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Article

Chemical Compositions of Essential Oils and Antimicrobial Activity of *Elettariopsis triloba* from Vietnam

Le Thi Huong ^{1*}, Le Duy Linh ¹, Isiaka Ajani Ogunwande ^{2*}

¹ School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghe An Province 4300, Vietnam

² Foresight Institute of Research and Translation, Eleyele, Ibadan, Nigeria

* Corresponding Author: lehuong223@mail.com; isiakaogunwande@gmail.com (Le Thi Huong; Isiaka Ajani Ogunwande)

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Abstract: The herb, *Elettariopsis triloba* (Gagnep.) Loes. (syn. *Amomum trilobum* Gagnep. or *Elettariopsis trilobum* (Gagnep.)), has been used ethnomedicinally for the treatment of rabies, inflammation and amelioration of microbial infections. We report herein the chemical constituents and antimicrobial activity of the rhizome essential oil of *E. triloba* from north-central Vietnam. The technique of gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) was used to analyze the oil sample while the microdilution assay was employed to determine the antimicrobial efficacy. Monoterpene compounds including camphene (23.2%), fenchyl acetate (12.7%), bornyl acetate (10.6%), α -pinene (5.7%) and limonene (5.1%) were the main constituents of the rhizome essential oil. The essential oil displayed antimicrobial activity only towards Gram-positive bacteria of *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579 with minimum inhibitory concentration (MIC) values of 16.0 μ g/mL, 256 μ g/mL and 32.0 μ g/mL, respectively. The oil also inhibited the growth of the yeast, *Candida albicans* ATCC 10231, with a MIC value of 16.0 μ g/mL. The chemical constituents and antimicrobial activity of essential oil from *E. triloba* were being reported for the first time and validation of the ethnomedicinal usage of the plant.

Keywords: *Elettariopsis triloba*, monoterpenes, Gram-positive bacteria.

Introduction

The genus *Elettariopsis* was in the family Zingiberaceae in the major group Angiosperms (Flowering plants), which has now been subsumed into the genus *Amomum* ¹. The various species are native to Southeast Asia, southern China and New Guinea ². *Elettariopsis triloba* (Gagnep.) Loes. (syn. *Amomum trilobum* Gagnep. or *E. trilobum* (Gagnep.)) is a small herb with slender and wide-creeping rhizomes bearing leaf shoots at 8 -15 cm intervals ³. The roots are not tuberous. The leaf shoots bear 1-5 leaves. The leaf-sheaths

are closely clasping to form a distinct pseudo-stem up to 35 cm tall. The glabrous leaves are lamina lanceolate in the shape of dimensions 30 x 5-8 cm. In *E. triloba* the inflorescence is a compact head borne at the end of a short scape ³. The bracts are broadly pointed, up to 3 x 1.8 cm, each subtending 1 or 2 flowers ³. It is known in Vietnamese as Tieu Dau ba thùy. The plant can be found in Lang Sôn (Huu Ling), Thái Nguyên (Đông Hy), Nghe An (Pù Hoat Nature Reserve), Hà Tĩnh (Vĩ Quang National Park: Doc De), Thừa Thiên-Hue (Xuân Loc), Đà Nang Provinces, HỒ

Chi Minh City and Malaysia². In ethnomedicine, *E. triloba* was previously used to cure rabies, inflammatory-related ailments and amelioration of microbial infections². The authors are not aware of any information about the chemical composition and biological activities of both the volatile and non-volatile extracts of *E. triloba*. However, recent information revealed that the compositions and biological activities of essential oils from some other *Elettariopsis* species have been reported. The chemical constituents of essential oils of *E. wandokthong*⁴, *E. elan*⁵, *E. smithiae*⁶, *E. rugosa*⁶ and *E. curtissii*⁷ were dominated by monoterpene compounds whose identities varied from one species to another. These monoterpenes include camphene and fenchyl acetate^{4,6}, geraniol⁵, geranial and neral⁶, β -pinene⁷ and β -phellandrene^{6,7}. In addition, previous studies also reported an abundance of aliphatic aldehydes, hydrocarbons and carboxylic acids in the essential oils of *E. curtissii*^{8,9} and *E. slahmong*^{10,11}. In addition, essential oil of *E. curtissii* was reported to inhibit the growth of Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli*^{8,9}.

