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Essential Oils of *Zingiber ottensii* Valet. and *Zingiber zerumbet* (L.) Sm. from Sabah, Malaysia

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ABSTRACT The essential oils of *Zingiber ottensii* Valet. and *Zingiber zerumbet* (L.) Sm. from Sabah, Malaysia obtained by hydrodistillation of the fresh rhizomes were analyzed by a combination of capillary GC and GC–MS. Twenty-eight and eighteen components were identified from the rhizomes of *Z. ottensii* and *Z. zerumbet* respectively. The components were determined by comparing their Retention Indices with those reported in the literature and their mass spectral data with those from the mass spectral database. The most abundant component of *Z. ottensii* and *Z. zerumbet* rhizome oil was zerumbone representing 37% and 73% of the total oil respectively. Other major components of *Z. ottensii* were terpinen-4-ol (16.8%), α-humulene (10.9%) and sabinene (7.2%) whilst that of *Z. zerumbet* were α-humulene (5.9%), camphene (2.8%) and caryophyllene oxide (2.7%).

ABSTRAK Minyak pati daripada rizom segar *Zingiber ottensii* (lempoyang hitam) dan *Zingiber zerumbet* (lempoyang) dari Sabah, Malaysia diperolehi melalui penyulingan hidro. Minyak pati yang diperolehi dianalisis dengan menggunakan kaedah KG dan KG-SJ. Dua puluh lapan dan lapan belas komponen kimia masing-masing dikenalpasti daripada rizom *Zingiber ottensii* dan *Zingiber zerumbet.* Komponen-komponen ini dikenalpasti dengan membandingkan data Indeks Penahanan komponen kimia tersebut dengan yang dilaporkan oleh kajian-kajian terdahulu dan perbandingan spektrum jisim komponen kimia dengan spektrum jisim rujukan daripada pangkalan data. Komponen kimia utama bagi minyak pati *Z. ottensii* dan *Z. zerumbet* adalah zerumbon yang mana masing-masing mengandungi 37% dan 73% daripada minyak pati total. Komponen kimia utama yang lain bagi minyak pati *Z. ottensii* adalah terpinen-4-ol (16.8%), α-humulene (10.9%) and sabinene (7.2%) manakala bagi minyak pati *Z. zerumbet* pula adalah α-humulene (5.9%), camphene (2.8%) and caryophyllene oxide (2.7%).

(*Zingiber ottensii*, *Zingiber zerumbet*, rhizome oils, zerumbone)

INTRODUCTION

Zingiber ottensii Valet. and *Zingiber zerumbet* (L.) Sm. locally known as *Lempoyang hitam* and *lempoyang* respectively, are two Zingiberaceous plants measuring less than 2 m in height. *Z. ottensii* is native to South East Asia and is only known in cultivation. *Z. ottensii* and *Z. zerumbet* are quite similar in their general morphology especially at their juvenile stage. However, in mature plants *Z. ottensii* differ from *Z. zerumbet* in having their basal stems coloured dark red, light orange flowers with pink blotches and purplish rhizomes in cross sections. The rhizomes of *Z. ottensii* are pungent and used traditionally as post partum medicine. Studies by other researchers showed that the rhizome oils of *Z. ottensii* from Johor, Malaysia also contained zerumbone as the major component and other components such as terpinen-4-ol, α-humulene

and sabinene were present in quite appreciable amount [1].

Z. zerumbet is probably indigenous to India and later became widespread in South East Asia. Unlike *Z. ottensii*, *Z. zerumbet* is a more variable species with several variants in Malaysia including wild and semi-wild variants. These variants differ slightly in their overall inflorescence shapes with white to pale lemon yellow flowers and deep yellow median patch on the lip. The rhizomes of *Z. zerumbet* are also pungent and are sometimes used as a spice or consumed to increase appetite. In traditional medicine the rhizomes are used as treatment for ulcers, inflammation, stomach aches, diarrhoea, asthma, rheumatism etc. [2, 3 and 4]. A number of studies have been done on the rhizome oil of *Z. zerumbet* by researchers from Vietnam [5], Polynesia Island [6], Reunion Island [3] and India [7]. Results showed that zerumbone was the major component in their rhizome oils of *Z. zerumbet.*

Zerumbone has been recently reported to have the capability to suppress colonic tumor marker formation in rats, induces apoptosis in colon cancer cell lines and expression of proinflammatory cytokine genes in human colon adenocarcinoma cell lines [8, 9 and 10].

