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Original Article

First report from Indonesia, phytochemical composition of essential oils from leaves and fruits of *Zanthoxylum avicennae* (Lam.) DC.

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Abstract

Zanthoxylum avicennae (Lam.) DC. [Karangean] is a widely distributed plant in Southeast Asia and has potential in the pharmaceutical and food flavour industry with fruits and leaves rich in essential oils. The plant materials of *Z. avicennae* (Lam.) DC. [Karangean] were a plant collection at Bali Botanical Garden which collected from West Nusa Tenggara, on June 15th 1993. The essential oils were extracted by hydrodistillation from fresh fruits and leaves of *Z. avicennae* and the phytochemical composition were analysed by Gas Chromatography-Mass Spectrometry (GC-MS). The yields of essential oils were 2.75% from fruit oil and 1% from leaf oil. Respectively, a total of 41 compounds were identified from the fruit oil and 11 compounds from the leaf oil representing 91.1% and 99.81% of the total oils composition. The major compounds of the fruit oil were 1.8-cineol (12.34%), 1-para-menthen-8-yl acetate (6.24%), l-Limonene (6.09%), Cyclopropane (5.06%), Hexane (3.84%), 3-Methyl-2-(2-methyl-2-butenyl)-furan (3,74%) and predominant compouns of the leaf oil were Estragol (87.13%), 1.8-cineol (4.28%), Trans-Anethole (3.51%), Myrtenyl Acetate (0.65%), isopiperitenone (0.62%). The highest contents of these essential oils were monoterpene and sesquiterpene and these componentshad desirable properties for use in industrial companies. Therefore, the essential oils of *Z. avicennae* [Karangean] from West Nusa Tenggara contained chemical compositions which had potential to be developed for various human needs.

Keywords: Essential oil, GC-MS, hydrodistillation, phytochemical, *Zanthoxylum avicennae* (Lam.) DC.

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Introduction

Zanthoxylum avicennae (Lam.) DC [Karangean] is characterized as a small tree to 13 m, diocious, and evergreen, belonging to the *Rutaceae* family. It is distributed mainly in dry forests, thickets and on open slopes at an altitude of up to 1630 m above sea level, in India, China, Taiwan, Cambodia, Laos, Vietnam, Thailand, Malaysia, South Java, Lesser Sunda Islands, the Philippinesand the Moluccas (Cho, 2012; Liu et al., 2014; Chen et al., 2015).

Z. avicennae is locally called Karangean (Java, Indonesia), Ying Bu Bo (Taiwan), pepper, local pepper or small-leaf pepper and used for various purposes by traditional people in many countries (Wu et al., 2017). In China, *Z. avicennae* [Karangean] is an important folk medicine plant for rheumatism, abdominal pain, jaundice, chronic hepatitis and the common cold (Cho et al., 2012; Liu et al., 2014). Leaves, fruits and seeds are also used as spices and flavouring agents (PROSEA, 2016). While in Vietnam, people use a decoction of *Z. avicennae* [Karangean] stems in folk medicine as a digestive tonic (Dai et al., 2012). In Java, the leaves and fruits are applied as flavour; and the hard, dense, heavy reddish wood of *Z. avicennae* [Karangean] is used to make small tool handles (PROSEA, 2016).

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Like other species of the genus *Zanthoxylum*, *Z. avicennae* [Karangean] contains some chemical components essential oil and extract which has a high potential for pharmaceutical, anticancer, pesticide, spice, and the food flavor industry (Chen et al., 2015). The volatile leaf oil and extract of *Z. avicennae* [Karangean] had strong anti-cancer (Dung et al., 2013; Wu et al., 2017), antibacterial and antifungi activity on plant pathogenic fungi (Lin et al., 2014; Zeng et al., 2015). While its fruit essential oil contained citral, 1-octanol, 4-methyl-6-acetoxyhexanal and linalool which show strong antifungi activities (Chen et al.1990). Other studies reported that several chemical components, especially Diosmetin and Methyl4-hydroxybenzoate were isolated from leaves of *Z. avicennae* [Karangean] (Cho et al., 2012), Neolignans, a coumarinolignan, lignan derivatives, a chromenewere extracted from the stem wood of *Z. avicennae* [Karangean] (Chen at al., 2008) and 8-Formylalloxanthoxyletin, Alloxanthoxyletin and Xanthoxyletin which were extracted from the stem bark of *Z. avicennae* [Karangean] (Chen et al., 2015) had high potential to be developed for the prevention and treatment of various inflammatory diseases.

Some previous research on the chemical contents of *Z. avicennae* [Karangean] extract and essential oil from different geographic regoins had been frequently reported, for example from Vietnam (Dai et al., 2012), and China (Cheng et al., 1990, Liu et al., 2014, Zhang et al., 2012, Lin et al., 2014, Zheng et al., 2015), but interestingly, most of the chemical compositions found were significantly different. Indonesia, especially the Lesser Sunda Islands is included in the *Z. avicennae* [Karangean] distribution area and so far, the research of the chemical content of its essential oil and its potential has never been reported. Therefore, this study aimed to collect more information on the chemical composition of essential oils of *Z. avicenna* [Karangean] from Batulanteh Mountain, West Nusa Tenggara, Indonesia.

