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Article

Essential Oils of Lauraceae: Antimicrobial Activity and Constituents of *Phoebe macrocarpa* C.Y. Wu Leaf Essential Oil from Vietnam

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Abstract: The present paper reports for the first time the chemical composition and antimicrobial activity of essential oil from the leaves of *Phoebe macrocarpa* C. Y. Wu. The yield of the essential oil was 0.28% (w/w). The essential oil features monoterpene hydrocarbons (3.3%), oxygenated monoterpenes (2.8%), sesquiterpene hydrocarbons (31.9%) and oxygenated sesquiterpenes (46.0%). The major compounds of the essential oil were β-caryophyllene (16.6%) and spathulenol (12.6%). The essential oil showed moderate antimicrobial activity towards *Enterococcus faecalis* ATCC299212 with minimum inhibitory concentration (MIC) value of 128 µg/mL, *Staphylococcus aureus* ATCC25923 (MIC 256 µg/mL) and *Bacillus cereus* ATCC14579 (MIC 1280 µg/mL). The essential oil did not display anti-candidal activity.

Keywords: *Phoebe macrocarpa,* Lauraceae, Essential oil, Sesquiterpenes, Antimicrobial activity.

Introduction

In continuation of an on-going research on the chemical constituents and biological activities of essential oils from poorly studied species of Lauraceae family of plants in particular [1-5] and other families in general [6-9] grown in Vietnam, we report herein the results of the investigation on the essential oil of *Phoebe macrocarpa* C. Y. *Phoebe macrocarpa* Wu (syn. *Phoebe poilanei* Kostermans), is a large tree, usually 15-20 m tall, with blackish-brown trunk of about 40-60 cm d.b.h. The bark is thick and densely yellowish brown. The leaves blades are elliptic-oblanceolate or oblanceolate, thinly leathery and sparsely yellowish brown. The fruits are ellipsoid or suboblong, leathery and hairy on both surfaces. Flowering takes place from April to May, while fruiting occurs from October to December [10]. The plant is known locally in Vietnamese as Re trắng quả. *Phoebe hekouensis* Bing Liu, W.Y. Jin, L.N. Zhao & Y. Yang was thought to resemble *P. macrocarpa* C.Y. Wu, but differs from the latter by the tepals being longer, 9-13 mm long (vs. ca. 4 mm) [10].

The lack of information on the phytochemical constituents and biological studies on the volatile

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and non-volatile constituents of *P. macrocarpa* led to this study. However, the chemical constituents and biological activities of essential oils from some other *Phoebe* plants from Vietnam and other parts of the world have been the subject of literature discussion. The constituents occurring in higher amounts in the leaf essential oil of *P. paniculata* from Vietnam were β-caryophyllene (12.1%), germacrene D (9.2%) and sabinene (8.8%) while bicyclogermacrene (15.5%), germacrene D (13.9%), sabinene (7.0%) and β-caryophyllene (7.0%) were the main compounds of *P. tovayana* leaf essential oil [1]. The major compounds found in the essential oil of *P. angustifolia* from Vietnam were *n*-hexacadecanoic acid (13.0%) and spathulenol (17.0%) [2]. Another investigated sample reported the abundance of α-pinene (26.9%) and β-pinene (20.8%) in the leaf essential oil of *P. angustifolia* also from Vietnam [3]. The leaves of *P. formosana* from Taiwan afforded essential oil whose major constituents were α-humulene (16.8%), τ-cadinol (8.9%), α-pinene (8.4%), α-cadinol (8.1%) and β-caryophyllene (8.0%) [11]. The essential oil exhibited moderate growth suppression against Gram-positive bacteria with minimum inhibitory concentration (MIC) values of 250 to 375 µg/mL [11]. The significant compounds identified in the leaf essential oil of *P. bournei* from China included α-copaene (5.4%), α-muurolene (7.3%), δ-cadinene (11.4%), *trans*-calamenene (5.2%), and the essential oil had significant inhibitory activity against *Epidermophyton floccosum* and *Microsporum gypseum*, potential antitumor activity and promotes glucose uptake by adipocytes [12]. The chemical composition of essential oils from bark and leaf of *P. zhennan* contained α-calacorene, τ-cadinol, β-eudesmol and δ-cadinene as major compounds, which are responsible for the DPPH radical-scavenging activity [13]. The essential oil of *P. porphyria* collected from northwestern Argentina (Yungas area) consists mainly of 1,8-cineole (10.5%), β-caryophyllene (19.3%) and spathulenol (17.1%) [14]. The essential oils from *P. hui* leaves had δ-cadinene (12.3%), α-eudesmol (8.2%), α-selinene (7.0%), γ-muurolene (6.7%), β-selinene (5.7%) and β-eudesmol (5.3%), in abundant, and a mixture of these oils exhibited significant antitumor properties [15]. The leaf essential oil of *P. sheareri* was analysed from China and contained torreyol (10.6%), δ-cadinene (5.7%), α-cedrol (5.4%) and β-eudesmol (5.0%) [16].

