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Article

Chemical Compositions of Essential Oils and Antimicrobial Activity of *Piper albispicum* C. DC. from Vietnam

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Abstract: The chemical constituents and antimicrobial activities of essential oils isolated from the leaf and stem of *Piper albispicum* C. DC. were reported. The main compounds of the leaf essential oil were chavicol acetate (16.3%), bicyclogermacrene (13.8%), sabinene (8.6%), b-pinene (7.7%) and a-pinene (7.3%). On the other hand b-pinene (15.6%), a-pinene (14.6%) and bicyclogermacrene (13.2%) were the significant compounds of the stem essential oil. The leaf oil displayed the best antimicrobial activity towards *Pseudomonas aeruginosa* ATCC27853 with the Minimum inhibitory concentration (MIC) value of 5.82 µg/mL. Both essential oils exhibited similar activities against *Enterococcus faecalis* ATCC299212 (MIC's, 9.07 µg/mL and 9.81 µg/mL, respectively) and *Candida albicans* ATCC 10231 (MIC's, 10.66 µg/mL and 10.91 µg/mL, respectively). However, the stem oil exhibited pronounced activity against *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC14579, with MIC values of 10.22 µg/mL and 10. 44 µg/mL, respectively. However, the studied essential oils did not inhibit the growth of *Escherichia coli* ATCC 25922 and *Salmonella enterica* ATCC13076. This is the first report on the chemical constituents and antimicrobial activity of the essential oil of *P. albispicum*.

Keywords: *Piper albispicum,* Essential oil, Monoterpenes, Sesquiterpenes, *Pseudomonas aeruginosa*

Introduction

Piper is the largest genus of the Piperaceae family comprising of 2000 species [1]. The species of this genus have diverse consumption rates, biological activities and are used in pharmacopeia throughout the world. They are also used in folk medicine for the treatment of many diseases in several countries including Vietnam. In particular, the essential oils obtained from the *Piper* species are considered important sources of biologically active substances. For example, the tissue essential oils of *P. caldense* showed antibacterial activity against tested microorganisms except *Enterococcus faecalis* [2]. The essential oil of *P. divaricatum* tissues and its main compound, safrole, were active against *Litsera monocytogenes, Salmonella typhimurium* and *Pseudomonas aeruginosa* [3]. In addition, 1-butyl-3,4-methylenedioxybenzene and terpinolene were reported to be responsible

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for the oviposition deterrent activity of essential oil from *P. corcovadensis* [4].

While working on a guide to new plants in Kẻ Gỗ Natural Reserve and adjacent areas, *Piper albispicum* C. DC, was collected that does not match any of the previously known *Piper* plants. The plant, *P. albispicum* is widely used in Vietnam for food preservation, treatment of microbial infection and killing of several microbes [5]. The lack of information on the phytochemical constituents and biological studies on *P. albispicum* led to this study. To our knowledge, there are no published reports on the antimicrobial activity and chemical compositions of the essential oils from *P. albispicum*. As part of the systematic study of volatile constituents of *Piper* species from Vietnam [6-8], in the present study, the chemical constituents of essential oils from the leaf and stem of *P. albispicum* were identified. Also, the medicinal property of the plant, using the antimicrobial activity of the essential oils was tested against a panel of microorganisms. This is part of the ongoing extensive research aimed at the characterization of the volatile constituents and antimicrobial activity of the poorly described species of Vietnamese flora [9,10].

