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Article

Essential Oils of *Piper pendulispicum* C. DC. and *Piper hymenophyllum* Miq.: Chemical Composition and Anti-microbial activity

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Abstract: From hydro-distillation and GC-FID/MS (gas chromatography-flame ionization detection/ mass spectrometry) analysis, chemical compositions in essential oils of two Vietnamese *Piper* plants *Piper pendulispicum* C. DC. and *P. hymenophyllum* Miq. were identified. Essential oils of *P. pendulispicum* leaf and stem are highlighted with the predominant amount of sesquiterpene hydrocarbons (73.21-78.06%), in which bicyclogermacrene (12.17-12.54%) and *β*-caryophyllene (12.10-14.51%) were the principal compounds. Monoterpene hydrocarbons (87.44%) are the main chemical class of *P. hymenophyllum* leaf oil, in which *α*-pinene (52.92%) and *β*-pinene (26.65%) are the principal compounds. These obtained essential oils with the MIC (minimum inhibitory concentration) values of 16-128 µg/mL are better than the positive control streptomycin ((MIC 128-256 µg/mL) in antimicrobial assay against three Gram-positive bacteria *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579. *P. pendulispicum* essential oils (MIC 16-32 µg/mL) are also comparable to the other standard cycloheximide (MIC 32 µg/mL) against the yeast *Candida albicans* ATCC 10231. In general, *P. pendulispicum* essential oil is better than *P. hymenophyllum* leaf oil for antimicrobial treatment.

Keywords: *Piper pendulispicum*, *Piper hymenophyllum*, essential oil, chemical composition, antimicrobial activity.

Introduction

The genus *Piper*, which is the largest in the Piperaceae family, has an estimated 700 species in the tropics and 1300 species in the Neotropics [1]. *Piper* species have been utilized as medicinal plants with various purposes, including spices, fragrances, oils, pesticides, hallucinogens, and medications [2,3]. The most traded spice in the world is pepper. White pepper is derived from the ripened fruit of *P. nigrum*, whereas black pepper is originated from its unripe fruit.

Many bioactive secondary metabolites, such as

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alkaloids, flavonoids, amides, propenylphenols, lignans, neolignans, and terpenoids, have been isolated as a result of extensive research into the chemistry of *Piper* species[4]. Significantly, chemical identifications and pharmacological evaluations of essential oils obtained from the genus *Piper* are also a big content in scientific studies. The principal volatile components identified in *Piper* essential oils are monterpene hydrocarbons, sesquiterpene hydrocarbons, and their oxygenated derivatives [5]. *P. aduncum* var. *ossanum* leaf essential oil containing mainly piperitone (20.1%), viridiflorol (13.0), and camphor (13.9%) showed potential antibacterial activity against *Staphylococcus aureus* [6]. The leaf essential oil of *Piper* sp. aff. *aereum*, *P. bredemeyeri*, and *P. oblanceolatum* inhibited the growth of *Bacterium cereus* [7]. *P. diospyrifolium* leaf essential oil demonstrated remarkably antiprotozoal activity against axenic amastigote forms of *Leishmania amazonensis* [8]. *Piper* essential oils have also established good effects in anticholinesterase, anti-inflammatory, antinociceptive, anticancer, etc [5].

Piper pendulispicum C. DC. (locally named: Tiêu gié thòng) and *Piper hymenophyllum* Miq. (locally named: Tiêu màng) are two perennial *Piper* species of Vietnam [9]. To date, no phytochemical report on essential oil of *P. pendulispicum*. In the meantime, the fruit essential oil of Indian *P. hymenophyllum* was associated with the presence of the major compounds phytol (21.87%), dihydroterpineol (17.42%), α-terpineol (13.93%), and *trans*-piperitol (9.66%) [10]. The current study aims to report chemical compositions in the leaf and stem oils of *P. pendulispicum*, and in the leaf oil of *P. hymenophyllum*, collected from central Vietnam. The obtained essential oils were further subjected to antimicrobial experiments.

Materials and methods

Reagents

All chemicals were pure products supplied by Sigma-Aldrich (USA) company. They included Mueller-Hinton agar, DMSO (dimethylsulfoxide), streptomycin, and cycloheximide.