The aim of the present study was to analyze and report for the first time, the chemical compounds identified in the essential oil hydrodistilled from the leaves of *P. macrocarp*a growing wild in Vietnam, and to evaluate the antimicrobial activity.

Materials and methods

All experimental procedures used in this study were similar to those described earlier in our previous published studies [3-9]. These includes method of collection of the plant sample, isolation of essential oils from the plant, determination of the constituents of the essential oils and antimicrobial study.

Procedure for collection of P. macrocarpa leaves from Pù Hoạt Nature Reserve and preservation of sample

The leaves of *P. macrocarpa* used in this study were obtained from Pù Hoạt Nature Reserve (GPS: 19°35′19″N, 104°43′7″E) situated in NgheAn Province, Vietnam. The collection of the leaves from plants growing in the reserve was achieved in December, 2019. The amount of sample collected was 2.31 kg. The botanical identification and confirmation of the plant was done by Prof. Dr. Huong, L.T., School of Natural Science Education, Vinh University, Vietnam. In case of future reference, a voucher specimen with code LTH 881 was preserved at the plant specimen room, Vinh University, Vietnam, as indicated in our previous studies [5,6].

Obtaining essential oil from the leaves of P. macrocarpa

The process began initially with the cleaning of the leaves thoroughly by removing unwanted particles in accordance with procedures reported previously [5]. Secondly, the cleaned leaves were chopped in locally made grinder, prior to the extraction of the essential oil. The weight obtained from the chopped leaves of P.

macrocarpa was 2 kg. Thirdly the sample was then divided into three parts so that the essential oils can be extracted in triplicate. Thereafter, the leaves sample were packed inside a 5-L flask, and enough quantity of distilled water (3.5-L) was also added in order that the samples will be submerged inside the flask. The flask was then connected to the distillation unit (Clevenger-type apparatus) according to the Vietnamese Pharmacopoeia specification [17] as described in previous report [5]. The essential oils were allowed to distill for 3 h after the whole apparatus was connected to a heating mantle maintained at normal pressure. Lastly, at the end of the experiment, the hydrodistilled essential oils were collected separately into clean and previously weighed sample bottles. The hydrodistillation was achieved in triplicate from each of the samples.

Preservation of esential oils and calculation of percentage yields

In accordance with our laboratory procedures, the essential oils were refrigerated at 4°C as reported in previous studies [5,6]. The yield (%) of the essential oils was calculated by dividing the mass (g) of the essential oil over the mass (g) of the pulverized leaves of *P. macrocarpa*, as described previously [5].

Determination of the constituents of the essential oils

The constituents present in the essential oils and the individual percentages were determined using the techniques of gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS), as described in previous studies [3-5].

During the GC analysis, the Gas chromatograph (HP 7890A Plus) used was from Agilent Technologies, USA. The GC was equipped with HP-5MS column of dimension 30 m x 0.25 mm and film thickness of 0.25 µm, which was connected with flame ionization detector (FID). 1.0 mL of diluted essential oil (1% n-hexane) was injected into the GC column, using the split ratio of 10:1 at the inlet pressure of 6.1 kPa. Helium was used as the carrier gas under the flow rate of 1 mL/min, conducted at normal pressure. The injector and detector temperatures were set at 250°C and 260°C, respectively. The column temperature programmed condition starts from 40°C and held isothermally for 2 min. It was allowed to rise to 220°C, and then held isothermally for 10 min. The analysis was performed in triplicate in accordance with normal specification [1-9]. The quantification of the constituents of each essential oil was done by using the calibration curves generated from the analyses of representative standard compounds from each class, as reported previously [3-5].

