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Article

Compositions and Antimicrobial Activity of Essential Oils from the Leaves of *Beilschmiedia fordii* Dunn. and *Lindera glauca* (Siebold & Zucc.) Blume from Vietnam

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Abstract: *Beilschmiedia fordii* Dunn. and *Lindera glauca* (Siebold & Zucc.), of the Lauraceae family have been used in ethnomedicine for the amelioration of diseases especially microbial infections. The compositions and antimicrobial activity of essential oils from the leaves of *B. fordii* and *L. glauca* were reported. The main constituents of the leaf essential oil of *B. fordii* were the monoterpene hydrocarbons namely α-pinene (45.1%), camphene (18.9%), β-pinene (16.5%) and limonene (9.0%). On the other hand, the sesquiterpenes, β-caryophyllene (29.2%), α-humulene (18.0%) and caryophyllene oxide (14.6%) were the significant compounds of *L. glauca* leaf essential oil. The essential oil from the leaves of *B. fordii* exhibited promising antimicrobial activity against *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC14579, as well as anti-candidal action towards *Candida albicans* ATCC 10231, with minimum inhibitory concentration (MIC) of 16.0 µg/mL. This is the first report on the chemical constituents and antimicrobial activity of essential oils from *B. fordii* from any part of the world. To the best of our knowledge, the antimicrobial activity of *L. glauca* leaf essential oil was being reported for the first time.

Keywords: Lauraceae, *Beilschmiedia fordii*, *Lindera glauca,* Essential oil composition, monoterpenes, sesquiterpenes, antimicrobial activity.

Introduction

Beilschmiedia is a genus of trees and shrubs in the family Lauraceae. Most of its species grow in tropical climates, but a few of them are native to temperate regions, and they are widespread in tropical Asia, Africa, Madagascar, Australia, New Zealand, North America, Central America, the Caribbean, and South America [1]. *Beilschmiedia fordii* Dunn (Vietnamese name: Chắp ford; Két ford) is a tree that grows up to 6-18 m tall and 15-50 cm in diameter. The leaves are generally opposite with petiole of 1-2 cm long. The leaf blade is abaxially greenish and adaxially dark green [2]. The bark is green while the flowers are yellow-green. The fruits are ellipsoid in shape of about 1.4-1.8 cm long. Flowering and fruiting takes place between June and December [2]. The plant is used in Vietnam for the treatment of malaria and infections [2]. However, no report could be seen on the chemical constituents and biological activity of essential oil from *B. fordii*. *Lindera*, a core genus containing more than 100

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species, is a member of the Litseeae tribe under the Lauraceae family. *Lindera glauca* (Sieb. et Zucc.) is a shrub or small tree of about 5 m tall and usually multi-stemmed. It is known in Vietnamses as Liên đàn mốc. The deciduous mature leaves has upper surface green and lower surface with some greenish white hairs [3]. All parts of the plant are aromatic. A finding suggests that extracts of *L. glauca* stem could be useful for developing potential therapeutic agents with protective effects against oxidative stress [4]. The stem wood from *L. glauca* was known to contain sterols such as β-sitosterol, 7-ketositosterol, 7-β-hydroxysitosterol, and daucosterol [5]. Also, the flavonoids-lindeglaucol, lindeglaucone, cilicicone B, tamarixetin 3-O-α-L-rhamnoside, procyanidin A2, cinnamtannin B1, cinnamtannin D1, and procyanidin A1, were isolated from *L. glauca* heartwood [6].

