Malaysian Journal of Science **19**: 77-81 (2000)

Studies on essential oil and antibacterial activities from *Elettariopsis smithiae* aff. Kam.

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ABSTRACT The essential oil from *Elettariopsis smithiae* was prepared from fresh leaves, rhizomes and roots and the chemical components were determined by GC-MS. The main component of the root and rhizome was cis-β-ocimene. More than 20 compounds were detected from the leaves and the major components were found to be farnesal (28.62%) and cis, trans-farnesal (20.63%).

ABSTRAK Minyak pati daripada *Elettariopsis smithiae* diperolehi daripada daun, rizom dan akar segar dan komponen-komponen kimianya telah ditentukan dengan menggunakan GC-MS. Komponen terbanyak yang didapati pada akar dan rizom adalah cis-β-ocimene. Lebih daripada 20 sebatian dikesan dalam daun manakala komponen terbanyak adalah farnesala (28.62%) dan cis, trans-farnesala (20.63%).

(*Elettariopsis smithiae,* farnesal, cis-β-ocimene, trans-farnesal)

INTRODUCTION

Elettariopsis smithiae aff. Kam. (Zingiberaceae) is a wild ginger species which is sometimes known by the village folks as *pijat-pijat*. It is a small herb up to 1 m. tall with creeping rhizomes and radical inflorescence [1]. Most plants of this species emit a strong bed-bug odour from the crushed leaves and rhizomes, however there are slight variations in their fragrance among wild populations in lowland forests. This species was first described from Malaysia but its distribution may extend to Southern Thailand. The volatile components of these plants showed a significant antimicrobial activity and chemical analysis of the oil components was conducted.

EXPERIMENTAL

Fresh specimens of *E. smithiae* were obtained from the Rimba Ilmu Botanic Garden (Accession no. RI-3480) for analysis. A substantial amount of the roots, rhizomes and leaves were washed, cut into small pieces and steam distilled. The distillate was separated and the oil was injected into GC-MS and checked with thin layer chromatography by comparison with standards obtained from TCI (Tokyo Kasei Organic Chemicals, Japan). The GC-MS used is Shimadzu QP-5000 GC-MS (Japan). The parameters of the GC-MS for the roots and rhizomes were as follows: Column: Altech Econo-cap (SE30) 25m. length, 0.22mm. I.D. Column initial temperature is 60°C, programme rate is 5°C/min to 250°C. Carrier gas used was Helium with flow rate of 1ml/min. Interface temperature: 280°C, Injector temperature: 280°C, split mode with split ratio of 50. For the leaves, the flow rate was 0.7 ml/min, while other parameters remain the same.

The antimicrobial activities were done on 10g of Mueller-Hinton agar dissolved in 400ml of distilled water and autoclaved at 121°C, 15psi for 15 minutes. The organisms tested were *Staphylococcus aureus* and *Escherichia coli* with gentamicin as the control antibiotic.

RESULTS AND DISCUSSION

The GC-MS spectrum of the analysis were as in Figures 1, 2 and 3. The components of the roots, leaves and rhizomes were shown in Tables 1, 2 and 3. The major component of the roots and rhizomes was cis-β-ocimene (29.53% and 26.97% respectively). The roots also contain a high amount of bicyclo heptan-2-ol,1,3,3-trimethyl-acetate (13.50%). Whereas the rhizomes contain a

high quantity of cis-2-methylene-3-[1-methylethenyl]-cyclohexanol acetate (23.60%). A total of 28 volatile components were detected from the leaves and the main components were found to be farnesal (28.62%) and cis, trans-farnesal (20.63%).

A high quantity of limonene (8.35%) were detected in the rhizomes, and this might be the contributing factor in the highly aromatic feature of the rhizomes. The other components found from the roots were α-phellandrene, β-myrcene, 3-carene and camphene. The rhizomes also contain camphene, ocimenol and isocaryophyllene. Limonene, α-phellandrene, β-myrcene, 3-carene and camphene have also been detected in the leaves, roots and rhizomes of *Elettariopsis triloba* [2]. Camphene have been reported in other zingiberaceous species such as *Zingiber officinale*, *Alpinia galanga, Alpinia zerumbet, Curcuma mangga*, *Curcuma xanthorhiza* and *Curcuma aeruginosa* [2,3,4,5]. α-Pinene which is only present in the leaves of *Elettariopsis smithiae* was detected in leaves, roots and rhizomes of *Elettariopsis triloba* [2]. α-pinene and β-pinene are relatively common in some Zingiberaceae species [3,4,5,6,7].

