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Chemical Composition of *Actinodaphne pilosa* Essential Oil From Vietnam, Mosquito Larvicidal Activity, and Antimicrobial Activity

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Abstract

Leaves of *Actinodaphne pilosa* were collected at 2 different seasons from the Pù Hoạt Nature Reserve, Vietnam. The leaf samples were hydrodistilled to give essential oils, which were analyzed by gas chromatography (GC)–mass spectrometry and GC-flame ionization detection. The major components in the essential oils were α-pinene, (*Z*)-β-ocimene, (*E*)-β-ocimene, β-caryophyllene, germacrene D, bicyclogermacrene, and spathulenol. The essential oils were screened for antimicrobial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, as well as mosquito larvicidal activity against *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*. *Actinodaphne pilosa* leaf essential oils showed broad antimicrobial activity (minimum inhibitory concentration = 32, 64, 64, 16, and 16 μg/mL against *E. faecalis*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and *C. albicans*, respectively) and excellent larvicidal activity (24-hour 50% lethal concentration = 19.0, 24.7, and 48.1 μg/mL against *A. aegypti*, *A. albopictus*, and *C. quinquefasciatus*, respectively).

Keywords

essential oil composition, antibacterial, antifungal, larvicidal, β-caryophyllene, germacrene D, bicyclogermacrene

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Actinodaphne pilosa (Lour.) Merr. (syn. *Actinodaphne cochinchinensis* Meisn., *Laurus pilosa* Lour., *Machilus hainanensis* Merr., *Machilus pilosa* [Lour.] Nees, *Tetranthera pilosa* [Lour.] Spreng.) (Vietnamese names, Bộp lông, Bánh dày) is a member of the Lauraceae and is a shrub or small tree (4-12 m tall) that ranges from southern China (Guangdong, Guangxi, Hainan) as well as Laos and Vietnam.[1-3] The leaves and bark of this tree have been used medicinally to treat skin infections, coughs, rheumatism, and swelling.[2]

There are numerous problematic emerging and re-emerging infectious diseases. Examples include bacterial infections such as *Escherichia coli* O157:H7,[4] vancomycin-resistant *Staphylococcus aureus*[5]; fungal infections by *Trichosporon* spp.,[6] *Candida auris*[7]; as well as arthropod-borne viral infections such as dengue,[8] yellow fever,[9] chikungunya,[10] or Zika.[11] It is imperative to identify new agents to combat the microorganisms themselves or the vectors responsible for their transmission. In this work, we present the chemical composition, antimicrobial activity, and mosquito larvicidal activity of the leaf essential oil of *A. pilosa* from north-central Vietnam.

Results and Discussion

Essential Oil Composition

Two different leaf samples of *A. pilosa* (samples 763L and 821L) were collected from the Pù Hoạt Nature Reserve in

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April and August 2019, respectively. The leaves were hydrodistilled to give essential oils in 0.37% and 0.41% yield, respectively, and were analyzed by gas chromatography–mass spectrometry (GC–MS) and gas chromatography-flame ionization detection (GC-FID) (Table 1).

The major components in Vietnamese *A. pilosa* leaf essential oils were α-pinene (6.0%, 7.2%), (*Z*)-β-ocimene (14.3%, 10.1%), (*E*)-β-ocimene (10.4%, 6.5%), β-caryophyllene (14.9%, 9.0%), germacrene D (12.0%, 16.2%), bicyclogermacrene (11.0%, 15.9%), and spathulenol (1.0%, 6.2%). The leaf essential oil composition of *A. pilosa* from Guangdong, China, has been reported.[12] The major components were viridiflorene (ledene, 12.7%), γ-muurolene (12.3%), germacrene D (11.6%), β-caryophyllene (10.7%), and globulol (5.9%). Thus, there are major qualitative and quantitative differences between the samples from Vietnam and China. The minor differences in composition between the two samples from Vietnam may be due to the phenological state; sample 763L was collected during the flowering stage (April 2019), while sample 821L was collected during the fruiting stage (August 2019).