The chemical constituents of essential oils from the fruits of *L. glauca* have been widely reported. The main compounds of hydrodistilled oil from China were found to be n-carpric acid (25.39%), germacrene A (10.71%), n-dodecanole acid (10.08%), while oil extracted with petroleum ether had abundance of camphene (17.55%), 3,6,6-trimethyl-2-norpinene (16.85%), capric acid, ethyl ester (13.61%), eucalyptol (8.10%) [7]. In a previous report, ocimene (77.99%) was identified as the main compound of *L. glauca* fruit oil [8]. The contents of β-ocimene which was identified as the major constituent of essential oils of *L. glauca* analysed in Jigongshan mountain, Henan, China, were 12.99% (middle of July), 37.40% (middle of August) and 30.30% (middle of September) [9]. However, the authors are aware of only one report on the chemical constituents of essential oil from the leaves of *L. glauca*, in which germacrene D (45.56%), (+)-ledene (5.76%) and caryophyllene (5.75%) were the dominant compounds [10]. Previous reports have shown that essential oils from the fruits of *L. glauca* were reported to exhibit antimicrobial activities. The essential oil distilled from the fruit of *L. glauca* exhibited antifungal properties than the oil obtained by solvent extraction, with MIC between 0.03-0.5 mL/L for pathogenic fungi species and 1.0-1.5 mL/L for moulds [7]. The fruit essential oil of *L. glauca* inhibited the growth of *Shigella flexneri* with minimum inhibitory concentration (MIC) of 0.156 µg/mL and bactericidal concentration (MBC) of 0.312 µg/mL [11].

In the past years, researcher has turned attention to the screening of plant products including essential oils as source of developing new therapeutic agents for the amelioration of diseases including microbial infections. Essential oils are well known for their various biological and pharmacological effects. The observed biological activities are normally related to the terpenes constituents of the essential oils [12]. Recent articles have described the potential of essential oils from plants grown in Vietnam [13-15] and other parts of the world as antimicrobial agents [16].

This work aimed to detect and report the chemical compounds of leaves essential oils of *B. fordii* and *L. glauca* grown in Vietnam and to evaluate antimicrobial activity to develop natural antimicrobials.

Materials and methods

Harvesting of the leaves of B. fordii and L. glauca

The leaves of *B. fordii* and *L. glauca* were harvested from their respective trees by picking with hands. During the process of handpicking, 2.5 kg and 2.3 kg of *B. fordii* and *L. glauca,* respectively, were collected from Pù Hoạt Nature Reserve (GPS: 19°35′19″ N, 104°43′7″ E), Vietnam. The harvesting was done during the month of December 2019. The harvested leaves were identified by Dr. Le Thi Huong of Faculty of Biology, College of Education, Vinh University, Vietnam. Moreover, voucher specimens of *B. fordii* (LTH 846) and *L. glauca* (LTH 882) were deposited in the plant specimen room, Vinh University, Vietnam, as indicated in our previous studies [13-15].

Preparation of the leaves of B. fordii and *L. glauca for isolation of essential oils*

Before essential oils were isolated from the plant samples by hydrodistillation, particles and debris materials were removed by picking with hands in accordance with procedures as done

previously [13-15]. Thereafter, the leaves samples were pulverised to expose the surface area for adequate oil extraction. Then, the grinded leaves of both plants (2 kg each) was divided separately into three separate parts so that the isolation of essential oils may be accomplished in triplicate steps.

Isolation of essential oils from the leaves of B. fordii and L. glauca

The method of hydrodistillation was utilized in obtaining essential oils from the leaves of *B. fordii* and *L. glauca*, using the steps described previously [13-15]. The first step was to introduce the pulverized leaves sample into a 5L flask. Thereafter, 3.5 L of distilled water was added to ensure that water covered the sample completely and that the samples were immersed in the flask. Then, the flask was connected to the Clevenger-type apparatus designed according to an established procedure [17] as described in previous studies [13-15]. The essential oils were allowed to be distilled for 3 h. The process of hydrodistillation was conducted at normal pressure (760 mm/Hg). When the hydrodistillation process was completed, the essential oils were collected separately into clean and previously weighed sample bottles through the tap connected to the receiver arm of the apparatus. The obtained essential oils were refrigerated at 4°C as reported in previous studies [13-15]. The hydrodistillation was achieved in triplicate from each of the samples. The yields (%) of the essential oils were calculated by dividing the masses (g) of the essential oils over the masses (g) of the pulverized leaves of each plant as described previously [13-15].

Comprehensive analysis of the constituents of the essential oils

The nature and percentages of the constituents of the essential oils from the leaves of *B. fordii* and *L. glauca* were determined with the aids of gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The steps involved were enumerated in previous studies [13-15].

