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ISOLATION AND IDENTIFICATION OF ESSENTIAL OILS FROM BITTER GINGER (Zingiber amaricans BL.), FRAGRANT GINGER (Zingiber aromaticum Val.) AND SHAMPOO GINGER (Zingiber zerumbet (L.) Smith) RHIZOMES GROWN IN JUMAPOLO KARANGANYAR CENTRAL JAVA INDONESIA

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ABSTRACT

Bitter ginger (*Zingiber amaricans* BL.), fragrant ginger (*Zingiber aromaticum* Val.) and shampoo ginger (*Zingiber zerumbet* (L.) Smith) rhizomes essential oils from Jumapolo Karanganyar Central Java Indonesia had been isolated by the steam distillation method, and the components had been identified by Gas Chromatography and Mass Spectroscopy (GC-MS) method. Fourteen, fifteen, and twelve components were identified in bitter ginger, fragrant ginger, and shampoo ginger essential oils rhizomes. The chemical composition of all types of essential oils varies, both qualitatively and quantitatively. The same components between *Z. amaricans* BL., *Z. aromaticum* Val., and *Z. zerumbet* (L.) Smith was zerumbone, α-humulene, β-selinene dan (-)-caryophyllene oxide. The major constituent in *Z. amaricans* BL., *Z. aromaticum* Val., and *Z. zerumbet* (L.) Smith oils were zerumbone. *Z. zerumbet* (L.) Smith oil presented the highest quantity of zerumbone (77.62%).

Key words: *Zingiber amaricans* BL., *Z. aromaticum* Val., *Z. zerumbet* (L.) Smith, essential oil, zerumbone

PENDAHULUAN

Familia Zingiberaceae includes approximately 47 genus and more than 1400 species distributed across South and South East Asia. The genus *Zingiber* spp. Contains approximately 80 species; there are three kinds of species in the genus *Zingiber* spp. that had been cultivated in Karanganyar, Central Java Indonesia, bitter ginger (*Zingiber amaricans* BL.), fragrant ginger (*Zingiber aromaticum* Val.) and shampoo ginger (*Zingiber zerumbet* (L.) Smith]. In Indonesia, *Z. amaricans* BL., *Z. aromaticum* Val. and *Z. zerumbet* (L.) Smith are commonly called *lempuyang emprit*, *lempuyang wangi* and *lempuyang gajah* [1, 2].

Since ancient times, the rhizome from these species had been used by people in Indonesia as spices, vegetables, and raw materials of traditional medicine (jamu) to treat various types of diseases. In the past few years, traditional medicine increasingly popular and

sought after by the community, along with the development of the movement back to nature. There are limited publications on *Z. amaricans* BL and *Z. aromaticum* Val. *Z. zerumbet* (L.) Smith has been used continuously as a subject for further investigations [3, 4].

The rhizome of bitter ginger (Z. amaricans BL.) is smaller in size, yellow with a bitter taste, and increased appetite. In West Java Indonesia, the rizhome of bitter ginger is usually as fresh vegetable [4, 5]. Rhizome of fragrant ginger (*Z. aromaticum* Val.) is greenish-colored and fraganted flesh [4]. N-hexana extract of fragrant ginger tested that suppressed Helicobacter pylori's growth with MIC (Minimal Inhibitory Concentration) 1.25 mg/ml [6]. Rhizome of the shampoo ginger [*Z. zerumbet* (L.) Smith] is bigger, yellow fleshed and smell aromatic. Shampoo ginger, in particular, has exhibited as anti-inflammatory, antidiabetic, anticancer, antimicrobial, analgesic, antioxidant and antiviral. *Z. amaricans* BL., *Z. aromaticum* Val. and *Z. zerumbet* (L.) In addition, Smith exhibited as antibacterial activity against *Mycoplasma gallisepticum* [2-4].

