

# ANALYSIS OF ESSENTIAL OILS OF *ETLINGERA SPHAEROCEPHALA* VAR. *GRANDIFLORA* BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETRY

(Analisis Minyak Pati Daripada *Etingera Sphaerocephala* Var. *Grandiflora* Dengan Kromatografi Gas Dua Dimensi - Spektrometri Jisim Masa Terbang)

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## Abstract

Hydrodistillation using a Clevenger-type apparatus of the *Etingera sphaerocephala* var. *grandiflora* rhizomes, stem, leaves and whole plant yielded oils of respective 0.03, 0.02, 0.17 and 0.05%. Forty two compounds (or 97%), 32 (81%), 40 (63%) and 36 (80%) of the rhizome, stem, leaf and whole plant oils gave good matches in GCXGC-TOFMS analysis. The major components in the rhizome oil: 1,8-cineole (16.8%),  $\alpha$ -phellandrene (12.7%) and  $\beta$ -trans-ocimene (8.9%); the stem: 1,8-cineole (17.4%),  $\alpha$ -phellandrene (9.7%) and 1S- $\alpha$ -pinene (9.5%); the leaf:  $\alpha$ -phellandrene (12.3%) and diprene (10.3%); and in the whole:  $\beta$ -pinene (12.2%),  $\alpha$ -pinene (8.6%), *p*-menth-1-en-8-ol (8.5%) and  $\alpha$ -phellandrene (8.5%). Monoterpenes constituted the richest components (76% average) in all of the four oils followed by sesquiterpenes (4%) and non-terpenes (0.2%). Three clusters of 1,8-cineole and  $\alpha$ -phellandrene;  $\beta$ -pinene and 1,8-cineole; and  $\alpha$ -phellandrene were obtained in cluster and principal component analyses.

**Keywords:** *Etingera sphaerocephala* var. *grandiflora*, essential oils, GCXGC-TOFMS

## Abstrak

Penyulingan air dengan radas jenis-Clevenger rizom, batang, daun dan keseluruhan tumbuhan menghasilkan minyak masing-masing sebanyak 0.03, 0.02, 0.17 dan 0.05%. Empat puluh dua sebatian (atau 97%), 32 (81%), 40 (63%), dan 36 (80%) minyak rizom, batang, daun dan keseluruhan tumbuhan memberikan padanan yang baik dalam analisis KGXKG-SJMP. Komponen utama dalam minyak rizom: 1,8-sineol (16.8%),  $\alpha$ -felandrena (12.7%) dan  $\beta$ -trans-osimena (8.9%); batang: 1,8-sineol (17.4%),  $\alpha$ -felandrena (9.7%) dan 1S- $\alpha$ -pinena (9.5%); daun:  $\alpha$ -felandrena (12.3%) dan diprena (10.3%); dan dalam keseluruhan:  $\beta$ -pinena (12.2%),  $\alpha$ -pinena (8.6%), *p*-ment-1-en-8-ol (8.5%) dan  $\alpha$ -felandrena (8.5%). Monoterpena menjuzukkan komponen terkaya (purata 76%) dalam kesemua empat minyak diikuti seskuiterpena (4%) dan bukan-terpena (0.2%). Tiga kluster 1,8-sineol dan  $\alpha$ -felandrena;  $\beta$ -pinena dan 1,8-sineol; dan  $\alpha$ -felandrena diperolehi dalam analisis kluster dan komponen utama.

**Kata kunci:** *Etingera sphaerocephala* var. *grandiflora*, minyak pati, GCXGC-TOFMS

## Introduction

There are 151 Zingiberaceae species belonging to 18 genera found in Peninsular Malaysia [1]. The largest Zingiberaceae genus is *Alpinia* (23 species), whereas *Etingera* (10) ranks sixth. The number of *Etingera* species has now increased to 15 [2]. *Etingera* species are tall forest plants, with larger species reaching up to 6 m in height [3]. Holttum [4] described *E. sphaerocephala* var. *grandiflora* by its subterranean inflorescence with flowers appearing at soil level; its stature is 2.5 m; its leaves when young also suffused purple below; its labellum is 6 cm or more in length and 2.7 cm wide, the base is red in colour. *Etingera sphaerocephala* var. *grandiflora* can be found in many parts of the Peninsular Malaysia and Borneo [5], mainly in lowland forests and at moderate elevation on the mountains. No uses have ever been recorded for *E. sphaerocephala* var. *grandiflora* [6].