Plant essential oils are complex mixtures of natural compounds, which have application as agro-feed, industrial crops and medicinal properties. Although chemical compositions of essential oils differ among species, the main compounds identified in essential oils often belong to the family of terpenes, which are highly lipophilic and low molecular weight, and thus, capable of disrupting cell membrane, causing cell death or inhibiting sprouting of microbes [11]. The chemical components of essential oils are affected by factors such as geographical location, environment, and stage of maturity and method of extraction [11,12]. This chemical difference is directly correlated to dissimilarity in biological activities. Essential oils and their compounds exhibit activity by firstly destroying the microbial cytoplasmic wall to enhance permeability and passage of large protons and ions [9-12]. The rhizome essential oil of *Alpinia kwangsiensis* was active against *Enterococcus faecalis* ATCC299212 with a minimum inhibitory concentration (MIC) value of 1.60 µg/mL, while the leaf oil displayed pronounce activity towards *Bacillus cereus* ATCC14579 (MIC 3.20 µg/mL). All the essential oils inhibited the growth of *Staphylococcus aureus* ATCC25923 and *Candida albicans* ATCC10231, with MIC value of 6.40 µg/mL [11]. The leaf oil of *Uvaria hamiltonii* demonstrated notable antimicrobial activity against *Enterococcus faecalis* ATCC299212 with a minimum inhibitory concentration (MIC) value of 7.99 µg/mL and *Bacillus cereus* ATCC14579 (MIC 5.67 µg/mL) while *Fissistigma kwangsiense* showed the most potent activity towards *Pseudomonas aeruginosa* ATCC27853 and *C. albicans* ATCC10231, with MIC values of 3.45 µg/mL and 16.45 µg/mL, respectively [12]. Essential oil from *P. beetle* inhibited the growth of *Malassezia furfur* (ATCC 14521 and VNF01) and *M. globosa* (VNG02) by 100% and 40%, respectively [13]. The essential oils of *P. caldesne* displayed moderate antimicrobial activity towards some microorganisms including *Escherichia coli* ATCC25922, *S. aurues* ATCC 6538 and *Bacillus subtilis* ARCC 6633 [2].

Materials and methods

Plant collection

The leaves (2.2 kg) and stem bark (2.1 kg) of *P. albipiscum* were from Kẻ Gỗ Natural Reserve (GPS: 18°17'18'' N; 105°21'40'' E), Vietnam, in May 2021. The plant samples were identified by Dr. Le Thi Huong of the Faculty of Biology, College of Education, Vinh University, Vietnam. A voucher specimen number LTH 755 was deposited in the plant specimen room, Vinh University, Vietnam.

Hydrodistillation of essential oils

Before hydrodistillation, the leaves and stems of *P. albispicum* were cleaned by handpicking debris and other undesirable materials to obtain 2 kg of each sample. Afterward, the samples were ground into coarse particles using a locally made grinder. Each of the collected plant materials was divided into three parts to ensure that each of the hydrodistillation was repeated three times. The samples were separately introduced into a 5 L flask after which distilled water (3.5 L) was

added until it covered the sample completely. Essential oils were obtained by hydrodistillation which was carried out in a Clevenger-type distillation unit designed according to an established procedure [14] as described in previous studies [6-12]. The distillation time was 3 h and was conducted at normal pressure. The volatile oils distilled over water were collected separately by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4°C) until the moment of analysis. The experiment was conducted in triplicate. The essential oils yield (%) was calculated by mass (g) of the essential oil divided by the mass (g) of the dried rhizomes of the plant.

Instrumental analysis of the hydrodistilled essential oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with an FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 mm, Agilent Technology). The analytical conditions were: carrier gas He (1 mL/min), injector temperature, 250°C; detector temperature 260°C; column temperature programmed from 40°C (held 2 min isothermally) and rise to 220°C (10 min hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume of the oil-injected was 1.0 mL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. Quantification was done using the calibration curves generated from the analyses of representative standard compounds from each class [6-12].

An Agilent Technologies HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 mm) 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 analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70eV; emission current 40 mA; acquisitions scan mass range of 45-350 amu at a sampling rate of 1.0 scan/s.

Identification of the constituents of the essential oils

The identification of constituents of essential oils from the GC/MS spectra of *P. albipiscum* was performed based on a comparison of retention indices (RI Exp.) with reference to a homologous series of *n*-alkanes (C_6-C_{40}), under identical experimental conditions. In some cases, co-injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition in literature [15] as described recently [6-12].