Sample collection

The fresh leaf and stem of *P. pendulispicum* were collected from Hatinh province, Vietnam in 05/2021, while the fresh leaf of *P. hymenophyllum* was gathered from Nghean province, Vietnam in 08/2022. The collected plants were identified and authenticated by the co-author Nguyen Thi Huong. The voucher specimens Huong-856 (*P. pendulispicum*) and Huong-927 (*P. hymenophyllum*) have been deposited Faculty of Biology, Vinh University.

Hydro-distillation of essential oils

The fresh leaf and stem (2.5 kg, each) were promptly divided into pieces and hydro-distilled for 3.0 h using a Clevenger apparatus to produce yellow essential oils. The extraction yields for *P. pendulispicum* leaf oil (0.23%, v/w), *P. pendulispicum* stem oil (0.2%, v/w), and *P. hymenophyllum* leaf (0.34%, v/w) were obtained following fresh materials.

Chemical analysis of essential oils

The procedures for gas chromatography with flame ionization detection (GC-FID) were followed [11–14]: Injector temperature of 260°C, detector temperature of 270°C, column temperature program: 65°C (3 min hold), increase to 230°C (4°C/min), 230°C (10 min hold), the inlet pressure of 6.0 kPa, split mode injection (split ratio, 10:1), and 1.1 L injection volume, and Agilent Technologies HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm).

GC-MS, or gas chromatography-mass spectrometry, was carried out similarly: HP-5 MS (30 m x 0.25 mm, film thickness 0.25 mm) column, HP 5973 MSD mass detector, He carrier gas (1.1 mL/min), MS ionization voltage of 70 eV, emission current of 40 mA, acquisitions range of 40-400 amu, and a sampling rate of 1.0 scan/s are all features of the Agilent Technologies HP 7890A Plus Chromatograph (Santa Clara, CA, USA). The identical conditions that applied to GC-FID were used to operate the GC. The chemical components of essential oils were identified using retention indices (RI) based on a series of *n*-alkanes, co-injection with pure compounds (Sigma-Aldrich, St. Louis, MO, USA), identified essential oil components, MS library search (NIST 17 and Wiley Version 10), and comparison with the literature MS fragmentation [11–14]. The relative percentage (%) of each compound was calculated based on the GC peak area (FID response) and without the application of correction factors.

Microorganisms

The antimicrobial activity of the oil samples was carried out using the broth dilution method [15,16]. Six pathogenic bacteria and one yeast were used, consisting of three Gram-positive bacteria *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), and *Bacillus cereus* (ATCC 14579), three Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella enterica* (ATCC 13076), and the yeast *Candida albicans* (ATCC 10231).

Antimicrobial activity

The concentration ranges that were chosen for the investigation were based on our earlier research [15,16], which showed that the tested essential oil was effective at the chosen concentration ranges. The final serial concentrations by 2-fold dilution ranged from 512 to 16 g/mL from an essential oil stock solution produced with DMSO (1%). After that, they were put on 96-well plates. Standardized Mueller-Hinton broth bacteria were cultured at a concentration of 5 x 10^5 CFU/mL. A positive control was the final row of well plates, which contained only antibiotics and no essential oils. As a negative control, DMSO (1%) was used (no antimicrobial agent). As reference compounds for antibacterial and anti-yeast activities, respectively, streptomycin and cycloheximide were used. The experiments were carried out three times. The MIC value displayed the result (the lowest dose at which bacterial growth is totally inhibited).

Results and discussion

Based on the GC-FID/MS analytical data, a total of 60 compounds were identified in *P. pendulispicum* leaf oil (0.23% yield, v/w), which represented 99.21% (Table 1). As can be seen, this oil was dominated by sesquiterpene hydrocarbons (78.06%), followed by oxygenated sesquiterpenes (9.76%), monoterpene hydrocarbons (7.74%), nonterpenic compounds (2.96%), and oxygenated monoterpenes (0.69%). Bicyclogermacrene (12.54%), β-caryophyllene (12.10%), α-gurjunene (11.66%), and α-copaene (9.00%) were the principal compounds. Some compounds have reached amounts of greater than 1.00%, such as viridiflorene (5.00%), α-cubebene (4.69%), α-pinene (3.64%), limonene (1.47%), α-humulene (1.38%), β-pinene (1.23%), and isoledene (1.02%).