The present communication reports the essential oil components of the rhizome oils of *Z. ottensii* and *Z. zerumbet* from Sabah, East Malaysia.

MATERIALS AND METHODS

The samples of fresh rhizomes of *Z. ottensii* (MZ 21) and *Z. zerumbet* (MZ 4) were collected from Sabah Agricultural Park (Tenom), East Malaysia in August 2004. The samples were identified by Professor Dr. Halijah Ibrahim and the voucher specimens were deposited in the herbarium of Institute of Biological Sciences, University of Malaya. The fresh rhizomes were sliced into small pieces and immediately soaked in distilled water. The soaked sample was distilled in a Clevenger-type apparatus for eight to ten hours. The oily layer obtained was separated from the water layer and subsequently dried by using anhydrous sodium sulphate. The yields of the oils were calculated based on the weight of the fresh plant material.

The oils were injected (injection volume: 1.0 μL; split mode; split ratio: 1:10) into a Shimadzu GC 14A equipped with a FID detector using fused silica capillary column ZB-1 (30 m, ID 0.25 mm, 0.25 μm film thickness) with helium as a carrier gas at a flow rate of 50 cm^3 min^{-1}. The column temperature was programmed initially at 60°C for 10 min, then increased 3°C min^{-1} to 230°C and was kept isothermally for one minute. The chromatogram of *Z. zerumbet* and *Z. ottensii* are shown in Figure 1 and Figure 2, respectively.

GC-MS analysis was performed using a Hewlett Packard GCMSD 5890 series II mass spectrometer (70 eV direct inlet; injection volume: 1.0 μL; split mode; split ratio: 1:10) on fused silica capillary column ZB-1 (30 m, ID 0.25 mm, 0.25 μm film thickness) with helium as carrier gas at a flow rate of 50 cm^3 min^{-1}. The column temperature was programmed similar to that for the GC programme.

The total ion chromatogram obtained was auto integrated by Chemstation and the constituents were identified by comparison with published mass spectra database [11]. Retention indices were determined from the gas chromatogram by logarithmic interpolation between bracketing alkanes using a homologous series of n-alkanes as standards and in accordance with established method [12]. The total ion chromatograms of *Z. zerumbet* and *Z. ottensii* are shown in Figure 3 and Figure 4, respectively.

RESULTS AND DISCUSSION

The physical characteristics of the essential oils from the water distillation of the fresh rhizomes of the *Zingiber* species studied are shown in Table 1. The list of chemical constituents identified from the rhizome of these two *Zingiber* species is tabulated in Table 2. The constituents were identified by matching their mass spectra and retention indices with reference libraries [3, 13-15].