Various researches have published the chemical compounds of Zingiber rhizomes essential oils. The composition of chemical components was variously based on growing location. Zerumbone and phytosterol mixtures were isolated from *Z. amaricans* BL. rhizome collected from Yogyakarta Indonesia. Zerumbon was isolated as the major constituent of hexane, dichloromethane and methanol extracts. Phytosterols were isolated as the minor compounds of hexane and dichloromethane extracts. The mixture of phytosterols consisted of β-sitosterol, cholesterol, campesterol and stigmasterol [7]. The major compounds of *Z. zerumbet* rhizome oil from Vietnam were sabinene (14.6%), camphene (16.3%) and (Z)-citral (26.1%), respectively. This oil had little zerumbone content (1.2%) [8]. Thirty compounds were identified in the essential oil of *Z. zerumbet* (L.) Smith rhizome from Bangladesh with the major compounds being in α-caryophyllene (19.0%) and zerumbone (46.8%), respectively [9]. Twenty seven compounds were identified in *Z. aromaticum* Val. rhizome essential oil from Tawangmangu, Karanganyar Central Java Indonesia. The main components were α-terpinolene (27.19%) and zerumbon (31.05%) [10]. Research results from these rhizomes from various regions showed differences in essential oils' chemical components [7-10].

Jumapolo Karanganyar, Central Java was one of the centers for producing empon-empon in Indonesia. The empon-empon planted included bitter ginger, fragrant ginger and shampoo ginger, too. Jumapolo is located at an altitude of 450 m above sea level, has different a climate, agroecology and soil fertility conditions with the growing location some of Zingiber rhizomes that have been researched above. This allows the formation of chemical components that are different from other places. So far, Zingiber cultivation as a medicinal plant is still used as a side job. There was no scientific study on the chemical content, including the chemical compound of Zingiber rhizomes essential oil growing in Jumapolo, causes the plant to be of less economic value in this area. There was no development in the mindset of farmers in Jumapolo to make Zingiber the main commodity that could support the family economy, encouraging the author to

conduct research specifically on Zingiber that grows in Jumapolo. Various natural products from essential oils had been discovered to exhibit useful medicinal substances against diverse human ailments. To develop the use of essential oils from empon-empon in Jumapolo, especially bitter ginger, fragrant ginger and shampoo ginger which had not been studied much so far, it was necessary to carry out research to determine about the chemical components in essential oils of bitter ginger, fragrant ginger and shampoo ginger rhizomes that were cultivated in Jumapolo Karanganyar, Central Java Indonesia.

METHODS

1. Plant Materials

Zingiber amaricans BL., *Zingiber aromaticum* Val. and *Zingiber zerumbet* (L.) Smith .

2. Research Methods

a. Collection of Plant

Z. amaricans BL., *Z. aromaticum* Val. and *Z. zerumbet* (L.) Smith were purchased from Jumapolo Karanganyar Central Java Indonesia. The plant material was identified and authenticated in the Laboratory of Plant Taxonomy, Gadjah Mada University Indonesia.

b. Preparation of Sample

Fresh rhizomes of *Z. amaricans* BL., *Z. aromaticum* Val. and *Z. zerumbet* (L.) Smith were harvested, collected, sorted and washed thoroughly under running water. The rhizomes were sliced transversely with a thickness of 1-2 mm and dried at 40^{O}C with a drying machine. The rhizomes were pulverized to course powder using a mechanical grinder.

c. Extraction of Essential Oil

500 g of the *Z. amaricans* BL. rhizome powder, 500 g of the *Z. aromaticum* Val. rhizome powder and 500 g of the *Z. zerumbet* (L.) Smith rhizome powder was isolated by steam distillation. The oils thus obtained were pulled out the water by anhydrous sodium sulphate and placed in a glass bottles at low temperature for further studies [11, 12].

