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Article

Lindera Essential Oils from Vietnam: Antimicrobial Activity and Constituents of the Leaf of *Lindera chunii* Merr. (Lauraceae) Essential Oils and Chemotaxonomy

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Abstract: *Lindera chunii* Merr. is an important member of the Lauraceae family of plants which are known for their medicinal values. In this research article, the chemical constituents and antimicrobial activity of essential oils from the leaves of *L. chunii* grown in Vietnam were reported. The essential oil was obtained by hydrodistillation of the leaves of *L. chunii* while the constituents were determined by using gas chromatography (GC) and gas chromatography-mass (GC/MS) spectrometry methods. The antimicrobial activity was evaluated using a microdilution broth susceptibility assay. The constituents occurring in higher amounts in the leaf essential oil of *L. chunii* were mainly the monoterpene hydrocarbons comprising terpinolene (13.0%), α-pinene (8.0%), β-pinene (7.6%), camphene (7.3%), β-phellandrene (7.1%) and myrcene (5.0%). The leaf essential oil of *L. chunii* inhibited the growth of *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923 and *Salmonella enterica* ATCC13076, each with minimum inhibitory concentration (MIC) value of 64.0 µg/mL. The corresponding median lethal concentration (IC_{50}) values were 19.45 µg/mL, 20.45 µg/mL and 18.78 µg/mL, respectively. The essential oil also displayed anti-candidal activity towards *Candida albicans* ATCC10231 with MIC value of 64.0 µg/mL, and IC_{50} value of 23.12 µg/mL. The antimicrobial activity of *L. chunii* essential oil was reported for the first time as a confirmation of the traditional uses of the plant in the treatment of microbial infections. Furthermore, the paper discusses the chemotaxonomy of *Lindera* oil samples.

Keywords: Antibacterial activity, anti-candidal activity, chemotaxonomy, *Lindera chunii,* minimum inhibitory concentration.

Introduction

Lindera is a genus of about 80–100 species of flowering plants in the family Lauraceae, mostly native to eastern Asia [1]. In Vietnam, *Lindera* is represented by over 20 species spread throughout the country. Majority of *Lindera* species are aromatic plants that produce fragrant principles [1]. *Lindera chunii* Merrill, (Vietnamse name: Ô đước chun) is a shrub or small tree about 6 m tall. The white or golden leaves are

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alternate while the leaf blades are elliptic or narrowly elliptic of dimension 5-10 × 1.5-4 cm. The male flowers have pedicels 2-3 mm, densely brown pubescent, with involucral trace at the base, while the female flowers have perianth tube funnel form about 1 mm in diameter. The glabrous fruits are ellipsoid, 8-10 × 6-7 mm. Flowering occurs from February-March, while fruiting takes place from August to September [2]. The phytochemical compounds isolated from *L. chunii* include lindenanolides E-G, lindechunines A and B, lindenanolide H, hernandonine, laurolistine, 7-oxohernangerine and lindechunine A [3]. In addition, (2E,3R,4S)-2-dodecylinene-3-hydroxy-4-ethoxy-4-methylbutanolide and (2E,3R,4S)-2-tetradecylinene-3-hydroxy-4-ethoxy-4-methylbutanolide were also isolated from the roots of *L. chunii* [4]. The compounds hernandonine, laurolistine, 7-oxohernangerine and lindechunine A showed significant anti-human immunodeficiency virus type 1 (HIV-1) integrase activity with IC_{50} values of 16.3, 7.7, 18.2 and 21.1 μM, respectively [3]. Lindechunisin A, a sesquiterpenoid-geranyl benzene conjugate, was isolated from the roots of *L. chunii*. The cytotoxicity evaluation revealed that lindechunisin A showed moderate cytotoxic activity against the HeLa cell line with IC_{50} value of 52.60 ± 4.22 μM [5]. In a previous report, essential oils from the flowers, leaves and stems of *L. chunii* growing wild in China were obtained by microwave-assisted hydrodistillation and the major constituents consists of germacrene B (0.7-43.2%), viridiflorene (trace to 14.6%), globulol (6.3-11.6%), α-cadinol (1.7-8.6%) and τ-cadinol (0.5-7.3%). This study shows that the chemical compositions of the essential oils from the different organs of *L. chunii* are very variable [6].