In the GC analysis, the Gas chromatograph (HP 7890A Plus, Agilent Technologies, USA) equipped with HP-5MS column of dimension 30 m x 0.25 mm and film thickness of 0.25 µm was used. The GC was also fitted with flame ionization detector (FID). 1.0 mL of each of the essential oils was injected separately into the GC column at the split ratio of 10:1, using the inlet pressure of 6.1 kPa. The analysis was conducted at normal pressure with Helium as carrier gas under the flow rate of 1 mL/min. The injector and detector were operated with temperatures set at 250°C and 260°C, respectively. The column temperature programmed condition starts from 40°C and held isothermally for 2 min, allowed to rise to 220°C, and then hold isothermally for 10 min, at 4°C/min. The analysis was performed in triplicate for each of the sample of essential oil. The quantification of the constituents of each essential oil was done by using the calibration curves generated from the analyses of representative standard compounds from each class, as reported previously [13-15].

The GC column and operating conditions for the GC/MS experiment were the same as described above for GC analysis. The GC chromatograph was interfaced with a Mass spectrometer (HP 5973 MSD, Agilent Technologies, USA). The GC/MS data were acquired with the ionization voltage set at 70 eV and emission current of 40 mA. The acquisitions scan mass range was 45-350 amu with sampling rate of 1.0 scan/s as reported previously [13-15].

The constituents of the studied essential oils were identified from their respective spectra by comparison of MS fragmentation patterns with those of known compounds in literature [18] as described recently [13-15]. The retention indices (RI Exp.) of each compound was also compared with reference to a homologous series of n-alkanes (C_6-C_{40}), run under identical experimental GC conditions as with samples. For some compounds, co-injection with known compounds under the same GC conditions was also used [13-15].

Test for the antimicrobial activity

The different microbes used for the study of antimicrobial effect of the essential oils were the American Type Culture Collection species. They are *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*,

Pseudomonas aeruginosa, *Salmonella enterica* and *Candida albicans*. The choice of investigated concentrations was based on our previous reports on similar investigations where essential oils have been found to be active within specific concentration range [13-15,19-21]. The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay as previously described [13-15,19-21].

The procedures were described in previously reported studies [13-15,19-21]. The bacteria were maintained in double-strength Mueller-Hinton broth or double-strength tryptic soy broth, while the fungi were grown in double-strength Sabouraud dextrose. Both bacteria and fungi were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The stock solutions of each of the essential oils were prepared in dimethylsulfoxide. The dilution steps were accomplished from 16,384 to 2 µg/mL (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3 and 2^1 µg/ mL) in sterile distilled water placed in the micro-test tubes. Thereafter, the solutions were transferred to 96-well microtiter plates. The negative controls (containing no antimicrobial agent) were sterile distilled water and medium. The standard drugs used as positive controls include streptomycin (antibacterial), nystatin and cycloheximide (anticandidal). The solutions were allowed to incubate at 37°C for 24 h. Thereafter, the minimum inhibitory concentration (MIC) values were recorded to well with the lowest concentration of agents completely inhibiting the growth of microorganisms as reported recently [13-15,19-21]. The IC_{50} values were determined by the percentage of microorganisms that inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and Rawdata computer software (Brussels, Belgium) according to the following equations:

$$\%\ \text{Inhibition} = \frac{OD_{control(-)} - OD_{test\ agent}}{OD_{control(-)} - OD_{control(+)}} \times 100\%$$

$$IC_{50} = High_{conc} - \frac{(High_{inh\%} - 50\%) \times (High_{conc} - Low_{conc})}{(High_{inh\%} - Low_{inh\%})}$$

where OD is the optical density, control(–) are the cells with medium but without antimicrobial agent, test agent corresponds to a known concentration of antimicrobial agent, control(+) is the culture medium without cells, $High_{conc}$/Low_{conc} is the concentration of test agent at high concentration/low concentration, and $High_{inh\%}$/$Low_{inh\%}$ is the % inhibition at high concentration/% inhibition at low concentration).

Statistical analysis

The differences between the mean values obtained for experimental groups were calculated as a mean of standard deviation (SD) of three independent measurements using Microsoft Excel program 2003 as described previously [13-15,19-21].