d. GC-MS Analysis

The essential oils extracted from rhizomes were analyzed by a SHIMADZU QP-500 Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS condition were: EI (Electron Impact) ionization, injection temperature 280^{o}C, detector temperature 280^{o}C, Rtx-MS column (30m x 0.32 mm x 0.25 µm), temperature of column 70^{o}C–280^{o}C, carrier gas was helium [12, 13].

e. Identification of compounds

The components of essential oils were identified by comparing the mass spectra with those recorded in the *National Institute of Standarts and Tecnology* (NIST) library

RESULTS AND DISCUSSION

The yield of the essential oils from *Z. amaricans* BL., *Z. aromaticum* Val. and *Z. zerumbet* (L.) Smith were varying from 1.6 to 4.4% v/w (Table 1).

Table 1. Yields and color of essential oils

Parameter	*Z. amaricans* BL.	*Z. aromaticum* Val.	*Z. zerumbet* (L.) Smith
Yield (% v/w)	1.6	2.8	4.4
Colour	Yellow pale	Dark yellow	Yellow

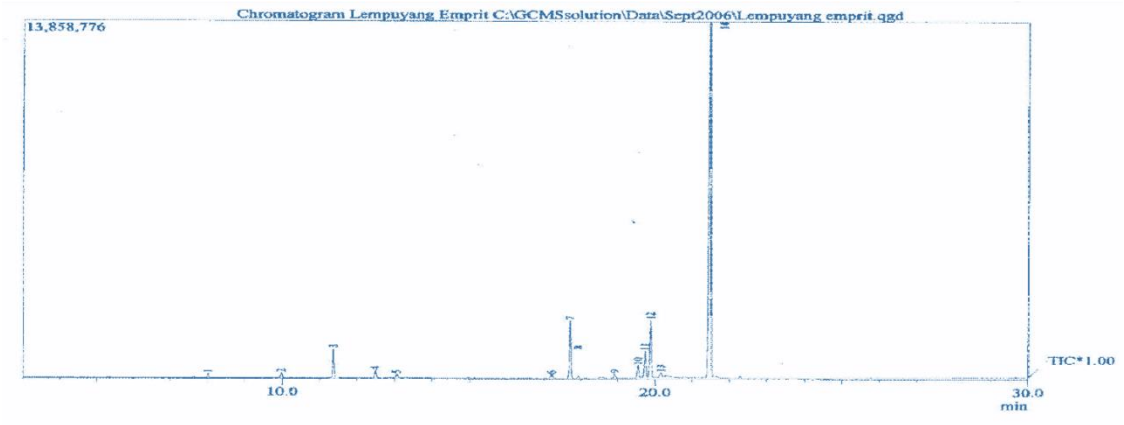

Figure 1. Gas Chromatogram of *Z. amaricans* BL. essential oil

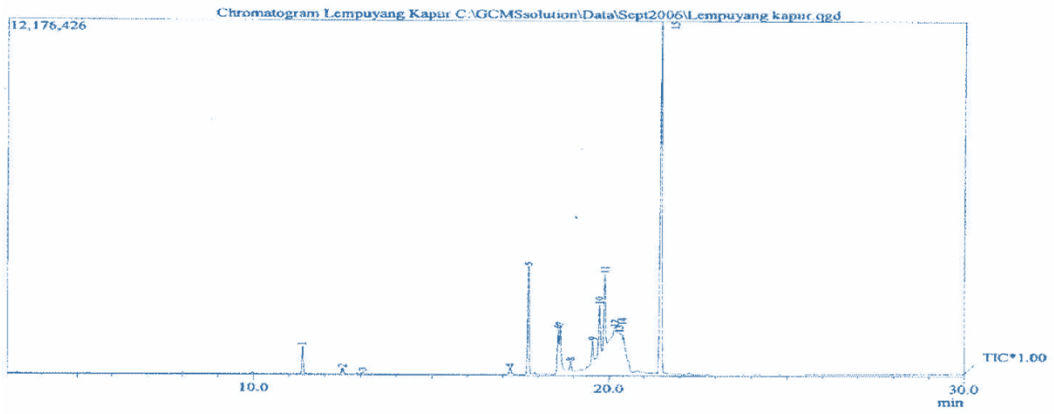

Figure 2. Gas Chromatogram of *Z. aromaticae* Vahl. essential oil

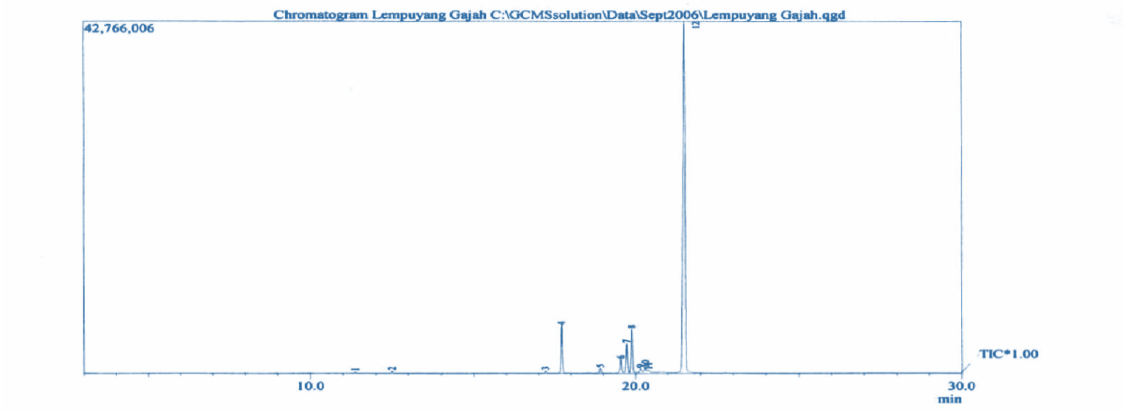

Figure 3. Gas Chromatogram of *Z. zerumbet* (L.) Smith. essential oil