To date, several studies have been carried out on *Etingera* essential oils. Lechat-Vahirua et al. [7] found methyl eugenol (47.4%) and (*E*)-methyl isoeugenol (18.2%) as major components in the rhizome essential oil of *Etingera cevuga*. The respective dry flower and flower axis essential oils of the Brazilian *E. elatior* contained dodecanol (42.5, 34.6%), dodecanal (14.5, 21.5%) and  $\alpha$ -pinene (22.2, 6.3%) as their major constituents [8].  $\beta$ -Pinene (19.2%), caryophyllene (15.4%) and (*E*)- $\beta$ -farnesene (27.9%) represented the major components of the leaf essential oil of

the Malaysian *E. elatior* whereas 1,1-dodecanediol diacetate (34.3%) and (*E*)-5-dodecene (27%) largely dominated the stem essential oil. The flower and rhizome essential oils of the Malaysian *E. elatior* comprised the major constituents of 1,1-dodecanediol diacetate and cyclododecane [9]. Nine components were identified from the rhizome essential oil of the Thai *Etingera punicea* from which the phenolic compound of methyl chavicol (95.7%) represented the major constituent [10].

## Materials and Methods

### Plant Material

Rhizome, stem, leaf and whole plant samples of the *Etingera sphaerocephala* var. *grandiflora* were collected in January 2009 from Genting Peras, Hulu Langat, Selangor, Malaysia and kept fresh in the freezer. A voucher specimen of WYA 386 for the plant was deposited at the Universiti Kebangsaan Malaysia Herbarium.

### Isolation Procedure

Each of the fresh parts and the whole plant of the *Etingera sphaerocephala* var. *grandiflora* was cut into small pieces, blended in distilled water and hydrodistilled in a Clevenger-type apparatus for 3 hours. The volatile oils obtained were dried over anhydrous sodium sulfate and stored in a cold room. Each of the oils was dissolved in dichloromethane for analysis using GCXGC-TOFMS.

### GCXGC-TOFMS System

#### GCXGC

GCXGC analyses were carried out using Agilent 6890N GC equipped with a LECO thermal modulator (technology under license from Zoex Corporation). Two columns were employed: Rtx-5MS (30 m, 0.10 mm id, film thickness 0.25  $\mu$ m) and DB-WAX (1 m, 0.1 mm id, film thickness 0.1  $\mu$ m). Operating conditions for both columns were as follows: initial oven temperature, 45  $^{\circ}$ C for 2 min, then to 230  $^{\circ}$ C (for Rtx-5MS) and 50  $^{\circ}$ C for 2 min, then to 235  $^{\circ}$ C (for DB-WAX) at 6  $^{\circ}$ C/min then held for 5 min; inlet temperature, 230  $^{\circ}$ C; carrier gas, 1 ml/min He; injection size, 1.0  $\mu$ l splitless; modulator temperature, 30  $^{\circ}$ C offset from main oven; modulation frequency, 5 s with a 1 s hot pulse time.

#### MS

The GC-TOF-MS software of the LECO Pegasus was used to find all peaks in the raw GCXGC chromatogram. Significant operating parameters: ionization voltage, EI at 70 eV; source temperature 200  $^{\circ}$ C; scan mass range, 50-400 U; acquisition rate, 100 spectra/s

### Compounds Identification

Compounds were identified by computer using their MS data compared to the NIST mass spectral library. The components which have similarity, reverse and probability of more than 800, 800 and 1000 respectively were considered as good matches.

### Statistical Analysis

The percentage composition of the major components of the essential oils was used to determine the relationship among different parts using hierarchical cluster analysis (SPSS software computer package). The cluster analysis was constructed on the basis of agglomerative grouping and average linkage between the groups employing the clustering method based on squared Euclidean distances.