In our previous communications, the chemical compositions and antimicrobial activity of essential oils from some *Lindera* species such as *L. glauca* [7] and *L. rufa* [8] were reported. The main compounds of *L. glauca* were β-caryophyllene (29.2%), α-humulene (18.0%) and caryophyllene oxide (14.6%) [6], while camphor (67.5%) was the singly abundant compound of *L. rufa* [8]. In the literature, the chemical components and biological activities of essential oils from several *Lindera* plants around the world have been reported (Table 1) [9-28]. The results indicated that there are both intra-species and inter-species variations in their chemical constituents. For example, β-caryophyllene, the main compound of *L. glauca* essential oil from Vietnam [7] was found in much lower quantities in the previous study from China [24-27], and Japan [28]. Also, α-humulene and caryophyllene oxide which occurred in larger quantities in samples from Vietnam [7] were not identified in the previous studies [24-28]. Moreover, compounds such as n-carpric acid [24], germacrene A [24], n-dodecanole acid [24], epishyobunol acetate [24], capric acid [24], ethyl ester [24], β-ocimene [25-28], germacrene D [27], (+)-ledene [27], aciphyllene [28], *trans*-2-hexenoic acid [28], *cis*-3-hexenol [27-28], and *trans*-2-hexenal [28], amongst others present in the previous studies were conspicuously absent in the Vietnamese sample of *L. glauca*.

In view of the diversity of *Lindera* species and their essential oils, this paper reports the essential oil constituents and antimicrobial activity of *L. chunii* harvested from Vietnam. The present study is part of the continued studies on the exposition of the compositional pattern and antimicrobial activities of essential oils from the genus Lauraceae family of plants grown in Vietnam [7,8,29,30].

Materials and methods

All experimental methods follow the pattern used in our previous studies and are described as such [7,8,29,30].

Harvesting and collection of the leaves of L. chunii

This study was performed with the leaves of *L. chunii* plant harvested by handpicking and collected from Pù Huống Natural Reserve (Bình Chuẩn Commune), Con Cuông District, located in Vietnam (GPS: 19035′19″N, 104043′7″E). The plants were identified by Prof. Dr. Huong, L.T., and voucher specimens LTH 893 were preserved in the plant specimen room, Vinh University. The amount of sample collected in each case was over 2.0 kg.

The essential oils of L. chunii

As mentioned earlier, the method of hydro-distillation follows the procedure described

Table 1. Compounds previously identified in the essential oils of some *Lindera* from various parts of the world[a]

Species	Parts	Origin	Main compounds	References
L. queenslandica	Leaves	Australia	β-elemene (21.4%), α-copaene (17.9%), β-caryophyllene (7.4%) and α-humulene (9.0%)	9
L. neesiana	Leaves	India	methyl chavicol (83.76%) and safrole (11.86%)	10
	Branch	"	myristicin (69.99%) and 1,8-cineole (17.97%)	10
	Fruit	Italy	*Z*-citral (15.08%), *E*-citral (11.89%), eucalyptol (8.75%), citronellal (6.72%)	11
L. nacusua	Leaves	''	caryophyllene oxide (8.79%), hexahydrofarnesylacetone (6.83%)	12
Lindera aggregate	Root tubers	''	α-longifolene (15.13%), bornyl acetate (11.49%), α-eudesmol (9.14%), α-pinene (7.88%)	13
L. fragrans	Leaves	China	spathulenol (27.63%), ledol (6.81%)	14
L. pulcherrima	Leaves	India	furanodienone (49.1%), curzerenone (17.4%),	15
L. pericarppa	Leaves	Malaysia	β-caryophyllene (52.1%), α-copaene (31.4%)	16
	Root	''	limonene (55.4%)	16
L. obtusiloba	Bark	Korea	α-cadinol (11.8%), hedycaryol (9.8%), α-eudesmol (9.7%), caryophyllene (6.4%)	17
	Mesocarp	China	α-humulene (21.45%), myrcene (20.60%), 5-dodecanolide (15.29%), lauric acid (8.74%),	18
	Leaves	''	caryophyllene (7.37%), elemol (5.06%),	18
	''	China	α-cadinol (11.8%), hedycaryol (9.7%), α-eudesmol (9.7%)	19
L. communis	''	China	(*E*)-β-ocimene (69.3%)	19
L. thomsonii	''	China	α-pinene (20.2%), β-pinene (12.7%)	19
L. strychnifolia	''	Japan	lindestrene (1.0%-36.1%), lindenenyl acetate (tr- 19.9%), β-selinene (1.8-54.1%), β-pinene (0.8%-16.7%), δ-cadinene (1.0%-11.2%)	20
	''	China	sesquithuriferol (35.9%), 14-oxy-α-muurolene (16.5%), 1,8-cineole (5.3%)	21

table 1. (continued).