The chromatograms from the result of identification with GC instrument showed that *Z. amaricans* BL had 14 peaks (Figure 1.), *Z. aromaticae* Vahl. had 15 peaks (Figure 2.) and *Z. zerumbet* (L.) Smith. had 12 peaks (Figure 3.). The chromatograms from the result of identification with GC instrument showed that *Z. amaricans* BL had 14 peaks *Z. aromaticum* Val. has 15 peaks, and *Z. zerumbet* (L.) Smith has 12 peaks. The volatile compounds were displayed in Table 2, Table 3 and Table 4, along with their retention time, molecule weight and percentage of area.

Table 2. Compound identified in the essential oil of *Z. amaricans* BL. by GC-MS instrument

No	Retention Time (minute)	Molecular Weight	% Area	Compound
1	8.029	136	0.59	Champene
2	9.982	154	0.72	1.8-Cineole
3	11.375	154	3.96	Linalool L
4	12.495	152	1.12	Champhor
5	13.064	154	0.57	Terpinene-4-ol
6	17.213	204	0.50	Trans-Caryophyllene
7	17.713	204	9.41	α-Humulene
8	17.934	198	0.35	Isotetradecane
9	18.915	178	0.79	Cyclohexane
10	19.547	220	2.09	(-)-Caryophyllene oxide
11	19.727	204	4.39	β-Selinene
12	19.884	220	9.66	Humulene oxide
13	20.159	154	0.80	Isogeraniol
14	21.465	218	65.06	Zerumbone

Z. amaricans BL. afforded yellow pale oil obtained in yield of 1.6% (v/w). Zerumbone (65.06%), humulene oxide (9.66%) and α-humulene (9.41%) were the main component of *Z. amaricans* BL. essential oil with zerumbone was the major constituent. On the other hand, β-selinene (4.39%) and linalool L (3.96%) were also identified in sizeable amount.