## Results and Discussion

The hydrodistillation of the rhizomes of *Etingera sphaerocephala* var. *grandiflora* gave a pale yellowish viscous oil in 0.03% yield (w/w). The same proved true for the stem and whole plant oils but they gave 0.02 and 0.05% yields. The leaves produced a colorless non-viscous oil in 0.17% yield. With the yield ratio for the leaves: whole plant: rhizomes: stem of 8.5: 2.5: 1.5: 1, the leaves contained far more oil than the others.

GCXGC-TOFMS analysis of the *Etlingera sphaerocephala* var. *grandiflora* rhizome, stem, leaf and whole plant oils has shown the presence of respective 42, 32, 40 and 36 components which comprised of 97, 81, 63 and 80% of the total constituents of the oils (Table 1). There were 70 different compounds present in those four oils.

All the major compounds of the *Etlingera sphaerocephala* var. *grandiflora* oils as presented below were of the terpenic, whereby the rhizomes gave 1,8-cineole (16.8%),  $\alpha$ -phellandrene (12.7%) and  $\beta$ -*trans*-ocimene (8.9%); the stem produced 1,8-cineole (17.4%),  $\alpha$ -phellandrene (9.7%) and 1*S*- $\alpha$ -pinene (9.5%); the leaves yielded  $\alpha$ -phellandrene (12.3%) and diprene (10.3%); and the whole plant gave  $\beta$ -pinene (12.2%),  $\alpha$ -pinene (8.6%), *p*-menth-1-en-8-ol (8.5%) and  $\alpha$ -phellandrene (8.5%) (Figure 1). From the above eight main components, it is obvious that  $\alpha$ -phellandrene was found in all four oils whereas 1,8-cineole occurred only in the rhizomes and the stem. The major compounds of the Malaysian *Etlingera elatior* leaf oil were also of the terpenic, comprising of (*E*)- $\beta$ -farnesene (27.9%),  $\beta$ -pinene (19.2%) and caryophyllene (15.4%). Notice that the major  $\beta$ -pinene found in the whole plant oil of the *Etlingera sphaerocephala* var. *grandiflora* was also a main component in the Malaysian *Etlingera elatior* leaf oil. On the other hand, the flower and flower axis oils of the Brazilian *Etlingera elatior* each contained one terpenic compound of  $\alpha$ -pinene (22.2 and 6.3%) out of three major components besides the non-terpenic dodecanol (42.5 and 34.6%) and dodecanal (14.5 and 21.5%). Coincidentally, the oils of the Brazilian *Etlingera elatior* and the *Etlingera sphaerocephala* var. *grandiflora* whole plant contained a similar major component,  $\alpha$ -pinene. Other main compounds of the *Etlingera* volatile oils were all non-terpenic such as methyl eugenol (47.4%) and (*E*)-methyl isoeugenol (18.2%) of the *Etlingera cevuga* rhizome oil; 1,1-dodecanediol diacetate (34.3%) and (*E*)-5-dodecene (27%) of the Malaysian *E. elatior* stem oil; 1,1-dodecanediol diacetate (47.3%) and cyclododecane (34.5%) of the Malaysian *E. elatior* rhizome oil; cyclododecane (40.3%) and 1,1-dodecanediol diacetate (24.4%) of the Malaysian *E. elatior* flower oil; and methyl chavicol (95.7%) of the Thai *Etlingera punicea* rhizome oil.

Out of the 70 different components that occurred in the four oils, 16 (23%) were found in each of the oils. They were  $\alpha$ -phellandrene, *o*-cymene,  $\alpha$ -terpinolene,  $\beta$ -pinene, camphene,  $\beta$ -*cis*-ocimene,  $\alpha$ ,*p*-dimethylstyrene, 1,8-cineole, *p*-menth-1-en-8-ol, 4-terpineol, linalool, borneol, *exo*-fenchol,  $\alpha$ -humulene, *epi*- $\beta$ -santalene and 3,5-dimethyloctane.  $\alpha$ -Santalene and copaene were available in all of the oils of *E. sphaerocephala* var. *grandiflora* except in the rhizomes. 3-Pinanone and myrtenal were found in the all oils of *E. sphaerocephala* var. *grandiflora* but not in the stem. Limonene,  $\delta$ -cadinene,  $\alpha$ -muurolene and  $\alpha$ -bergamotene were present in all of the oils of *E. sphaerocephala* var. *grandiflora* except in the leaves.

