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Piper hainanense Hemsl. and *P. thomsonii* (C.DC.) Hook.f.: Essential Oil Compositions, Antimicrobial and Mosquito Larvicidal Activities

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Abstract

Background and Objectives: The genus *Piper* (family Piperaceae) includes aromatic plants widely used as spices and in traditional medicine. Essential oils from *Piper* species are known for their antimicrobial and pesticidal properties. This study aims to characterize the chemical profiles of the stem bark and leaf essential oils from two Vietnamese *Piper* species, *Piper hainanense* and *P. thomsonii*, and evaluate their biological activities. **Methods:** Essential oil components were identified using gas chromatography-mass spectrometry (GC-MS). Antimicrobial activity was assessed using the broth microdilution method, while mosquito larvicidal activity was evaluated against fourth instar larvae of *Aedes aegypti*. **Results:** The major constituents of *P. hainanense* essential oils were sabinene (14.4-15.9%), δ-selinene (6.7-14.6%), β-pinene (13.5-13.9%), β-selinene (5.9-12.4%), α-pinene (7.0-9.0%), and β-elemene (6.1-6.6%). In *P. thomsonii* stem bark essential oil, elemicin (23.8%), spathulenol (14.5%), and caryophyllene oxide (7.4%) predominated, while its leaf essential oil contained elemicin (23.0%), β-pinene (15.6%), and γ-elemene (13.6%). Antimicrobial assays revealed that *P. thomsonii* essential oils exhibited strong antifungal activity against *Aspergillus niger* (MIC = 32 μg/mL). *P. hainanense* essential oils demonstrated strong mosquito larvicidal activity, with 24-h LC_{50} values of 26.72–32.57 μg/mL and LC_{90} values of 34.15–43.48 μg/mL. **Conclusion:** *P. hainanense* and *P. thomsonii* essential oils exhibit potential as natural agents for creating antimicrobial medications and mosquito-control strategies. These results serve as a foundation for additional investigation into the medicinal and insecticidal uses of essential oils from *Piper*.

Keywords

Piperaceae, *Piper* species, essential oils, terpene derivatives, biological activities

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Introduction

Piper, or pepper, is a genus of about 2000 herbaceous vines or small trees in the family Piperaceae.[1] They are frequently found in the understory of lowland tropical forests but also occur in clearings and higher-elevation life areas.[1] Due to its pungency and ability to lend a unique flavor to a variety of cuisines worldwide, pepper is the most often used spice and condiment in the world. Originally used primarily as a spice and medicinal plant, its uses have expanded to include fresh and processed vegetables, spices, dried forms, food coloring, ornamental plant breeding, and the creation of extracts for the pharmaceutical and cosmetics industries.[2] Among species, *black pepper* (*P. nigrum* L.) can be seen as the "King of spices".[2] Alkaloid derivatives, together with flavonoids, amides, phenolic acids, lignans, and terpenoids, are the main secondary metabolites found in *Piper* crude extracts.[3]

It was noted that *Piper* species are a good reservoir of essential oils, and monoterpene hydrocarbons, sesquiterpene

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hydrocarbons, and their oxygenated derivatives were the principal classes.[4] Essential oil from Cuban *P. aduncum* aerial parts were reported to contain camphor (17.1%), viridiflorol (14.5%), and piperitone (23.7%).[5] β-Caryophyllene achieved the highest percentages of 10.9–11.2% in essential oils of Malaysian *P. officinarum* leaves and stems.[6] Bicyclogermacrene (21.88%)/β-caryophyllene (20.69%) and myrcene (52.60%)/linalool (15.89%) were the main constituents in essential oil from the leaves of Brazilian *P. cernuum* and *P. regnellii*, respectively.[7] These oil samples showed antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* with inhibition zones of 12–15 mm.[7] The leaf essential oils from other Brazilian *Piper* species *P. permucronatum* and *P. hostmanianum* showed larvicidal activity against third-instar larvae of *Ae. aegypti* mosquitoes with LC_{50} values of 36 and 54 μg/mL, respectively.[8] *Piper* essential oils have also been documented to possess antiprotozoal, antinociceptive, anti-inflammatory, and cytotoxic activities.[1]