Because of the potentials of *Elettariopsis* species as sources of biologically active products, the present paper reports for the first time the chemical constituents and antimicrobial activity of essential oils hydrodistilled from the rhizomes of *E. triloba*. This is in continuation of our ongoing extensive research aimed at the characterization of the chemical constituents and biological activities of Vietnamese plants, the results of which were published recently¹²⁻¹⁸.

Materials and methods

Rhizomes of E. triloba and hydrodistillation of essential oil

Mature rhizomes of *E. triloba* were collected from wild-growing plants in north-central Vietnam. The place of the collection was Vu Quang National Park (GPS 18°17'15"N; 105°21'39"E) at an elevation of 140 m. The plant sample was identified and authenticated by Dr. L.T. Huong, School of Natural Science Education, Vinh University, Vietnam. A voucher specimen (LTH 744) was deposited in the plant specimen

room, Faculty of Agriculture, Forestry and Fishery, Nghe An, College of Economics, Vietnam. The amount of sample collected was 2.24 kg. the rhizomes were separated from debris and other unwanted materials by handpicking.

Fresh rhizomes were chopped to obtain 2.0 kg weight of sample which was subjected to hydrodistillation using a Clevenger-type apparatus as described in previous studies¹²⁻¹⁸.

Two kilograms (kg) of the rhizome of *E. triloba* was used for the hydrodistillation experiment. The sample was separately introduced into a 5 L flask after which distilled water was added until it covered the sample completely. The essential oil was obtained by hydrodistillation which was carried out in a Clevenger-type distillation unit designed according to an established procedure as described in previous studies¹²⁻¹⁸. The distillation time was 3 h and conducted at normal pressure. The volatile oil distilled over the water was collected separately by running through the tap in the receiver arm of the apparatus into a clean and previously weighed sample bottle. The oil was kept under refrigeration (4°C) until the moment of analysis. The experiment was conducted in triplicate. The essential oil yield (%) was calculated by mass (g) of the essential oil divided by the mass (g) of the dried rhizomes of the plant.

Analysis of the rhizome essential oil of E. triloba

The analysis of the chemical constituents of the rhizome essential oil of *E. triloba* was achieved using GC and GC/MS. Gas chromatographic (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (Agilent Technologies, Santa Clara, California, USA) of dimension 30 m x 0.25 mm with a film thickness of 0.25 μ m. The analytical conditions employed in the GC analysis were: carrier gas H₂ with a flow rate of 1 mL/min, while both the injector temperature (PTV: programmable temperature vaporization) and detector temperature were maintained at 250°C and 260°C, respectively. The column temperature from 60°C, with a 2 min hold, to 220°C (10 min hold) at a rate of 4°C/min. The essential oil (1.0 mL; 10 % *n*-

hexane solution) was injected using a split mode with a split ratio of 10:1, at inlet pressure was 6.1 kPa. Quantification was done using the calibration curves generated from the analyses of representative standard compounds from each class.

An Agilent Technologies (Santa Clara, California, USA) HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5MS column (dimension 30 m x 0.25 mm; film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis. The GC conditions were the same as those reported above for the GC/MS analysis. However, Helium was used as the carrier gas. The MS was operated at an ionization voltage of 70 eV with an emission current of 40 mA, with the acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

The identification of constituents of essential oils from the GC/MS spectra of *E. triloba* was performed based on a comparison of retention indices (RI Exp.) regarding a homologous series of *n*-alkanes ($\text{C}_4\text{-C}_{40}$), 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 literature¹⁹⁻²¹ as described recently¹²⁻¹⁸.

Antimicrobial test

The antimicrobial activity of the essential oils was evaluated using three strains of Gram-positive test bacteria which were *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, three strains of Gram-negative test bacteria namely, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* ATCC 13076, and one strain of yeast of *Candida albicans* ATCC 10231.

The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were determined by the microdilution broth susceptibility assay as described in our previous studies¹²⁻¹⁷. Stock solutions of the oil were prepared in dimethylsulfoxide. Dilution series were prepared from 16,384 to 2 $\mu\text{g/mL}$ (2^{14} , 2^{13} , 2^{12} , 2^{11} , 2^{10} , 2^9 , 2^7 , 2^5 , 2^3 and 2^1 $\mu\text{g/mL}$) 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 negative control. Streptomycin was used as the antibacterial standard, nystatin and cycloheximide were used as antifungal standards. After incubation at 37°C for 24 hours, 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:

$$\% \text{ inhibition} = \frac{\text{OD}_{\text{control}(-)} - \text{OD}_{\text{test agent}}}{\text{OD}_{\text{control}(-)} - \text{OD}_{\text{control}(+)}} \times 100\%$$

$$\text{IC}_{50} = \text{High}_{\text{conc}} - \frac{(\text{High}_{\text{inh}\%} - 50\%) \times (\text{High}_{\text{conc}} - \text{Low}_{\text{conc}})}{(\text{High}_{\text{inh}\%} - \text{Low}_{\text{inh}\%})}$$