On average, monoterpenes represented the most abundant constituents (76%) in all four oils followed by sesquiterpenes (4%) and non-terpenes (0.2%). Previous studies on the *Etlingera* essential oils found that they were not rich in monoterpenes, as shown in the *Etlingera cevuga* rhizome (20.1%); Brazilian *Etlingera elatior* flower (25.3%) and flower axis (6.8%); Malaysian *Etlingera elatior* leaf (38.8%), stem (4.8%), flower (7.5%) and rhizome (0.5%); and Thai *Etlingera punicea* rhizome (4.2%), compared to the current study on the *E. sphaerocephala* var. *grandiflora* rhizome (89.9%), stem (77%), leaf (61.2%) and whole plant (75.9%). Eight, four and fifteen compounds of the sesquiterpenes comprising 4.1, 1.2 and 6.8% of the whole components were identified in the *Etlingera cevuga*, *E. punicea* and *E. sphaerocephala* var. *grandiflora* rhizome oils whereas the Malaysian *Etlingera elatior* rhizome oil had no sesquiterpenes. The sesquiterpene percentages in the Malaysian *Etlingera elatior* leaf and stem oils (45.7 and 11.3%) were higher than those in the *E. sphaerocephala* var. *grandiflora* leaf and stem oils (1.1 and 4.2%). The sesquiterpenes were found in small percentages in the oils of Brazilian *Etlingera elatior* flower (3.2%), Malaysian *Etlingera elatior* flower (5.8%) and *E. sphaerocephala* var. *grandiflora* whole plant (3.7%) whereas the one found in Brazilian *Etlingera elatior* flower axis yielded 22.5%. The non-terpenes represented the high percentages in the essential oils from the *Etlingera cevuga* rhizome (72.9%); Brazilian *Etlingera elatior* flower (66.6%) and flower axis (65.2%); Malaysian *Etlingera elatior* stem (86.8%), flower (81.1%) and rhizome (82.8%); and Thai *Etlingera punicea* rhizome (95.7%). The non-terpenes which were found in the Malaysian *Etlingera elatior* leaf oil gave 3.5%. The non-terpene percentages were considerably very low in all parts of the *E. sphaerocephala* var. *grandiflora*.

The hierarchical cluster analysis of the major volatile constituents from the rhizomes, stem, leaves and whole plant grouped those oils into three main two-part clusters (Fig. 1 and Fig. 2). The first cluster is formed of oils of the rhizomes (16.8%) and stem (17.4%) contained 1,8-cineole as the main component. The second cluster constructed

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by oils of the leaves and whole plant comprised  $\alpha$ -phellandrene (12.3%) and  $\beta$ -pinene (12.2%) as the major components, while the third cluster consisted of oils of the rhizomes and leaves and contained 1,8-cineole (16.8%) and  $\alpha$ -phellandrene (12.3%) as the main components.

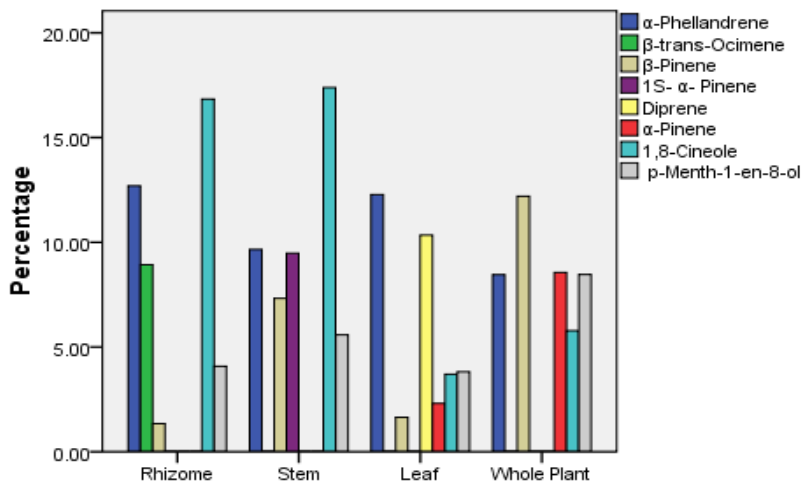


Figure 1: The percentages of the main compounds in the essential oils of the rhizomes, stem, leaves and whole plant of the *Etingera sphaerocephala* var. *grandiflora*.