Results and discussion

Compounds identified in the essential oil of B. fordii

The essential oil from the leaves of *B. fordii* was obtained in a yield of 0.18% (w/w; 1.82 g). The colour of the essential oil was light-yellow. The compounds identified in the essential oil were highlighted in Table 1. Twenty-four compounds accounting for 99.9% of the essential oil contents, were identified. The essential oil contained predominantly monoterpene hydrocarbons compounds (95.8%) with minor contents of oxygenated monoterpenes (1.7%), sesquiterpene hydrocarbons (0.9%) and oxygen-containing sesquiterpenes (1.5%). The main constituents of the leaf essential oil of *B. fordii* were α-pinene (45.1%), camphene (18.9%), β-pinene (16.5%) and limonene (9.0%). Except for myrcene (3.9%), all other compounds were identified in amount < 1%. Since no information exists on the chemical constituents of *B. fordii* volatiles, this result is the first of its kind. However, the compositional pattern of essential oil of *B. fordii* differs from data obtained for the essential oils of some other studied *Beilschmiedia* species. For example, β-caryophyllene, the main compound of *B. erythrophloia* [13], *B. robusta* [13], *B. yunnanensis* [13], *B. kunstleri* [22] and *B. erythrophloia* [23] leaf oils was found in insignificant quantity in *B. fordii*. Also, bicyclogermacrene and (*Z*)-β-ocimene that were found in *B. erythrophloia* [13], germacreme D which was present in *B. robusta* [13], 9-*epi*-(*E*)-caryophyllene that was identified in *B.*

Table 1. Nature and percentages of compounds of the leaves essential oils of *B. fordii* and *L. glauca* collected in Vietnam

S. No.	Compounds [a]	RI [b]	RI [c]	Percent composition [d]	
				B. fordii	*L. glauca*
1	Tricyclene	928	921	0.6 ± 0.00	-
2	α-Pinene	939	932	45.1 ± 0.01	0.7 ± 0.01
3	Camphene	955	946	18.9 ± 0.01	0.2 ± 0.00
4	Sabinene	979	978	0.4 ± 0.00	-
5	β-Pinene	984	982	16.5 ± 0.00	1.4 ± 0.01
6	Myrcene	992	990	3.9 ± 0.00	-
7	δ-3-Carene	1016	1014	-	0.1 ± 0.00
8	o-Cymene	1030	1020	0.3 ± 0.00	3.4 ± 0.00
9	Limonene	1034	1028	9.0 ± 0.00	0.4 ± 0.00
10	β-Phellandrene	1036	1030	0.2 ± 0.00	-
11	1,8-Cineole	1037	1032	0.7 ± 0.01	0.6 ± 0.00
12	(*E*)-β-Ocimene	1049	1044	0.3 ± 0.00	-
13	Terpinolene	1094	1089	0.5 ± 0.00	-
14	Linalool	1101	1100	0.2 ± 0.00	-
15	*trans*-Sabinol	1149	1151	0.2 ± 0.00	-
16	Terpinen-4-ol	1187	1187	0.2 ± 0.00	0.6 ± 0.00
17	α-Terpineol	1198	1197	0.3 ± 0.01	-
18	p-Cymen-8-ol	1190	1192	-	0.2 ± 0.00
19	Dihydroedulane	1301	1303	-	0.2 ± 0.00
20	Methyl geranate	1326	1330	0.1 ± 0.00	-
21	α-Cubebene	1360	1363	-	0.1 ± 0.00
22	Geranyl acetate	1384	1382	-	0.8 ± 0.01
23	α-Copaene	1389	1387	-	1.3 ± 0.01
24	β-Cubebene	1402	1400	-	1.7 ± 0.00
25	Longifolene	1403	1402	-	2.0 ± 0.00
26	β-Caryophyllene	1437	1437	0.3 ± 0.00	29.2 ± 0.01
27	Aromadendrene	1457	1454	0.2 ± 0.00	0.4 ± 0.00
28	α-Humulene	1472	1472	-	18.0 ± 0.01
29	9-*epi*-(*E*)-Caryophyllene	1479	1481	-	0.3 ± 0.00
30	β-Selinene	1505	1503	-	0.2 ± 0.00
31	Viridiflorene	1512	1510	-	0.3 ± 0.00
32	Bicyclogermacrene	1514	1512	0.4 ± 0.00	0.3 ± 0.00
33	δ-Cadinene	1537	1537	-	0.1 ± 0.00
34	*trans*-Calamenene	1539	1541	-	0.2 ± 0.00
35	(*E*)-Nerolidol	1569	1571	-	0.2 ± 0.00
36	(*Z*)-3-Hexenyl benzoate	1580	1581	-	0.2 ± 0.00
37	Palustrol	1589	1590	-	0.4 ± 0.00
38	Spathulenol	1598	1600	0.8 ± 0.00	4.6 ± 0.00
39	Caryophyllene oxide	1605	1605	0.2 ± 0.00	14.6 ± 0.01
40	Cubeban-11-ol	1613	1613	-	0.3 ± 0.00
41	Guaiol	1614	1612	0.3 ± 0.01	-
42	Humulene epoxide I	1620	1622	-	0.6 ± 0.00