Species	Parts	Origin	Main compounds	References
L. setchuenensis	‘’	‘’[b]	palmitic acid (26.6%), myristic acid (6.7%), phytol (6.6%), linoleic acid (5.4%), *cis*-13-octadecenal (5.4%)	22
L. umbellata	‘’	Japan	linalool	23
L. glauca	Fruits	China	*n*-carpric acid (25.39%), germacrene A (10.71%), odecanole acid (10.08%), epishyobunol acetate (7.29%)	24
	‘’	‘’	camphene (17.55%), 3,6,6-trimethyl-2-norpinene (16.85%), capric acid, ethyl ester (13.61%), eucalyptol (8.10%), and β-*cis*-ocimene (7.38%)	24
	‘’	‘’	ocimene (77.99%)	25
			β-ocimene (12.99%-37.40%)	26
	‘’	‘’	germacrene D and (+)-ledenec	27
	Neutral	Japan	α-guaiene, aciphyllene, δ-cadinenec	28
	Mesocarp	‘’	*trans*-β-ocimene, glaucic acidc	28
	Seed	‘’	decanoic acid lauric acidc	28
	Leaf	‘’	*trans*-2-hexenoic acid, *cis*-3-hexenol, *trans*-2-hexenal[c]	*28*

[a] hydrodistilled oil; [b] petroleum ether; [c] quantitative data not known

earlier [7,8,29,30]. By the conventional laboratory procedure, 2.0 kg of fresh and pulverized leaves of *L. chunii* divided into three parts each was used. The weight samples were carefully introduced into a clean 5 L round-bottomed flask. Distilled water was added until it covered the sample completely. The whole apparatus was connected to a heating mantle. Hydrodistillation was carried out with a Clevenger-type distillation unit designed according to the specification described previously. The distillation time was 4 h and conducted at normal pressure. The volatile oils were collected separately into clean weighed sample bottles. The oils were kept under refrigeration (4°C) until the moment of analysis.

Determination of the constituents of the essential oils

Gas chromatographic (GC) analysis was performed on an HP 7890A Plus Gas chromatograph equipped with a flame ionization detector (FID) and fitted with an HP-5MS column of dimension 30 m × 0.25 mm, and film thickness of 0.25 µm. The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (PTV: programmable temperature vaporization) 250°C, detector temperature 260°C, column temperature programmed from 60°C (isothermal 2 min hold) to 220°C (isothermal 10 min hold) at 4°C/min. Samples were injected using a split mode with a split ratio of 10:1. The volume injected was 1.0 µL. Inlet pressure was 6.1 kPa as described previously [7,8,29,30]. The relative amounts of individual components in the GC peak were done by an external standard method using calibration curves generated by running GC analysis of representative compounds.

An Agilent Technologies HP 7890A Plus

Chromatograph fitted with a fused silica capillary HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above except that He (1 mL/min) was used as the carrier gas. The Mass spectrometry was operated under conditions of ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 45-350 amu at a sampling rate of 1.0 scan/s, as described previously [7,8,29,30].

The identification of constituents of essential oil of *L. chunii* was performed on the basis of a comparison of retention indices (RI Exp.) with reference to a homologous series of n-alkane (C_4-C_{40}), under identical experimental conditions. Quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds. In some cases, co-injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition in literature [31,32] as described recently [7,8,29,30].

The study of the antimicrobial tests on L. chunii essential oil

The microorganisms employed in this procedure were prepared from the laboratory, Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam. The testing media for bacteria and fungi were described previously [8,29,30,33,34]. The minimum inhibitory concentration (MIC) and half maximal inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay as mentioned earlier [8,29,30,33,34]. The concentration of the essential oils was prepared by two-fold dilution from 1.6384 x 10^4 μg/mL to 2^1 μg/mL, as described previously [8,29,30,33,34] in a 2-fold dilution range in micro-test tubes from where they were transferred to 96-well microliter plates. Bacteria grown in double-strength Mueller-Hinton broth or double-strength tryptic soy broth and fungi grown in double-strength Sabouraud dextrose broth were standardized to 5 × 10^5 and 1 × 10^3 CFU/mL, respectively. The last row of the micro-test tubes containing only the serial dilutions of samples without microorganisms was used as a positive (no growth) control. Sterile distilled water and medium served as a negative (no antimicrobial agent) control. Streptomycin was used as the antibacterial standard while nystatin was used as the antifungal standard.