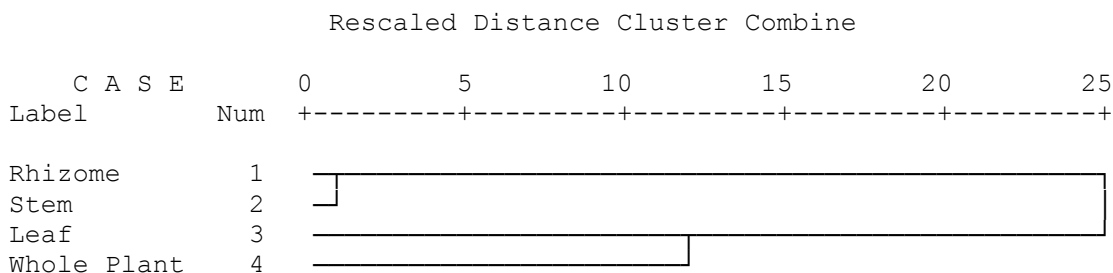


Figure 2: Dendrogram generated by hierarchical cluster analysis of the percentage composition of eight major components of the essential oils from rhizomes, stem, leaves and whole plant of *Etingera sphaerocephala* var. *grandiflora*, clustering method based on squared Euclidean distances.

Table 1: Components of essential oils obtained from the rhizomes, stem, leaves and whole plant of *Etlingera sphaerocephala* var. *grandiflora*.

Compound	Rhizome					Stem					Leaf					Whole plant							
	R.t. <sub>1</sub> R.t. <sub>2</sub>	S	R	P	%	R.t. <sub>1</sub> R.t. <sub>2</sub>	S	R	P	%	R.t. <sub>1</sub> R.t. <sub>2</sub>	S	R	P	%	R.t. <sub>1</sub> R.t. <sub>2</sub>	S	R	P	%			
<b>Monoterpene hydrocarbon</b>																							
$\alpha$ -Phellandrene	9.83333 0.730	919	929	6954	12.69	9.83333 0.720	922	922	7235	9.66	9.75 0.700	938	938	8759	12.28	9.75 0.670	934	934	8743	8.45			
$\beta$ - <i>trans</i> -Ocimene	8.16667 0.670	913	913	3633	8.93					-					-						-		
(3Z)-2,7-Dimethyl-3-octen-5-yne	8.25 0.710	813	821	1603	7.33					-					-	8.16667 0.710	838	845	1638	5.40			
Pseudolimonene	9.16667 0.740	811	827	2404	7.04					-	9.1666 0.750	813	828	2196	4.65						-		
Sabinene	9.16667 0.670	912	912	6176	5.27					-	9.25 0.670	900	901	5709	5.13							-	
<i>o</i> -Cymene	10.3333 0.740	947	952	5596	4.82	10.3333 0.760	942	947	5084	6.60	10.3333 0.740	935	944	5014	3.18	10.25 0.690	953	956	5648	4.72			
Limonene	10.3333 0.700	896	896	2564	3.50	10.3333 0.700	876	892	3612	4.70					-	10.3333 0.680	805	824	4483	1.46			
$\alpha$ -Terpinolene	11.8333 0.690	938	940	3220	2.00	11.8333 0.700	939	941	3987	2.22	11.8333 0.710	938	942	2756	1.90	11.8333 0.670	955	957	4265	2.01			
$\beta$ -Pinene	9.5 0.700	912	912	4821	1.34	9.16667 0.690	957	960	5609	7.33	9.50 0.710	919	910	5000	1.64	9.25 0.670	927	931	3910	12.2			
Camphene	8.58333 0.640	963	966	5823	1.21	8.58333 0.640	963	966	5983	1.30	8.58333 0.640	958	961	5416	0.56	8.58333 0.630	962	965	5754	0.96			
$\alpha$ -Terpine	10.0833 0.680	882	889	2006	1.08					-					-	10.0833 0.660	888	895	2012	0.55			
$\beta$ - <i>cis</i> -Ocimene	10.8333 0.710	929	929	3919	0.52	10.8333 0.730	896	896	2638	0.31	10.8333 0.720	892	892	2254	0.15	10.8333 0.690	902	902	2951	0.40			
$\alpha$ , <i>p</i> -Dimethylstyrene	11.8333 0.870	918	918	3568	0.23	11.8333 0.850	924	938	4153	0.38	11.8333 0.880	898	899	2482	0.30	11.8333 0.820	905	905	3910	0.27			
1 <i>S</i> - $\alpha$ -Pinene					-	8.25 0.680	931	931	3856	9.48					-							-	
Moslene					-	10.0833 0.680	874	874	3737	1.00					-							-	
Diprene					-					-	10.5 0.940	810	810	1655	10.34								-
Sabinane					-					-	8.25 0.820	893	893	2002	3.68								-
<i>D</i> -Limonene					-					-	10.3333 0.700	878	878	3255	2.37								-
$\alpha$ -Pinene					-					-	8.16667	968	968	5101	2.31	8.16667	939	939	3754	8.56			