Piper hainanense Hemsl. is native to Hainan, China, and Vietnam.[9,10] It grows to a height of 2–4 meters, has pink leaves, and a distinctive aroma. It was used in traditional medicine for headaches, dizziness, and shortness of breath. Phytochemical separation revealed that the main constituents of this species were cytotoxic benzenoids.[9] *Piper thomsonii* (C.DC.) Hook.f. is a climbing shrub, and grows primarily in the wet tropical biome of India, China, and Indo-China.[10] This communication briefly describes the chemical profiles of essential oils from the fresh stem barks and leaves of these two *Piper* species, collected from Central Vietnam. The studied essential oils have been investigated for their potential in antimicrobial experiments against the Gram bacteria, fungi, and yeast, and mosquito larvicidal activity against fourth instar larvae of *Ae. aegypti* mosquitoes.

Materials and Methods

Plant Materials

Piper hainanense fresh stem barks and leaves were collected in March 2023 from Chauly, Pu Huong Natural Reserve, Nghean, Vietnam (19°11′57″N, 105°60′14″E). *P. thomsonii* fresh stem barks and leaves were gathered in April 2022 from Dongvan, Pu Hoat Natural Reserve, Nghean, Vietnam (19° 48′28″'N, 105°40′54″E). Plant identification was confirmed by co-author Dr Do Ngoc Dai, and voucher specimens (11-PH for *P. hainanense* and 967-PT for *P. thomsonii*) were deposited at Nghe An College of Economics.

Due to differences in plant metabolism, growth stages, and environmental factors, the essential oil content may be impacted by the collections' March and April dates, which coincide with these species' growing seasons in Vietnam. Temperature, humidity, soil composition, and sunlight levels during these times can all affect the yield and chemical makeup of the essential oils. The biosynthesis of terpenoids and other secondary metabolites can be greatly impacted by these environmental factors, even though thorough investigations of seasonal variation were not carried out.

Hydro-Distillation of Essential Oils

The fresh stem barks and leaves were carefully washed and sliced into small pieces. These sliced materials have been subjected to hydro-distillation using a Clevenger-type apparatus with a duration of 3.5 h. These actions resulted in essential oils, characterized by yellow color. The extraction yields were 0.24, 0.22, 0.31, and 0.27% v/w (fresh weight) for the stem barks and leaves of *P. hainanense* and *P. thomsonii*, respectively. To eliminate any remaining water content, the gathered essential oils were dried over Na_2SO_4. The oils were then meticulously kept at 5 °C to preserve their integrity for further examinations.

The GC-MS Analysis

The GC-MS analytical procedure was conducted using a Shimadzu Technologies GCMS-QP2010 Plus chromatograph equipped with a fused silica Equity-5 capillary column (30 m length, 0.25 mm diameter, 0.25 μm film thickness, Supelco, USA).[11] The analytical conditions were optimized to ensure accurate and efficient separation of essential oil components, which are primarily volatile and thermally sensitive compounds. The carrier gas helium was chosen for its inert nature and stable flow rate (1.2 mL/min) to maintain consistent chromatographic performance. The injector and interface temperatures were set at 280 °C to ensure efficient volatilization and transfer of analytes. The column temperature program, ranging from 60 °C to 280 °C with gradual increases (5 °C/min), was designed to provide sufficient resolution of both low- and high-boiling-point compounds while minimizing thermal degradation. The split ratio of 10:1 was applied to balance sample concentration and column performance, preventing column overload while ensuring adequate sensitivity. The MS parameters, including an ionization voltage of 70 eV and a scanning mass range of 50–500 amu, were selected to achieve reliable fragmentation patterns for compound identification. Retention indices (RI) were determined by co-injecting compounds with a homologous series of *n*-alkanes (C_8–C_{38}) for accurate peak identification. Compound identification was based on comparisons with reference libraries (NIST 11 and WILEY 7), the Adams book,[12] and the NIST Chemistry WebBook.[13] The relative percentages of identified compounds were calculated from GC peak areas without correction factors, ensuring a reliable quantitative profile.