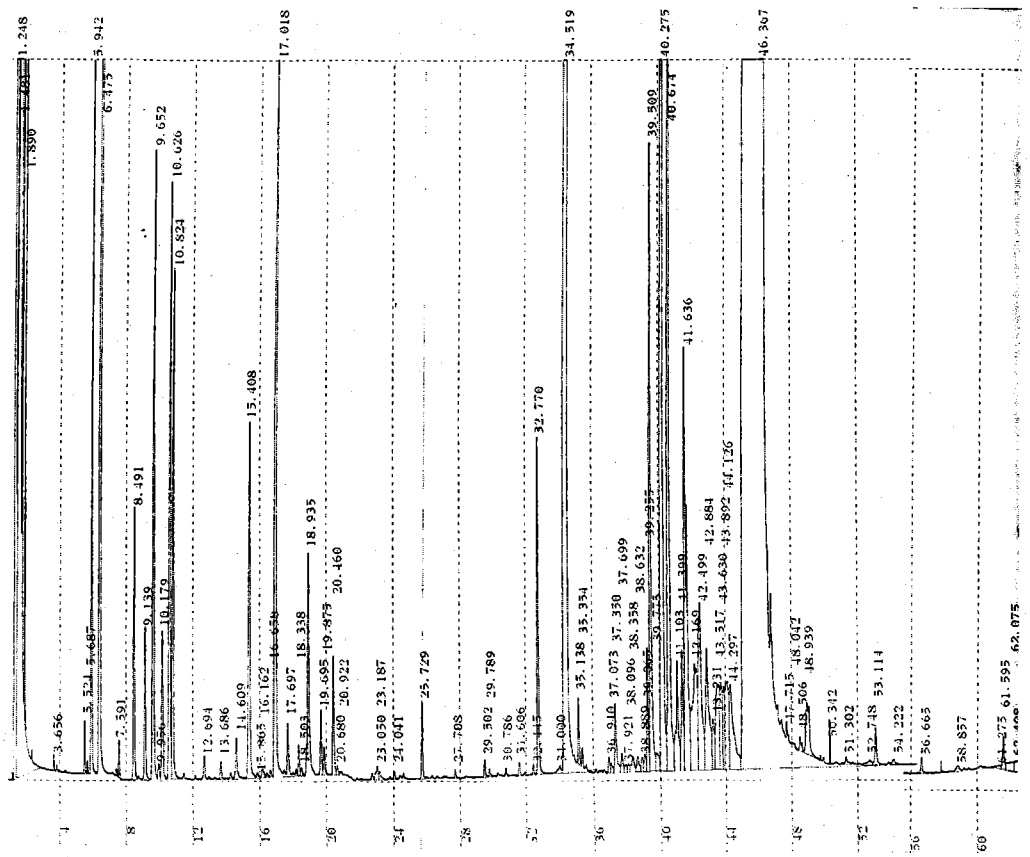

Figure 1. The chromatogram of the rhizome of *Z. zerumbet*

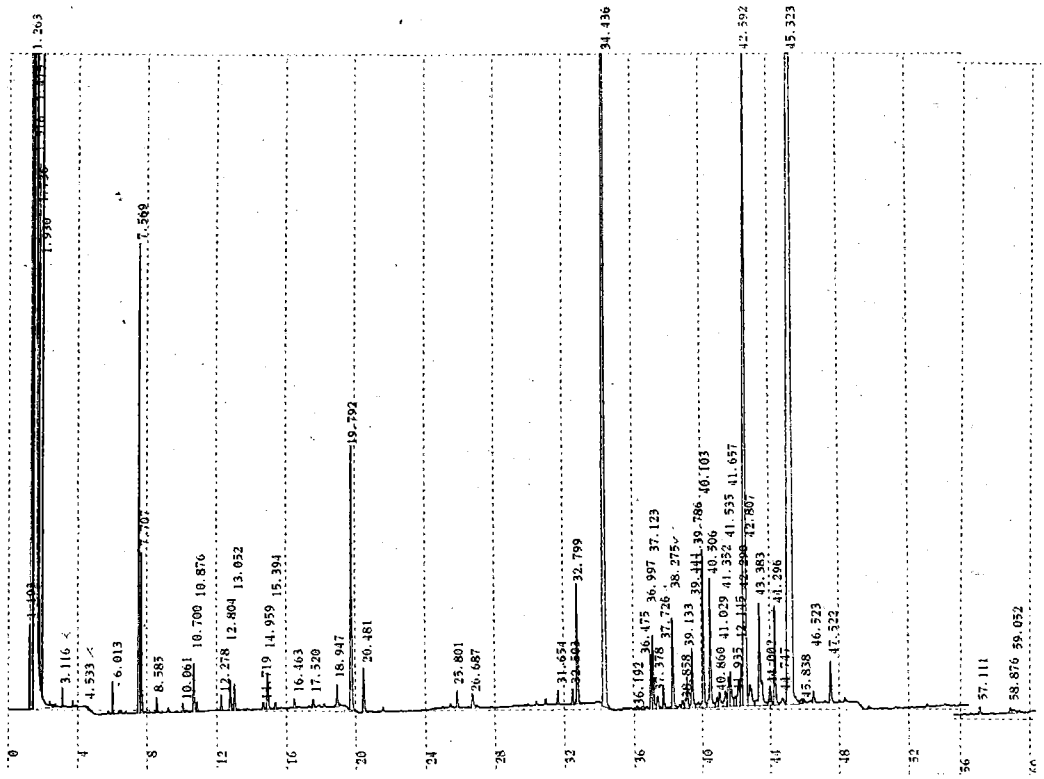

Figure 2. The chromatogram of the rhizome of *Z. ottensii*

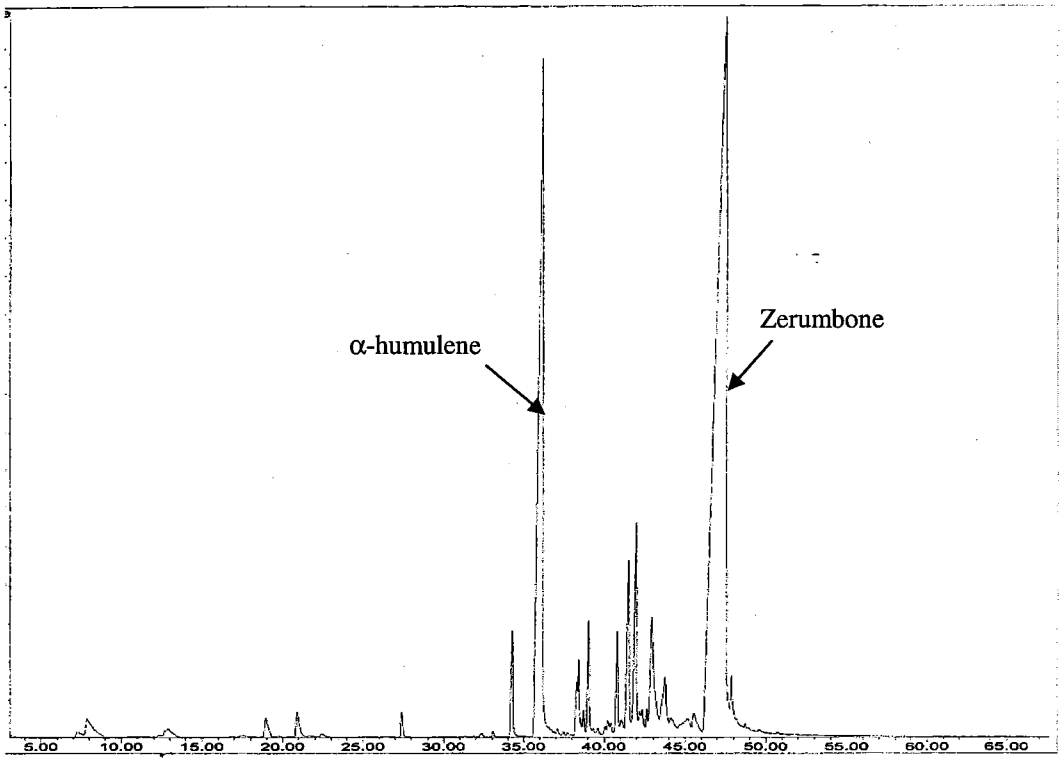

Figure 3. The total ion chromatogram of the rhizome of *Z. zerumbet*

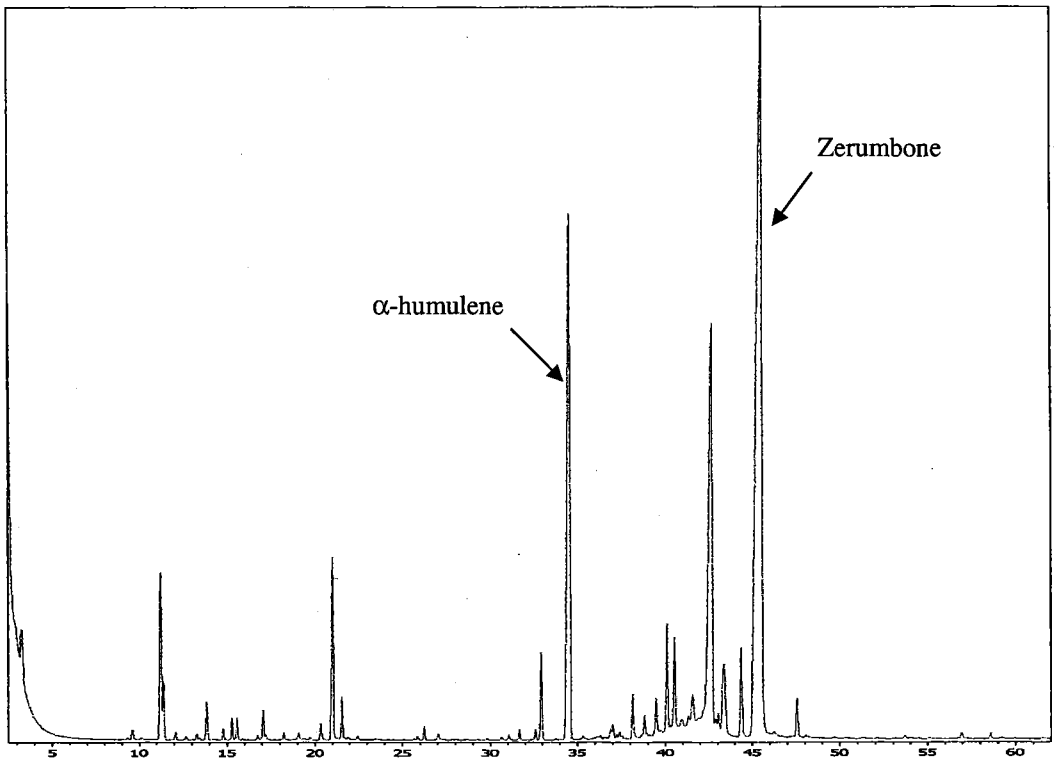

Figure 4. The total ion chromatogram of the rhizome of *Z. ottensii*

Table 1. The Yield and Characteristics of the Essential Oils from the Rhizomes of *Zingiber ottensii* and *Zingiber zerumbet*

Species	No. of Compounds Identified	Yield (%) (v/w)	Physical Characteristics
Z. ottensii	27	0.86	Light yellow, distinct sharp aromatic odour
Z. zerumbet	14	1.22	Light yellow, distinct sharp pungent odour