Antimicrobial activity assays

Antimicrobial strains

The antimicrobial activity of the essential oils was evaluated using three strains of Gram-positive test bacteria, *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579, three strains of Gram-negative test bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076 and one strain of yeast, *Candida albicans* ATCC10231. The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay as previously described [8-12].

Microdilution broth susceptibility assay

Stock solutions of the essential oils were prepared in dimethylsulfoxide. The choice of investigated concentrations was based on our previous reports on similar investigations where essential oils are active within a specific concentration range [8-12]. Dilution series were prepared from 16,384 to 2 µg/mL (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^{9}, 2^{7}, 2^{5}, 2^{3} and 2^{1} µg/ mL) in sterile distilled water in micro-test tubes from where they were transferred to 96-well microtiter plates. Bacteria were 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 CFU/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, while nystatin and cycloheximide were used as anticandidal standards. After incubation at 37°C for 24 h, 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 medium but without the antimicrobial agent, test agent corresponds to a known concentration of the antimicrobial agent, control(+) is the culture medium without cells, $High_{conc}/Low_{conc}$ 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).

Statistical analysis

Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD) of three independent measurements using the Microsoft Excel program 2003.

Results and discussion

The percentage yield and colour of the essential oils

The hydrodistillation experiment afforded light yellow essential oils in yields of 0.21% (w/w; 1.2 g) and 0.15% (w/w; 1.31 g), respectively, for the leaves and stem barks, with SD ± 0.01. The yields are consistent with data obtained for essential oils of other *Piper* plants from Vietnam and the rest of the world. Previously, *P. retrofractum, P. boehmeriaefolium* and *P. sarmentosum* leaf essential oils from Vietnam were obtained in yields of 0.20%, 0.20% and 0.25%, respectively [6]. The leaf and stem of *P. maclurei* afforded oils in yields of 0.25% and 0.20%, respectively [6]. *P. laosanum* leaf and stem were also obtained in yields of 0.21% and 0.16%, respectively, while *P. acre* leaf and stem essential oils from Vietnam were obtained in yields of 0.21% and 0.15%, respectively [7]. The yield for *P. majusculum* leaf was 0.15%, while *P. bvericaule* leaf had 0.17%, with 0.23% and 0.15% obtained for the leaf and stem of *P. harmandii* [8]. *P. betle* from Vietnam produced essential oil in a yield of 0.25%. Essential oil from the fruit of *P. guineense* grown in Nigeria was obtained in a yield of 1.34% [16]. The yields of essential oils from aerial parts of *P. abbreviatum, P. erecticaule* and *P. lanatum* oils analysed from Malaysia were 0.22%, 0.18% and 0.25%, respectively [17]. The difference in essential oil yields could be attributed to distinct plant species, geographical origins, climatic conditions, environmental conditions, and methods of extractions [8-12].

Chemical constituents of the essential oils

The chemical constituents of *P. albipiscum* essential oils are shown in Table 1. By using a combination of GC-FID and GC/MS with HP-5MS column, fifty and fifty-three compounds representing 97.4% and 99.5% of the contents of the essential oil were identified respectively in the leaf and stem bark of *P. albispicum* (Table 1). Monoterpene hydrocarbons (32.1% and 44.9%, respectively), oxygenated monoterpenes (29.4% and 6.4% respectively) and sesquiterpene hydrocarbons (29.7% and 44.3%, respectively) were the main classes of compounds identified in the essential oils. Both the leaf and stem bark essential oils consist of an equal quantity of oxygen-containing sesquiterpenes (ca. 2.8%). It could be seen that ubiquitous terpenes as defined in some other previously analysed *Piper* essential oils from Vietnam [6-8,13, 18,19], and other parts of the world [2-4,16,17], were also identified in the present oil samples of *P. albispicum.* The main constituents of leaf essential oil of

Table 1. Chemical compositions of the leaf and stem bark essential oils of *P. albispicum* collected in Vietnam