Methods

Study Area and Plant Materials

The fresh fruits and leaves of *Z. avicennae* [Karangean] were obtained from the Bali Botanical Garden plant collection in March 2016. This plant was collected from natural forest, Batulanteh Mountain, Sumbawa Regency, West Nusa Tenggara, Indonesia (Fig. 1) on June 15th, 1993, and planted at Bali Botanical Garden on December 15, 1993. Bali Botanical Garden is an ex-situ conservation centre for dry highland plants operated by the Indonesian Institute of Sciences (LIPI). This Botanical Garden is located in the mountain region of Bedugul, North Bali, at an altitude of 1250-1450 m above sea level with temperatures ranging between 18-20 °C and 70-90% humidity (Peneng & Andila, 2017). The plant was identified by a taxonomist of Bali Botanic Garden, Mr Ida Bagus Ketut Arinasa, M.Si and the herbarium voucher with number Put 55 was deposited in the herbarium of Tabanan Hortus Botanicus Baliense (THBB).

Figure 1. Location of Batulanteh Mountain, Sumbawa Regency, West Nusa Tenggara, Indonesia indicating the sampling sites of *Zanthoxylum avicennae* [Karangean] (Google Maps 2018).

Instrumentation

Water hydrodistillator trademark "Pudak Scientific Apparatus" was used to extract the essential oil. GC-MS equipment model Shimadzu GCMS-QP2010 was used to analyse the phytochemical composition of the essential oil.

Isolation of Essential Oil

Respectively, 100 g of fresh leaves and fruits of *Z. avicennae* [Karangean] were extracted by hydrodistillation for five hours using a Pudak Scientific apparatus. The aqueous emulsions were separated by a Duran Schott separator and produced a yellowish viscous essential oil. Duran Schott separator was used for separating hydrosols and essential oil after the distillation process. The distillate was poured into the Duran Schott separator and then the hydrosols and essential oil would separate passively due to the differential in density between the hidrosol (water) and the essential oils. The essential oil would be floating on the top of the liquid and and the hydrosol at the bottom. The hydrosol was flowing through the perforation when the faucet was opened. A cup could be placed below the separator to capture the dripping hydrosol. Then the essential oils were pipetted using a micropipette (Gilson Pipetman) through a hole in the top of the separator glass and inserted into a dark glass tube.

Determination of Extraction Yield (% yield)

The essential oil yields (%, w/w) from all fresh materials were calculated according to modification of Negreiros et al. (2015) method, as:

$$Yield\ (\%) = \frac{W1 \times 100}{W2}$$

Where W1 is the weight of the essential oil after hydro distillation, and W2 is the weight of the fresh plant material.

GC-MS Analytical Condition and Identification of Essential Oil Component

The chemical components of *Z. avicennae* essential oil were analysed using GC-MS equipment model Shimadzu GC-MS – QP2010 with the condition: The chromatopraphic column is Rtx 5ms, capillary column 60.0 m x 25 mm with 0.25 µm thickness. The carries gas is Helium UHP and detector is MS (Mass Spectrometer). The GC Setting: Column oven temperature is 50 °C for ±5 mins then raise the temperature to 280 °C. Injection temperature is 280 °C, Injection mode is split with total program time 50 mins. Its flow control mode is linear velocity, pressure is 101.0 KPa, total flow is 46.5 mL/min, column flow is 0.85 mL/min, linear velocity is 23.7 cm/sec, purge flow is 3.0 mL/min, split ratio is 1: 50 and its total sample injection is 1 µL. The mass spectrum (MS) settings : its ion source temperature is 200 °C, interface temperature is 280 °C, solvent cut time is 1.5 mins and its detector temperature is 280 °C.

Data Analysis

The compounds were identified by mass spectra fragmentation patterns and their identity was approved by comparing their retention time indices and their spectral data with those from computer library WILEY7.LIB and open published literature.

Results

Hydro distillation of the leaves and fruits of *Z. avicennae* [Karangean] harvested from *Batulanteh Mountain*, Sumbawa Regency, West Nusa Tenggara, Indonesia produced essential oils with the yields 1% (v/w) and 2.7% (v/w) according to fresh material. Both of the essential oils were yellowish and revealed the scent of citrus. The results of GC-MS analyses obtained from fruit and leaf essential oils were shown in Table 1 and 2. While the GC-MS chromatograms of them were performed in Figure 2A and 2B. Respectively, in all 41 chemical compounds were identified from the fruit essential oil of *Z. avicennae* [Karangean], representing 91.01% of the total fruit oil (of the total peak areas in chromatogram of fruit essential oil) and 11 chemical compounds from leaf essential oil, accounting for 99.81% of the total leaf oil (of the total of peak areas in chromatogram of leaf essential oil). These values were obtained by summing the relative concentrations of each component identified on the chromatograms. The percentage of relative concentration (for one component) is a quantitative analysis of chromatogram by comparing a peak area in the chromatogram with the total area of the peaks.