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Pinene hydrochloride	-	-	-	-	-	0.660	13.5833	928	936	7228	0.08	0.650	13.5833	898	903	5221	0.12						
3-Carene	-	-	-	-	-	0.670	14.8333	843	845	1442	0.04	0.630	-	-	-	-	-						
1 <i>R</i> - $\alpha$ -Pinene	-	-	-	-	-	0.710	-	-	-	-	-	8.16667	959	959	4468	8.03							
Thuja-2,4(10)-diene	-	-	-	-	-	-	-	-	-	-	-	8.66667	827	827	3147	0.16							
	(Sub-total 55.96%)						(Sub-total 42.98%)						(Sub-total 48.61%)						(Sub-total 53.29%)				
<b>Oxygenated monoterpene</b>																							
1,8-Cineole	10.5	856	886	8092	16.84	0.830	10.5	864	891	8515	17.39	0.810	10.4167	909	909	9499	3.70	10.5	879	906	8578	5.78	
$\beta$ -Terpinyl acetate	10.4167	899	901	4058	5.93	0.780	10.4167	893	897	4047	6.59	0.770	-	-	-	-	-	-	-	-	-	-	
<i>p</i> -Menth-1-en-8-ol	14.25	946	946	6693	4.08	0.910	14.25	948	948	6672	5.58	0.920	14.25	955	955	6806	3.82	14.25	947	947	6756	8.46	
3-Pinanone	13.9167	917	917	5957	2.18	0.750	-	-	-	-	-	0.930	13.9167	928	931	6769	1.71	13.9167	917	917	4760	1.22	
4-Terpineol	13.9167	886	886	5817	1.95	0.810	13.9167	901	901	6568	1.57	0.800	13.9167	919	919	6433	1.64	13.9167	838	849	5438	3.45	
Linalool	12.0833	896	896	7321	1.03	0.860	12.0833	898	898	7519	1.18	0.850	12.0833	899	899	7498	0.69	12.0833	890	890	7150	1.50	
Borneol	13.6667	920	920	3130	0.72	0.840	13.6667	944	944	3233	0.98	0.850	13.6667	934	934	3098	0.37	13.75	925	925	3076	1.12	
<i>exo</i> -Fenchol	12.4167	915	915	5904	0.29	0.810	12.4167	932	932	5858	0.69	0.800	12.4167	937	937	6307	0.20	12.5	923	923	5327	0.46	
<i>L</i> -Pinocarveol	13.0833	854	854	8669	0.29	0.830	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Myrtenol	14.4167	862	862	7201	0.23	0.900	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pinocarpone	13.5833	819	820	7097	0.19	0.760	-	-	-	-	-	-	-	-	-	-	-	13.6667	808	810	5052	0.36	
Myrtenal	14.4167	932	932	8994	0.17	0.780	-	-	-	-	-	14.4167	876	876	7771	0.04	14.4167	921	921	9000	0.19		
<i>cis</i> -Sabinol	-	-	-	-	-	-	-	-	-	-	-	14.5	865	865	3826	0.31	0.960	-	-	-	-	-	
(-)-Myrtenyl acetate	-	-	-	-	-	-	-	-	-	-	-	17.1667	888	888	6532	0.10	0.730	-	-	-	-	-	
Pinocarveol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13.0833	816	817	4217	0.09	

