) and *δ*-cadinol (2.05%). The detailed list of the identified compounds are presented in Table 1.

Table 1. Composition of the essential oil from the leaves of *L. fragrans*

No[#]	Compound	RI	RI*	Area[c] (%)	Identification
1	*(Z)*-3-Hexen-1-ol	875	875	0.06	MS[b], RI[a]
2	Linalool	1094	1096	0.09	MS[b], RI[a]
3	Fenchol	1118	1114	0.04	MS[b], RI[a]
4	(-)-*trans*-Pinocarveol	1129	1135	0.18	MS[b], RI[a]
5	4-Isopropylcyclohexanone	1142	1142	0.11	MS[b], RI[a]
6	Borneol	1148	1160	0.11	MS[b], RI[a]
7	Terpinen-4-ol	1161	1174	0.07	MS[b], RI[a]
8	*p*-Cymen-8-ol	1181	1179	0.18	MS[b], RI[a]
9	Cryptone	1185	1183	0.27	MS[b], RI[a]
10	*α*-Terpineol	1186	1189	0.33	MS[b], RI[a]
11	Myrtenol	1195	1194	0.26	MS[b], RI[a]
12	*cis*-Carveol	1228	1226	0.13	MS[b], RI[a]
13	Cuminal	1241	1238	0.14	MS[b], RI[a]
14	2-Isopropyl-4-methylanisole	1243	1244	0.07	MS[b], RI[a]
15	*D*-Carvone	1244	1246	0.04	MS[b], RI[a]
16	Phellandral	1257	1250	0.23	MS[b], RI[a]
17	Bornyl acetate	1285	1287	0.21	MS[b], RI[a]
18	*p*-Thymol	1289	1289	1.10	MS[b], RI[a]
19	Cumic alcohol	1292	1298	0.08	MS[b], RI[a]
20	*α*-cubenene	1342	1345	0.12	MS[b], RI[a]
21	*γ*-Pironene	1350	1350	0.31	MS[b], RI[a]
22	*β*-Caryophyllene	1419	1417	4.01	MS[b], RI[a]
23	10S,11S-Himachala-3(12),4-diene	1436	1436	0.11	MS[b], RI[a]
24	*α*-Bergamotene	1442	1440	0.83	MS[b], RI[a]
25	Humulene	1448	1444	1.42	MS[b], RI[a]
26	Dihydropseudoionone	1451	1451	0.22	MS[b], RI[a]

Table 1 continued..

27	*allo*-Aromadendrene	1460	1458	0.18	MS[b], RI[a]
28	*γ*-Muurolene	1479	1478	0.27	MS[b], RI[a]
29	Germacrene D	1481	1484	0.65	MS[b], RI[a]
30	*β*-Selinene	1489	1489	1.69	MS[b], RI[a]
31	Bicyclogermacrene	1492	1499	1.37	MS[b], RI[a]
32	*α*-Muurolene	1495	1500	0.20	MS[b], RI[a]
33	2,2,8,8-tetramethylnona-3,6-diyn-5-one	1504	1504	0.16	MS[b], RI[a]
34	*(Z)-α*-Bisabolene	1507	1506	0.36	MS[b], RI[a]
35	*δ*-Selinene	1508	1509	0.97	MS[b], RI[a]
36	*α*-Panasinsen	1512	1519	1.15	MS[b], RI[a]
37	*δ*-Cadinene	1523	1522	2.00	MS[b], RI[a]
38	1,6-Dimethyl-4-(1-methylethyl)-1,2,3,4,4a,7-hexahydronaphthalene	1547	1546	0.93	MS[b], RI[a]
39	Nerolidol	1563	1563	1.31	MS[b], RI[a]
40	Spathulenol	1567	1577	27.63	MS[b], RI[a]
41	Caryophyllene oxide	1582	1582	2.27	MS[b], RI[a]
42	Ledol	1605	1602	6.81	MS[b], RI[a]
43	(-)-Humulene epoxide II	1608	1608	2.32	MS[b], RI[a]
44	*β*-Spathulenol	1618	1619	0.25	MS[b], RI[a]
45	Caryophylladienol I	1639	1639	1.97	MS[b], RI[a]
46	Alloaromadendrene oxide I	1641	1641	0.12	MS[b], RI[a]
47	*δ*-Cadinol	1646	1644	2.05	MS[b], RI[a]
48	*β*-Eudesmol	1650	1649	2.55	MS[b], RI[a]
49	*α*-Cadinol	1652	1652	3.24	MS[b], RI[a]
50	(+)-*cis*-Limonene oxide	1660	1660	3.69	MS[b], RI[a]
51	Longifolenaldehyde	1668	1668	0.09	MS[b], RI[a]
52	(*Z*)-4-Hexadecen-6-yne	1687	1687	0.15	MS[b], RI[a]
53	(*E*,*E*)-Farnesol	1742	1742	0.11	MS[b], RI[a]
54	6,10,14-Trimethyl-2-pentadecanone	1847	1847	0.20	MS[b], RI[a]
55	Methyl hexadecanoate	1921	1922	0.10	MS[b], RI[a]
56	Diisobutyl phthalate	1940	1940	0.21	MS[b], RI[a]
57	(*E*,*E*,*E*)-Geranylgeraniol	2009	2009	0.33	MS[b], RI[a]
58	Luciferin aldehyde	2113	2113	0.07	MS[b], RI[a]
59	*n*-Octadecanoic acid methyl ester	2124	2124	0.12	MS[b], RI[a]
60	13-Tetradecen-1-yl acetate	2135	2137	0.05	MS[b], RI[a]
61	14-Methyl-8-hexadecyn-1-ol	2341	2341	0.09	MS[b], RI[a]
62	Octanedioic acid, 2-undecyl-1,8-dimethyl-ester	2354	2354	0.07	MS[b], RI[a]
	Total			76.45	

[#] Compounds are listed in order; RI, retention indices relative to n-alkanes (C8–C30) series on the DB-1 MS column; RI*, retention index from literature; MS, mass spectroscopy; [c]The content (%) of the individual components was calculated based on the peak area (FID response).