There were significant differences between the distribution of the components in the leaves, roots and rhizomes. The GC-MS analysis of the volatile components revealed the presence of high proportion of farnesal and cis, trans-farnesal in the leaves while none of these compounds were detected in the roots and rhizomes. Limonene was detected in the rhizomes and leaves, but not in the roots while α-phellandrene and γ-terpinene were found only in the roots and rhizomes. The high percentage of farnesal or cis, trans- farnesal in the leaves might be the contributing factor for its antimicrobial activity.

Table 1. Volatile components of leaves of *Elettariopsis smithiae* aff. elucidated by Gas Chromatography-Mass Spectrometry.

Pk. No.	Rt.	Mol. Wt	Mol Formula	Total %	Compound Name
1.	6.73	136	$C_{10}H_{16}$	1.10	trans-β-ocimene
2.	7.18	136	$C_{10}H_{16}$	0.26	camphene
3.	8.02	196	$C_{12}H_{20}O_2$	0.35	dihydrocarveol acetate
4.	8.18	136	$C_{10}H_{16}$	6.14	α-pinene
5.	10.45	136	$C_{10}H_{16}$	1.09	limonene
6.	23.37	152	$C_{10}H_{16}O$	0.47	cis, trans-citral[isomer]
7.	35.28	194	$C_{13}H_{22}O$	7.63	nerylacetone
8.	39.53	154	$C_{10}H_{18}O$	0.36	nerol
9.	41.23	190	$C_{12}H_{20}O_2$	0.65	6-dimethyl-bicyclo 3.1.1 heptane-2-methanol-6-acetate
10.	41.87	204	$C_{15}H_{24}$	0.30	α-farnesene
11.	42.48	222	$C_{15}H_{26}O$	1.66	[+]-nerolidol
12.	42.98	168	$C_{11}H_{20}O$	0.35	10-undecyn-1-ol
13.	43.27	170	$C_{10}H_{18}O_2$	0.50	Trans, trans-2,6-dimethyl-2,6-octadiene-1,8-diol.
14.	43.88	168	$C_{16}H_{16}O_2$	0.54	α-limonene diepoxide
15.	45.05	204	$C_{15}H_{24}$	1.25	[Z]-β-farnesene
16.	45.80	152	$C_{10}H_{16}O$	1.58	β-Citral
17.	46.85	204	$C_{15}H_{24}$	2.28	β-farnesene
18.	50.65	220	$C_{15}H_{24}O$	20.63	cis, trans-farnesal
19.	52.15	220	$C_{15}H_{24}O$	28.62	farnesal
20.	53.30	54	$C_{10}H_{18}O$	0.42	Methyl 2,2,3- trimethylcyclopentylketon,
21.	53.60	126	$C_8H_{14}O$	0.56	Methylheptone
22.	55.68	152	$C_{11}H_{20}$	0.60	4, 8-dimethyl-1,7-nonadiene
23.	56.43	154	$C_{10}H_{18}O$	0.92	7-methyl-3-methylene-6-octane-1-ol,
24.	57.65	224	$C_{15}H_{28}O$	0.88	2-pentadecyn-1-ol
25.	58.35	154	$C_{10}H_{18}O$	0.28	[R]-lavandulol
26.	64.18	154	$C_{10}H_{18}O$	0.84	[1R,2R,3R,15S]-[-]-isopinocampheol
27.	67.48	102	$C_6H_{14}O$	0.49	2, 3-dimethyl-2-butanol
28.	68.68	116	$C_8H_{16}O$	0.66	2-ethoxy-2-methyl butane

Table 2. Volatile components of roots of *Elettariopsis smithiae* aff. elucidated by Gas Chromatography-Mass Spectrometry.