The test was based on the assessment of growth through turbidimetry (use of optical density as a measure of growth). The cultures of tested microorganisms grown overnight are diluted and read on a spectrophotometer at 600 nm in comparison with McFarland reagents (Barium Sulphate) to obtain the microbial load as standardized culture. After incubation at 37°C for 24 h, the MIC values were determined to be the lowest concentration of essential oils which completely inhibited the growth of microorganisms. On the other hand, the IC_{50} values were measured by considering the percentage of microorganisms that inhibited growth based on the turbidity measurement data of the EPOCH2C spectrophotometer [35,36].

Statistical analysis

All results of chemical composition and antimicrobial experiments were repeated three times and are expressed as mean ± standard deviation (SD).

Results and discussion

The volatile constituents of *L. chunii* from Vietnam

The hydrodistillation of *L. chunii* leaf sample gave a light-yellow coloured essential oil in yield of 0.21% (w/w).

The volatile compounds present in the GC/MS spectra were shown in Table 2. The classes of compounds identified in the essential oil of *L. chunii* were monoterpene hydrocarbons (67.3%), oxygenated monoterpenes (0.9%), sesquiterpene hydrocarbons (23.5%) and oxygenated sesquiterpene (6.4%). Non-terpenoid compounds were present in lower quantities (1.1%). The compounds occurring in higher quantity were

Table 2. Chemical constituents of *L. chunii* leaf essential oil

No.	RT (min.)	Compounds[a]	RI (Exp.)	RI (Lit.)	*L. chunii*[b]
1	10.23	α-Thujene	930	921	0.7±0.01
2	10.51	α-Pinene	939	932	8.0±0.00
3	11.01	Camphene	956	941	7.3±0.00
4	11.71	Sabinene	979	961	4.7±0.01
5	11.89	β-Pinene	986	978	7.6±0.01
6	12.11	Myrcene	992	988	5.0±0.00
7	12.70	α-Phellandrene	1011	1008	0.6±0.00
8	12.92	δ-3-Carene	1016	1012	0.9±0.00
9	13.11	α-Terpinene	1022	1018	0.8±0.00
10	13.36	o-Cymene	1030	1022	0.3±0.00
11	13.53	Limonene	1035	1030	4.1±0.00
12	13.59	(*Z*)-β-Ocimene	1037	1035	2.1±0.00
13	13.65	(*E*)-β-Ocimene	1049	1047	4.0±0.00
14	14.03	β-Phellandrene	1036	1034	7.1±0.01
15	14.52	γ-Terpinene	1064	1056	1.1±0.01
16	15.61	Terpinolene	1094	1093	13.0±0.01
17	15.82	Linalool	1102	1100	0.1±0.00
18	18.79	Terpinene-4-ol	1188	1189	0.7±0.00
19	18.96	p-Cymene-8-ol	1190	1190	0.1±0.00
20	24.34	δ-Elemene	1348	1345	0.9±0.01
21	24.74	α-Cubebene	1360	1358	0.4±0.00
22	25.70	α-Copaene	1390	1392	0.3±0.00
23	28.13	β-Cubebene	1403	1401	0.7±0.00
24	26.86	α-Gurjunene	1426	1424	0.1±0.00
25	27.22	β-Caryophyllene	1437	1440	1.3±0.01
26	27.55	2-Phenylethyl butyrate	1448	1446	1.1±0.00
27	27.84	Aromadendrene	1453	1455	0.6±0.00
28	28.15	*cis*-Muurola-3,5-diene	1467	1470	0.3±0.00
29	28.30	α-Humulene	1472	1474	0.3±0.00
30	28.54	9-*epi*-(*E*)-Caryophyllene	1479	1481	0.2±0.00
31	28.81	*trans*-Cadina-1(6),4-diene	1488	1489	0.3±0.00
32	28.89	γ-Muurolene	1490	1490	0.2±0.00
33	29.01	α-Amorphene	1494	1493	0.2±0.00
34	29.13	Germacrene D	1498	1500	1.6±0.00
35	29.37	δ-Selinene	1506	1508	0.3±0.00
36	29.51	*trans*-Muurola-4(14),5-diene	1510	1512	0.7±0.00
37	29.64	Bicyclogermacrene	1515	1513	13.0±0.01
38	29.84	δ-Amorphene	1522	1523	0.2±0.00
39	30.13	γ-Cadinene	1531	1531	0.3±0.01

table 2. (continued).

