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Composition, Antibacterial and Antioxidant Activities of Essential Oils from *Ligusticum sinense* and *L. jeholense* (Umbelliferae) from China

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Abstract: The essential oils were obtained by hydro-distillation from the roots and rhizomes of two umbelliferous species *Ligusticum sinense* and *L. jeholense*. They were analyzed for their chemical composition by GC and GC-MS. 5-Oxo-*δ*-4-decahydrobenzindene (50.1%), ligustilide (16.4%), *β*-phellandrene (7.8%), myristicine (5.5%), and spathulenol (3.3%) were the major compounds of the 25 identified components which accounted for 96.6% of the total oil of *L. sinense*. *m*-Diaminobenzene (68.2%), ligustilide (10.1%), and *p*-vinylguaiacol (3.5%) were the major compounds of the 29 identified components which accounted for 92.7% of the total oil of *L. jeholense*. The essential oils were assayed to exhibit antibacterial activity with the MBC values ranged from 150 μg/mL to 350 μg/mL, MIC values from 62.5 μg/mL to 300 μg/mL, and IC_{50} values from 43.53 μg/mL to 197.49 μg/mL. Two oils inhibited both the DPPH radical scavenging and *β*-carotene-linoleic acid oxidation in a dose dependent manner. The antibacterial activity of *L. sinense* oil was stronger than that of *L. jeholense* oil. Contrarily, the antioxidant activity of *L. jeholense* oil was stronger than that of *L. sinense* oil. The results showed that the isolated essential oils have potential for development as natural antimicrobial or antioxidant agents.

Keywords: *Ligusticum sinense*; *Ligusticum jeholense*; essential oil; antibacterial activity; antioxidant activity.

1. Plant Source

Ligusticum sinense and *L. jeholense* are two umbelliferous plant species and widely distributed in China [1]. The roots and rhizomes of *Ligusticum* species have been used to relieve pain and cure cold as the traditional Chinese medicine [1].

The roots and rhizomes of *L. sinense* and *L. jeholense* were collected respectively in the Provinces of Hunan and Liaoning of China in April 2008. The materials were dried in the shade at

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room temperature. The taxonomical identifications were done by Prof. Quanru Liu of Beijing Normal University. The voucher specimens (BSMPMI-200804001 for *L. sinense* and BSMPMI-200804002 for *L. jeholense*) were deposited at the Herbarium of the Institute of Chinese Medicinal Materials, China Agricultural University.

2. Previous Studies

The essential oils from the roots and rhizomes of *Ligusticum* species were proved to have multi-beneficial properties such as analgesic, antipyretic, anti-inflammatory, anticonvulsive activities [2-4]. Previous studies on the essential oils of *L. sinense* and *L. jeholense* were focused on their chemical composition [5-7]. However, studies on their antimicrobial and antioxidant activities have not been reported.

3. Present Study

The dry roots and rhizomes (1 kg) of *L. sinense* and *L. jeholense* were submitted respectively to hydro-distillation in a Clevenger-type apparatus at 100 °C for 4 h. The distilled oil was extracted with diethyl ether and dried over anhydrous sodium sulfate. After filtration, the yield of the two essential oils was 0.7 g (0.07%, w/w) and 1.1 g (0.11%, w/w), respectively.

The composition of two essential oils was determined by the use of analytical GC (FID) and GC/MS techniques. The same column and analysis conditions were used for both GC and GC/MS. An Agilent 6890N Network GC system for gas chromatography was equipped with an HP-5MS column [30 m × 0.25 mm (5%-phenyl)-methylpolysiloxane capillary column, film thickness 0.25 µm], a split-splitless injector heated at 250 ºC and a flame ionization detector (FID) at 240 ºC. The oven temperature was programmed as follows: initial temperature 50 ºC for 1.50 min, increase 10 ºC/min up to 180 ºC, 2 min at 180 ºC, and then increase by 6 ºC/min up to 280 ºC, 10 min at 280 ºC. Helium (99.999%) was used as carrier gas at a flow rate of 1.0 mL/min. The injection volume was 1.0 µL (split ratio 1:20). GC/MS analyses were performed using an Agilent 6890N Network GC system with an Agilent 5973 Network mass selective detector, mass spectrometer in EI mode at 70 eV in m/e range 10-550 amu.