Antimicrobial Assay

Ten microbial strains were selected from the ATCC (American Type Culture Collection, USA) based on their relevance as model organisms representing Gram-positive bacteria (*Bacillus subtilis* ATCC 5230, *Staphylococcus aureus* ATCC 33591, *Clostridium sporogenes* ATCC 7955), Gram-negative bacteria

(*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853), fungi (*Aspergillus brasiliensis* ATCC 9642, *A. niger* ATCC 9587, *Fusarium oxysporum* ATCC 11739), and yeasts (*Candida albicans* ATCC 12354, *Saccharomyces cerevisiae* ATCC 4078). These strains were chosen due to their clinical and industrial significance, providing a comprehensive evaluation of the essential oils' antimicrobial spectrum. Positive controls included standard antibiotics (streptomycin and tetracycline for bacteria, nystatin for fungi and yeasts) to validate assay reliability. Negative controls consisted of media without essential oil to account for background microbial growth. Each assay was conducted in triplicate.[14,15]

Mosquito Larvicidal Assay

The mosquito species *Ae. aegypti* was selected due to its global significance as a vector for diseases such as dengue, Zika, and chikungunya, making it a priority target for vector control strategies.[16] The reporting of this study conforms to ARRIVE 2.0 guidelines.[17] Mosquito eggs were hatched in tap water overnight, and the larvae were fed on a mixture of yeast and cat food (1:3 w/w). Daily, the water was replaced with fresh overnight tap water, and dead larvae were removed. Adult mosquitoes were fed on a 10% glucose solution and sucked blood from white mice. All stages of mosquito development were placed under laboratory conditions: temperature 25 °C, relative humidity $70 \pm 5\%$, 12 h/12 h light/dark cycle. The larvicidal activity was assessed using a previously described protocol, where aliquots of essential oils were added to water containing 20 fourth-instar larvae. Permethrin served as the positive control (its detailed doses were in Table S1), while a separate set of controls consisting solely of ethanol functioned as the negative control. Mortality rates were recorded at 24-h and 48-h post-exposure, during which no dietary supplements were provided. The trials were conducted at a controlled temperature of 25 ± 2 °C. The test was performed with five different concentrations (100, 50, 25, 12.5, and 6 μg/mL). The dead larvae were enumerated, and the average percentage mortality was subsequently calculated.

All tests were conducted at a controlled temperature of 25 ± 2 °C to minimize variability. Five concentrations of essential oils (100, 50, 25, 12.5, and 6 μg/mL) were tested in triplicate, and average mortality rates were calculated. Mortality data were analyzed using log-probit analysis (Minitab® 19), providing robust estimates of LC_{50} and LC_{90} values with 95% confidence intervals to ensure statistical reliability.

$$\text{Mortality (\%)} = \frac{A - B}{B} \times 100$$

In this analysis, A denotes the survival percentage in the control larvae population, while B represents the percentage of survival in the treated larvae population.

Results

Chemical Profile of Essential Oils

The hydro-distillation of *Piper hainanense* stem barks produced a yellow essential oil with a yield of 0.24% v/w. A total of 40 compounds were identified, accounting for 99.6% of the oil (Table 1 and Fig. S1). Monoterpene hydrocarbons (46.8%) and sesquiterpene hydrocarbons (33.3%) dominated the chemical profile, followed by non-terpenic compounds (8.8%), oxygenated sesquiterpenes (6.7%), and oxygenated monoterpenes (4.0%). The major components were sabinene (14.4%), β-pinene (13.9%), α-pinene (7.0%), δ-selinene (6.7%), β-elemene (6.1%), and β-selinene (5.9%).

The extraction of *P. hainanense* leaves also resulted in yellow essential oil, yielding 0.22% v/w. A list of 25 identified compounds was tabulated in Table 1 and Fig. S2, representing 99.7%. The main chemical classes were monoterpene hydrocarbons (46.1%) and sesquiterpene hydrocarbons (47.5%), whereas their oxygenated derivatives accounted for 2.7 and 3.4%, respectively. The studied sample was characterized by sabinene (15.9%), δ-selinene (14.6%), β-pinene (13.5%), β-selinene (12.4%), α-pinene (9.0%), and β-elemene (6.6%). Some other compounds were associated with significant percentages, such as α-humulene (4.0%), β-phellandrene (3.5%), (*E*)-nerolidol (2.9%), and (*E*)-caryophyllene (2.8%).