S. No.	Compounds [a]	RI [b]	RI [c]	Percent composition [d]	
				Leaf	Stem
1	α-Thujene	930	921	0.3	0.3
2	α-Pinene	939	932	7.3	14.6
3	Camphene	955	946	0.3	0.4
4	Sabinene	979	978	8.6	3.7
5	β-Pinene	984	982	7.7	15.6
6	Myrcene	992	990	0.8	1.4
7	α-Phellandrene	1010	1004	1.2	1.0
8	α-Terpinene	1022	1014	0.5	0.5
9	o-Cymene	1030	1020	1.2	1.2
10	Limonene	1034	1028	1.0	2.0
11	β-Phellandrene	1036	1030	2.4	1.6
12	1,8-Cineole	1037	1032	1.4	0.5
13	(*Z*)-β-Ocimene	1038	1036	-	0.4
14	(*E*)-β-Ocimene	1049	1044	0.1	0.2
15	γ-Terpinene	1063	1056	0.6	0.7
16	Terpinolene	1094	1089	0.1	0.3
17	Sabinene	1103	1101	4.3	2.7
18	Terpinen-4-ol	1187	1187	0.9	1.0
19	Chavicol	1263	1261	5.8	0.5
20	*trans*-Linalool oxide acetate	1291	1294	0.7	-
21	δ-Elemene	1348	1347	0.3	0.2
22	Chavicol acetate	1354	1352	16.3	1.7
23	α-Cubebene	1360	1363	0.3	0.4
24	α-Copaene	1389	1387	1.3	2.0
25	β-Cubebene	1402	1400	1.7	1.7
26	*cis*-β-Elemene	1403	1401	-	1.1
27	α-Gurjunene	1425	1422	0.3	0.9
28	β-Caryophyllene	1437	1437	1.4	5.3
29	β-Gurjunene	1445	1443	0.4	1.5
30	Aromadendrene	1457	1454	0.2	0.3
31	(Z)-β-Farnesene	1461	1459	-	0.2
32	*cis*-Muurola-3,5-diene	1466	1460	0.2	0.3
33	α-Humulene	1472	1472	1.5	4.3
34	Ishwarane	1485	1484	1.7	1.3
35	*trans*-Cadina-1(6),4-diene	1488	1486	0.2	-
36	γ-Curcumene	1489	1488	-	1.7
37	γ-Muurolene	1491	1489	0.2	-

Table 1 cont.

S. No.	Compounds [a]	RI [b]	RI [c]	Percent composition [d]	
				Leaf	Stem
38	ar-curcumene	1492	1490	-	0.5
39	Germacrene D	1498	1498	3.9	4.9
40	β-Selinene	1505	1503	0.4	0.8
41	*trans*-Muurola-4(14),5-diene	1510	1508	-	0.8
42	Bicyclogermacrene	1514	1512	13.8	13.2
43	β-Curcumene	1521	1519	-	0.4
44	γ-Cadinene	1530	1528	0.2	0.3
45	δ-Cadinene	1537	1537	1.3	1.5
46	*cis*-Calamenene	1539	1541	0.2	0.1
47	*trans*-Cadina-1,4-diene	1548	1550	0.2	0.2
48	*trans*-Cadinene ether	1562	1563	0.4	-
49	Germacrene B	1577	1574	0.5	0.4
50	Scapanol	1595	1596	0.2	-
51	Spathulenol	1598	1600	0.4	0.4
52	Viridiflorol	1605	1608	0.3	0.6
53	Guaiol	1614	1612	0.2	0.4
54	1,10-di-*epi*-Cubenol	1647	1645	0.2	0.4
55	1,2-Diacetoxy-4-allylbenzene	1652	1650	3.4	1.1
56	*epi*-α-Muurolol	1661	1658	0.3	-
57	α-Muurolol	1662	1664	-	0.3
58	α-Cadinol	1674	1670	0.3	0.4
59	α-Eudesmol	1676	1678	-	0.3
Total				97.4	99.5
Monoterpene hydrocarbons (Sr. No. 1-11, 13-17)				32.1	44.9
Oxygenated monoterpenes (Sr. No. 12, 18-20,22)				29.4	6.4
Sesquiterpene hydrocarbons (Sr. No. 21, 23-47, 49)				29.7	44.3
Oxygenated sesquiterpenes (Sr. No. 48, 50-54,56-59)				2.8	2.8
Others (Sr. No. 55)				3.4	1.1