Table 2. The Chemical Constituents of Rhizome Oils of *Z. ottensii* and *Z. zerumbet*

Chemical Constituents	Retention Indices		Percentage (%)		Method of Identification
	Sample	Reference	*Zingiber ottensii*	*Zingiber zerumbet*	
α-pinene	941	942 (a)	0.23	0.66	MS, RI
camphene	954	954 (a)	-	2.81	MS, RI
sabinene	976	972 (a)	4.12	-	MS, RI
β-pinene	979	978 (a)	1.34	-	MS, RI
myrcene	992	991 (b)	0.16	0.30	MS, RI
α-phellandrene	998	998 (a)	t	-	MS, RI
δ-3-carene	1013	1013 (a)	-	0.76	MS, RI
α-terpinene	1021	1016 (a)	0.11	-	MS, RI
1,8-cineole	1032	1025 (c)	0.57	-	MS, RI
(E)- β-ocimene	1056	1044 (a)	0.19	-	MS, RI
γ-terpinene	1064	1057 (a)	0.36	-	MS, RI
trans-sabinene hydrate	1067	1068 (a)	0.32	-	MS, RI
terpinolene	1088	1081 (c)	0.09	-	MS, RI
cis-sabinene hydrate	1091	1092 (a)	0.45	-	MS, RI