Pk. No.	Rt.	Mol. Wt	Mol. Formula	Total %	Compound Name
1.	5.41	136	$C_{10}H_{16}$	29.53	cis-β-ocimene
2.	5.68	136	$C_{10}H_{16}$	3.74	camphene
3.	6.19	136	$C_{10}H_{16}$	0.45	[-]-β-pinene
4.	6.29	136	$C_{10}H_{16}$	2.07	β-pinene
5.	6.60	136	$C_{10}H_{16}$	3.49	β-myrcene
6.	6.93	136	$C_{10}H_{16}$	5.76	α-phellandrene
7.	7.13	136	$C_{10}H_{16}$	7.71	3-carene
8.	7.24	136	$C_{10}H_{16}$	0.36	[+]-2-carene
9.	7.32	134	$C_{10}H_{14}$	1.46	β-cymene
10.	7.56	136	$C_{10}H_{16}$	10.94	cyclohexene, 1-methyl-5-[1-methylethenyl]-
11.	8.29	136	$C_{10}H_{16}$	2.41	γ-terpinene
12.	9.08	136	$C10H_{16}$	1.23	terpinolene
13.	12.67	196	$C_{12}H_{20}O_2$	13.50	bicyclo heptan-2-ol,1,3,3-trimethyl-acetate.
14.	13.21	164	$C_{11}H_{16}O$	0.50	2-isopropyl-1-methoxy-4-methylbenzene
15.	14.41	196	$C_{12}H_{20}O_2$	2.20	isopulegyl acetate
16.	16.17	196	$C_{12}H_{20}O_2$	0.78	α-terpinyl acetate
17.	18.38	204	$C_{15}H_{24}$	5.13	isocaryophyllene
18.	19.23	204	$C_{15}H_{24}$	0.59	α-caryophyllene
19.	19.97	204	$C_{15}H_{24}$	0.59	β-chamigrene
20.	20.04	204	$C_{15}H_{24}$	0.82	α-guaiene
21.	22.23	220	$C_{15}H_{24}O$	1.92	caryophyllene oxide
22.	22.67	220	$C_{15}H_{24}O$	0.99	4,4-dimethyltricyclo [2, 5] trideca-8-en-1-ol
23.	23.99	222	$C_{15}H_{26}O$	0.73	[-]-globulol

Table 3. Volatile components of rhizomes of *Elettariopsis smithiae* aff. elucidated by Gas Chromatography-Mass Spectrometry.

Pk. No.	Rt	Mol. Wt	Mol. Formula	Total %	Compound Name
1.	5.39	136	$C_{10}H_{16}$	26.97	cis-β-ocimene
2.	5.67	136	$C_{10}H_{16}$	1.00	camphene
3.	6.18	136	$C_{10}H_{16}$	0.36	[-]-β-pinene
4.	6.28	136	$C_{10}H_{16}$	0.80	β-pinene
5.	6.59	136	$C_{10}H_{16}$	6.90	β-myrcene
6.	6.93	136	$C_{10}H_{16}$	0,27	α-phellandrene
7.	7.31	134	$C_{10}H_{14}$	0.50	β-cymene
8.	7.54	136	$C_{10}H_{16}$	8.35	limonene
9.	8.28	136	$C_{10}H_{16}$	1.75	γ-terpinene
10.	9.08	136	$C_{10}H_{16}$	0.87	terpinolene
11.	9.63	154	$C_{10}H_{18}O$	0.67	[1R]-endo-[+]-fenchyl alcohol
12.	10.78	154	$C_{10}H_{18}O$	0.31	borneol
13.	11.37	154	$C_{10}H_{18}O$	0.74	1-methyl-4-[1-methylethyl]-2-cyclohexen-1-ol,
14.	11.68	154	$C_{10}H_{18}O$	2.19	ocimenol
15.	12.66	196	$C_{12}H_{20}O_2$	8.77	1, 1, 3, 3-trimethyl- Bicyclo heptan-2-ol-acetate.
16.	14.45	154	$C_{10}H_{18}O$	23.60	cis-1-methyl-4-[1-methylethyl]-2-cyclohexen-1-ol
17.	15.43	194	$C_{12}H_{20}O_2$	0.64	cis-2-methylene-3-[1-methylethenyl]-cyclohexanol acetate,
18.	16.18	194	$C_{21}H_{20}O_2$	0.58	isopulegyl acetate
19.	18.38	204	$C_{15}H_{24}$	3.13	isocaryophyllene
20.	19.24	204	$C_{15}H_{24}$	0.46	α-caryophyllene
21.	20.31	204	$C_{15}H_{24}$	0.74	germacrene B
22.	22.24	220	$C_{15}H_{24}O$	0.97	caryophyllene oxide
23.	22.37	250	$C_{15}H_{22}O_3$	0.49	1,6-dimethyl-9-[1-methylethylidene]-5,12-dioxatricyclo[4, 6]dodecan-8-one
24.	22.54	346	$C_{22}H_{34}O_3$	0.31	17-[acetyloxy]-[4.β.]-kauran-18-al,