[a] Elution order on HP-5MS column; [b] Experimental retention indices; [c] Literature retention indices on HP-5MS column as seen in NIST [15]; [d] means of three replicate values, SD (±) omitted to avoid congestion; S. No: serial number; - not identified

P. albispicum were chavicol acetate (16.3%), bicyclogermacrene (13.8%), sabinene (8.6%), β-pinene (7.7%) and α-pinene (7.3%). The leaf oil had sizeable amount of chavicol (5.8%), linalool (4.3%), germacrene D (3.9%), 1,2-diacetoxy-4-allylbenzene (3.4%) and b-phellandrene (2.4%). On the other hand, β-pinene (15.6%), α-pinene (14.6%), bicyclogermacrene (13.2%) and b-caryophyllene (5.3%) were the significant compounds of the stem bark essential oil. The bark oil also features significant quantity of germacrene D (4.9%), a-humulene (4.3%), sabinene (3.7%), linalool (2.7%), limonene (2.0%) and a-copaene (2.0%). To the best of the authors' knowledge, this is the first report on the chemical constituent of essential oils from *P.*

albispicum grown in Vietnam and any other parts of the world.

The essential oil compositions of essential oils from *Piper* species from Vietnam and other parts of the world have been characterized. Although a majority of them contained ubiquitous terpenes, however, the identities of these compounds differ from each other. However, other chemical classes of compounds have been identified as well. Table 2 indicates the representative compounds of some *Piper* essential oils analysed from Vietnam and other parts of the world. It is well known that essential oils of *Piper* plants from Vietnam exhibited chemical variability and various chemotyic forms of some species have been delineated [8,9,10,16]. For example, six chemotypic forms of essential oils of *P. guineense* are known [16]. The main compounds of *P. retrofractum, P. boehmeriaefolium* from Vietnam were completely different from samples elsewhere [6]. The essential oils of *P. divaricatum* from Brazil contained methyl eugenol/eugenol chemotype [3] and 1-butyl-3,4-methylenedioxybenzene chemotype [24]. This variation in the chemical constituents of essential oils of *P. albispicun* with other members of the genus may be attributed to the nature of each plant species, differences in the place of origin, ecological conditions and plant parts used.

Result of the antimicrobial test on the essential oils

The essential oils from *P. albispicum* were screened against a panel of microorganisms (Table 3). The MIC was used to determine

Table 2. Representative compounds of some *Piper* essential oils from Vietnam and other parts of the world

Species	Parts	Origin	Main constituents	References
P. caldense	Root	Brazil	pentadecane (35.7%) and valencene (10.5%)	2
″	Stem	″	terpinen-4-ol (18.5%) and α-terpineol (15.3%)	2
″	Leaf	″	α-cadinol (19.0%)	2
Piper divaricatum	LeafFruits/ Stem	″	safrole (98%, 87% and 83%, respectively)	3
Piper corcovadensis	″	″	1-butyl-3,4-methylenedioxybenzene (30.62%) and terpinolene (17.44%)	4
P. retrofractum	Leaf	Vietnam	benzyl benzoate (14.4%), myrcene (14.4%) and bicycloelemene (9.9%)	6
P. boehmeriaefolium	″	″	α-copaene (28.3%) and α-pinene (7.4%)	6
P. sarmentosum	″	″	benzyl benzoate (49.1%), benzyl alcohol (17.9%) and 2-hydroxy-benzoic acid phenylmethyl ester (10.0%)	6
P. maclurei	″	″	(*E*)-cinnamic acid (37.4%) and (*E*)-nerolidol (19.4%)	6
″	Stem	″	(*Z*)-9-octadecanoic acid methyl ester (28.0%), (*E*)-cinnamyl acetate (17.2%) and phytol (12.2%)	6
P. laosanum	Leaf	″	α-curcumene (12.0 %), germacrene D (6.3 %) and sabinene (6.1 %)	7

Table 2 cont.