The second oil sample of this plant, *P. pendulispicum* stem essential oil (0.2% yield, v/w), included 55 identified compounds, which accounted for 98.57% (Table 1). Sesquiterpene hydrocarbons still reached the highest amount of 73.21%. The remaining chemical classes were oxygenated sesquiterpenes (16.84%), monoterpenes hydrocarbons (6.36%), nonterpenic compounds (1.19%), and oxygenated monoterpenes (0.97%). The major compounds in this oil have composed of β-caryophyllene (14.51%), bicyclogermacrene (12.17%), α-gurjunene (9.74%), guaiol (7.07%), and α-copaene (6.99%). The percentages of several other compounds were found to be greater than 1.00%, such as germacrene D (5.40%), *cis*-calamenene (5.10%), and viridiflorene (4.24%). As compared with similar studies, Sesquiterpene hydrocarbons were also characteristics of various *Piper* species, such as the essential oils of Indian *P. betleoides* leaf (79.08%), Brazilian P. *dilatatum* aerial part (87.7%), Egyptian *P. nigrum* fruit (30.66%) [17-19]. Various minor compounds were detected in the leaf essential oil and *vice versa*. α-Thujene, hexyl acetate, chavicol, β-bourbonene, α-maalinene, *allo*-aromadendrene, ishwarane, *cis*-β-guaiene, aromadendra-4,9-diene, *epi*-α-eudesmol, α-muurolol, and cadalene were only found in the leaf oil, whereas α-phellandrene, cyclosativene, β-selinene, cubebol, rosifoliol, 5-guaiene-11-ol, isospathulenol, and β-eudesmol have only appeared in the stem essential oil.

The leaf essential oil from *P. hymenophyllum* (0.34% yield, v/w) was accompanied by the appearance of 24 compounds, corresponding to 99.83%. Monoterpene hydrocarbons (87.44%) were characteristic of this sample, followed by sesquiterpene hydrocarbons (8.57%), oxygenated monoterpenes (3.28%), and oxygenated

Table 1. Chemical compositions in the rhizome essential oils of two *Piper* plants

No.	Compounds[a]	RT	RI[b]	RI[c]	Concentration (%) A	B	C
1	α-Thujene	9.86	930	930	0.11	-	-
2	α-Pinene	10.13	939	939	3.64	1.15	52.92
3	Camphene	11.00	956	955	-	-	1.01
4	Sabinene	11.33	978	979	0.14	0.14	1.18
5	β-Pinene	11.50	984	985	1.23	1.81	26.65
6	Myrcene	11.74	992	992	0.27	0.32	3.76
7	α-Phellandrene	12.33	1010	1010	-	0.46	-
8	Hexyl acetate	12.37	1011	1007	0.54	-	-
9	o-Cymene	13.00	1030	1022	0.73	0.46	-
10	Limonene	13.15	1034	1024	1.47	1.89	1.01
11	β-Phellandrene	13.20	1035	1025	0.15	0.13	0.48
12	1,8-Cineole	13.66	1038	1026	-	-	0.16
13	β-(E)-Ocimene	14.02	1048	1044	-	-	0.12
14	γ-Terpinene	14.52	1063	1054	-	-	0.15
15	Linalool	15.51	1103	1095	0.69	0.97	2.41
16	Terpinen-4-ol	18.79	1185	1174	-	-	0.23
17	α-Terpineol	19.21	1197	1186	-	-	0.17
18	Chavicol	21.09	1263	1247	0.13	-	-
19	2-Undecanone	22.21	1294	1293	0.59	0.16	-
20	Bornyl acetate	22.55	1295	1284	-	-	0.47
21	δ-Elemene	23.95	1348	1335	0.56	0.25	-
22	Chavicol acetate	24.10	1352	1350	0.70	0.11	-
23	α-Cubebene	24.37	1360	1360	4.69	2.91	-
24	Cyclosativene	25.07	1382	1369	-	0.12	-
25	Isoledene	25.21	1386	1374	1.02	0.73	-
26	α-Copaene	25.34	1390	1382	9.00	6.99	0.17
27	β-Bourbonene	25.67	1400	1387	0.56	-	0.16
28	β-Cubebene	25.74	1402	1388	0.52	0.78	-
29	*cis*-β-Elemene	25.79	1404	1396	0.59	0.28	0.17
30	α-Gurjunene	26.49	1426	1409	11.66	9.74	0.30
31	β-Caryophyllene	26.87	1438	1417	12.10	14.51	5.49
32	β-Copaene	27.10	1445	1430	0.74	0.43	-
33	β-Gurjunene	27.26	1450	1431	0.19	0.13	-
34	α-Maalinene	27.35	1453	1436	0.12	-	-
35	Aromadendrene	27.46	1457	1439	0.75	0.42	-
36	*allo*-Aromadendrene	27.57	1460	1447	0.22	-	-
37	*cis*-Muurola-3,5-diene	27.77	1466	1448	0.28	0.33	-
38	α-Humulene	27.93	1472	1452	1.38	1.35	0.33
39	9-epi-(E)-Caryophyllene	28.16	1479	1464	0.49	0.41	-
40	Ishwarane	28.33	1485	1465	0.23	-	-
41	γ-Gurjunene	28.46	1489	1475	1.24	1.30	-
42	γ-Muurolene	28.53	1491	1478	0.93	0.36	-
43	Germacrene D	28.77	1498	1487	2.73	5.40	0.77