The GC/MS analysis involves a GC chromatograph which was interfaced with a Mass spectrometer (HP 5973 MSD, Agilent Technologies, USA). The GC column and operating conditions for the GC/MS experiment were the same as described above for GC analysis. The data in the GC/MS experiment was acquired with the ionization voltage set at 70 eV and emission current of 40 mA. The acquisitions scan mass range was 45-350 amu with sampling rate of 1.0 scan/s as reported previously [5].

The individual constituents present in the essential oil were identified by comparison of the Mass spectral data with MS fragmentation patterns of known compounds in literature [18] as described recently [3-9]. The retention indices (RI Exp.) of each compound was also compared with reference to a homologous series of n-alkanes (C_6-C_{40}), run under identical experimental GC conditions as with samples. Also, co-injection with known compounds under the same GC conditions was also used to identify some compounds [3-6].

Study of the antimicrobial activity of the essential oil

The source of strains of microbes used for the study was Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam, as mentioned previously [5,6]. The testing media used for the bacteria and fungi were Mueller-Hinton Agar (MHA) and Sabouraud Agar (SA), respectively, as mentioned previously [5,6]. The bacteria and yeast used for the test were the same as described previously [5,6]. They were *Enterococcus faecalis* ATCC 299212,

Staphylococcus aureus ATCC 25923, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076 and *Candida albicans* ATCC 10231. The procedures employed were described previously in our various reports [3-9]. The concentrations used for experiment were chosen based on previous reports of similar studies where studied essential oils exhibited activity within the specific concentration range [5,6]. The activity levels namely- the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay as described previously [5-9].

The bacteria and fungi were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The stock solutions of each of the essential oils were prepared in dimethylsulfoxide. The dilution steps were accomplished 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 placed in the micro-test tubes. The solutions were allowed to incubate at 37 °C for 24 h. The negative control (containing no antimicrobial agent) was the sterile distilled water and medium. The standard drugs used as positive controls include streptomycin (antibacterial), nystatin and cycloheximide (anti-candidal). Thereafter, the MIC values were recorded to be the well with the lowest concentration of essential oil completely inhibiting the growth of microorganisms as reported recently [5-9]. 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 Rawdata 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 antimicrobial agent, test agent corresponds to a known concentration of 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

The differences between the mean values obtained for experimental groups were calculated as a mean of standard deviation (SD) of three independent measurements using Microsoft Excel program 2003 as described previously [5,6].

Results and discussion

The yield of the essential oil from the leaves of *P. macrocarpa* was 0.28% (w/w; 1.27 g). The essential oil sample was light-yellow coloured. The nature and percentages of each compound identified in the essential oil could be seen in Table 1. The 41 components of the essential oil from the leaves of *P. macrocarpa* comprised of monoterpene hydrocarbons (3.3%), oxygenated monoterpenes (2.8%), sesquiterpene

Table 1. Volatile constituents of the leaves of *P. macrocarpa*

S. No.	RT (min)	Compounds [a]	RI [b]	RI [c]	Percentage composition [d]
1	10.51	α-Pinene	939	932	0.5 ± 0.00
2	11.02	Camphene	955	946	0.3 ± 0.00
3	11.90	β-Pinene	984	982	0.4 ± 0.00
4	13.37	o-Cymene	1030	1020	1.7 ± 0.01
5	13.53	Limonene	1034	1028	0.4 ± 0.00
6	19.66	Octyl acetate	1210	1208	1.4 ± 0.00
7	21.85	Geranial	1270	1272	0.4 ± 0.00
8	24.35	δ-Elemene	1348	1347	1.1 ± 0.00

table 1. (continued).