Herein, we were reported the chemical composition of the essential oil of the leaves of *L. fragrans* for the first time. Nearly half (47.87%) of the identified compounds were determined as alcohols and 25.13% of them as olefins of the essential oil. Among twenty two different kinds of alcohols type compounds spathulenol, ledol, *α*-cadinol, *δ*-cadinol and *β*-eudesmol are found to be most abundant ones. Apart from those, a hydrocarbon sesquiterpene *β*-caryophyllene was found as relatively high ratio (4.01%) in the essential oil.

Chemotaxonomic evaluation was done by a comprehensive investigation of reported literature on the *Lindera* species and compared with our data. Similar components and percentages were reported from *L. chunii* and *L. glauca* which are spathulenol, *β*-selinene, *γ*-muurolene, germacrene D and ledol [7, 8, 10]. Those data confirms the close chemotaxonomic relations between the species *L. fragrans*, *L. chunii* and *L. glauca*. On the other hand, when we compared with published data of *L. neesiana* and the *L. nacusua*, the major compounds were also determined as terpinen-4-ol, spathulenol and *β*-selinene, spathulenol, methyl hexadecanoate, respectively [4, 9].

Spathulenol exists widely in the genus *Lindera*, including *L. neesiana* (1.66%), *L. nacusua* (1.41%), *L. setchuenensis* (23.2%), *L. glauca* (0.05%) and *L. chunii* (3.3%). And the content of spathulenol reaches a maximum in *L. fragrans* with 27.63%. Besides the genus *Lindera*, some plants essential oil in which spathulenol as a major compound possess some biological activities including antiproliferative, anti-inflammatory, antimicrobial and so on [14]. Spathulenol proved to be highly effective biting deterrents against *A. stephensi* and *A. aegypti* [15]. The spathulenol was also the major constituent of the essential oil of *Psidium guineense* Sw. and inhibited *M. tuberculosis* at the MIC values of 231.9 µg/mL [16]. Another report delivered that spathulenol was a good candidate to be used in combination chemotherapy of MDR cancer [17]. We can clearly say that the amount oxygenated sesquiterpenoid spathulenol is differentiate in the genus, and the title species can be considered as spathulenol chemotype species. The other main compound *β*-caryophyllene, as active ingredient, widely exists in essential oil of plant species and its amount was found to be rich in *L. erythrocarpa* and *L. chunii*. Previous pharmacological studies have shown that the *β*-caryophyllene had the effects of anti-inflammatory, anti-anxiety and anti-depression [18, 19]. A separate study found *β*-caryophyllene appeared to be effective against *An. subpictus*, *Ae. albopictus* and *Cx. Tritaeniorhynchus* and promised to be eco-friendly larvicides against malaria [20].

Antimicrobial Activity Test: Method employed in the tests was given [21]in supporting information S2.The essential oil of *L. fragrans* leaf exhibited inhibition effect against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* with the inhibition zone at 8.31, 7.52, 7.69 mm, respectively, compared with that of amoxicillin all at 7.00 mm. While the essential oil of *L. fragrans* leaf reveals no obvious bacteriostasis for *Pseudomonas aeruginosa*. These results reveal the new potential application of *L. fragrans* in the treatment of some infection diseases.

Antioxidant Activity Test: Method employed in the tests was given [22] in supporting information S3.The antioxidant capacity of *L. fragrans* essential oil was presented in Table 2. We found that the essential oil possessed weak antioxidant activity against DPPH with the IC_{50} values of 33.01 mg/mL, lower than trolox with the IC_{50} values of 23.33 mg/mL . However, no obvious antioxidant activity was found in the ABTS assay and *β*-Carotene bleaching assay.

Table 2. Antioxidant potential of the essential oil from the leaves of *L. fragrans* [a].

	Essential oil	**BHA**	**Trolox**
DPPH (IC_{50} mg/mL)	33.01 ±2.77	0.0062 ±0.00030	23.33 ±0.92
ABTS (IC_{50} mg/mL)	7.73 ±3.61	0.023 ±0.0074	0.051 ±0.0013
β-Carotene bleaching (IC_{50} µL/mL)	28.94 ±8.35	0.019 ±0.0028	4.2 ±0.89

[a]Values expressed are means ± SD of three parallel measurements ($p<0.05$).

As a conclusion, this is the first report on the chemical composition of leaf essential oil of *L. fragrans*. The species was determined as a spathulenol chemotype and it may be considered as a new source of spathulenol. While the essential oil showed remarkable antimicrobial activity, it did not show effective antioxidant activity.

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Supporting Information

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