table 1. (continued).

No.	Compounds[a]	RT	RI[b]	RI[c]	Concentration (%)		
					A	B	C
44	*cis*-β-Guaiene	28.98	1505	1488	0.24	-	-
45	β-Selinene	29.03	1507	1490	-	1.07	-
46	*allo*-Aromadendr-9-ene	29.04	1507	1490	0.94	-	-
47	Viridiflorene	29.21	1513	1496	5.00	4.24	-
48	Bicyclogermacrene	29.28	1515	1500	12.54	12.17	1.34
49	γ-Cadinene	29.73	1530	1513	0.22	0.21	-
50	Cubebol	29.80	1533	1514	-	0.26	-
51	δ-Cadinene	29.94	1537	1522	1.81	2.37	-
52	*cis*-Calamenene	29.99	1539	1528	5.76	5.10	-
53	*trans*-Cadina-1,4-diene	30.27	1548	1533	0.60	1.08	-
54	α-Calacorene	30.63	1561	1544	0.53	0.41	-
55	Elemol	30.75	1565	1548	0.44	1.50	-
56	(E)-Nerolidol	30.93	1571	1561	0.94	0.87	-
57	β-Calacorene	31.23	1581	1564	0.17	0.12	-
58	Aromadendra-4,9-diene	31.55	1591	1577	0.12		-
59	Spathulenol	31.76	1598	1578	0.90	0.46	0.32
60	Viridiflorol	31.96	1605	1592	1.07	1.32	
61	Guaiol	32.23	1615	1601	2.89	7.07	
62	Caryopheyllene oxide	32.30	1617	1602	-	-	0.22
63	Rosifoliol	32.46	1623	1603	-	0.12	-
64	Ledol	32.55	1626	1608	0.38	0.71	-
65	5-Guaiene-11-ol	33.03	1633	1613	-	0.13	-
66	1-epi-Cubenol	33.15	1647	1630	0.33	0.39	-
67	1,2-Diacetoxy-4-allylbenzene	33.29	1652	1637	1.00	0.92	-
68	Isospathulenol	33.45	1658	1637	-	0.16	-
69	*epi*-α-Cadinol	33.52	1660	1638	0.12	0.72	-
70	*epi*-α-Eudesmol	33.58	1662	1639	0.26	-	-
71	α-Muurolol	33.64	1664	1640	0.42	-	-
72	β-Eudesmol	33.87	1673	1649	-	0.12	-
73	α-Cadinol	33.91	1674	1650	0.46	0.80	-
74	α-Eudesmol	33.96	1676	1652	0.27	1.03	-
75	Bulnesol	34.27	1687	1670	0.28	1.18	-
76	Cadalene	34.50	1695	1675	0.13	-	-
	Total				99.21	98.57	99.83
	Monoterpene hydrocarbons (Sr. no. 1-7, 9-14)				7.74	6.36	87.44
	Oxygenated monoterpenes (Sr. no. 15-17, 20)				0.69	0.97	3.28
	Sesquiterpene hydrocarbons (Sr. no. 21, 23-49, 51-54, 57-58, 76)				78.06	73.21	8.57
	Oxygenated sesquiterpenes (50, 55-56, 59-66, 68-75)				9.76	16.84	0.54
	Nonterpenic compounds (8, 18, 19, 22, 67)				2.96	1.19	-

a Elution order on HP-5MS column

b Retention indices on HP-5 column

c Literature retention indices (see references) sesquiterpenes (0.54%). Among identified compounds, two isomers α-pinene and β-pinene were predominant with 52.92, and 26.65%, respectively. Several other compounds have also possessed significant percentages, consisting of β-caryophyllene (5.49%), myrcene (3.76%), linalool (2.41%), bicyclogermacrene (1.34%), sabinene (1.18%), and camphene and limonene (1.01%). Apparently, chemical compositions in *P. hymenophyllum* essential oils collected from Vietnam and India are quite different [10]. This may be due to collection time, and part use, especially geographic factors.