The GC-MS data of essential oil (yellow color, 0.31% yield, v/w) from *P. thomsonii* stem barks contained 26 identified compounds, corresponding to 95.1% (Table 1 and Fig. S3). Four chemical classes consisted of oxygenated sesquiterpenes (35.7%), non-terpenic compounds (29.5%), sesquiterpene hydrocarbons (18.2%), and monoterpene hydrocarbons (11.7%). Elemicin (23.8%), spathulenol (14.5%), caryophyllene oxide (7.4%), and β-pinene (6.4%) were the principal compounds.

The hydro-distillation of *P. thomsonii* fresh leaves produced a yellow essential oil with a yield of 0.27 v/w. From the GC-MS analysis, 37 compounds were identified, which represented 97.2% (Table 1 and Fig. S4). The main chemical classes comprised non-terpenic compounds (30.0%), monoterpene hydrocarbons (25.4%), oxygenated sesquiterpenes (19.8%), and sesquiterpene hydrocarbons (19.5%). Meanwhile, oxygenated monoterpenes were the remaining class with 1.4%. This leaf essential oil was characterized by elemicin (23.0%), β-pinene (15.6%), γ-elemene (13.6%), silphiperfol-5-en-3-one B (6.7%), 4,6-dimethoxy-5-vinyl-1,2-benzodioxide (5.9%), and α-pinene (5.3%). Some others were also significant, such as spathulenol (3.9%), β-elemene (2.5%), and limonene (2.1%).

Antimicrobial Activity

Four studied essential oils have been subjected to antimicrobial activity, and the result is outlined in Table 2. *P. hainanense* stem bark essential oil only showed moderate activity against the fungus *A. niger* with the MIC value of 128 μg/mL, but failed

Table 1. Chemical Compositions of the Studied Essential Oils (%).