No.	RT (min.)	Compounds[a]	RI (Exp.)	RI (Lit.)	*L. chunii*[b]
40	30.29	δ-Cadinene	1537	1537	1.9±0.01
41	30.41	Zonarene	1541	1542	0.1±0.00
42	30.61	*trans*-Cadina-1,4-diene	1548	1550	0.2±0.00
43	31.06	Elemol	1563	1561	1.3±0.01
44	31.24	(*E*)-Nerolidol	1569	1571	0.3±0.00
45	32.07	Spathulenol	1597	1596	0.4±0.00
46	32.29	Viridiflorol	1604	1602	0.5±0.00
47	32.54	Guaiol	1613	1615	1.6±0.01
48	33.38	1-*epi*-Cubenol	1646	1646	0.4±0.01
49	33.84	*epi*-α-Muurolol	1659	1661	0.2±0.00
50	33.90	α-Muurolol	1663	1663	0.3±0.00
51	34.20	α-Cadinol	1672	1670	0.6±0.00
52	34.25	β-Eudesmol	1674	1674	0.3±0.00
53	34.58	Bulnesol	1685	1597	0.5±0.01
Total					99.2
Monoterpene hydrocarbons (Sr. No. 1-16)					67.3
Oxygenated monoterpene (Sr. No. 17- 19)					0.9
Sesquiterpene hydrocarbons (Sr. No. 20-25, 27-42)					23.5
Oxygenated sesquiterpenes (Sr. No. 43-53)					6.4
Non-terpenes (Sr. No. 26)					1.1

[a]Elution order on HP-5MS column

[b]Standard deviation

RI (Exp.) Retention indices on HP-5MS column

RI (Lit.) Literature retention indices 32

the monoterpene hydrocarbons represented by terpinolene (13.0%), α-pinene (8.0%), β-pinene (7.6%), camphene (7.3%), β-phellandrene (7.1%) and myrcene (5.0%). Other monoterpene compounds that were identified above included sabinene (4.7%), limonene (4.1%), (*E*)-β-ocimene (4.0%), (*Z*)-β-ocimene (2.1%) and γ-terpinene (1.1%). The quantitatively significant sesquiterpene compounds present in the essential oil were: bicyclogermacrene (13.0%), δ-cadinene (1.9%), germacrene D (1.6%), guaiol (1.6%), β-caryophyllene (1.3%), and elemol (1.3%).

Only one report described the composition of essential oils from the flowers, leaves and stems of *L. chunii* from China obtained by microwave-assisted distillation [5]. Both quantitative and qualitative variations could be observed in the chemical compositions of essential oil from the leaves of *L. chunii* grown in Vietnam and China[5]. The leaf essential oil of *L. chunii* from China is composed mainly of sesquiterpene hydrocarbons (59.1%) and oxygenated sesquiterpenes (34.2%), while monoterpene hydrocarbons (67.3%) and sesquiterpene hydrocarbons (23.5%) predominates in the sample under investigation from Vietnam. The content of monoterpene hydrocarbons in China sample was 1.3%, while 6.4% of oxygenated sesquiterpenes were present in the Vietnam oil sample. The monoterpene hydrocarbons comprising of terpinolene, α-pinene, β-pinene, camphene, β-phellandrene and myrcene constitute the bulk of leaf oil of *L. chunii* from Vietnam. However, the sesquiterpene compounds mainly germacrene B, globulol and ledol, which form the main compounds of leaf oil from China were not identified in the Vietnam oil

sample under investigation. Conversely, all the major compounds of the oil sample from Vietnam were also conspicuously absent in the Chinese oil sample. It is interesting to note that viridiflorene and β-cadinene, the main compounds of the flower of *L. chunii* from China, were not present in the leaf oil under investigation. Likewise, τ-cadinol and globulol, which occurred in higher amounts in the flower oil from China, were also not identified in the leaf oil from Vietnam. As previously mentioned, these observations clearly indicate that the chemical compositions of the essential oils from the different organs of *L. chunii* are very variable.