25.	22.67	222	$C_{14}H_{22}O_2$	0.92	3,3,8,8-tetramethyl-tricyclo4octane-5-carboxylicacid methyl ester
26.	22.83	232	$C_{10}H_{10}C_{12}O_2$	1.11	1,1-dichloro-2-methyl-3-[4,4-difformyl-1,3-butadien-1-yl] cyclopropane
27.	23.44	204	$C_{15}H_{24}$	0.27	4,11,11-trimethyl-8-methylene bicyclo undec-4-ene,
28.	23.93	222	$C_{15}H_{26}O$	0.80	α-bisabolol
29.	24.00	222	$C_{15}H_{26}O$	0.77	elemol

Table 4. The antimicrobial activities of *E. smithiae* aff.

	Staphylococcus aureus		*Escherichia coli*	
Sample (*E. smithiae* aff.)	**Antimicrobial activity**	**Diameter of inhibition (cm.)**	**Antimicrobial activity**	**Diameter of inhibition (cm.)**
Leaves (100µg)	+++	1.8	-	-
Rhizomes (100µg)	-	-	-	-
Roots (100µg)	-	-	-	-
Gentamicin (25µg)	++++	2.5	-	-

\+ : Weak activity
++ : Medium activity.
+++ : High activity
++++: Very high activity.

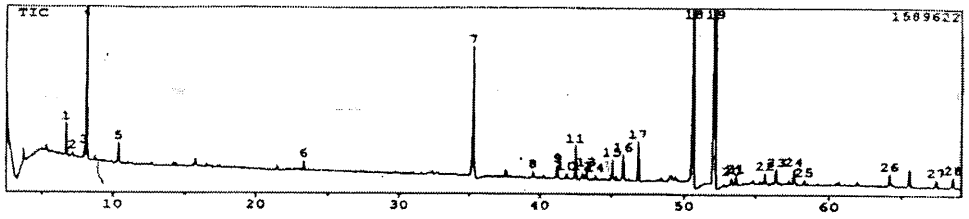

Figure 1 : GCMS spectrum of leaves of *E.smithiae* aff.

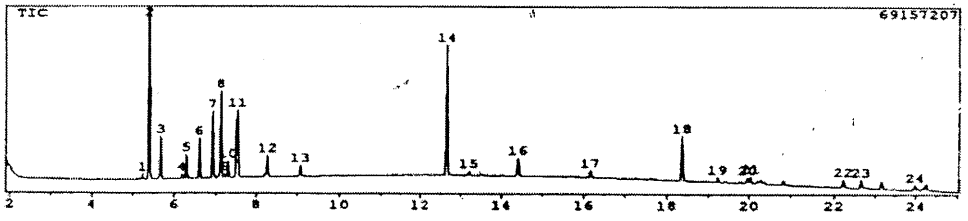

Figure 2 : GCMS spectrum of roots of *E.smithiae* aff.

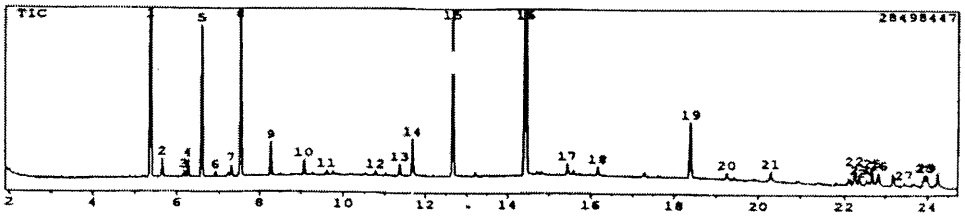

Figure 3 : GCMS spectrum of rhizomes of *E.smithiae* aff.

The antimicrobial test showed that the leaves of *E smithiae* aff. were positive on *S. aureus*, and negative on *E. coli*. The roots and rhizomes of this species do not show any activity on *S. aureus* and *E. coli*.

Acknowledgment This research was supported by the University of Malaya (F330/96) and partially supported by the Government of Malaysia.

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