RT	RI_E	RI_L	Compound	*P. hainanense* stem barks	*P. hainanense* leaves	*P. thomsonii* stem barks	*P. thomsonii* leaves	Identification
5.67	900	900	*n*-Nonane	0.2				RI and MS
6.49	925	924	*α*-Thujene	0.6	0.5			RI and MS
6.72	933	932	*α*-Pinene	**7.0**	**9.0**	1.4	**5.3**	RI and MS
7.16	946	946	Camphene			0.8	1.7	RI and MS
8.07	974	969	Sabinene	**14.4**	**15.9**		0.3	RI and MS
8.18	978	974	*β*-Pinene	**13.9**	**13.5**	**6.4**	**15.6**	RI and MS
8.61	991	988	*β*-Myrcene	2.5	1.6		0.4	RI and MS
9.11	1005	1002	*α*-Phellandrene	0.8	0.3			RI and MS
9.57	1016	1014	*α*-Terpinene	1.2	0.5			RI and MS
9.84	1023	1022	*o*-Cymene			0.9		RI and MS
10.01	1027	1024	Limonene			2.2	2.1	RI and MS
10.06	1028	1025	*β*-Phellandrene	3.5	3.5			RI and MS
10.13	1030	1026	1,8-Cineole		0.8			RI and MS
10.81	1047	1044	(*E*)-*β*-Ocimene	0.5	0.5			RI and MS
11.25	1058	1054	*γ*-Terpinene	1.9	0.8			RI and MS
12.48	1088	1086	Terpinolene	0.5				RI and MS
12.97	1100	1095	Linalool	1.8	1.0		0.2	RI and MS
13.69	1116	1114	*endo*-Fenchol	0.4	0.3			RI and MS
14.65	1138	1135	*trans*-Pinocarveol				0.4	RI and MS
16.37	1177	1174	Terpinen-4-ol	1.8	0.6			RI and MS
17.21	1196	1194	Myrtenol				0.5	RI and MS
21.18	1286	1284	Bornyl acetate				0.3	RI and MS
21.30	1289	1285	Safrole	2.4				RI and MS
21.53	1294	1293	2-Undecanone	0.5				RI and MS
22.22	1310	1310	Isoamyl benzyl ether	0.4				RI and MS
23.43	1338	1338	Bicycloelemene	2.2	0.5		0.6	RI and MS
25.09	1377	1374	*α*-Copaene	0.3		1.0		RI and MS
25.48	1386	1387	*β*-Bourbonene	1.2	1.0			RI and MS
25.83	1394	1389	*β*-Elemene	**6.1**	**6.6**	4.4	2.5	RI and MS
26.94	1421	1417	(*E*)-Caryophyllene	3.7	2.8	3.8	1.6	RI and MS
27.34	1430	1430	*β*-Copaene	0.6				RI and MS
27.52	1435	1434	*γ*-Elemene	0.2		3.4	**13.6**	RI and MS
27.72	1439	1439	Aromadendrene			0.9		RI and MS
27.97	1445	1442	6,9-Guaiadiene	0.3				RI and MS
28.36	1455	1452	*α*-Humulene	3.0	4.0	1.8	0.4	RI and MS
28.67	1463	1457	Croweacin	4.8				RI and MS
28.69	1463	1460	Dehydro-aromadendrane			1.2		RI and MS
29.50	1483	1480	Germacrene D	2.3	3.8		0.4	RI and MS
29.66	1487	1483	*α*-Amorphene			1.7	0.4	RI and MS
29.74	1489	1489	*β*-Selinene	**5.9**	**12.4**			RI and MS
30.04	1496	1492	*δ*-Selinene	**6.7**	**14.6**			RI and MS
30.08	1497	1497	Sandalore			2.5	0.8	RI and MS
30.56	1509	1505	*β*-Bisabolene	0.2	1.8			RI and MS
31.18	1525	1522	*δ*-Cadinene	0.6				RI and MS
32.19	1551	1548	Elemol				0.7	RI and MS
32.51	1559	1555	Elemicin			**23.8**	**23.0**	RI and MS
32.51	1559	1550	Silphiperfol-5-en-3-one B				**6.7**	RI and MS
32.77	1565	1561	(*E*)-Nerolidol	3.1	2.9	3.2	0.8	RI and MS
33.18	1576	1572	*γ*-Asarone	0.5				RI and MS
33.30	1579	1577	Spathulenol	0.9		**14.5**	3.9	RI and MS
33.51	1584	1582	Caryophyllene oxide			**7.4**	0.8	RI and MS
33.85	1593	1592	Viridiflorol			1.1		RI and MS
34.28	1604	1608	3,4,5-Trimethoxybenzaldehyde			0.9	1.1	RI and MS
34.5	1610	1608	Humulene epoxide II			1.4		RI and MS
34.67	1615	1607	Platyphyllol	0.5				RI and MS
35.25	1630	1628	1-*epi*-Cubenol	0.2				RI and MS

(Continued)

Table 1. Continued

RT	RI_E	RI_L	Compound	*P. hainanense* stem barks	*P. hainanense* leaves	*P. thomsonii* stem barks	*P. thomsonii* leaves	Identification
35.27	1631	1630	Muurola-4,10(14)-dien-1-β-ol				0.5	RI and MS
35.61	1640	1639	*allo*-Aromadendrene epoxide				0.3	RI and MS
35.88	1647	1646	Agarospirol				0.7	RI and MS
36.09	1653	1653	4,6-Dimethoxy-5-vinyl-1,2-benzodioxide			1.7	**5.9**	RI and MS
36.22	1656	1651	Pogostol	0.4				RI and MS
37.21	1683	1675	(*E*)-Asarone	1.6	0.5			RI and MS
37.68	1696	1694	(*Z*)-γ-Atlantone				0.4	RI and MS
38.35	1714	1713	Longifolol				0.4	RI and MS
38.52	1719	1717	(*Z*)-α-Atlantone			2.3	1.1	RI and MS
39.20	1738	1728	*iso*-Longifolol				0.4	RI and MS
40.13	1764	1759	Benzyl benzoate			3.1		RI and MS
40.21	1767	1767	(*Z*)-Lanceol			1.3	0.6	RI and MS
40.32	1770	1767	13-Hydroxy-valencene			2.0	1.4	RI and MS
41.81	1812	1811	Vetivenic acid				0.3	RI and MS
45.84	1933	1933	Isohibaene				1.1	RI and MS
Total				99.6	99.7	95.1	97.2	
Monoterpene hydrocarbons				46.8	46.1	11.7	25.4	
Oxygenated monoterpenes				4.0	2.7		1.4	
Sesquiterpene hydrocarbons				33.3	47.5	18.2	19.5	
Oxygenated sesquiterpenes				6.7	3.4	35.7	19.8	
Diterpene hydrocarbons							1.1	
Non-terpenic compounds				8.8		29.5	30.0	