Species	Parts	Origin	Main constituents	References
″	Stem	″	sabinene (14.9 %), benzyl salicylate (14.3 %) and (*E*)-nerolidol (9.3 %)	7
P. acre	Leaf/Stem	″	(*E*)-nerolidol (22.7 % and 15.6 %), sabinene (19.5 % and 19.9 %) and δ-cadinene (12.4 % and 13.5 %)	7
P. majusculum	Leaf	″	β-caryophyllene (20.7%), germacrene D (18.6%) and β-elemene (11.3%)	8
P. harmandii	Leaf/Stem	″	sabinene (14.5% vs. 16.2%), benzyl benzoate (20.0% vs. 29.4%) and benzyl salicylate (14.1% vs. 24.3%). α-Cadinol (17.0%) present in the leaf	8
P. brevicaule	Leaf/Stem	″	sabinene (17.9% vs. 13.5%), benzyl benzoate (20.5% vs. 32.5%) and β-eudesmol (13.8% vs. 8.4%)	8
P. betle	Leaf	″	eugenol acetate (38.66%) and eugenol (30.28)	13
P. abbreviatum	Leaf	Malaysia	spathulenol (11.2%), (*E*)-nerolidol (8.5%) and β-caryophyllene (7.8%)	17
P. erecticaule	″	″	β-caryophyllene (5.7%) and spathulenol (5.1%)	`7
P.guineense	Fruit	Nigeria	linalool (52.2%)	
P. lanatum	″	″	borneol (7.5%), β-caryophyllene (6.6%) and α-amorphene (5.6%)	17
P. bavinum	Leaf	″	bicyclogermacrene (10.6%), globulol (5.7%) and ledene (5.1%)	18
P. nigrum	″	″	3-carene (29.21%), D-limonene (20.94%), caryophyllene (15.05%), and β-pinene (9.77%)	19
P. lolot	Leaves, stems and rhizomes	″	β-caryophyllene (26.1-30.9%). The rhizome oil contained bornyl acetate (10.0%) as second major component	20
P. arborescens	Leaf	Malaysia	β-phellandrene (24.3%), sabinene (16.3%), α-pinene (10.4%) and terpinen-4-ol (7.2%)	21
″	Stem	″	β-phellandrene (20.4%), methyl eugenol (11.0%) and β-caryophyllene (9.0%)	21
P. pierrei	Leaf	Vietnam	α-methylbenzyl cinnamate (28.0%) and an isomeric methylbenzyl cinnamate (18.1%)	22
P. amaalgo	″	Ecuador	β-phellandrene (20.42%) and spathulenol (10.34%)	23

Table 3. Antimicrobial activity of *P. albispicum* leaf and stem bark essential oils

Microorganisms	MIC (µg/mL) [a]		IC_{50}(µg/mL) [a]	
	Leaf	Stem	Leaf	Stem
Enterococcus faecalis ATCC299212	9.07	9.81	32.0	32.0
Staphylococcus aureus ATCC25923	23.35	10.22	64.0	32.0
Bacillus cereus ATCC14579	55.62	10.44	128.0	32.0
Escherichia coli ATCC 25922	-	-	-	-
Pseudomonas aeruginosa ATCC27853	5.82	11.05	16.0	32.0
Salmonella enterica ATCC13076	-	-	-	-
Candida albicans ATCC 10231	10.66	10.91	64.0	64.0

