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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF *Piper minutistigmum*

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Piper is an economically crucial genus in the family Piperaceae. About 700 and 1300 species were found in tropical and neotropical zones, respectively [1]. It is recognizable that *Piper* plants, especially their seeds, have often been used as spices, perfumes, insecticides, hallucinogens, and pharmaceuticals [2]. Black pepper derives from the unripe fruit of *P. nigrum*, whereas white pepper comes from the ripened fruit [3]. The phytochemicals flavonoids, lignans, amides, terpenoids, and alkaloids can be seen as the main chemical classes in *Piper* species [1, 4].

There have been many advances in the phytochemical analysis of *Piper* essential oils using the GC-MS (gas chromatography-mass spectrometry) analytical approach. Generally, the terpenoids monoterpene and sesquiterpene derivatives were predominant in *Piper* essential oils [1–4]. In Vietnam, about 45 accepted *Piper* species were recorded, and essential oils of about 30 species were studied [5]. For instance, *P. longum* leaf essential oil was associated with the appearance of fonenol (40.5%) and elemol (8.2%) [6]. Antimicrobial activity of *P. albispicum* leaf and stem oils was due to the role of bicyclogermacrene (13.2–13.8%), α-pinene (7.3–14.6%), and β-pinene (7.7–15.6%) [7].

Piper minutistigmum C. DC., which is a new *Piper* species, was discovered in Central Vietnam in 2019 [8]. The current study first describes the chemical composition in the leaf and stem oils of this species, collected from Pu Mat National Park, Nghean, Vietnam. The obtained oils were further taken into antimicrobial consideration.

Hydro-distillation of a powder of the fresh leaves resulted in a yellow oil with a yield of 0.31%. A total of 43 compounds were identified, which accounted for 89.2% (Table 1). The individual peak in the resulting chromatograms were identified by comparison of the retention times of *n*-alkanes series with bibliographic Ris [9, 10].

Chemical classes in this essential oil included sesquiterpene hydrocarbons (37.7%), monoterpene hydrocarbons (32.3%), oxygenated sesquiterpenes (17.7%), and oxygenated monoterpenes (1.5%). Spathulenol (12.4%), β-pinene (11.3%), germacrene D (10.1%), α-terpinolene (8.9%), α-pinene (8.6%), and bicyclogermacrene (6.2%) can be seen to be the principal compounds. Several other compounds also reached significant percentages, consisting of β-gurjunene (4.8%), γ-elemene (4.7%), limonene (1.8%), β-cubebene (1.8%), β-elemene (1.7%), caryophyllene oxide (1.5%), bornyl acetate (1.4%), γ-cadinene (1.2%), and isospathulenol (1.1%).

Regarding the stem oil (yellow, 0.25% yield), 47 compounds were identified, which represented 94.9%. Sesquiterpene hydrocarbons (57.5%) and nonterpenic compounds (24.7%) were the main chemical classes, followed by monoterpene hydrocarbons (9.2%), oxygenated sesquiterpenes (3.2%), oxygenated diterpenes (0.2%), and oxygenated monoterpenes (0.1%). The stem oil was dominated by β-caryophyllene (14.6%), bicyclogermacrene (12.8%), germacrene D (12.3%), benzyl benzoate (11.6%), bicycloelemene (8.9%), and apiole (8.4%) (Table 1). This essential oil was also characterized by the presence of other compounds with significant amounts, such as myrcene (2.1%), α-humulene (1.9%), (*E*)-β-ocimene (1.8%), dodecanal (1.8%), and δ-cadinene (1.5%).