RT: Retention time, RI_E: Experimental retention indices, RI_L: Literature retention indices, Bold: major compounds with ≥ 5.00%.

to inhibit the remaining microbial strains (MIC > 256 μg/mL). *P. hainanense* leaf essential oil controlled the growth of the Gram (+) bacterium *B. subtilis* and the yeast *S. cerevisiae* with the same MIC value of 64 μg/mL, compared to that of the standards streptomycin (MIC 4 μg/mL) and nystatin (MIC 8 μg/mL). This sample was found to inhibit the Gram (+) bacterium *C. sporogenes*, the Gram (–) bacteria *E. coli* and *P. aeruginosa*, the fungi *A. niger,* and *F. oxysporum*, and the yeast *C. albicans* with the same MIC value of 128 μg/mL, but it did not show activity against the Gram (+) bacterium *S. aureus* and the fungus *A. brasiliensis*.

P. thomsonii stem bark essential oil suppressed the proliferation of the Gram (+) bacteria *B. subtilis*, *C. sporogenes*, and *S. aureus* with the corresponding MIC values of 64, 256, and 128 μg/mL, when the leaf essential oil exerted MIC values of 64, > 256, and 64 μg/mL, respectively. The stem bark essential oil was inactive against two tested Gram (–) bacteria, whereas the leaf essential oil exerted MIC values of 128–256 μg/mL. Both studied essential oils exhibited the same MIC values of 32 and 64 against the fungi *A. niger* and *F. oxysporum*, respectively. The stem bark sample outstripped the leaf sample in the antimicrobial assay against the fungus *A. brasiliensis*. In contrast, the leaf essential oil was superior to the stem bark essential oil against two yeasts *C. albicans* and *S. cerevisiae* (Table 2).

Mosquito Larvicidal Activity

The studied essential oils have been further taken into mosquito larvicidal consideration against *Ae. aegypti* fourth instar larvae. For Table 3, essential oils from *P. hainanense* stem barks and leaves were associated with 24-h LC_{50} values of 26.74–32.57 μg/mL and 24-h LC_{90} values of 34.15–43.38 μg/mL. In the meantime, the larvicidal activity of *P. thomsonii* stem bark and leaf essential oils was accompanied by 24-h LC_{50} values of 50.03–55.12 μg/mL and 24-h LC_{90} values of 77.08–80.13 μg/mL.

Considering the 48-h treatment, LC_{50} values of 26.37–31.08 μg/mL and LC_{90} values of 34.34–41.35 μg/mL were assigned to *P. hainanense* stem bark and leaf essential oils. *P. thomsonii* stem bark and leaf essential oils were responsible for LC_{50} values of 48.94–51.54 μg/mL and LC_{90} values of 62.16–76.94 μg/mL.

Discussion

There is a difference in chemical profiles between essential oils from *P. hainanense* stem barks and leaves. Various compounds were found in one sample. For instance, non-terpenic compounds were only found in the stem bark essential oil. β-Selinene and δ-selinene in the leaf essential oil were higher two times than those of the stem bark essential oil. Regarding *P. thomsonii* essential oils, monoterpene hydrocarbons were higher in the leaf essential oil, whereas oxygenated sesquiterpenes in the stem bark essential oil were higher. In addition, oxygenated monoterpenes and diterpene hydrocarbons were only found in the leaf essential oil. Spathulenol and caryophyllene oxide were higher in the stem bark essential oil, while α-pinene, β-pinene, γ-elemene, and

Table 2. Antimicrobial Activity of the Studied Essential Oils.