Table 3. A compound identified in the essential oil of *Z. aromaticum* Val. by GC-MS instrument

No	Time Retention (minute)	Molecular Weight	% Area	Compound
1	11.372	154	1.81	Linaool L
2	12.491	152	0.42	Camphor
3	13.064	154	0.15	3-Terpinen-4-ol
4	17.211	204	0.44	Trans-Caryopyllene
5	17.711	204	8.36	α-Humulene
6	18.557	180	3.34	4-Isoprophenyl-4,7-dimethyl-1-oxa-spiro-(2.5) octane
7	18.619	168	4.45	Dihydronopol
8	18.914	178	1.20	Cyclohexane
9	19.546	220	2.49	(-)-Caryophyllene oxide
10	19.728	204	7.55	β-Selinene
11	19.883	154	10.52	Isogeraniol
12	20.165	262	13.52	Cyclohexene
13	20.300	148	5.48	Methyl chavicol
14	20.383	222	8.83	β-Eudesmol
15	21.461	218	31.45	Zerumbone

Z. aromaticum Val. afforded dark yellow oil obtained in yield of 2.8% (v/w). Zerumbone (31.45%), cyclohexene (13.52%) and isogeraniol (10.52%) were the dominant component of *Z. aromaticum* Val. oil with zerumbone, which zerumbone was the major constituent. In addition, there is a significant amount of β-eudesmol (8.83%), α-humulene (8.36%), β-selinene (7.55%) and methyl chavicol (5.48%).

Table 4. A compound identified in the essential oil of *Z. zerumbet* (L.) Smith by GC-MS instrument

No	Time Retention (minute)	Molecular Weight	% Area	Compound
1	11.376	154	0.15	Linalool L
2	12.495	152	0.30	Camphor
3	17.215	204	0.30	Trans-Caryophyllene
4	17.716	204	6.52	α-Humulene
5	18.915	178	0.74	Cyclohexane
6	19.550	220	1.96	(-)-Caryophyllene oxide
7	19.732	204	4.23	β-Selinene
8	19.889	220	6.42	Humulene oxide
9	20.157	222	0.69	1-H-Cycloprop(e)-azulen-4-ol
10	20.308	204	0.73	1,5-Cycloundecadiene
11	20.400	222	0.34	β-Eudesmol
12	21.515	218	77.62	Zerumbone

Z. zerumbet (L.) Smith afforded yellow oil obtained in a yield of 4.4% (v/w). Zerumbone (77.62%), α-humulene (6.52%) and humulene oxide (6.42%) were the main component of *Z. zerumbet* (L.) Smith. Essential oil with zerumbone was the major constituent. In addition, β-selinene (4.23%) were also identified in sizeable amount.