Varieties of terpenoids have been reported from *Lindera* species (Table 1) [9-28]. Additionally, β-caryophyllene and (*E*)-nerolidol were reported as major constituents from the leaf oil of *L. benzoin* [37]. The oil of leaves of *L. benzoin* var. *benzoin* is notably high in 6-methyl-5-hepten-2-one (1.94% in Oregon to 34.83 ± 9.69% in Delaware), β-caryophyllene (15.26% in Oregon to 48.44 ± 1.35% in Delaware), and/or (*E*)-nerolidol (10.20% in Oregon to 12.05 ± 2.02% in Delaware). The oil of the twigs comprised of 1,8-cineole (45.41 ± 0.35% in Delaware), while the oil of the fruits consists mainly of α-phellandrene (64.62 ± 0.66% in Delaware) and β-phellandrene (11.23+0.17% in Delaware)[37]. The major constituents of *L. angustifolia* oils included ocimene, myrcene and β-elemene 38. Among the *Lindera* species grown in Japan, the leaf oil of *L. umbellata* was shown to possess carvone, linalool, and 1,8-cineole as the major constituents while the leaf oil of *L. sericea* was mainly dominated by 1,8-cineole, limonene, and α-pinene. Bornyl acetate, α-pinene, and camphene were reported as the major constituents of *L. sericea* var. *glabrata* [39]. The main compounds of *L. erythrocarpa* were nerolidol, caryophyllene and α-humulene [40]. It is therefore probable to observe that *Lindera* essential oils exhibited chemical variability. These variations in the compositional patterns of *Lindera* essential oils may also be explained by the nature of the plant parts, and differences in the ecological and climatic conditions at the points of collection among others.

However, some essential oils have nearly similar compositional patterns in their major constituents. The essential oils of the leaf oil of *L. benzoin* [37], the leaf of *L. benzoin* var. *benzoin* [37] and *L. erythrocarpa* [40], may be classified as belonging to β-caryophyllene/(*E*)-nerolidol chemotype. The leaves oil of *L. communis* [19] and fruits of *L. glauca* from China [25,26] may be classified into ocimene chemotype. Another chemotype of *L. communis* exists with (-)-spathulenol as the main compound [41]. Therefore, in view of the present analysis, the essential oils of *L. chunii* analysed so far can be discerned into two chemical groups of monoterpenes chemotype (Vietnam) and sesquiterpene chemotype (China).

The result of the antimicrobial activity of the leaf essential oil of L. chunii

Although no report exists on the antimicrobial activity of *L. chunii* leaf essential oils, the essential oils from other *Lindera* plants are known for their antimicrobial potential. The leaf essential oil of *L. chunii* exhibited moderate antimicrobial activity against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* ATCC 13076, as well as anti-candidal action towards *Candida albicans* ATCC 10231 with MIC value of 64.0 µg/mL (Table 3). The obtained IC_{50} values were 19.45 µg/mL, 20.45 µg/mL, 23.12 µg/mL, and 18.78 µg/mL, respectively. Lesser activity was observed against *Bacillus cereus* ATCC14579 with MIC and IC_{50} values of 128.0 µg/mL and 45.34 µg/mL, respectively. Overall, the studied essential oils displayed moderate antimicrobial activity with most MIC values less than 100 µg/mL, when considered the criteria for determining the antimicrobial efficacy of natural substances [42]. Recent findings indicated that substances with MIC values ≤ 100 µg/mL may be considered to be of good antimicrobial activity [42]. Thus, *L. chunii* essential oils should be considered a promising antimicrobial agent because the essential oil displayed antibacterial activity with most MIC < 100 µg/mL. However, the essential oil of *L. chunii* did not display antimicrobial activity towards *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Streptomycin, the

Table 3. Antimicrobial activity of leaf essential oil of *L. chunii*

Microorganisms	MIC μg/mL[a]	IC_{50} μg/mL[a]
Enterococcus faecalis ATCC 29212	64.0±0.01[b]	19.45±0.00
Staphylococcus aureus ATCC 25923	64.0±0.00[c]	20.45±0.00
Bacillus cereus ATCC 14579	128.0±0.01[d]	45.34±0.00
*Candida albican*s ATCC 10231	64.0±0.00[f]	23.12±0.00
Escherichia coli ATCC 25922	-	-
Salmonella enterica ATCC 13076	64.0±0.00[e]	18.78±0.00
Pseudomonas aeruginosa ATCC 27853	-	-