table 1. (continued).

S. No.	Compounds [a]	RI [b]	RI [c]	Percent composition [d]	
				B. fordii	*L. glauca*
43	Humulene epoxide II	1632	1634	-	5.3 ± 0.01
44	Humulene epoxide III	1653	1655	-	0.7 ± 0.00
45	α-Cadinol	1674	1670	-	0.2 ± 0.00
46	Bulnesol	1685	1684	0.2 ± 0.00	-
47	14-Hydroxyl-9-epi-(*E*)-caryophyllene	1688	1690	-	0.2 ± 0.00
	Total			99.9	90.0
	Monoterpene hydrocarbons (S. No. 1-10, 12, 13)			95.8	6.2
	Oxygenated monoterpenes (S. No. 11, 14, 20,22)			1.7	2.4
	Sesquiterpene hydrocarbons (S. No. 21, 23-34)			0.9	54.1
	Oxygenated sesquiterpenes (S. No. 35, 37-47)			1.5	27.1
	Others (S. No. 36)			-	0.2

[a] Elution order on HP-5MS column; [b] Experimental retention indices; [c] Literature retention indices on HP-5MS column as seen in NIST [18]; [d] means of three replicate values, SD (±); Sr. No, serial number; - not identified

yunnanensis [13], as well as α-humulene the main compound of *B. robusta* [13] and *B. erythrophloia* [23], were conspicuously absent in the studied *B. fordii* leaf oil. Also, β-eudesmol which constitutes the bulk of the leaf and bark essential oil of *B. mangayi* [22] was not identified in *B. fordii* leaf oil. Moreover, phenylpropanoids that were present in *B. miersii* [24] leaf oils were not identified in *B. fordii* under investigation.

A noteworthy observation is that the high contents of α-pinene and β-pinene in the essential oil of *B. fordii* confers similarity with the compositional pattern of essential oils from the leaves of *B. pendula* from Costa Rica [25] and *B. tarairie* from United States [26]. However, *B. pendula* and *B. tarairie* contained β-caryophyllene and germacrene D, respectively, that were not identified in *B. fordii*. The difference in essential oil compositions could be attributed to distinct plant species, geographical origins, climatic conditions, environmental conditions, and methods of extractions [2,14,15,19-21].

Major and minor constituents of essential oil of L. glauca

The hydrodisitllation of the leaf of *L. glauca* afforded essential oil with a yield of 0.21% (w/w; 2.21 g). The essential oil was light-yellow coloured. Thirty-four constituents equalling 90.0% of the content were identified (Table 1). Monoterpene hydrocarbons (6.2%), oxygenated monoterpenes (2.4%), sesquiterpene hydrocarbons (54.1%), oxyenated sesquiterpenes (27.1%) and non-terpenes (0.2%) were the classes of compounds identified in the essential oil. The sesquiterpenes, β-caryophyllene (29.2 %), α-humulene (18.0%) and caryophyllene oxide (14.6%) were the main constituents of *L. glauca* leaf essential oil. Other sesquiterpenes present in significant amount were humulene epoxide II (5.3%) and spathulenol (4.6%), while o-cymene (3.4%) was the only monoterpene compound identified above 1%. Expectedly, the compositional pattern of the leaf oil differs from those of the fruit oils reported so far in the literature [7-11,30]. The main compounds of *L. glauca* fruit oils including n-carpric acid, germacrene A, n-dodecanole acid, 3,6,6-trimethyl-2-norpinene (16.85%), capric acid, ethyl ester [7], and ocimene [8,9], were not identified in the leaf oil under study. However, camphene and eucalyptol were present in insignificant quantity in the leaf oil when compared with previous studies on the fruit essential oil [7].