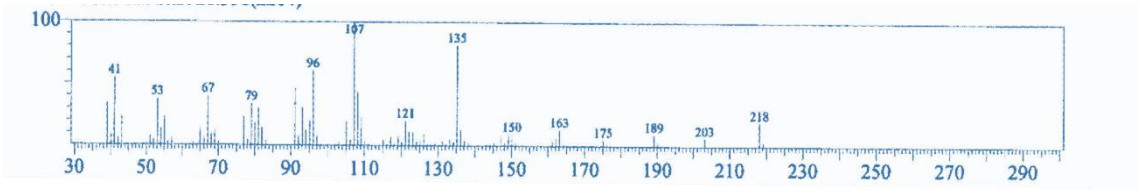

Figure 4. Mass spectrum of zerumbone

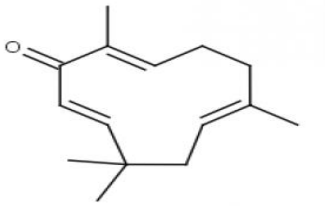

Figure 5. The structure of zerumbone

The same components between *Z. amaricans* BL., *Z. aromaticum* Val. and *Z. zerumbet* (L.) Smith was zerumbone, α-humulene, β-selinene dan caryophyllene oxide. Zerumbone showed 65.6% in *Z. amaricans* BL. oil, 31.45% in *Z. aromaticum* Val. oil and 77.62% in *Z. zerumbet* (L.) Smith oil respectively. The mass spectrum and the structure of zerumbone can be seen in Figure 4. and Figure 5. The relative mass of zerumbone is 218. The structural formula of zerumbone is $C_{15}H_{22}O$ and the IUPAC name of zerumbone is (2E,6E,10E)-2,6,9,9-tetramethylcycloundeca-2,6,10-trien-1-one**.** Zerumbone indicated immunomodulatory, anti-inflammatory, antipyretic, antibacterial, antioxidant, anti-cancer and anti-neoplastic effects. Zerumbone has indicated its anti-cancer effects by making significant suppression of proliferation, survival, angiogenesis, invasion, and metastasis through the molecular modulation of another pathway such as NF-κB, Akt, and IL-6/JAK2/STAT3 (interleukin-6/janus kinase-2/signal transducer and activator of transcription 3) and their downstream target proteins [14]. Zerumbone also affects angiogenesis and acts as an antitumor drug in the treatment of cancer, showing selective toxicity toward varied cancer cell lines [15].

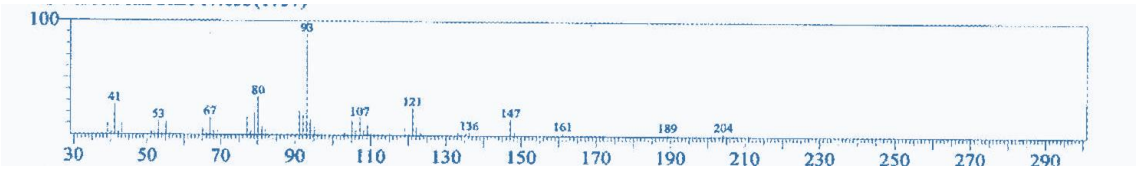

Figure 6. Mass spectrum of α-humulene

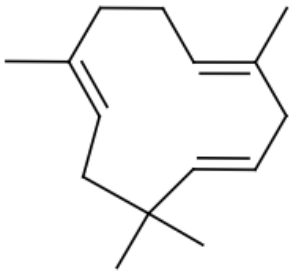

Figure 7. The structure of α-humulene

α-Humulene showed 9.41% in *Z. amaricans* BL oil, 8.36% in *Z. aromaticum* Val. oil and 6.52% in *Z. zerumbet* (L.) Smith oil, respectively. The relative mass of α-humulene is 204. The structural formula of α-humulene is $C_{15}H_{24}$. The IUPAC name of α-humulene is (1E,4E,8E)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene. The mass spectrum and the structure of α-humulene can be seen in Figures 6 and 7. α-Humulene is also known as α-caryophyllene. α-humulene extracted from *Salvia officinalis* essential oil inhibits tumour cell growth. S. officinalis essential oil was obtained by hydro-

distillation and fractionated with column chromatography. GC-MS evaluated the essential oil and its fractions. The cytotoxic effect was analyzed in cellular lines of breast cancer MCF-7, colon cancer HCT-116, and murine macrophage RAW264.7 cell lines by the MTT test. The sub-subfraction F1.1.1 of *Salvia officinalis* essential oil containing α-humulene presented the largest effect on RAW264.7 and HCT-116 with IC_{50} values of 41.9 and 77.3 mu g/ml, respectively [16].