	(Sub-total 33.90%)					(Sub-total 33.98%)					(Sub-total 12.58%)					(Sub-total 22.63%)				
<b>Sesquiterpene hydrocarbon</b>																				
$\alpha$ -Humulene	19.8333	943	943	8066	1.35	19.8333	940	940	7820	1.05	19.8333	935	935	7443	0.26	19.9167	945	945	7860	1.44
	0.690					0.680					0.670					0.600				
$\delta$ -Cadinene	21.25	882	891	4419	0.80	21.25	869	875	4498	0.46						21.25	879	885	5071	0.12
	0.670					0.670										0.580				
Caryophyllene	19.1667	891	891	1813	0.76					-					-					-
	0.680																			
$\alpha$ -Muuroolene	20.3333	887	890	2150	0.72	20.3333	878	881	2190	0.24					-	20.4167	879	882	4412	0.16
	0.670					0.670										0.580				
$\alpha$ -Gurjunene	18.75	883	895	2145	0.67					-					-					
	0.650																			
$\alpha$ -Bergamotene	19.4167	908	915	2630	0.41	19.4167	918	923	3162	0.35					-	19.5	925	930	3271	0.46
	0.670					0.670										0.590				
$\gamma$ -Muuroolene	21.0833	881	885	2517	0.39	21.0833	875	879	2521	0.13					-					-
	0.680					0.680														
$\gamma$ -Gurjunene	20.6667	886	893	1374	0.29	20.6667	891	896	1945	0.15					-					-
	0.670					0.670														
Calamenene	21.25	846	847	5523	0.28					-					-					-
	0.700																			
<i>trans</i> - $\alpha$ -Bergamotene	20.9167	822	845	1777	0.27					-					-					-
	0.690																			
$\alpha$ -Cubebene	18.25	872	876	3643	0.22					-					-					-
	0.660																			
<i>epi</i> - $\beta$ -Santalene	19.6667	867	870	3071	0.20	19.6667	913	913	6424	0.14	19.6667		906	5030	0.05	19.75	922	922	6667	0.20
	0.670					0.670					0.660	906				0.580				
$\alpha$ -Calacorene	21.5833	815	886	5478	0.13					-					-					-
	0.720																			
$\alpha$ -Santalene					-	19.1667	883	887	3562	0.59	19.1667	921	925	5613	0.28	19.1667	938	942	7830	0.55
						0.670					0.670					0.590				
$\alpha$ -Selinene					-	20.5	898	906	1781	0.18					-					-
						0.680														
Copaene					-	18.25	894	895	5825	0.16	18.25	876	877	5012	0.04	18.3333	883	884	5999	0.21
						0.650					0.650					0.580				
( <i>Z,E</i> )- $\alpha$ -Santalene					-					-	19.4167	916	929	7579	0.20					-
											0.670									
$\beta$ -Bisabolene					-					-	20.9167	848	848	2817	0.13					-
											0.690									
$\beta$ -Santalene					-					-	20	912	912	4975	0.04					-
											0.660									
Cyperene					-					-					-	18.8333	868	874	2065	0.11