linalool	1096	1097 (a)	0.09	0.53	MS, RI
camphor	1124	1126 (a)	-	1.31	MS, RI
isoborneol	1156	1157 (a)	0.29	-	MS, RI
borneol	1157	1154 (a)	-	0.35	MS (d), RI
terpinen-4-ol	1170	1170 (a)	3.16	0.08	MS, RI
α-terpineol	1181	1178 (a)	0.56	0.04	MS, RI
bornyl acetate	1276	1278 (a)	0.19	0.09	MS, RI
α-ylangene	1368	1368 (a)	t	-	MS, RI
α-copaene	1369	1369 (a)	t	-	MS, RI
β-elemene	1391	1384 (c)	0.15	-	MS, RI
α-gurjunene	1410	1413 (a)	0.17	-	MS, RI
β-caryophyllene	1417	1417 (a)	1.32	-	MS, RI
α-humulene	1454	1447 (a)	18.32	5.93	MS, RI
elemol	1541	1540 (a)	1.21	-	MS, RI
(Z)-nerolidol	1561	1553 (a)	0.48	-	MS, RI
(E)-nerolidol	1570	1564 (b)	-	0.93	MS, RI
caryophyllene oxide	1568	1574 (c)	0.81	2.72	MS, RI
Unidentified oxygenated sesquiterpene	1645	-	13.15	-	-
zerumbone	1714	-	36.68	73.08	MS (d)