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TABLE 1. Essential Oils of *P. minutistigmum* Leaves and Stems, %

Compound	RI	Leaves	Stems	Compound	RI	Leaves	Stems
α-Pinene	939	8.6	0.2	Germacrene D	1485	10.1	12.3
Camphene	953	0.5	1.0	*cis*-Cadina-1,4-diene	1496	–	0.2
β-Pinene	980	11.3	0.1	Bicyclogermacrene	1500	6.2	12.8
Myrcene	990	0.6	2.1	Lepidozene	1502	0.7	–
α-Phellandrene	1006	–	0.1	β-Bisabolene	1506	0.6	–
α-Terpinene	1017	0.1	0.1	γ-Cadinene	1514	1.2	0.4
Limonene	1032	1.8	1.3	*trans*-γ-Bisabolene	1516	0.3	–
(*Z*)-β-Ocimene	1043	0.2	0.4	β-Maaliene	1522	–	0.1
(*E*)-β-Ocimene	1052	0.1	1.8	δ-Cadinene	1525	0.8	1.5
γ-Terpinene	1061	0.1	0.1	α-Calacorene	1546	0.2	–
α-Terpinolene	1090	8.9	1.7	Elemol	1550	0.3	0.2
Undecane	1100	–	0.6	Germacrene B	1561	–	0.5
Alloocimene	1128	0.1	0.2	Germacrene D-4-ol	1574	0.2	–
Camphor	1145	–	0.1	Spathulenol	1578	12.4	0.7
Decanal	1186	–	0.1	Caryophyllene oxide	1583	1.5	–
Myrtenal	1209	0.1	–	Neoisolongifolene	1588	–	0.9
Bornyl acetate	1289	1.4	0.1	Dillapiole	1589	–	0.8
Bicycloelemene	1327	–	8.9	Benzene, 1,2,3,4-tetramethoxy-5-(2-propenyl)	1591	–	1.2
α-Longipinene	1347	–	0.3	Viridiflorol	1593	–	0.7
α-Cubebene	1351	0.1	0.1	Salvial-4(14)-en-1-one	1595	0.5	–
Cyclosativene	1371	–	0.3	Guaiol	1601	0.5	–
α-Ylangene	1375	0.5	–	Aromadendrene epoxide	1623	0.6	–
Isoledene	1376	–	0.3	Isospathulenol	1636	1.1	–
α-Copaene	1377	0.3	1.4	τ-Muurolol	1646	0.9	–
β-Bourbonene	1385	0.3	0.2	β-Eudesmol	1651	–	0.5
β-Cubebene	1388	1.8	–	α-Cadinol	1654	–	0.4
Dodecanal	1390	–	1.8	Apiole	1671	–	8.4
β-Elemene	1391	1.7	1.1	Benzyl benzoate	1760	–	11.6
α-Gurjunene	1412	0.5	0.1	Phytol	2125	–	0.2
β-Caryophyllene	1419	–	14.6	Total		89.2	94.9
β-Gurjunene	1434	4.8	–	Monoterpene hydrocarbons		32.3	9.2
γ-Elemene	1437	4.7	–	Oxygenated monoterpenes		1.5	0.1
Aromadendrene	1441	–	0.3	Sesquiterpene hydrocarbons		37.7	57.5
α-Humulene	1454	1.6	1.9	Oxygenated sesquiterpenes		17.7	3.2
3,7-Guaiadiene	1461	0.1	–	Oxygenated diterpenes		–	0.2
Valencene	1473	0.2	–	Non-terpenic compounds		–	24.7
γ-Muurolene	1480	0.7	–				

RI: Retention indices relative to *n*-alkanes (C_7–C_{30}) on an HP-5 MS column.

As can be seen, nonterpenic compounds were absent in the leaf oil, but they were predominant in the stem oil. Various compounds, especially some major compounds, were found in the leaf oil, but were not observed in the stem oil and vice versa. Spathulenol, β-pinene, α-pinene, and α-terpinolene were the main compounds in the leaf oil, but they were absent or present as trace amounts in the stem oil. Similarly, β-caryophyllene, benzyl benzoate, bicycloelemene, and apiole were only found in the stem oil. These major chemical compounds were also found in various Vietnamese *Piper* essential oils. For instance, the principal compounds in Hatinh–Vietnam *P. retrofractum* leaf oil were benzyl benzoate (14.4%), myrcene (14.4%), bicycloelemene (9.9%), bicyclogermacrene (7.0%) [9]. Benzyl benzoate accounted for 49.1% in Hue-Vietnam *P. sarmentosum* leaf oil [11]. β-Caryophyllene (19.2%) was one of the main compounds in the leaf and stem oils of Quangnam-Vietnam *P. cambodianum* [5].