S. No.	RT (min)	Compounds[a]	RI[b]	RI[c]	Percentage composition [d]
9	24.75	α-Cubebene	1360	1363	1.2 ± 0.01
10	25.53	Geranyl acetate	1384	1383	2.4 ± 0.00
11	25.71	α-Copaene	1389	1387	3.7 ± 0.00
12	26.08	β-Bourbonene	1401	1401	0.3 ± 0.00
13	26.14	β-Cubebene	1402	1403	5.4 ± 0.01
14	26.87	Cyperene	1426	1426	0.3 ± 0.00
15	27.23	β-Caryophyllene	1437	1437	16.6 ± 0.01
16	27.47	β-Gurjunene	1445	1443	0.5 ± 0.01
17	27.51	*trans*-α-Bergamotene	1446	1444	0.5 ± 0.00
18	27.84	Aromadendrene	1457	1454	2.3 ± 0.00
19	28.31	α-Humulene	1472	1472	3.0 ± 0.01
20	28.55	9-*epi*-(*E*)-Caryophyllene	1479	1477	0.4 ± 0.00
21	28.89	γ-Muurolene	1491	1489	1.2 ± 0.01
22	29.01	α-Amorphene	1494	1491	0.3 ± 0.00
23	29.14	Germacrene D	1498	1498	1.0 ± 0.01
24	29.34	β-Selinene	1505	1503	0.5 ± 0.00
25	29.50	γ-Amorphene	1511	1508	0.3 ± 0.00
26	29.56	Viridiflorene	1512	1510	1.7 ± 0.01
27	29.61	Bicyclogermacrene	1514	1512	5.0 ± 0.00
28	30.09	γ-Cadinene	1530	1528	0.8 ± 0.00
29	30.29	δ-Cadinene	1537	1537	1.0 ± 0.00
30	30.33	*trans*-Calamenene	1538	1535	0.7 ± 0.00
31	30.78	α-Cadinene	1553	1555	0.2 ± 0.00
32	31.25	(*E*)-Nerolidol	1569	1571	3.8 ± 0.00
33	31.84	Palustrol	1589	1591	1.6 ± 0.00
34	32.09	Spathulenol	1597	1599	12.8 ± 0.02
35	32.32	Caryophyllene oxide	1605	1608	4.3 ± 0.00
36	32.56	Cubeban-11-ol	1614	1612	0.5 ± 0.00
37	33.77	Eudesma-4(15),7-dien-1β-ol	1656	1652	2.3 ± 0.00
38	34.22	α-Cadinol	1674	1670	0.5 ± 0.00
39	34.33	*cis*-Calamenen-10-ol	1676	1672	0.5 ± 0.00
40	34.56	α-Eudesmol	1676	1678	4.7 ± 0.01
41	34.69	*trans*-Calamenen-10-ol	1685	1687	0.9 ± 0.00
	Total				85.4
	Monoterpene hydrocarbons (Sr. No. 1-5)				3.3
	Oxygenated monoterpenes (Sr. No. 7, 10)				2.8
	Sesquiterpene hydrocarbons (Sr. No. 8, 9, 11-31)				31.9
	Oxygenated sesquiterpenes (Sr. No. 32-41)				46.0
	Others (Sr. No. 6)				1.4

[a] Elution order on HP-5MS column; [b] Experimental retention indices; [c] Literature retention indices on HP-5MS column as seen in NIST [18]; [d] means of three replicate values; Sr. No, serial number; RT, retention times on HP-5MS column

hydrocarbons (31.9%) and oxygen-containing sesquiterpenes (46.0%). The amount of non-terpene present in the essential oil was 1.4%. The major compounds of the essential oil of *P. macrocarpa* comprised of β-caryophyllene (16.6%) and spathulenol (12.6%), β-cubebene (5.4%), bicyclogermacrene (5.0%), α-eudesmol (4.7%) and caryophyllene oxide (4.3%). The minor constituents of the essential oil includes (*E*)-nerolidol (3.8%), α-copaene (3.7%), α-humulene (3.0%), geranyl acetate (2.4%), aromadendrene (2.3%), and eudesma-4(15),7-dien-1β-ol (2.3%). Monoterpene compounds comprising of monoterpene hydrocarbons (3.3%) and oxygenated monoterpenes (2.8%) were identified in lower quantities in the essential oil. This is the first report on the essential oil from the leaves of *P. macrocarpa* dominated by sesquiterpene compounds.