Where OD is the optical density, control(-) are the cells with medium but without an antimicrobial agent, test agent corresponds to a known concentration of the antimicrobial agent, control(+) is the culture medium without cells, $\text{High}_{\text{conc}}/\text{Low}_{\text{conc}}$ is the concentration of test agent at high concentration/low concentration, and $\text{High}_{\text{inh}\%}/\text{Low}_{\text{inh}\%}$ is the % inhibition at high concentration/% inhibition at low concentration).

Statistic analysis

Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD, \pm) of three and four independent measurements, respectively for the chemical constituents and larvicidal test, using Microsoft excel program 2003.

Results and discussion

The percentage yield and colour of the essential oil

The average yield of the essential oil was 0.35 % (w/w). The yield was obtained with SD of ± 0.01 for the essential oil. The essential oil was yellow-colored.

Nature and percentages of chemical compounds identified in the essential oil

Using a combination of GC-FID and GC/MS with HP-5MD column, fifty-eight compounds representing 97.4 % of the oil contents were identified in *E. triloba* (Table 1). The main classes of compounds present in the oil were monoterpene hydrocarbons (52.3 %), oxygen-containing monoterpenes (28.4 %), sesquiterpene hydrocarbons

(8.8 %) and oxygen-containing sesquiterpenes (7.9 %), as seen in Table 1. The constituents occurring in higher amounts in the rhizome essential oil of *E. triloba* were camphene (23.2 %), fenchyl acetate (12.7 %), bornyl acetate (10.6 %), sabinene (6.1 %), α -pinene (5.7 %) and limonene (5.1 %). To the best of the authors' knowledge, no studies on the oils of *E. triloba* have ever been reported and this report represents the first of its kind in this regard.

Monoterpene hydrocarbons were the main classes of compounds identified in the essential oil of the rhizome of *E. triloba*, in agreement with the composition of previously analysed essential oils of *E. wandokthong* ⁴, *E. elan* ⁵, *E. smithiae* ⁶, *E. rugosa* ⁶ and *E. curtissii* ⁷. However, the identities of these compounds differ from each

Table 1. Chemical composition of the rhizome essential oil of *E. triloba* from Vu Quang National Park, Vietnam

No.	RT (min)	Compounds ^a	RI (Exp.)	RI (Lit.)	Concentration ^b
1	9.81	Tricyclene	928	922	0.4
2	9.87	α -Thujene	930	926	0.4
3	10.14	α -Pinene ^c	939	932	5.7
4	10.65	Camphene ^c	956	954	23.2
5	11.34	Sabinene ^c	979	972	6.1
6	11.51	β -Pinene	984	980	1.3
7	11.75	Myrcene	992	992	0.9
8	12.34	α -Phellandrene	1010	1008	0.3
9	12.54	δ -3-Carene	1016	1014	1.0
10	12.74	α -Terpinene	1022	1020	0.7
11	13.02	o-Cymene	1030	1026	1.1
12	13.17	Limonene	1034	1030	5.1
13	13.22	β -Phellandrene	1036	1034	3.2
14	13.28	1,8-Cineole	1038	1036	0.8
15	13.67	(<i>E</i>)- β -Ocimene	1049	1049	0.2
16	14.16	γ -Terpinene	1064	1064	2.1
17	15.22	Terpinolene	1094	1092	0.6
18	15.52	Linalool	1103	1100	0.6
19	18.13	Borneol	1177	1175	0.4
20	18.48	Terpinene-4-ol	1187	1184	0.8
21	18.93	α -Terpineol	1200	1198	0.1
22	19.89	Fenchyl acetate	1228	1220	12.7
23	20.63	Carvacrol methyl ether ^c	1250	1250	0.2
24	22.18	Bornyl acetate	1294	1291	10.6
25	22.27	Isobornyl acetate	1297	1297	0.5
26	22.67	Terpinen-4-ol acetate	1307	1304	0.2

table 1. (continued).