The obtained oils have been further subjected to antimicrobial experiments, and the results are outlined in Table 2. Both oil samples showed the same MIC value of 16 μg/mL against the Gram-positive bacterium *E. faecalis*.

TABLE 2. Antimicrobial Activity of *P. minutistigmum* Essential Oils (MIC, μg/mL)

Microbial strains	Leaves	Stems	Streptomycin	Microbial strains	Leaves	Stems	Streptomycin
E. faecalis	16	16	32	*P. aeruginosa*	N.i.	128	128
S. aureus	64	32	128	*S. enterica*	N.i.	256	256
B. cereus	16	32	256	*C. albicans*	128	128	
E. coli	N.i.	N.i.	256				

N.i.: no inhibition.

Two tested oils (MIC 16–64 μg/mL) were comparable with the standard streptomycin (MIC 128–256 μg/mL) in antimicrobial treatment against two Gram-positive bacteria, *S. aureus* and *B. cereus*. Regarding the Gram-negative bacteria, the leaf oil was inactive against *E. coli*, *P. aeruginosa*, and *S. enterica*, whereas the stem oil only exhibited an MIC value of 256 μg/mL against the bacterium *S. enterica*. The two samples also controlled the growth of the fungus *C. albicans* with an MIC value of 128 μg/mL, compared with that of the standard cycloheximide (MIC 32 μg/mL). The current result matches well with those of previous studies, in which Vietnamese *Piper* essential oils seem to be potential antimicrobial agents. Our previous record indicated that the leaf oils of *P. pendulispicum* and *P. hymenophyllum* successfully inhibited three Gram-positive bacteria, *E. faecalis*, *S. aureus*, and *B. cereus*, with an MIC value of 16–128 μg/mL [1]. *P. albispicum* leaf oil was strongly active against *E. faecalis*, *P. aeruginosa*, and *C. albicans* with an MIC value of about 5–11 μg/mL [7].

Collectively, phytochemical analysis of essential oils of *P. minutistigmum* leaves and stems, collected from Central Vietnam, were first identified using the GC/FID-MS analytical method. Sesquiterpene hydrocarbons (37.7–57.5%) were the main chemical class in both the leaf and stem oils. Spathulenol (12.4%), β-pinene (11.3%), germacrene D (10.1%), α-terpinolene (8.9%), α-pinene (8.6%), and bicyclogermacrene (6.2%) were characteristic compounds of the leaf oil, whereas β-caryophyllene (14.6%), bicyclogermacrene (12.8%), germacrene D (12.3%), benzyl benzoate (11.6%), bicycloelemene (8.9%), and apiole (8.4%) were the main agents of the stem oil. Both oils showed strong antimicrobial activity against three Gram-positive bacteria, *E. faecalis*, *S. aureus*, and *B. cereus*, with an MIC value of 16–64 μg/mL.

Materials. The fresh leaves and stems were collected from Pu Mat National Park, Nghean Province, Vietnam (18°97′04″N and 104°80′06″E) in June 2022. The plant materials were identified by our co-author Dr. Do Ngoc Dai. The voucher specimens PML-2022 (leaves) and PMS-2022 (stems) were deposited at Nghean University of Economics.

Hydro-Distilled Procedure. *P. minutistigmum* fresh leaves (1.5 kg) were hydro-distilled using a Clevenger apparatus with water for 3.0 h, to produce a yellow oil (0.31%, w/w). The obtained oil was dried over anhydrous Na_2SO_4 before analysis. The same procedure was applied to the stems (1.5 kg), to afford a yellow oil (0.25%).

GC/FID-MS Analysis. The procedure was the same as in our previous reports [12–14].

Antimicrobial Activity. Seven microbacterial strains were used in this study, including three Gram-positive bacteria, *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and *Bacillus cereus* ATCC14579, three Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076, and one yeast *Candida albicans* ATCC 10231. The experimental method was described in our previous works [1, 3, 12–14].

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