MS = Mass Spectrum, RI = Retention Indices
t = trace (less than 0.01%)
Percentages were calculated based on results obtained from gas chromatography on column ZB-1 (equivalent to SE 30).
a = Davies, N. W. 1990
b = Jantan, I. et al. 1999
c = Jimmy, C. M. et al. 2003
d = Adams, R.P. 2001

Table 3. Comparison of the essential oils of *Z. ottensii* from Sabah and Johor, Malaysia

Chemical Constituents	Percentage (%) *Z. ottensii*	
	Sabah	Johor
α-thujene	-	0.66
α-pinene	0.23	1.65
camphene	-	0.22
sabinene	4.12	7.20
β-pinene	1.34	5.08
myrcene	0.16	1.56
α-phellandrene	t	0.52
δ-3-carene	-	1.30
α-terpinene	0.11	3.64
1,8-cineole	0.57	3.34
(E)- β-ocimene	0.19	-
γ-terpinene	0.36	5.13
trans-sabinene hydrate	0.32	-
terpinolene	0.09	1.25
cis-sabinene hydrate	0.45	-
linalool	0.09	0.36
isoborneol	0.29	-
terpinen-4-ol	3.16	16.81
α-terpineol	0.56	2.30
bornyl acetate	0.19	-
α-ylangene	t	-
α-copaene	t	0.08
β-elemene	0.15	0.17
α-gurjunene	0.17	0.12
β-caryophyllene	1.32	1.46
α-humulene	18.32	10.93
β-bisabolene	-	0.31
β-sesquiphellandrene	-	1.50
elemol	1.21	-
(Z)-nerolidol	0.48	-
caryophyllene oxide	0.81	-
humulene epoxide II	-	2.91
β-eudesmol	-	0.94
zerumbone	36.68	25.63

t = trace (less than 0.01%)

Table 4. Comparison of the essential oils of *Z. zerumbet* from Malaysia, Vietnam, Reunion Island, Polynesia Island and India

Chemical Constituents	Percentage (%)				
	Z. zerumbet				
	Malaysia	Vietnam	Reunion Island	Polynesia Island	India
tricyclene	-	t	0.5	-	-
α-thujene	-	t	0.1	0.1	-
α-pinene	0.66	0.7	3.5	1.1	1.3
camphene	2.81	3.1	13.8	4.1	4.4
heptanol	-	-	-	-	0.1
sabinene	-	t	-	0.1	-
β-pinene	-	0.1	0.1	0.3	2.4
β-myrcene	0.30	0.2	0.6	-	0.3
2-octanol	-	-	-	-	0.1
p-cymene	-	t	0.9	0.4	0.1
limonene	-	0.4	1.3	0.5	0.8
α-phellandrene	-	0.1	1.3	0.1	-
δ-3-carene	0.76	0.2	1.0	-	-
1,8-cineole	-	0.8	3.2	1.3	7.1
α-terpinene	-	-	0.1	-	-
fenchone	-	t	0.1	-	-
2-nonanone	-	-	-	-	0.3
terpinolene	-	t	0.2	-	-
linalool	0.53	0.4	1.3	0.4	1.1
camphor	1.31	1.2	3.8	2.2	12.8
camphene hydrate	-	t	-	-	-
citronella	-	-	0.2	-	-
isoborneol	-	-	0.2	-	8.9
neoborneol	-	t	-	-	-
borneol	0.35	0.2	0.2	2.3	4.9
2-nonanol	-	-	-	-	1.9
terpinen-4-ol	0.08	0.1	0.2	0.2	0.7
p-cymen-8-ol	-	-	-	-	0.1
α-terpineol	0.04	0.2	0.1	0.4	1.3
decanol	-	-	-	-	0.1
bornyl acetate	0.09	t	0.1	0.6	0.3
δ-elemene	-	-	-	-	0.2
2-undecanol	-	-	-	-	0.2
β-elemene	-	-	-	-	3.3
β-caryophyllene	-	0.3	1.4	0.3	0.5
γ-elemene	-	-	-	-	1.7
α-humulene	5.93	4.2	14.4	0.5	0.3
12-norcaryophyllen-2-one	-	0.4	-	-	-
ar-curcumene	-	-	-	-	0.4
β-selinene	-	-	-	-	1.0
zingiberene	-	-	-	-	0.5
β-selinene	-	-	-	-	0.8
(E,E)- α-farnesene	-	-	-	0.1	-
β-bisabolene	-	-	-	0.1	-
δ-cadinene	-	-	0.1	-	-
(Z)-nerolidol	-	-	0.1	-	-
(E)-nerolidol	0.93	0.1	2.1	0.1	-