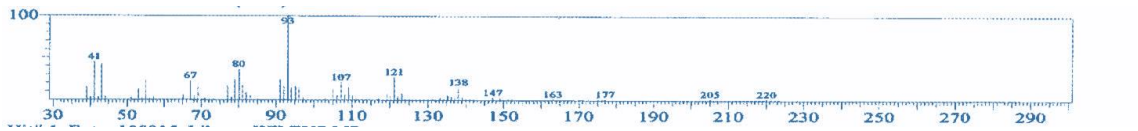

Figure 8. Mass spectrum of Figure 4. Mass spectrum of β-selinene

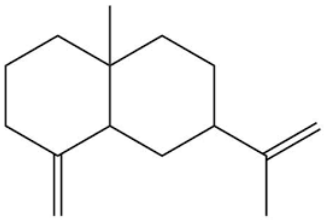

Figure 9. The structure of β-selinene

β-Selinene presented 4.39% in *Z. amaricans* BL. oil, 7.55% in *Z. aromaticum* Val. oil and 4.23% in *Z. zerumbet* (L.) Smith oil, respectively. The relative mass of β-selinene is 204. The structural formula of β-selinene is C_{15} H_{24}. The IUPAC name of β-selinene is (3*R*,4*aS*,8*aR*)-8*a*-methyl-5-methylidene-3-prop-1-en-2-yl-1,2,3,4,4*a*,6,7,8-octahydronaphthalene. The mass spectrum and the structure of β-selinene can be seen in Figure 8. and Figure 9. β-selinene was the major component of *Callicarpa macrophylla* leaves essential oil (37.51%), *Callicarpa macrophylla* pre ripe seeds and fruits oils (44.66%) and *Callicarpa macrophylla* ripe seeds and fruits oils (57.01%), respectively [17].

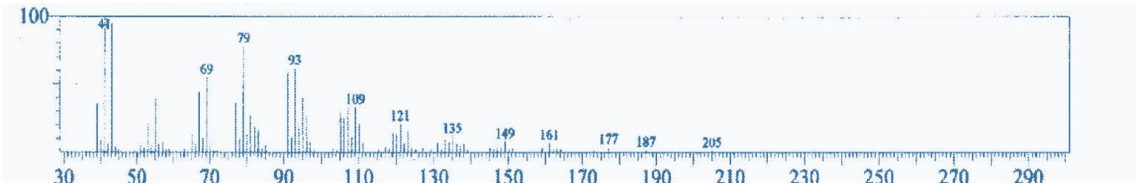

Figure 10. Mass spectrum of caryophyllene oxide

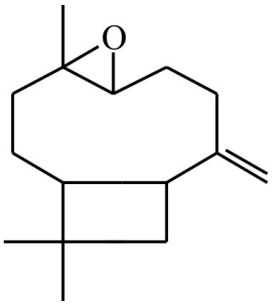

Figure 11. The structure of caryophyllene oxide

Caryophyllene oxide indicated 2.09% in *Zingiber amaricans* BL oil, 2.49% in *Z. aromaticum* Val. oil and 1.96% in *Z. zerumbet* (L.) Smith oil respectively. The relative mass of caryophyllene oxide is 220. The structural formula of caryophyllene oxide is $C_{15}H_{24}O$. The IUPAC name of caryophyllene oxide is 4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.04,6]dodecane. The mass spectrum and the structure of caryophyllene oxide can be seen in Figures 10. and 11. Caryophyllene oxide has potent cholinesterase inhibitory activities and antioxidant properties [18].

CONCLUSION

Fourteen, fifteen and twelve components were identified in bitter ginger, fragrant ginger and shampoo ginger essential oils rhizomes, respectively. The compositions of all essential oils varied qualitatively and quantitatively. The same components between *Z. amaricans* BL., *Z. aromaticum* Val., and *Z. zerumbet* (L.) Smith were zerumbone, α-humulene, β-selinene dan (-)-caryophyllene oxide. The most mayor constituent in *Z. amaricans* BL., *Z. aromaticum* Val., and *Z. zerumbet* (L.) Smith oils were zerumbone. *Z. zerumbet* (L.) Smith oil presented the highest quantity of zerumbone (77.62%).

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