Microbial strains		Minimum Inhibitory concentration (MIC: µg/mL)						
		P. hainanense stem barks	*P. hainanense* leaves	*P. thomsonii* stem barks	*P. thomsonii* leaves	Streptomycin	Tetracycline	Nystatin
Gram (+)	*B. subtilis*	>256	64	64	64	4		
	C. sporogenes	>256	128	256	>256	8		
	S. aureus	>256	>256	128	64	8		
Gram (–)	*E. coli*	>256	128	>256	256		4	
	P. aeruginosa	>256	128	>256	128		4	
Fungi	*A. niger*	128	128	32	32			8
	A. brasiliensis	>256	>256	128	256			8
	F. oxysporum	>256	128	64	64			8
Yeasts	*C. albicans*	>256	128	256	128			4
	S. cerevisiae	>256	64	256	64			8

4,6-dimethoxy-5-vinyl-1,2-benzodioxide predominated in the leaf essential oil.

This is the first time that essential oils from Vietnamese *P. hainanense* were chemically analyzed. Hao et al (2018) reported that *β*-elemene (10.49%), *β*-pinene (8.37%), (*E*)-*β*-farnesene (7.23%), and *α*-pinene (7.14%), were identified as the main compounds of *P. hainanense* leaf essential oil, collected from Hainan island, China.[18] The current study matches well with the previous publications since monoterpenes, sesquiterpenes, and their oxygenated derivatives were also predominant in other Central Vietnamese *Piper* essential oils. As an example, *P. laosanum* leaf essential oil collected from Thanhhoa, Vietnam, contained α-curcumene (12.0%), germacrene D (6.3%), sabinene (6.1%) and spathulenol (5.1%).[19] γ-Elemene (12.7%), valeranone (9.3%), and ishwarone (6.0%) were the major compounds in essential oil from *P. betle* f. *densum* leaves, another species derived from Pu Hoat Natural Reserve.[20]

The dominance of compounds such as sabinene, elemicin, and *β*-pinene suggests that these species have evolved to produce specific metabolites with strong ecological and biological roles. The higher levels of oxygenated sesquiterpenes in *P. thomsonii* stem bark oil might be linked to its defensive strategies against microbial pathogens or herbivores. Significant findings, such as the presence of silphiperfol-5-en-3-one B, highlight the potential for discovering unique bioactive compounds in these essential oils, which could have implications for antimicrobial and pesticidal applications.

In general, *P. thomsonii* essential oils were better than *P. hainanense* essential oils in antimicrobial treatment, as well as the leaf essential oil is superior to the stem bark essential oil. Especially, *P. thomsonii* essential oils showed strong activity against the

Table 3. Mosquito Larvicidal Activity of the Studied Essential Oils (LC_{50} and LC_{90}: µg/mL).

Samples	LC_{50} (95% confidence levels)	LC_{90} (95% confidence levels)	χ^2	*p*
24-h treatment (*Ae. aegypti*)				
P. hainanense stem barks	26.74 (25.36-28.83)	34.15 (31.32-39.80)	0.551	.908
P. hainanense leaves	32.57 (30.27-35.39)	43.38 (39.87-48.44)	3.443	.328
P. thomsonii stem barks	55.12 (43.63-33.72)	80.13 (66.14-89.33)	1.297	.832
P. thomsonii leaves	50.03 (46.53-53.74)	77.08 (70.11-87.57)	1.388	.846
Permethrin (control)	0.00064 (0.00054-0.00074)	0.00248 (0.00197-0.00337)	13.46	.009
48-h treatment (*Ae. aegypti*)				
P. hainanense stem barks	31.08 (28.92-33.91)	41.35 (37.81-46.76)	4.125	.248
P. hainanense leaves	26.37 (24.96-28.32)	34.34 (31.58-39.34)	0.917	.821
P. thomsonii stem barks	51.54 (40.11-43.77)	62.16 (34.98-40.51)	0.923	.912
P. thomsonii leaves	48.94 (45.45-52.66)	76.94 (69.83-87.53)	0.884	.927