Since the authors are not aware of any previously published data on the volatile and non-volatile compounds of *P. macrocarpa*, this is the first result of its kind. However, the compositional pattern of essential oil of *P. macrocarpa* shows considerable differentiation and similarity with some other studied *Phoebe* species. Firstly, sesquiterpene compounds as seen in *P. macrocarpa* were also the dominant class of compounds in the leaf oil of *P. paniculata* and *P. tovayana* from Vietnam [1]. Secondly, the identities of these sesquiterpenes differ from one species to another. For example, β-caryophyllene and germacrene D occurred in *P. paniculata,* while bicyclogermacrene and germacrene D were present in *P. tovayana* [1]. Although spathulenol was a major compound of *P. macrocarpa* (this study) and *P. angustifolia* from Vietnam [2], *n*-hexacadecanoic acid present in *P. angustifolia* [2] was not identified in *P. macrocarpa*. However, α-pinene and β-pinene, the abundant monoterpene compounds in the leaf essential oil of *P. angustifolia* from Vietnam [3], occurred in much lower quantities in *P. macrocarpa* and other reported *Phoebe* essential oils [1,2]. It should be noted that sesquiterpene was the dominant class of compounds in the essential oils of *Phoebe* plants from other parts of the world. These include the leaf essential oil of *P. formosana* from Taiwan [11], the leaf essential oil of *P. bournei* from China [12], the bark and leaf essential oils of *P. zhennan* from China [13], the leaf essential oil of *P. porphyria* from north-western Argentina[14], as well as the leaf essential oils of *P. hui* [15] and *P. sheareri* from Korea [16].

Data on antimicrobial activities of the essential oil

The results are presented in Table 2. The leaf essential oil of *P. macrocarpa* displayed moderate antimicrobial activity against three of the seven tested microorganisms. The results of the antimicrobial activities of the essential oil indicate that the essential oil showed activity against *E. faecalis* ATCC 299212 and *B. cereus* ATCC 14579 with MIC value of 128 µg/mL. The observed activity was twice the value recorded against *S. aureus* ATCC 25923, with MIC of 256 µg/mL. The essential oil did not inhibit the growth of Gram-negative organisms and also showed no anti-candidal properties. The results in this study are in agreement and comparable with data reported on the antimicrobial activity

Table 2. Results of the antimicrobial activity of the essential oil of *P. macrocarpa*

Microorganisms	MIC (µg/mL) [a]	IC_{50}(µg/mL) [a]
Enterococcus faecalis ATCC 299212	128 ± 0.00	37.9 ± 0.01
Staphylococcus aureus ATCC 25923	256 ± 0.02	99.7 ± 0.01
Bacillus cereus ATCC 14579	128 ± 0.01	45.7 ± 0.01
Escherichia coli ATCC 25922	-	-
Pseudomonas aeruginosa ATCC 27853	-	-
Salmonella enterica ATCC 13076	-	-
Candida albicans ATCC 10231	-	-

[a] means of three replicate values; -No activity

of essential oils of other *Phoebe* plants analyzed from other parts of the world. The essential oil of *P. macrocarpa* exhibited moderate growth suppression against Gram-positive bacteria like *P. formosana* from Taiwan [11], but with lower MIC values than the latter. In addition, *P. macrocarpa* seems to be more active toward the microorganisms than previously reported *P. angustifolia* from Vietnam.

The chemical constituents and antimicrobial activity of *P. macrocarpa* essential oils are being reported for the first time. It is believed that the constituents present in the studied essential oils might have influenced the observed antimicrobial activity of *P. macrocarpa*. A known theory fact that the chemical compounds identified in the essential oils were reported previously to possess antimicrobial activity. It was reported β-caryophyllene demonstrated selective antibacterial activity against *S. aureus* [19], *P. aeruginosa* [20] and *B. cereus* [21], as well as pronounced anti-fungal activity [19]. Spathulenol also showed pronounced antimicrobial action against *S. aureus* [22].

Conclusions

The present study determined the volatile composition of the leaf essential oil of *P. macrocarpa* to be dominated by β-caryophyllene (16.6%) and spathulenol (12.6%). In addition, the essential oil from the leaves of *P. macrocarpa* displayed moderate antimicrobial activity against *E. faecalis* ATCC 299212, *S. aureus* ATCC 25923 and *B. cereus* ATCC 14579, with MIC values of 128, 256 and 128 µg/mL, respectively. In effect, *P. macrocarpa* can be exploited further for volatile contents and its antimicrobial activity.

Competing interests

The authors declare that no competing interest exists.

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