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Germacrene D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.560	21.0833	864	893	2261	0.07
															0.590					
	(Sub-total 6.49%)					(Sub-total 3.45%)					(Sub-total 1.00%)					(Sub-total 3.32%)				
<b>Oxygenated sesquiterpene</b>																				
(±)- <i>trans</i> -Nerolidol	21.9167	917	917	4964	0.22	21.9167	932	932	5064	0.59										
	0.800					0.820														
Humulene oxide II	22.9167	823	841	2891	0.10	22.9167	820	826	3607	0.11										
	0.740					0.740														
Guaiol					-						22.6667	875	877	4184	0.10	22.75	856	859	3049	0.08
											0.770					0.660				
Nerolidol					-						21.9167	911	922	6255	0.04	22	912	919	5621	0.31
											0.800					0.700				
	(Sub-total 0.32%)					(Sub-total 0.70%)					(Sub-total 0.14%)					(Sub-total 0.39%)				
<b>Non-terpenic compound</b>																				
1,13-Tetradecadiene	27.1667	944	951	2487	0.09															
	0.810																			
3,5-Dimethyloctane	11	855	903	2437	0.04	11	870	913	2306	0.13	11	838	907	2344	0.04	11	851	899	2508	0.13
	0.690					0.680					0.680					0.660				
Decamethylcyclopentasiloxane					-	13.25	802	802	8895	0.16	13.25	810	810	9272	0.04					
						0.850					0.870									
Hexadecane					-	16.0833	878	883	2365	0.05	16.0833	875	880	2970	0.02					
						0.690					0.690									
( <i>Z</i> )-3-Hexenol					-						6.66667	924	924	4315	0.22					
											1.080									
2-Hexenal					-						6.66667	939	939	7381	0.06					
											0.940									
Undecane					-						12.0833	859	859	2071	0.01					
											0.680									
	(Sub-total 0.13%)					(Sub-total 0.34%)					(Sub-total 0.39%)					(Sub-total 0.13%)				
<b>Total</b>	96.80%					81.45%					62.72%					79.76%				

<sup>R.t.</sup><sub>1</sub> retention time in the first dimension (min).

<sup>R.t.</sup><sub>2</sub> retention time in the second dimension (min).

S = similarity, R = reverse, P = probability.



### Conclusion

In comparison, the leaves of *Etilingera sphaerocephala* var. *grandiflora* yielded more oil percentage than others. The major compounds in each of the four oils of *E. sphaerocephala* var. *grandiflora* noticeably differed. These oils were rich in monoterpenes compared to the oils of *E. cevuga*, *E. elatior* and *E. punicea*.

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### References

1. Turner, I.M. (1995). A catalogue of the vascular plants of Malaya. *Gardens' Bulletin Singapore*. 47(2): 642-655.
2. Lim, C.K. (2001). Taxonomic notes on *Etilingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the "Achasma" taxa and supplementary notes on the "Nicolaia" taxa. *Folia Malaysian*. 2(3): 141-178, 209-210.
3. Khaw, S.H. (2001). The genus *Etilingera* (Zingiberaceae) in Peninsular Malaysia including a new species. *Gardens' Bulletin Singapore*. 53: 191- 239.
4. Holttum, R.E. (1950). The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin Singapore*. 13(1): 1-249.
5. Smith, R.M. (1986). New combinations in *Etilingera* Gisek (Zingiberaceae). *Notes Royal Botanic Garden Edinburgh*. 43: 243-254.
6. Poulsen, A.D. (2006). *Etilingera of Borneo*. Natural History Publications (Borneo). Kota Kinabalu
7. Lechat-Vahirua, I., Francois, P., Menut, C., Lamaty, G., Bessiere, J.M. (1993). Aromatic plants of French Polynesia. I. Constituents of three Zingiberaceae: *Zingiber zerumbet* Smith, *Hedychium coronarium* Koeing and *Etilingera cevuga* Smith. *Journal of Essential Oil Research*. 5: 55-59.
8. Zoghbi, M.D.G.B., Andrade, E.H.A. (2005). Volatiles of *Etilingera elatior* (Jack) R.M.Sm. and *Zingiber spectabile* Griff.: two Zingiberaceae cultivated in the Amazon. *Journal of Essential Oil Research*. 17: 209-211.
9. Faridahanim Mohd Jaafar, Che Puteh Osman, Nor Hadiani Ismail, Khalijah Awang (2007). Analysis of essential oils of leaves, stems, flowers and rhizomes of *Etilingera elatior* (Jack) R. M. Smith. *Malaysian Journal of Analytical Sciences*. 11: 269-273.
10. Tadtong, S., Wannakhot, P., Poolsawat, w., Athikomkulchai, S., Ruangrunsi, N. 2009. Antimicrobial activities of essential oil from *Etilingera puniceae* rhizome. *J Health Res*. 23(2): 77-79.