The essential oil components were identified by comparison of their mass spectra with NIST 2002 library data of the GC-MS system, as well as by comparison of their retention indices (RI) with the relevant data in the literature [5-10]. The identified components along with their RI values and percentage composition are summarized in Table 1. In the oil of *L. sinense*, 25 compounds were identified accounting for 96.6% of the oil, and 29 compounds were identified in the oil of *L. jeholense* which represented 92.7% of its total composition. The main components identified from the *L. sinense* oil were 5-oxo-*δ*-4-decahydrobenzindene (50.1%), ligustilide (16.4%), *β*-phellandrene (7.8%), myristicine (5.5%), spathulenol (3.3%), *p*-vinylguaiacol (2.6%), and dictyopterene A (2.3%). The main components from the *L. jeholense* oil were *m*-diaminobenzene (68.2%), ligustilide (10.1%), *p*-vinylguaiacol (3.5%), and apiol (2.0%). Comparing the chemical composition of these two *Ligusticum* species oils, they all contained *α*-pinene, *β*-myrcene, *α*-phellandrene, *p*-cymene, *β*-phellandrene, *γ*-terpinene, 4-carene, *β*-linalool, terpinen-4-ol, *α*-terpineol, phellandral, *p*-vinylguaiacol, *β*-cedrene, paeonol, valencene, *γ*-selinene, myristicine, 5-allyl-1,2,3-trimethoxybenzene, spathulenol, and ligustilide. Previous study reported that *L. sinense* essential oil mainly contained 3-isobutylidenphthalide, limonene, 3-methyl-butylisovalerate, and 2-methylbenzone, and *L. jeholense* essential oil mainly contained β-phellandrene, 4-terpinyl acetate and ligustilide [5]. This result confirms that the chemical composition of the oils of *Ligusticum* can vary depending on different geographical environment, cultivar type, growth season and physiological age of the plants besides the method of oil isolation [11].

Table 1. Chemical composition of the essential oils from *L. sinense* and *L. jeholense*.

RI[a]	Compound[b]	Peak area (%)	
		L. sinense oil	*L. jeholense* oil
927	*A*-Pinene	1.7	0.2
978	*β*-Myrcene	0.1	t
990	*A*-Phellandrene	t	t
1010	*p*-Cymene	0.4	t
1015	*β*-Phellandrene	7.8	0.8
1047	*γ*-Terpinene	0.7	0.5
1080	4-Carene	0.2	t
1089	*β*-Linalool	0.5	t
1174	Terpinen-4-ol	1.0	0.9
1188	*A*-Terpineol	0.4	0.3
1280	Phellandral	0.8	0.4
1289	Borneyl acetate	0.2	-
1322	*p*-Vinylguaiacol	2.6	3.5
1358	2,6-Dimethyl-2,6-octadiene	-	0.1
1369	6-Methylhydrocoumarin	-	0.2
1400	*β*-Elemene	-	0.2
1412	Eugenyl methyl ether	-	0.2
1428	*β*-Cedrene	t	0.1
1451	Paeonol	1.3	0.1
1461	Isocaryophillene	-	0.4
1486	A-Curcumene	0.1	-
1493	*β*-Helmiscapene	0.3	-
1500	Valencene	t	0.7
1509	*γ*-Selinene	t	0.5
1529	Myristicine	5.5	0.8
1560	5-Allyl-1,2,3-trimethoxybenzene	0.9	1.0
1580	Citronellyl propionate	-	0.3
1587	Spathulenol	3.3	0.9
1601	*β*-Guaiene	-	0.3
1688	Ligustilide	16.4	10.1
1696	Apiol	-	2.0
1703	Dictyopterene A	2.3	-
1762	5-Oxo-*δ*-4-decahydrobenzindene	50.1	-
1763	*m*-Diaminobenzene	-	68.2
	Total identified	96.6	92.7
	Monoterpene hydrocarbons	10.9	1.6
	Oxygenated monoterpenoids	8.8	2.9
	Sesquiterpene hydrocarbons	1.7	1.9
	Oxygenated sesquiterpenoids	3.3	0.9
	Phenylpropanoids	2.6	3.7

t: trace < 0.05%; -: not detected; a: RI indicates the retention indices calculated against C_8-C_{40} *n*-alkanes on the HP-5MS column; b: The identified constituents are listed in their order of elution.

