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

(Analisis Minyak Pati Daripada *Etlingera 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 *Etlingera 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%), α-phellandrene (12.7%) and β-trans-ocimene (8.9%); the stem: 1,8-cineole (17.4%), α-phellandrene (9.7%) and 1*S*-α-pinene (9.5%); the leaf: α-phellandrene (12.3%) and diprene (10.3%); and in the whole: β-pinene (12.2%), α-pinene (8.6%), p-menth-1-en-8-ol (8.5%) and α-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 α-phellandrene; β-pinene and 1,8-cineole; and α-phellandrene were obtained in cluster and principal component analyses.

Keywords: Etlingera 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%), α-felandrena (12.7%) dan β-trans-osimena (8.9%); batang: 1,8-sineol (17.4%), α-felandrena (9.7%) dan 1S-α-pinena (9.5%); daun: α-felandrena (12.3%) dan diprena (10.3%); dan dalam keseluruhan: β-pinena (12.2%), α-pinena (8.6%), p-ment-1-en-8-ol (8.5%) dan α-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 α-felandrena; β-pinena dan 1,8-sineol; dan α-felandrena diperolehi dalam analisis kluster dan komponen utama.

Kata kunci: Etlingera 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 *Etlingera* (10) ranks sixth. The number of *Etlingera* species has now increased to 15 [2]. *Etlingera* 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. *Etlingera 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 *Etlingera* 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 *Etlingera* 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 α -pinene (22.2, 6.3%) as their major constituents [8]. β -Pinene (19.2%), caryophyllene (15.4%) and (*E*)- β -farnesene (27.9%) represented the major components of the leaf essential oil of

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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 *Etlingera 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 *Etlingera 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 Hebarium.

Isolation Procedure

Each of the fresh parts and the whole plant of the *Etlingera 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 dicholoromethane 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 μ m) and DB-WAX (1 m, 0.1 mm id, film thickness 0.1 μ m). Operating conditions for both columns were as follows: initial oven temperature, 45 °C for 2 min, then to 230 °C (for Rtx-5MS) and 50 °C for 2 min, then to 235 °C (for DB-WAX) at 6 °C/min then held for 5 min; inlet temperature, 230 °C; carrier gas, 1 ml/min He; injection size, 1.0 μ l splitless; modulator temperature, 30 °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 °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 *Etlingera 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%), α-phellandrene (12.7%) and β-trans-ocimene (8.9%); the stem produced 1,8-cineole (17.4%), α-phellandrene (9.7%) and 1S-α-pinene (9.5%); the leaves yielded αphellandrene (12.3%) and diprene (10.3%); and the whole plant gave β-pinene (12.2%), α-pinene (8.6%), p-menth-1-en-8-ol (8.5%) and α -phellandrene (8.5%) (Figure 1). From the above eight main components, it is obvious that α 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)-\(\theta\)-farnesene (27.9%), β -pinene (19.2%) and caryophyllene (15.4%). Notice that the major β -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 α-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%). Coincidently, the oils of the Brazilian Etlingera elatior and the Etlingera sphaerocephala var. grandiflora whole plant contained a similar major component, α-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 α -phellandrene, o-cymene, α -terpinolene, β -pinene, camphene, β -cis-ocimene, α -p-dimethylstyrene, 1,8-cineole, p-menth-1-en-8-ol, 4-terpineol, linalool, borneol, exo-fenchol, α -humulene, epi- β -santalene and 3,5-dimethyloctane. α -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, δ -cadinene, α -muurolene and α -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 α -phellandrene (12.3%) and β -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 α -phellandrene (12.3%) as the main components.

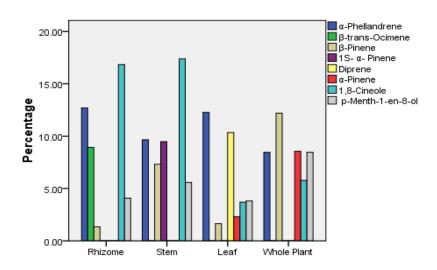


Figure 1: The percentages of the main compounds in the essential oils of the rhizomes, stem, leaves and whole plant of the *Etlingera sphaerocephala* var. *grandiflora*.

Rescaled Distance Cluster Combine

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 *Etlingera 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.