[a]Standard deviation
[b]MIC value 0.28 μg/mL
[c]MIC value 0.36 μg/mL
[d]MIC value 1.28 μg/mL
[e]Cycloheximide MIC value of 1.40 μg/mL
[f]Nystatine MIC value of 2.20 μg/mL
- No activity; Streptomycin

standard antimicrobial agent for gram-positive bacteria displayed antimicrobial activity with MIC values in the range of 0.28 μg/mL to 1.35 μg/mL, while Cycloheximide for Gram-negative bacteria had MIC value of 3.20 μg/mL; and nystatin, an anti-candidal agent showed activity at MIC of 2.20 μg/mL. This is the first report on the antimicrobial activity of the essential oils of *L. chunii* essential oil.

The antimicrobial activity of *L. chunii* leaf oils was comparable to data available on the activity of essential oils from other *Lindera* plants. Literature information revealed that *Lindera* essentials selectively inhibited the growth of different microorganisms. For example, the leaf oil of *L. chunii* (MIC, 64.0 μg/mL) in this study displayed stronger antimicrobial activity towards *E. faecalis* than previously analysed *L. glauca* [8] (MIC, 256.0 μg/mL) oil from Vietnam. Although both essential oils showed similar activity to *B. cereus* (MIC, 64.0 μg/mL), *L. glauca* exhibited pronounced action than *L. chunii* against *S. aurues* (MIC, 32.0 vs. 64.0 μg/mL, respectively) and *C. albicans* (MIC, 32.0 vs. 64.0 μg/mL, respectively). Unlike *L. glauca,* the leaf oil of *L. chunii* showed moderate activity to *Salmonella enterica* ATCC 13076 (MIC, 64.0 μg/mL). Like *L. chunii* under investigation, the essential oil of *L. neesiana* fruit [11] and *L. nacusua* [12] leaf showed significant antimicrobial activity against *S. aureus* and *C. albicans.* The leaf essential oil of *L. fragrans* showed a remarkable inhibition effect against *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans* [14]. The essential oil of *L. pulcherimma* was effective against *S. aureus* [15], while the order of antimicrobial activity of *L. setchuenensis* is in the order *S. aureus* > *C. albicans* > *E. coli* [22]. The fruit essential of *L. glauca* [24] from China exhibited only antifungal properties. The essential oil from the leaf oil of *L. communis* [41] was reported to have exhibited antifungal and antibacterial activities against several fungi pathogen species and bacterial species. The leaf oil of *L. erythroderma* showed excellent antibacterial activities against drug-susceptible and resistant skin pathogens such as *Propionibacterium acnes*, *S. epidermidis*, and *Malassezia furfur*, which are acne-causing bacteria [43].

It is well known that the constituents present in any substance may have a direct correlation with biological activity. Thus, the antimicrobial activities of the essential oil of *L. chunii* could well suit the major compounds or synergy among the constituents present in the essential oil. Although terpinolene, the major compound of *L. chunii* was known to possess weak antimicrobial activity [44], it has contributed significantly to the antifungal properties of some essential oils [45].

Therefore, the antimicrobial activity of *L. chunii* essential oil may well be due to the presence of synergy between terpinolene and other constituents of the essential oil such as α-pinene and β-pinene which are known with various degrees of antibacterial activity [46].

Conclusion

The results of the investigation of essential oil of *L. chunii* leaf sample indicate an abundance of terpinolene (13.0%), α-pinene (8.0%), β-pinene (7.6%), camphene (7.3%), β-phellandrene (7.1%) and myrcene (5.0%). The compositional pattern showed the essential oils from *L. chunii* exhibited chemical variability. The leaf essential oil of *L. chunii* exhibited antimicrobial activity towards *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Salmonella enterica* ATCC 13076, each with MIC value of 64.0 µg/mL. The essential oil also displayed anti-candidal activity towards *Candida albican*s ATCC 10231 with a MIC value of 64.0 µg/mL. The observed antimicrobial effects are an indication that *L. chunii* essential oil may be considered for further investigation as a renewable green microbial agent.

Competing interests

The authors declare that no competing interest exists.

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