Four Gram-negative (*Agrobacterium tumefaciens* ATCC 11158, *Escherichia coli* ATCC 29425, *Pseudomonas lachrymans* ATCC 11921 and *Xanthomonas vesicatoria* ATCC 11633), and three Gram-positive (*Bacillus subtilis* ATCC 11562, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus haemolyticus* ATCC 29970) bacteria were selected for antibacterial activity assay. The minimum inhibitory concentration (MIC), minimum bacteriocidal concentration (MBC), and median

inhibitory concentration (IC_{50}) of two essential oils were determined according to the method described in our previous reports [12-14].

The antibacterial potency of two essential oils was assessed quantitatively by MBC, MIC and IC_{50} values which were shown in Table 2. The MIC values of the essential oil of *L. sinense* on test bacteria ranged from 62.5 μg/mL to 200 μg/mL, MBC values from 150 μg/mL to 250 μg/mL, and IC_{50} values from 43.53 μg/mL to 135.58 μg/mL. Of them, both *B. subtilis* and *A. tumefaciens* were the most sensitive with their IC_{50} values as 43.53 μg/mL and 49.35 μg/mL, respectively.

The MIC values of the essential oil of *L. jeholense* on test bacteria ranged from 100 μg/mL to 300 μg/mL, MBC values from 150 μg/mL to 350 μg/mL, and IC_{50} values from 77.67 μg/mL to 197.49 μg/mL. Of them, *S. aureus* was the most sensitive with IC_{50} value as 77.67 μg/mL. Antibacterial activity of *L. sinense* oil was stronger than that of *L. jeholense* oil.

Table 2. Antibacterial activity of the essential oils from *L. sinense* and *L. jeholense*.

Test bacterium	Essential oil of *L. sinense* (μg/mL)			Essential oil of *L. jeholense* (μg/mL)			Streptomycin sulfate (μg/mL)
	MBC	MIC	IC_{50}	MBC	MIC	IC_{50}	IC_{50}
A. tumefaciens	200	100	49.35±1.04	350	250	184.09±3.47	8.34±0.09
E. coli	200	175	132.89±1.17	300	250	151.16±0.78	10.47±0.31
P. lachrymans	250	200	135.58±2.66	200	150	112.16±1.51	9.01±0.09
X. vesicatoria	150	100	62.53±0.23	200	150	120.48±0.34	11.62±0.19
B. subtilis	150	62.5	43.53±0.47	350	300	191.99±1.64	4.98±0.06
S. aureus	200	175	128.05±1.50	150	100	77.67±0.23	78.60±0.61
S. haemolyticus	250	150	90.89±1.21	300	250	197.49±2.87	7.75±0.16

The essential oils were subjected to the screening for their antioxidant activity by two complementary tests, namely DPPH free radical scavenging and *β*-carotene-linoleic acid oxidation inhibition assays [15,16]. The antioxidant activity results of the oils and positive control (BHT) were shown in Table 3. The ability of the oils to scavenge DPPH radical was determined on the basis of their concentrations, with IC_{50} values of 407.38 μg/mL for *L. sinense* and 177.04 μg/mL for *L. jeholens*, respectively. The scavenging DPPH radical capability of *L. jeholense* oil was stronger than that of the essential oil of *L. sinense*. The two essential oils inhibited *β*-carotene bleaching in a dose dependent variable with the IC_{50} values of 224.64 μg/mL for *L. sinense* and 117.84 μg/mL for *L. jeholense*, respectively (Table 3). Both two oils showed stronger *β*-carotene bleaching inhibitory activity by comparing with the results from the DPPH inhibition assay. Similarly, the *β*-carotene bleaching inhibitory capability of *L. jeholense* oil was stronger than that of the essential oil of *L. sinense*.

The present investigation has provided additional data supporting the future utilization and development of *Ligusticum* essential oils as antibacterial and antioxidant agents. The underlying antimicrobial and antioxidant mechanisms of the essential oils as well as their active components need to be further studied and clarified.

Table 3. Antioxidant activity of the essential oils from *L. sinense* and *L. jeholense*.

Assay	*L. sinense* oil IC_{50} (μg/mL)	*L. jeholense* oil IC_{50} (μg/mL)	BHT IC_{50} (μg/mL)
DPPH inhibition	407.38 ± 11.55	177.04 ± 2.53	25.66 ± 0.42
β-Carotene bleaching	224.64 ± 3.13	117.84 ± 3.37	31.46 ± 0.68

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