

## CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE LEAF ESSENTIAL OIL OF *Beilschmiedia danhkyyii*

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*Beilschmiedia* is a genus of flowering plants in the family Lauraceae. It is composed of about 300 species of evergreen trees and shrubs that are often found in tropical and subtropical areas of Asia, Africa, and the Pacific Islands [1]. *Beilschmiedia* species were used for the treatment of various disorders, such as uterine tumors, rubella, female genital infections, rheumatism, colon, digestive disorders, malaria, headache, as well as bacterial and fungal infections [2]. The plants of this genus are a good reservoir of essential oils, in which terpenes are the main phytochemical class. The principal compounds of the essential oils of *B. madang* leaves and barks are  $\delta$ -cadinene (17.0–20.5%),  $\beta$ -caryophyllene (6.7–10.3%),  $\alpha$ -cubebene (11.3–15.6%), and  $\alpha$ -cadinol (5.8–10.6%) [3]. *B. madang* bark essential oil showed antifungal activity against *Aspergillus niger* and *A. fumigatus* with the same MIC value of 62.5  $\mu$ g/mL [3]. In another report, *B. glabra* leaf essential oil containing  $\beta$ -eudesmol (15.4%),  $\beta$ -selinene (12.2%), caryophyllene oxide (8.1%), and  $\gamma$ -gurjunene (5.2%) demonstrated strong antimicrobial activity against *Candida glabrata*, with an MIC value of 31.3  $\mu$ g/mL [4].

*Beilschmiedia danhkyyii* B. H. Quang, V. H. Nguyen & Tagane was identified as a new species in 2021 [1]. This species is a medium tree characterized by ferruginous hairy terminal buds, branchlets, and abaxial surfaces of the lamina, sub-opposite leaves, adaxially impressed midrib, and secondary veins, short inflorescence 2–3 cm long, and large ellipsoid fruits 5–8 cm long [1]. *B. danhkyyii* can be found in the Central Highlands of Vietnam [1]. The current study first reports the chemical profile of its leaf essential oil and an experimental consideration of this oil in an antimicrobial assay.

*B. danhkyyii* fresh leaves were collected from Vu Quang National Park (around 18°21'57"N and 105°18'27"E), Hatinh, Vietnam in November 2020. The Latin name was confirmed by our co-author Dr. Le Thi Huong. The voucher specimen BD-2023 has been deposited at the Faculty of Biology, Vinh University. The fresh leaf powder (2.0 kg) was hydro-distilled using a Clevenger apparatus for 3.5 h. By this procedure, the yellow essential oil obtained was then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and maintained at 4°C for further analyses. The extraction yield of 0.56% w/v was an average value of three times.

Chemical compositions in the essential oil were determined using the GC-FID/MS (gas chromatography-flame ionization detection/mass spectrometry) apparatus, an Agilent 7890A gas chromatograph with an Agilent 5975C mass detector fitted with an HP-5MS fused silica column (60 m  $\times$  0.25 mm with 0.25- $\mu$ m fixed phase). The running conditions were performed as previously described [5–8]. Chemical compounds were determined by comparing their retention indices relative to *n*-alkanes (C<sub>7</sub>–C<sub>30</sub>) with those in the previous reports, and by matching the mass spectra with the NIST 21, MassFinder 4.0, and Wiley GC-MS library [5–8]. The relative percentage of each compound was calculated as the GC peak area relative to the total peak area.

Hydro-distillation of *B. danhkyyii* fresh leaves resulted in a yellow essential oil with 35 identified compounds, which represented 97.7% (Table 1). The essential oil was dominated by monoterpene hydrocarbons (30.7%), and oxygenated monoterpenes (54.3%), whereas the other chemical classes included sesquiterpene hydrocarbons (8.4%), oxygenated sesquiterpenes (3.4%), and non-terpenic compounds (0.9%). The essential oil obtained was characterized by the percentages of two major compounds, linalool (45.9%) and 1,8-cineole (23.3%). The amounts of some other compounds were also significant, including  $\alpha$ -santalene (4.2%),  $\alpha$ -terpineol (3.2%), *p*-cymene (3.1%), spathulenol (2.3%),  $\beta$ -santalene (2.1%),  $\alpha$ -*trans*-bergamotene (1.3%),  $\alpha$ -pinene (1.2%), and  $\beta$ -pinene (1.0%). The remaining compounds were obtained in minor amounts of less than 1.0%.