The high content of sesquiterpene compounds in the leaf oil of *L. glauca* from Vietnam was in agreement with the previous study of essential oil analysed from China [11]. However, the identity

of the sesquiterpenes differs from each other. Firstly, β-caryophyllene, the main compound of the present investigated oil sample was found in much lower quantity in the previous study, while, α-humulene and caryophyllene oxide were also not identified in the previous study [11]. Moreover, germacrene D and (+)-ledene present in the previous study [11] were conspicuously absent in the oil sample under investigation. The difference in the compositional pattern of essential oil from the leaves of *L. glauca* analysed from Vietnam and China [11] could be attributed to geographical origins, climatic conditions, environmental conditions, and methods of extractions [2,14,15,19-21,27].

Previous reports from several parts of the world have shown that *Lindera* essential oils consist of diversed terpene and non-terpene compounds. For example, sesquiterpene compounds constitute the bulk of essential oils from the leaves of some *Lindera* species. The sesquiterpene, spathulenol (27.63%) was the main compound of *L. fragrans* from China [27], while caryophyllene oxide (8.79%), hexahydrofarnesyl acetone (6.83%) and β-selinene (5.02%) were identified in the leaf oil of *L. nacusua* also from China [28]. The high content of β-caryophyllene (32.1%) in the leaf of *L. pipericarpa* from Malaysia [29] may confers similarity with *L. glauca* under study, although the latter contained lower quantity of α-copaene and nerolidol than the former. On the other hand, other chemical classes of compounds that were also found to predominate in the essential oils of *Lindera* plant included the oxygenated monoterpenes camphor and linalool which are the main compounds of *L. rufa* grown in Vietnam [30] and *L. umbellata* [31] from Japan, , respectively, while the fatty acids, myristic acid (26.6%) was present in the leaf oil of *L. setchuenensis* [32]. It could be postulated that essential oils from *Lindera* species exhibited chemical variability.

Analysis of antimicrobial test results

Antimicrobial activity of B. fordii leaf essential oil

The data obtained for the antimicrobial activity of the studied essential oils were indicated in Table 2. The essential oil from the leaf of *B. fordii* displayed antimicrobial activity against Gram-positive bacteria *S. aureus* and *B. cereus*, and anti-candial action towards *C. albicans*, with MIC value of 16.0 µg/mL. The IC_{50} values were 5.67, 5.12 and 4.89 µg/mL, respectively. In addition, the oil also showed much lower activity towards *E. faecalis* with MIC value of 32.0 µg/mL and IC_{50} value of 10.34 µg/mL. The essential oil did not displayed activity against the Gram-negative microorganisms of *E. coli*, *P. aeruginosa* and *S. enterica*. Although no report exists on the antimicrobial activity of *B. fordii* essential oil, the essential oils of some *Beilschmiedia* were known to have exhibited antimicrobial properties. For example, the essential oil of *B. miersii* [24] displayed moderate antimicrobial activities towards *Pseudomonas* species with MIC value > 100 µg/mL, while the studied *B. fordii* essential oil did not inhibited the growth of *P. aeruginosa*. The leaf essential oil of *B. pulverulenta* exhibited strong antimicrobial activity against all Gram-positive bacteria with MIC values each of 62.5 µg/mL [33], which was in

Table 2. Data on the antimicrobial activity of *B. fordii* and *L. glauca* leaf essential oils

Microorganisms	MIC (µg/mL) [a]		IC_{50}(µg/mL) [a]	
	B. fordii	*L. glauca*	*B. fordii*	*L. glauca*
Enterococcus faecalis	32.0 ± 0.00	256.0 ± 0.00	10.34 ± 0.00	99.34 ± 0.01
Staphylococcus aureus	16.0 ± 0.00	32.0 ± 0.01	5.67 ± 0.00	9.39 ± 0.00
Bacillus cereus	16.0 ± 0.01	64.0 ± 0.00	5.12 ± 0.00	21.45 ± 0.01
Escherichia coli	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Salmonella enterica	-	-	-	-
Candida albicans	16.0 ± 0.00	32.0 ± 0.01	4.89 ± 0.00	19.00 ± 0.01