		Rhizon					Stem					Leaf				W	Whole plant				
Compound	R.t. 1 R.t. 2	S	R	P	%	R.t. 1 R.t. 2	S	R	P	%	R.t. 1 R.t. 2	S	R	P	%	R.t. 1 R.t. 2	S	R	P	%	
Monoterpene hydrocarbon	-					-					-					-					
α-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	
β-trans-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					-	
o-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	876	892	3612	4.70	0.710				-	10.3333 0.680	805	824	4483	1.46	
α-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	
β-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	
α-Terpine	10.0833 0.680	882	889	2006	1.08	0.010				-	0.010				-	10.0833 0.660	888	895	2012	0.55	
β-cis-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	
α, <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> - α-Pinene	0.070				-	8.25 0.680	931	931	3856	9.48	0.000				-	0.020				-	
Moslene					-	10.0833 0.680	874	874	3737	1.00					-					-	
Diprene					-	0.000				-	10.5 0.940	810	810	1655	10.34					-	
Sabinane					-					-	8.25 0.820	893	893	2002	3.68					-	
D-Limonene					-					-	10.3333 0.700	878	878	3255	2.37					-	
α-Pinene					-					-	8.16667	968	968	5101	2.31	8.16667	939	939	3754	8.56	

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Pinene hydrochloride 3-Carene					-					-	0.660 13.5833 0.670 14.8333	928 843	936 845	7228 1442	0.08 0.04	0.650 13.5833 0.630	898	903	5221	0.12
1 <i>R</i> -α-Pinene					-					-	0.710				-	8.16667	959	959	4468	8.03
Thuja-2,4(10)-diene					-					-					-	0.740 8.66667	827	827	3147	0.16
	(Sub-total 55.96%)							(Sub-total 42.98½)					(Sul	o-total 4	8.61%)	0.640		ub-total 53.29%)		
Oxygenated monoterpene																				
1,8-Cineole	10.5 0.830	856	886	8092	16.84	10.5 0.810	864	891	8515	17.39	10.4167 0.710	909	909	9499	3.70	10.5 0.760	879	906	8578	5.78
β-Terpinyl acetate	10.4167 0.780	899	901	4058	5.93	10.4167 0.770	893	897	4047	6.59					-					-
p-Menth-1-en-8-ol	14.25 0.910	946	946	6693	4.08	14.25 0.920	948	948	6672	5.58	14.25 0.930	955	955	6806	3.82	14.25 0.820	947	947	6756	8.46
3-Pinanone	13.9167 0.750	917	917	5957	2.18	0.520				-	13.9167 0.740	928	931	6769	1.71	13.9167 0.710	917	917	4760	1.22
4-Terpineol	13.9167 0.810	886	886	5817	1.95	13.9167 0.800	901	901	6568	1.57	13.9167 0.82	919	919	6433	1.64	13.9167 0.750	838	849	5438	3.45
Linalool	12.0833 0.860	896	896	7321	1.03	12,0833 0.860	898	898	7519	1.18	12.0833 0.850	899	899	7498	0.69	12.0833 0.800	890	890	7150	1.50
Borneol	13.6667 0.840	920	920	3130	0.72	13.6667 0.850	944	944	3233	0.98	13.6667 0.840	934	934	3098	0.37	13.75 0.790	925	925	3076	1.12
exo-Fenchol	12.4167 0.810	915	915	5904	0.29	12.4167 0.800	932	932	5858	0.69	12.4167 0.800	937	937	6307	0.20	12.5 0.760	923	923	5327	0.46
L-Pinocarveol	13.0833 0.830	854	854	8669	0.29	0.000				-	0.000				-	0.700				-
Myrtenol	14.4167 0.900	862	862	7201	0.23					-					-					
Pinocarvone	13.5833 0.760	819	820	7097	0.19					-					-	13.6667 0.710	808	810	5052	0.36
Myrtenal	14.4167 0.780	932	932	8994	0.17					-	14.4167 0.790	876	876	7771	0.04	14,4167 0.720	921	921	9000	0.19
cis-Sabinol	0.700				-					-	14.5 0.960	865	865	3826	0.31	0.720				-
(-)-Myrtenyl acetate					-					-	17.1667 0.730	888	888	6532	0.10					-
Pinocarveol					-					-					-	13.0833	816	817	4217	0.09