spathulenol	-	-	-	-	1.0
caryophyllene oxide	2.72	1.5	5.2	1.2	0.9
curzerenone	-	-	-	-	14.4
humulene epoxide I	-	3.8	-	2.9	0.3
humulene epoxide II	-	3.3	2.7	4.4	-
humulene epoxide III	-	-	-	2.4	-
T-cadinol	-	-	-	0.5	-
β-eudesmol	-	0.2	-	0.7	-
germacrone	-	-	-	-	1.0
humulenol-I	-	-	-	0.2	-
humulenol-II	-	-	-	0.9	-
zerumbone	73.08	72.3	37.0	65.3	12.6
humulene dioxide	-	-	-	0.3	-

t = trace (less than 0.01%)

Twenty seven constituents comprising 88.2% of the rhizome oil of *Z. ottensii* were identified, of which four were oxygenated sesquiterpenes (39.2%), six sesquiterpene hydrocarbons (20.0%), nine monoterpene hydrocarbons (6.6%) and eight oxygenated monoterpenes (5.6%). Zerumbone was the most abundant constituent (36.7%), followed by α-humulene (18.3%), sabinene (4.1%) and terpinen-4-ol (3.2%). Other compounds were present in less than 3.0%. However, the percentages of chemical constituents of *Z. ottensii* rhizome oil of Sabah were slightly different from those reported from Johor, West Malaysia [1] whereby the content of zerumbone present as a major component was slightly lower (25.6%), followed by terpinen-4-ol (16.8%), α-humulene (10.9%) and sabinene (7.2%). A comparison of the essential oils of *Z. ottensii* from Sabah and Johor is shown in Table 3. Some compounds present in the Sabahan species [(E)- β-ocimene, *trans*-sabinene hydrate, *cis*-sabinene hydrate, isoborneol, bornyl acetate, α-ylangene, elemol, (Z)-nerolidol and caryophyllene oxide] were not detected in the Johor species. Similarly, there were components present in the Johor species [α-thujene, camphene, δ-3-carena, β-bisabolene, β-sesquiphellandrene, humulene epoxide II and β-eudesmol] which were not detected in *Z. ottensii* from Sabah [1].

Fourteen components comprising 89.6% of the rhizome oil of *Z. zerumbet* were identified. The total oil consisted mainly of three oxygenated sesquiterpenes (76.7%), one sesquiterpene hydrocarbons (5.9%), four monoterpene hydrocarbons (4.5%) and six oxygenated monoterpenes (2.4%). The major component of the oil was zerumbone comprising 73.1% of the total oil. Thus, the rhizome of *Z. zerumbet* is a good source of zerumbone. Other constituents present in the oil in significant amount were α-humulene (5.9%), camphene (2.8%) and caryophyllene oxide (2.7%). The proportions of these components were quite similar to those reported for specimens from Vietnam oil [5]. However, humulene epoxide I and II representing 3.8% and 3.3% of the Vietnamese oil respectively were not detected in the Sabah species. A comparison of the essential oils of *Z. zerumbet* from Malaysia, Vietnam, Reunion Island, Polynesia Island and India is shown in Table 4. The results showed that the Malaysian sample produces the most amount of zerumbone. The rhizome oils of *Z. zerumbet* from the Polynesian and Reunion Island were different from the Malaysian specimen [6, 3]. The major components in the Polynesian oil were zerumbone (65.3%), humulene epoxide II (4.4%) and camphene (4.2%), whilst that of the Reunion oil contained zerumbone (37.0%), α-humulene (14.4%) and camphene (13.8%). The rhizome oil of *Z. zerumbet* from India differs from those reported here as the former comprised of curzerenone (14.4%), camphor (12.8%) and zerumbone (12.6%) as the major components [7].

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