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TABLE 1. Chemical Compositions in the Essential Oil of *B. danhkyii* Leaves

Compound	RI	%	Compound	RI	%
$\alpha$ -Thujene	930	0.1	Myrtenal	1206	0.2
$\alpha$ -Pinene	939	1.2	Nerol	1230	0.1
Camphene	955	0.5	Geraniol	1255	0.2
Sabinene	978	0.5	Bornyl acetate	1294	0.7
$\beta$ -Pinene	984	1.0	$\alpha$ -Santalene	1432	4.2
Myrcene	991	0.3	( <i>E</i> )-Caryophyllene	1437	0.2
$\delta$ -3-Carene	1016	0.1	$\alpha$ - <i>trans</i> -Bergamotene	1446	1.3
<i>p</i> -Cymene	1029	3.1	<i>epi</i> - $\beta$ -Santalene	1460	0.4
Limonene	1034	0.5	$\beta$ -Santalene	1472	2.1
1,8-Cineole	1038	23.3	$\beta$ -Bisabolene	1517	0.2
( <i>E</i> )- $\beta$ -Ocimene	1048	0.1	Elemicine	1559	0.6
<i>trans</i> -Linalool oxide (furanoid)	1077	0.3	( <i>E</i> )-Nerolidol	1569	0.4
<i>cis</i> -Linalool oxide (furanoid)	1093	0.3	Spathulenol	1597	2.3
Linalool	1103	45.9	Caryophyllene oxide	1605	0.7
<i>trans</i> -Sabinol	1148	0.3	Total		97.7
$\delta$ -Terpineol	1174	0.3	Monoterpene hydrocarbons		30.7
Borneol	1175	0.8	Oxygenated monoterpenes		54.3
Terpinen-4-ol	1185	1.9	Sesquiterpene hydrocarbons		8.4
<i>p</i> -Cymen-8-ol	1190	0.1	Oxygenated sesquiterpenes		3.4
$\alpha$ -Terpineol	1198	3.2	Nonterpenic compounds		0.9
Methyl chavicol	1204	0.3			

RI: Retention index relative to *n*-alkanes (C<sub>7</sub>–C<sub>30</sub>) on HP-5MS column.

TABLE 2. Antimicrobial Activity of the Essential Oil Studied (IC<sub>50</sub>/MIC,  $\mu$ g/mL)

Microbial strains	Essential oil		Streptomycin	
	IC <sub>50</sub>	MIC	IC <sub>50</sub>	MIC
<i>Enterococcus faecalis</i> ATCC299212	5.89	16	50.34	64
<i>Staphylococcus aureus</i> ATCC25923	77.89	256	45.24	32
<i>Bacillus cereus</i> ATCC14579	38.98	128	20.45	32
<i>Escherichia coli</i> ATCC25922	Inactive	Inactive	9.45	256
<i>Pseudomonas aeruginosa</i> ATCC27853	189.56	256	41.46	128
<i>Salmonella enterica</i> ATCC13076	Inactive	Inactive	45.67	128

Monoterpene and sesquiterpene derivatives are also characteristic of various *Beilschmiedia* essential oils. For instance, the major compounds of *B. alloiophylla* leaf essential oil were germacrene D (18.9%), *cis*- and *trans*- $\beta$ -ocimene (9.3–18.8%),  $\alpha$ -pinene (11.8%), and bicyclogermacrene (9.1%), whereas *B. brenesii* leaf essential oil contained sesquiterpenes germacrene D (19.3%),  $\beta$ -caryophyllene (13.4%),  $\alpha$ -copaene (9.0%),  $\alpha$ -humulene (8.1%), and  $\delta$ -cadinene (5.8%) [9]. Among the identified compounds in the essential oil of *B. pulverulenta* aerial parts, eugenol reached the highest percentage of 45.3% [10]. *B. tonkinensis* leaf essential oil, collected from Vietnam, was composed of bicyclogermacrene (23.3%), (*E*)-caryophyllene (21.9%), caryophyllene oxide (9.9%), and spathulenol (6.0%) [11].

The essential oil has been subjected to an antimicrobial assay, following the micro-dilution method that was previously described [12]. With IC<sub>50</sub>/MIC values of 5.89  $\mu$ g/mL/16  $\mu$ g/mL, the sample was better than the standard compound streptomycin against the Gram-positive bacterium *E. faecalis* (Table 2). The oil sample also inhibited the growth of two Gram-positive bacteria, *S. aureus* and *B. cereus*, with IC<sub>50</sub>/MIC values of 38.98–77.89  $\mu$ g/mL/128–256  $\mu$ g/mL. Regarding Gram-negative bacteria, *B. danhkyii* leaf essential oil only exhibited inhibitory activity against the bacterium *P. aeruginosa* with IC<sub>50</sub>/MIC values of 189.56  $\mu$ g/mL/256  $\mu$ g/mL, but did not show antimicrobial activity against two bacteria, *E. coli* and

*S. enterica*. In the last case, the essential oil was found to be inactivated to the growth of the fungus *C. albicans*. Our current result matches well with previous results as *Beilschmiedia* essential oils are appropriate for antimicrobial treatments. The essential oil of *B. pulverulenta* aerial parts possessed the same MIC value of 62.5 µg/mL against the bacteria *B. subtilis*, *S. aureus*, and *E. faecalis* [10]. The essential oil from *B. fordii* leaves exhibited promising antimicrobial activity against *S. aureus* and *B. cereus*, as well as anti-candidal action toward *C. albicans*, with the MIC value of 16.0 µg/mL [13]. *B. tonkinensis* leaf essential oil also strongly suppressed the bacterium *P. aeruginosa* and the fungus *Aspergillus niger*, with MIC values of 16 and 32 µg/mL respectively [10].

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