No.	RT (min)	Compounds ^a	RI (Exp.)	RI (Lit.)	Concentration ^b
27	23.98	δ -Elemene	1348	1342	0.3
28	24.25	α -Terpinyl acetate	1357	1353	1.3
29	25.02	<i>endo</i> -Isocamphanyl acetate ^c	1380	1382	0.2
30	25.79	<i>cis</i> - β -Elemene	1404	1401	0.2
31	26.84	β -Caryophyllene	1427	1420	0.5
32	27.11	γ -Elemene	1445	1434	0.2
33	27.93	α -Humulene	1452	1452	0.4
34	28.16	9- <i>epi</i> -(<i>E</i>)-Caryophyllene ^c	1479	1474	0.3
35	28.53	γ -Muurolene	1491	1488	0.2
36	28.64	α -Amorphene	1494	1490	0.2
37	28.77	Germacrene D	1498	1498	2.9
38	29.24	Bicyclogermacrene	1514	1512	1.7
39	29.36	β -Bisabolene	1518	1515	0.1
40	29.47	δ -Amorphene	1522	1522	0.1
41	29.73	γ -Cadinene	1530	1530	0.6
42	29.93	δ -Cadinene	1537	1535	0.4
43	30.76	Elemol	1565	1562	0.8
44	30.93	(<i>E</i>)-Nerolidol	1571	1574	0.7
45	31.14	Germacrene B	1578	1578	0.7
46	31.76	Spathulenol	1598	1596	0.6
47	32.23	Guaiol	1615	1612	0.5
48	32.60	Cedrol	1628	1626	0.3
49	33.17	1- <i>epi</i> -Cubenol ^c	1648	1644	0.5
50	33.29	γ -Eudesmol ^c	1652	1652	0.5
51	33.55	<i>epi</i> - α -Muurolol ^c	1661	1662	0.3
52	33.91	β -Eudesmol	1664	1666	1.0
53	33.97	α -Eudesmol	1676	1672	0.8
54	34.13	α -Turmerone ^c	1682	1680	0.4
55	34.28	Bulnesol	1687	1688	0.3
56	34.63	γ -Bicyclopofarnesal ^c	1699	1700	0.3
57	35.07	Curlone ^c	1716	1714	0.2
58	38.08	γ -Bicyclohomofarnesal ^c	1809	1809	0.7
		Total			97.4
		Monoterpene hydrocarbons (Sr. No. 1-13, 15-17)			52.3
		Oxygen-containing monoterpenes (Sr. No. 14, 18-26, 28, 29)			28.4
		Sesquiterpene hydrocarbons (Sr. No, 27, 30-42, 45)			8.8
		Oxygen-containing sesquiterpenes (Sr. No. 43, 44, 46-58)			7.9

RT = Retention Time (min) on HP-5MS column (see Fig. 1)

^a Elution order on the HP-5MS column

^b Standard deviation was insignificant and excluded from the Table to avoid congestion

^c Further identification by co-injection with known compounds

RI (Exp.) = Retention Index calculated with respect to a homologous series of *n*-alkanes on a HP-5MS column

RI (Cal.) = Retention Index from NIST databases

other. The major chemical compounds of the rhizome essential oil of *E. triloba* namely camphene and fenchyl acetate were found to be present in higher quantities in the rhizome of *E. wandokthong*⁴, roots and rhizomes of *E. elan*⁵ as well as the roots and rhizomes of *E. smithiae*⁶ and *E. rugosa*⁶. Interestingly, β -pinene and β -phellandrene which were the most abundant compounds in the rhizomes essential oil of *E. rugosa*⁶ as well as the leaf, root and rhizomes essential oils of *E. curtissii*⁷ were identified in a lower amount in the rhizome essential oil of *E. triloba*. Moreover, geraniol found in the leaf oil of *E. elan*⁵, as well as geranial and neral, the most abundant compounds of *E. smithiae*⁶ leaf essential oil⁶, were conspicuously absent in the rhizome essential oil of *E. triloba*. In addition, several aliphatic aldehydes, hydrocarbons and carboxylic acids are characteristics of the volatile contents of the leaves and rhizomes of *E. curtissii*^{8,9} and rhizomes of *E. slahmong*^{10,11}, were also absent in *E. triloba*. The compositional pattern of essential oils from *Elletatropis* plants studied so far could be classified into three groups;

i. Oils containing monoterpene hydrocarbons represented by *E. wandokthong* (rhizomes), *E. elan* (roots and rhizomes), *E. smithiae* (roots and rhizomes), *E. rugosa* (roots and rhizomes), *E. curtissii* (leaf, root and rhizomes) and rhizomes of *E. triloba* (present study);

ii. Oils with abundance of oxygenated monoterpenes seen in leaves of *E. elan* and *E. smithiae*;

iii. Oils consisting of aliphatic aldehydes, hydro-

carbons and carboxylic acids found in the leaves and rhizomes of *E. curtissii* and rhizomes of *E. slahmong*.