Antimicrobial Activity

The leaf essential oils of *A. pilosa* were screened for antimicrobial activity against 3 Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*), 2 Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and 1 yeast (*Candida albicans*) (Table 2). Sample 763L was particularly active with minimum inhibitory concentration (MIC) values of 32, 64, 64, 16, and 16 μg/mL against *E. faecalis*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and *C. albicans*, respectively.

It is not clear what compound(s) may be responsible for the antimicrobial activities in the complex *A. pilosa* essential oils. (*Z*)- and (*E*)-β-Ocimene do not appear to be particularly active.[13–16] On the other hand, several investigations have shown β-caryophyllene[17,18] and germacrene D[16] to be broadly antimicrobial. In addition, essential oils rich in both bicyclogermacrene and β-caryophyllene have shown pronounced antimicrobial activity.[19,20] In addition to the antimicrobial activities of these sesquiterpene hydrocarbons, synergistic effects may also be responsible for the antimicrobial activities observed for *A. pilosa* leaf essential oil.

Mosquito Larvicidal Activity

The *A. pilosa* leaf essential oils were screened for mosquito larvicidal activity against *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* (Table 3). Based on the criteria of Dias and Moraes,[21] the leaf essential oil of *A. pilosa* shows good larvicidal activities, particularly against the *Aedes* larvae.

The larvicidal activities of *A. pilosa* leaf essential oil can probably be attributed to the high concentrations of the sesquiterpene hydrocarbons β-caryophyllene, germacrene D, and bicyclogermacrene. β-Caryophyllene[22–24] and germacrene D[25,26] have shown larvicidal activity against *A. aegypti*. In addition, the leaf essential oil of *Lantana camara*, rich in bicyclogermacrene (19.5%) and β-caryophyllene (16.7%), has shown larvicidal activity against *A. aegypti*.[27] On the other hand, (*Z*)- and (*E*)-β-ocimene are relatively inactive; essential oils rich in these components have shown only marginal larvicidal activity.[28,29]

Conclusions

The leaf essential oil of *A. pilosa* can be considered to be active and shows promise as a potential antimicrobial agent and as an alternative insecticidal agent against mosquito larvae.

Materials and Methods

Plant Material

Leaves of *A. pilosa* were collected from the Pù Hoạt Nature Reserve in April 2019, flowering stage (sample 763L: 19°32'10"*N*, 104°41'28"E, elev. 870 m), and August 2019, fruiting stage (sample 821L: 19°42'19"*N*, 104°49'40"E, elev. 640 m). The plant was identified by Do N. Dai and voucher specimens (763 and 821) have been placed in the plant specimen room, Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics. In each case, 2 kg of the fresh leaves were shredded and hydrodistilled for 4 hours using a Clevenger apparatus to give the essential oils.

GC Analysis

GC-FID analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with an FID and fitted with HP-5ms column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (programmed temperature vaporizing) 250°C, detector temperature 260°C, column temperature programmed from 60°C (2 minutes hold) to 220°C (10 minutes hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μL. Inlet pressure was 6.1 kPa.

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-FID analysis. The conditions were the same as described above with He (1 mL/min) as the carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisition scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

Compound identification was carried out by comparison of the retention indices (RI), which were determined with respect to a homologous series of *n*-alkanes, under identical

Table 1. Chemical Compositions (%) of *Actinodaphne Pilosa* Leaf Essential Oils From Pù Hoạt Nature Reserve, Vietnam.