Till now, there have been plenty of studies on chemical identifications of Vietnamese *Piper* essential oils.

3-Carene (35.21%), β-caryophyllene (10.05%), and pinene (9.17%) were the main constituents of Vungtau-Vietnamese *P. nigrum* seed oil [20]. *P. arboricola* stem essential oil from central Vietnam was rich in *α*-pinene (19.3%) and β-pinene (26.9%) [21]. Our recent report also identified these two isomers accounted for about 15.0% in the stem oil of Hatinh-Vietnamese *P. albispicum* [1]. Therefore, Vietnamese *Piper* medicinal plants, especially *P. hymenophyllum*, seem to be a good reservoir of pinene derivative. The obtained essential oils have been further subjected to antimicrobial activity. As shown in Table 2, both three oil samples are excellent to inhibit the growth of three Gram-positive bacteria *E. faecalis*, *S. aureus*, and *B. cereus* with MIC values of 16-128 µg/mL. Obviously, this result is better than those of the positive control streptomycin (MIC 128-256 µg/mL). However, these essential oils did not inhibit the Gram-negative bacteria. This phenomenon can be explained that the cell wall of the Gram-negative bacteria is a complex envelope, which is structurally formulated by the cytoplasmic membrane, the periplasm and the outer membrane [22]. In addition, the leaf and stem oils of *P. pendulispicum* with MIC values of 16-32 µg/mL were comparable to the positive control cycloheximide (MIC 32 µg/mL) against the yeast *C. albicans*, whereas the leaf oil of *P. hymenophyllum* showed moderate activity with the MIC value of 128 µg/mL. Collectively, due to the high amount of sesquiterpene hydrocarbons,

Table 2. Antimicrobial activity of the studied *Piper* essential oils

Samples	Minimum inhibitory concentration (MIC, µg/mL)						
	Gram (+)			Gram (-)			Yeast
	E. faecalis	***S. aureus***	***B. cereus***	***E. coli***	***P. aeruginosa***	***S. enterica***	***C. albicans***
P. pendulispicum leaf	16	64	64	-	-	-	16
P. pendulispicum stem	32	32	64				32
P. hymenophyllum leaf	64	128	128				128
Streptomycin	256	128	128	32	256	128	-
Cycloheximide	-	-	-	-	-	-	32

-: inactive

P. pendulispicum essential oils are better than *P. hymenophyllum* essential oil in antimicrobial activity. This is the first time *P. pendulispicum* essential oils are applied to antimicrobial assay. In the meantime, Vietnamese *P. hymenophyllum* essential oils seem better than Indian *P. hymenophyllum* essential oils since the Indian fruit oil of this plant showed MIC values of 625 µg/mL against *B. cereus*, *S. aureus*, and *C. albicans* [10]. In another approach, Vietnamese *Piper* essential oils are outstanding agents in antimicrobial treatments. For instance, the leaf and stem essential oils of *P. albispicum* successfully controlled *E. faecalis* and *C. albicans* with MIC values of less than 11 µg/mL [1]. Hence, it is expected to get more *in vitro* and *in vivo* research using Vietnamese *Piper* essential oils.

Conclusions

This study first describes the chemical compositions of two Vietnamese *Piper* essential oils. Sesquiterpene hydrocarbons (73.21-78.06%) were characteristics of essential oils of *P. pendulispicum* leaf and stem, whereas monoterpene hydrocarbons (87.44%) represented *P. hymenophyllum* leaf oil. Bicyclogermacrene (12.17-12.54%) and β-caryophyllene (12.10-14.51%) were predominant in *P. pendulispicum* essential oil, whereas α-pinene (52.92%) and β-pinene (26.65%) were the major compounds in *P. hymenophyllum* essential oil. Both three obtained oils showed potential antimicrobial effects against Gram-bacteria *E. faecalis*, *S. aureus*, and *B. cereus*, and the yeast *C. albicans* with MIC values of 16-128 µg/mL. It is necessary to demand more phytochemical and pharmacological research, to aim to highlight the great values of these two species and other analogous *Piper* plants.

Competing interests

No potential conflict of interest was reported by the authors.

Supplementary data

Figures S1-S3.

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