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18.8333

868

874

2065

0.11

(Sub-total 33.90%) (Sub-total 33.98%) (Sub-total 12.58%) (Sub-total 22.63½) Sesquiterpene hydrocarbon 19.9167 α-Humulene 19.8333 943 943 8066 1.35 19.8333 940 940 7820 1.05 19.8333 935 935 7443 0.26 945 945 7860 1.44 0.690 0.680 0.670 0.600 0.80 δ-Cadinene 21.25 882 891 4419 21.25 869 875 4498 0.46 21.25 879 885 5071 0.12 0.670 0.670 0.580 19.1667 891 891 1813 0.76 Caryophyllene 0.680 α-Muurolene 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 0.580 895 2145 α- Gurjunene 18.75 883 0.67 0.650 19.5 915 0.41 923 930 α-Bergamotene 19.4167 908 2630 19.4167 918 3162 0.35 925 3271 0.46 0.670 0.670 0.590 γ- Muurolene 21.0833 881 885 2517 0.39 21.0833 875 879 2521 0.13 0.680 0.680 γ-Gurjunene 893 1374 0.29 20.6667 1945 0.15 20.6667 886 891 896 0.670 0.670 Calamenene 21.25 846 847 5523 0.28 0.700 0.27 trans-α-Bergamotene 20.9167 822 845 1777 0.690 α-Cubebene 18.25 872 876 3643 0.22 0.660 *epi*-β-Santalene 0.20 19.6667 19.75 922 922 19.6667 867 870 3071 19.6667 913 913 6424 0.14 906 5030 0.05 0.20 6667 0.580 0.670 0.670 0.660 906 α-Calacorene 21.5833 815 886 5478 0.13 0.720 α-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 α-Selinene 20.5 898 906 1781 0.18 0.680 18.25 894 895 5825 0.16 18.25 876 5012 0.04 18.3333 883 884 5999 0.21 Copaene 877 0.650 0.650 0.580 (Z,E)- α -Santalene 19.4167 916 929 7579 0.20 0.670 β-Bisabolene 20.9167 848 848 2817 0.13 0.690 β-Santalene 20 912 0.04 912 4975 0.660

Cyperene

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Germacrene D					-					-					-	0.560 21.0833 0.590	864	893	2261	0.07	
		(Sub-total 6.49%)							o-total 3	.45%)		(Sub-total 1.00%)						(Su	b-total 3	3.32%)	
Oxygenated sesquiterpene	21.017	017	017	1061	0.22	21.0167	022	022	5064	0.50											
(±)-trans-Nerolidol	21.9167 0.800	917	917	4964	0.22	21.9167 0.820	932	932	5064	0.59					-					-	
Humulene oxide II	22.9167 0.740	823	841	2891	0.10	22.9167 0.740	820	826	3607	0.11					-					-	
Guaiol					-					-	22.6667 0.770	875	877	4184	0.10	22.75 0.660	856	859	3049	0.08	
Nerolidol					-					-	21.9167 0.800	911	922	6255	0.04	22 0.700	912	919	5621	0.31	
		(Sub-total 0.32½)							ub-total	0.70%)	(Sub-total 0.14 ½)								b-total (total 0.39%)	
Non-terpenic compound																					
1,13-Tetradecadiene	27.1667 0.810	944	951	2487	0.09					-					-					-	
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	
Decamethylcyclopentasiloxane	0.690				-	0.680 13.25	802	802	8895	0.16	0.680 13.25	810	810	9272	0.04	0.660				-	
Hexadecane					-	0.850 16.0833	878	883	2365	0.05	0.870 16.0833	875	880	2970	0.02					-	
(Z)-3-Hexenol					_	0.690				_	0.690 6.66667	924	924	4315	0.22					_	
											1.080										
2-Hexenal					-					-	6.66667 0.940	939	939	7381	0.06					-	
Undecane					-					-	12.0833	859	859	2071	0.01					-	
			(Sı	ıb-total	0.13%)			(S	ub-total	0.34%)	0.680		(Sı	ıb-total (0.39%)			(Su	b-total (0.13%)	
R.t. rotantian time in the	6° . 1°	• ,	,		96.80%					81.45%			•		2.72½			-		79.76%	

 $_{\rm R.t.}^{\rm R.t.}$, retention time in the first dimension (min). $_{\rm 2}^{\rm R.t.}$, retention time in the second dimension (min). $_{\rm 2}^{\rm R.t.}$ = similarity, $_{\rm 1}^{\rm R.t.}$ = reverse, $_{\rm 2}^{\rm R.t.}$ = probability.

Conclusion

In comparison, the leaves of *Etlingera 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 *Etlingera* 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 *Etlingera* (Zingiberaceae) in Peninsular Malaysia including a new species. *Gardens' Bulletin Singapore*. 53: 191-239.
- 4. Holttum, R.E. (1950). The Zingiberance of the Malay Peninsula. Gardens' Bulletin Singapore. 13(1): 1-249.
- 5. Smith, R.M. (1986). New combinations in *Etlingera* Gisek (Zingiberaceae). *Notes Royal Botanic Garden Edinburgh*. 43: 243-254.
- 6. Poulsen, A.D. (2006). Etlingera 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 *Etlingera cevuga* Smith. *Journal of Essential Oil Research*. 5: 55-59.
- 8. Zoghbi, M.D.G.B., Andrade, E.H.A. (2005). Volatiles of *Etlingera 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 *Etlingera elatior* (Jack) R. M. Smith. *Malaysian Journal of Analytical Sciences*. 11: 269-273.
- 10. Tadtong, S., Wannakhot, P., Poolsawat, w., Athikomkulchai, S., Ruangrungsi, N. 2009. Antimicrobial activities of essential oil from *Etlingera puniceae* rhizome. *J Health Res*, 23(2): 77-79.