No.	Compounds[a]	RI[b]	763L	821L
1	α-Thujene	930	0.2	0.5
2	α-Pinene	939	6.0	7.2
3	Camphene	955	0.7	0.4
4	Sabinene	978	0.8	0.8
5	β-Pinene	984	0.7	0.7
6	Myrcene	992	1.2	1.2
7	α-Phellandrene	1010	0.5	0.4
8	δ-3-Carene	1016	0.2	0.3
9	*o*-Cymene	1029	-	0.3
10	Limonene	1034	0.4	0.4
11	(*Z*)-β-Ocimene	1039	14.3	10.1
12	(*E*)-β-Ocimene	1050	10.4	6.5
13	γ-Terpinene	1063	0.2	0.1
14	Terpinolene	1094	0.7	0.6
15	Linalool	1103	0.5	0.6
16	*allo*-Ocimene	1132	0.3	0.1
17	Methyl salicylate	1204	0.2	-
18	δ-Elemene	1348	0.5	0.7
19	α-Cubebene	1360	0.2	0.2
20	Eugenol	1367	4.1	-
21	α-Copaene	1389	2.8	1.4
22	β-Cubebene	1402	2.4	0.7
23	*cis*-β-Elemene	1403	1.9	3.2
24	β-Caryophyllene	1438	14.9	9.0
25	β-Gurjunene	1445	-	0.2
26	α-Guaiene	1456	-	0.5
27	Guaia-6,9-diene	1457	0.6	-
28	(*Z*)-β-Farnesene	1461	0.2	0.5
29	*cis*-Muurola-4(14),5-diene	1466	0.2	-
30	α-Humulene	1471	1.8	1.3
31	Aromadendra-1(10),4-diene	1475	0.6	-
32	9-*epi*-(*E*)-Caryophyllene	1479	0.2	-
33	γ-Muurolene	1491	0.7	0.8
34	α-Amorphene	1494	0.3	0.5
35	Germacrene D	1499	12.0	16.2
36	*trans*-Muurola-4(14),5-diene	1510	-	0.4
37	Bicyclogermacrene	1514	11.0	15.9
38	δ-Amorphene	1521	0.2	-
39	γ-Cadinene	1529	-	0.2
40	Eugenyl acetate	1533	1.5	-
41	δ-Cadinene	1537	2.1	1.8
42	(*E*)-Dendrolasin	1583	0.2	-
43	Spathulenol	1598	1.0	6.2
44	Caryophyllene oxide	1605	0.7	1.7
45	Guaiol	1612	-	0.2
46	Cubeban-11-ol	1613	-	0.2
47	Guaia-6,10(14)-dien-4β-ol	1642	-	0.3
48	1-*epi*-Cubenol	1645	-	0.4
49	*epi*-α-Cadinol	1657	-	0.4
50	α-Cadinol	1673	0.2	0.4
51	Eudesma-4(15),7-dien-1β-ol	1684	-	0.5
	Monoterpene hydrocarbons		36.6	29.6

(Continued)

Table 1. Continued

No.	Compounds[a]	RI[b]	763L	821L
	Oxygenated monoterpenoids		0.5	0.6
	Sesquiterpene hydrocarbons		52.6	53.5
	Oxygenated sesquiterpenoids		2.1	10.3
	Benzenoid aromatics		5.8	0.0
	Total identified		97.6	94.0

RI, retention index.
[a]Compounds identified by comparison of the RI and the mass spectral fragmentation patterns found in the databases and the published literature.
[b]RI determined with reference to a homologous series of *n*-alkanes on an HP-5ms column.

chromatographic conditions, and the mass spectral fragmentation patterns found in the Wiley (Wiley 9th Version) and NIST 08 libraries (on ChemStation HP), or with those in the literature.[30] The concentrations of the chemical components were calculated based on the GC peak area (FID response) without using correction factors.

Antimicrobial Screening

The antimicrobial activity of the essential oils was evaluated using 3 strains of Gram-positive test bacteria, *E. faecalis* (ATCC 299212), *S. aureus* (ATCC 25923), *B. cereus* (ATCC 14579), 2 strains of Gram-negative test bacteria, *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), and 1 strain of yeast, *C. albicans* (ATCC 10231).