[a] means of three replicate values, SD (±); - No activity

agreement with the finding in the present study. However, the oil showed moderate activity against all tested fungi with MIC values each of 125 µg/mL, while *B. fordii* showed greater activity against the fungi. The leaf oil of *B. fordii* under investigation exhibited higher antimicrobial efficacy (MIC, 16.0 µg/mL) than the leaf oil of *B. madang* which inhibited the growth of *S. aureus* with MIC value of 125 µg/mL [34]. Likewise, *B. fordii* leaf oil inhibited the growth of *B. cereus* (MIC, 16.0 µg/mL) while *B. madang* leaf oil was active against *B. subtilis* (MIC, 125 µg/mL) [34]. The leaf oil of *B. fordii* also displayed stronger antifungal activity against *C. albicans* (MIC 16.0 µg/mL), while *B. penangiana* showed strong antifungal activity against *C. glabra* (MIC 31.3 µg/mL) [35]. It should be stated that essential oils from *Beilschmiedia* plants selectively inhibited the growth of different microorganisms.

Results of antimicrobial activity of L. glauca leaf essential oil

From Table 2, the leaf oil of *L. glauca* showed antimicrobial activity against *S. aureus* and anti-candidal activity towards *C. albicans* with MIC value of 32.0 µg/mL. The obtained IC_{50} values were 9.39 and 19.00 µg/mL, respectively. The essential oil of *L. glauca* displayed stronger activity against *S. aureus* (MIC, 32.0 µg/mL) and *C. albicans* (MIC, 32.0 µg/mL) than *B. cereus* (MIC, 64.0 µg/mL) and *E. faecalis* (MIC, 256.0 µg/mL). Moreover, the studied essential oil did not show any antimicrobial activity against the Gram-negative microorganisms of *E. coli*, *P. aeruginosa* and *S. enterica*.

Although no report exists on the antimicrobial activity of *L. glauca* leaf essential oil, the essential oils of the fruits were known to display manifest antifungal [7] and antibacterial [11] properties. The antimicrobial activity of *L. glauca* leaf oil was comparable to data available on the activity of essential oils from other *Lindera* plants. For example, the leaf oil of *L. glauca, L. fragrans* [27], *L. setchuenensis* [32] and *L. nacusua* [36] inhibited the growth of *S. aureus* and *C. albicans,* but not *P. aeruginosa*. However, *L. setchuenensis* [32] oil displayed activity towards *E. coli* [31] contrary to data obtained for *L. glauca* under investigation. The essential oil from the leaf oil of *L. communis* was reported to have exhibited antifungal and antibacterial activities against several fungi pathogen species and bacterial species with MIC between 0.08-0.8 mL/L [37].

Previous studies have revealed that the main compounds of an essential oil were mostly responsible for the biological property [19-21,27,31,33-38]. In some case, the synergistic antimicrobial effects of some other minor compounds have been reported [16,19-21,36,37]. It can be postulated that the constituents of the studied essential oils such as α-pinene, β-pinene, limonene, camphene β-caryophyllene, α-humulene and caryophyllene oxide contributed immensely to the observed activity. In fact, these compounds were reported to show potential antimicrobial activities towards a wide range of microorganisms [38].

Conclusions

The present study revealed the main constituents of the leaf essential oil of *B. fordii* to be α-pinene (45.1%), camphene (18.9%), β-pinene (16.5%) and limonene (9.0%), while β-caryophyllene (29.2%), α-humulene (18.0%) and caryophyllene oxide (14.6%) were the significant compounds of *L. glauca* leaf essential oil. The essential oil from the leaves of *B. fordii* exhibited promising antimicrobial activity against *S. aureus* and *B. cereus*, as well as anti-candidal action towards *C. albicans*, with MIC value of 16.0 µg/mL. The leaf oil of *L. glauca* showed moderate activity to the mentioned microorganisms. The present data provide information on further utilization of the studied essential oils.

Competing interests

The authors declare that no competing interest exists.

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