Result of the antimicrobial test on the essential oil

The essential oil from the rhizome of *E. triloba* was screened against a panel of microorganisms. The essential oil showed good antibacterial activity against the Gram-positive bacteria *E. faecalis* ATCC 299212, *B. cereus* ATCC 14579 and *S. aureus* ATCC 25923 with MIC values of 16.0, 32.0 and 256.0 $\mu\text{g/mL}$, respectively, and anti-candidal activity against *C. albicans* ATCC 10231 with MIC value of 16.0 $\mu\text{g/mL}$ (Table 2).

However, the rhizome essential oil of *E. triloba* was inactive against Gram-negative bacteria *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. enterica* ATCC 13076. The inability of the essential oil to inhibit the growth of Gram-negative bacteria is noteworthy. Several previous studies have shown that Gram-positive bacteria were more susceptible to essential oils than Gram-negative organisms¹²⁻¹⁷. This observation may be attributed to cell wall lipopolysaccharides in the Gram-negative organisms that inhibit the lipophilic essential oil components from diffusing into the cells^{22, 23}.

In comparison, the rhizome essential oil of *E. triloba* was effective against strains *S. aureus* and *B. subtilis* in agreement with the reported activity of *E. curtissii*⁹. On the contrary, the oils of *E. curtissii*^{8,9} inhibited the growth of Gram-

Table 2. Antimicrobial activity of rhizome essential oil of *E. triloba*

Sample	Gram (+)			Gram (-)		Yeast	
	<i>E. faecalis</i> ATCC 299212	<i>S. aureus</i> ATCC 25923	<i>B. cereus</i> ATCC 14579	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. enterica</i> ATCC 13076	<i>C. albicans</i> ATCC 10231
	MIC ($\mu\text{g/mL}$)						
<i>E. triloba</i>	16.0	256.0	32.0	-	-	-	16.0
Strep	16.0	256.0	32.0	-	-	-	NT
Nis	NT	NT	NT	-	-	-	8.0
Cyc	NT	NT	NT	-	-	-	32.0
	IC₅₀ ($\mu\text{g/mL}$)						
<i>E. triloba</i>	5.78	68.98	9.35	-	-	-	6.78

- No activity;

NT: Not tested

negative bacteria such as *P. aeruginosa* and *E. coli* when compared with *E. triloba*. The major components of the essential oil namely camphene, fenchyl acetate, bornyl acetate, α -pinene and limonene have shown moderate antimicrobial activities against *B. cereus*, *S. aureus*, *E. faecalis*, and *C. albicans*²⁴, and likely account for the observed antimicrobial activity of *E. triloba* rhizome essential oil. It is interesting to note that the rhizome essential oil of *Zingiber nitens* whose major constituents were camphene and bornyl acetate also displayed antimicrobial activity¹⁵. The other major constituents of *E. triloba* rhizome essential oil either alone or in combination with others have provided synergistic antimicrobial effects^{12-17, 25-27}. This was evident from data obtained from essential oils of some other medicinal plants analysed from other parts of the world^{28,29}.

Conclusions

In conclusion, essential oil from the rhizome of *E. triloba* from Vietnam was found to contain high contents of camphene, fenchyl acetate, bornyl acetate, α -pinene and limonene. The essential oil also showed antimicrobial activity against Gram-positive bacteria *E. faecalis* ATCC 299212, *B. cereus* ATCC 14579 and *S. aureus* ATCC 25923 with MIC values of 16.0, 32.0 and 256.0 $\mu\text{g/mL}$, respectively, and anticandidal activity against *C. albicans* ATCC 10231 with MIC value of 16.0 $\mu\text{g/mL}$. From the foregoing, the essential oil could be considered as a potential alternative source for the development of a formulation for controlling diseases.

Competing interests

The authors declare that they have no competing interests.

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