[a]Standard deviation in the range ± 0.00-0.01; - No activity

the minimum concentration that prevented the growth of test microbes. However, IC_{50} is that concentration that achieved 50% growth inhibition. In the present work, it may be useful in drug formulation for some applications including the use of essential oils as a component of antimicrobial packaging. The leaf and stem essential oils of *P. albispicum* showed an almost equal good antibacterial activity against the Gram-positive bacteria *E. faecalis* ATCC299212 with MIC values of 9.07 µg/mL and 9.81 µg/mL, respectively; and anticandidal activity against *C. albicans* ATCC10231 with MIC values of 10.66 µg/mL and 10.91 µg/mL, respectively. In this report, the leaf essential oil was the most active against the gram-negative bacteria, *P. aeruginosa* ATCC27853 with MIC value of 5.82 µg/mL, while the stem oil displayed pronounced activity towards *S. aurues* ATCC25923 (MIC 10.22 µg/mL) and *B. cereus* ATCC14579 (MIC 10.44 µg/mL). The essential oils did not exhibit any activity towards the Gram-negative bacteria of *E. coli* ATCC25922 and *S. enterica* ATCC13076. The potency of the essential oils against the tested microorganisms defined by IC_{50} is shown in Table 3. The leaf oil exhibited activity towards *P. aeruginosa* and *E. faecalis* with the least inhibitory concentration of 16.0 µg/mL and 32.0 µg/mL, respectively. The stem oil showed good activity towards *E. faecalis, S. aureus* and *B. cereus* with an IC_{50} value of 32.0 µg/mL. The reference compounds namely Streptomycin for gram-positive bacteria exhibited activity with MIC values in the range of 0.5-1.0 µg/mL, while cycloheximide used as antifungal had MIC values in the range of 1.2-3.7 µg/mL. Also, Nystatin an anticandidal compound displayed activity with MIC values in the range of 0.8-2.3 µg/mL. The IC_{50} values have also been considered in the range of 10-120 µg/mL [9-12]. No previous information exists on the antimicrobial activity of essential oils from *P. albispicum.*

The inactivity of the essential oils against Gram-negative bacteria was not unexpected. Several previous studies have shown Gram-positive bacteria to be more susceptible to essential oils than Gram-negative organisms [23]. This may be due to cell wall lipopolysaccharides in the Gram-negative organisms that inhibit the lipophilic essential oil components from diffusing into the cells. However, the ability of essential oils to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely reason for its lethal action [9-12]. The observed antimicrobial result of *P. albispicum* essential oils was in agreement with previous information that *Piper* essential oils from Vietnam [13] and other parts of the world [2-4,17,21,23] selectively inhibited the growth of different microorganisms.

The major components of the essential oil namely α-pinene, β-pinene, sabinene, chavicol acetate and bicyclogermacrene have shown antimicrobial activities against microorganisms, and likely account for the observed antimicrobial activity of *P. albispicum* essential oils. The

synergistic antimicrobial effects of some other minor compounds have been reported. In addition, α-and β-pinene, linalool, 1,8-cineol are ubiquitous monoterpenoids in conifer and the other aromatic plants, and each compound was widely tested against many organisms, and cancer cell lines [25].

Conclusions

This study found out that chavicol acetate (16.3%), bicyclogermacrene (13.8%), sabinene (8.6%), β-pinene (7.7%) and α-pinene (7.3%) were the main compounds of *P. albispicum*, while β-pinene (15.6%), α-pinene (14.6%) and bicyclogermacrene (13.2%) were the significant compounds of the stem essential oil. The essential oil displayed a varying degree of antibacterial and anti-candidal activities. The leaf oil was active towards *P. aeruginosa* ATCC27853 with the MIC value of 5.82 µg/mL. These results give significant information about the pharmacological activity of the essential oil, which suggests the benefits o human health, having the potential to be used as antimicrobial agents.

Competing interests

The authors declare that no competing interest exists.

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