MIC and half-maximal inhibitory concentration (IC_{50}) values were determined by the microdilution broth susceptibility assay. Stock solutions of the oil were prepared in dimethylsulfoxide. Dilution series were prepared from 16 384 to 2 μg/mL in sterile distilled water in micro-test tubes from where they were transferred to 96-well microtiter 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 colony-forming unit/mL, respectively. The last row, containing only the serial dilutions of the sample 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; nystatin and cycloheximide were used as antifungal standards. After incubation at 37°C for 24 hours, the MIC values were determined to be well with the lowest concentration of agents completely inhibiting the growth of microorganisms. 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 Raw data 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 the medium but without an antimicrobial agent, test agent corresponds to a known concentration of the antimicrobial agent, control(+) is the culture medium without cells, $High_{conc}$/

Table 2. Antimicrobial Activities of the Leaf Essential Oils of *Actinodaphne pilosa*.

Sample	Gram (+)			Gram (-)		Yeast
	*Enterococcus faecalis*ATCC 299212	*Staphylococcus aureus*ATCC 25923	*Bacillus cereus*ATCC 14579	*Escherichia coli*ATCC 25922	*Pseudomonas aeruginosa*ATCC 27853	*Candida albicans*ATCC 10231
	MIC (μg/ml)					
763L	32	64	64	-	16	16
821L	64	128	128	128	-	128
Strep	256	256	128	32	256	-
Nis	-	-	-	-	-	8
Cyc	-	-	-	-	-	32
	IC_{50} (μg/ml)					
763L	16.33	33.57	32.57	-	8.67	8.76
821L	23.56	45.68	46.77	45.67	-	45.68

IC_{50}, half-maximal inhibitory concentration; MIC, minimum inhibitory concentration.

Table 3. Mosquito Larvicidal Activity of *Actinodaphne pilosa* Leaf Essential Oil.

		24 hours		
Sample[a]	LC_{50} (95% limits)[b]	LC_{90} (95% limits)[b]	χ^2	*P* value
		Aedes aegypti		
763L	19.022 (16.94-21.33)	34.46 (30.76-39.92)	20.87	0.000
821L	14.78 (12.11-17.11)	38.37 (31.78-51.75)	1.539	0.215
Aedes albopictus				
763L	24.74 (22.75-27.28)	35.97 (32.36-42.03)	0.2447	0.885
821L	n.t.	n.t.	-	-
		Culex quinquefasciatus		
763L	48.06 (43.76-53.31)	76.75 (69.24-87.21)	8.839	0.032
821L	n.t.	n.t.	-	-
		48 hours		
		Aedes aegypti		
763L	11.76 (9.46-13.79)	26.81 (23.62-31.70)	20.80	0.000
821L	8.404 (4.815-11.035)	25.64 (21.15-35.11)	0.1033	0.748
		Aedes albopictus		
763L	22.75 (20.78-25.11)	34.53 (30.98-40.49)	0.1681	0.919
821L	n.t.	n.t.	-	-
		Culex quinquefasciatus		
763L	39.85 (35.59-44.90)	74.15 (66.06-85.60)	5.242	0.155
821L	n.t.	n.t.	-	-

n.t., not tested.
[a]Sample 763L (leaf essential oil collected in April 2019), sample 821L (leaf essential oil collected in August 2019).
[b]50% lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) in µg/mL.

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). Each of the antimicrobial screens was carried out in triplicate.

Larvicidal Screening

Mosquito colonies were obtained and maintained as previously described.[31] Larvicidal activity screening was carried out on 3rd instar larvae of *A. aegypti*, *A. albopictus*, and *C. quinquefasciatus* as previously described.[31] The data obtained were subjected to log-probit analysis to obtain 50% lethal concentration values, 90% lethal concentration values, and 95% confidence limits using Minitab 19 (Minitab, LLC, State College, PA, USA).

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