LC_{50}: 50% Lethal concentration, LC_{90}: 90% Lethal concentration.

fungus *A. niger*. The current result matches well with previous reports since *Piper* essential oils were known for bacterial inhibitions. *P. cernuum* leaf essential oil, gathered from Brazil in autumn, generated the MIC value of 48 μg/mL against *B. subtilis*.[21] Essential oils from the aerial parts of Malaysian *P. erecticaule* oil exhibited the best activity on *A. niger* (MIC 31.3 μg/mL), followed by *P. lanatum* aerial part essential oil (MIC 62.5 μg/mL).[22] *P. minutistigmum* is a species found in Pu Mat National Park, Nghean, Vietnam. Its leaf and stem bark essential oils exhibited strong activity against three Gram (+) bacteria *Enterococcus faecalis*, *S. aureus*, and *B. cereus* with MIC values of 16–64 μg/mL.[23]

A criterion can be applied to mosquito larvicidal activity: strong, $LC_{50} \leq 50$ μg/mL; moderate, $50 < LC_{50} \leq 100$ μg/mL; weak, $100 < LC_{50} \leq 750$ μg/mL, and inactive, $LC_{50} > 750$ μg/mL.[24] By this means, essential oils from *P. hainanense* stem barks and leaves showed strong activity, whereas essential oils from *P. thomsonii* stem barks and leaves were moderate. Previously, there have many attempts using *Piper* essential oils in larvicidal activity. Essential oils from the aerial parts of *P. caninum*, *P. longum*, *P. montium*, and *P. mutabile*, collected from Central Vietnam, exhibited good larvicidal activity against *Ae. aegypti* with 24-h LC_{50} and 24-h LC_{90} values less than 10 μg/mL.[25] As mentioned above, the leaf essential oils of Brazillian *P. permucronatum* and *P. hostmanianum* showed larvicidal activity against third-instar larvae of *Ae. aegypti* mosquitoes with 24-h LC_{50} values of 36 and 54 μg/mL, respectively.[8] Besides the antimicrobial, antioxidant, and tyrosinase inhibitory activities, *P. betle* leaf essential oil originating from Taiwan was responsible for mosquito larvicidal activity against *Ae. aegypti* with 2-h and 24-h LD_{50} values of 86 and 48 ppm, respectively.[26] Collectively, the current study provides useful evidence of the use of Vietnamese *Piper* essential oils in two *in vitro* biological models. However, it still lacks *in vivo* molecular mechanisms of action. Chromatographic separation of the main compounds, and their biological assays are also encouraged.

Conclusions

The current study provides a detailed chemical profile of essential oils from two Vietnamese *Piper* species, highlighting the dominance of monoterpene hydrocarbons, sesquiterpene hydrocarbons, and their oxygenated derivatives, along with some non-terpenic compounds. The major compounds in *P. hainanense* essential oils included sabinene, δ-selinene, β-pinene, β-selinene, α-pinene, and β-elemene. In contrast, *P. thomsonii* essential oils were characterized by high levels of elemicin, spathulenol, caryophyllene oxide, γ-elemene, and silphiperfol-5-en-3-one B. Biological evaluations revealed that *P. thomsonii* essential oils outperformed *P. hainanense* in antimicrobial assays, with strong inhibition of *A. niger*. Meanwhile, *P. hainanense* essential oils demonstrated potent larvicidal activity against *Ae. aegypti* larvae, highlighting their potential for mosquito control applications. To investigate their contributions to the reported activities, future research should concentrate on extracting and purifying the main bioactive substances, such as elemicin, silphiperfol-5-en-3-one B, and sabinene. Confirming these substances' and essential oils' safety and effectiveness requires testing them in *in vivo* models. Further understanding of their patterns of activity may also be obtained by examining the molecular mechanisms that underlie their biological effects. The study's encouraging findings point to the possibility of using these essential oils to create natural antimicrobials or insect repellents. Partnerships with the pharmaceutical and agricultural sectors could further investigate commercial uses, such as environmentally friendly insecticides and antifungal therapies.

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Ethical Approach

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