The main compounds of the fruit essential oil of *Z. Avicennae* [Karangean] were 1,8-cineol (12.34%), 1-p-menthen-8-yl acetate (6.24%), l-limonene (6.09%), cyclopropane (5.06%), hexane (cas) n-hexane (3.84%), 3-methyl-2-(2-methyl-2-butenyl)-furan (3.74%). While the predominant chemical components in the leaf oil were estragol (87.13%), 1,8-cineol (4.28%), trans-anethole (3.51%), myrtenyl acetate (0.65%), isopiperitenone (0.62%). The major components found in fruit and leaf essential oil of *Z. avicennae* (Karangean) were different, but some materials such as 1,8-Cineole, Isopiperitenone, Myrtenyl Acetate, β-elemene, 1-para-menthen-8-yl acetate were present in both of them.

Table 1. The result of GCMS analyses obtained from the fruit essential oil of *Zanthoxylum avicennae from Batulanteh Mountain,* West Sumbawa Regency,West Nusa Tenggara, Indonesia

Peak#	Compounds of the fruitessentiall oils of *Z. avicennae*	R.Time	SI	Relative Conc. %
	Monoterpene			
1	l-Limonene	12.494	96	6.09
2	1,8-Cineole	12.559	96	12.34
3	Alloocimene	13.345	93	1.16
4	Terpinen-4-ol	14.541	95	0.93
5	Terpineol	14.696	96	1.29
6	Myrtenil Acetate	16.018	96	0.72
7	Artemisia ketone	21.777	85	3.48
	Total of Monoterpene			**26.01**
	Triterpen			
8	Squalene	30.27	87	0.56
	Sesquiterpene			
9	β-Elemene	16.688	88	2.1
10	trans-Caryophyllene	17.057	95	1.26
11	α-Humulene	17.367	92	1.12
	Total of Sesquiterpene			**4.48**
	Other Compounds			
12	Myristic acid	19.508	74	0.66
13	Palmitic acid	20.876	94	2.97
14	n-Hexatriacontane	26.193	97	1.9
15	n-Tricosane	29.319	95	1.17
16	Cyclopropane	2.31	99	5.06
17	3-Methylpentane	3.281	95	0.73
18	n-Hexane	3.441	97	3.84
19	Ethyl acetate	3.742	98	2.82
20	Methylcyclopentane	3.842	96	3.58
21	3-Methyl-2-(2-methyl-2-butenyl)-furan	15.048	83	3.74
22	3,5-Heptadienal, 2-ethylidene-6-methyl-	15.15	82	0.67
23	(+)Isopiperitenone	15.625	81	2.41
24	1-para-menthen-8-yl acetate	16.229	96	6.24
25	Viridiflorene	18.132	77	0.76
26	2-Nitro-2-(3-oxobutyl) cycloheptanone	18.908	82	0.81
27	(-)-Caryophyllene oxide	19.1	79	1.06
28	Spathulenol	19.602	84	1.02
29	Eicosane, 2-phenyl-	19.882	72	0.6
30	2-Heptadecanone	20.452	97	1.32
31	7-Decen-2-one	21.702	85	1.5

32	Heptadecene-(8)-Carbonic Acid-(1)	22.149	92	1.91
33	2-Decene, 2,4-dimethyl-	22.271	82	1.93
34	2,4-Dinitro-6-(2-Butyl)-Phenyl-.beta.,.beta.Dimethylacrylsaeureester	22.702	88	1.88
35	6-Nitro-cylohexadecane-1,3-dione	22.895	83	1.34
36	n-Tricosane	23.174	94	0.87
37	n-Tetracosane	24.033	96	1.18
38	n-Pentatriacontane	25.024	97	1.74
39	Dioctyl phthalate	25.996	93	0.72
40	n-Hexatriacontane	31.425	87	2.21
41	n-Heptacosane	37.288	89	3.32
	Total of other compounds			**59.96**
	Total compounds			**91.1**

Note: R.Time: Retention Time, Conc. %: Concentration of each detected compounds of a total of essential oil, SI: Similarity Index

Table 2. The result of GCMS analyses obtained from leaf essential oil of *Zanthoxylum avicennae* [Karangean] *from Batulanteh Mountain*, West Sumbawa Regency,West Nusa Tenggara, Indonesia

Peak#	Compounds of the leaf essential oils of *Z. avicennae*	R.Time	SI	Relative
	Monoterpene			
1	1,8-Cineole	12.571	97	4.28
2	α-Pinene	10.874	98	0.51
3	α-Ocimene	12.7	96	0.48
4	Estragol	15.013	85	87.13
5	8 isopropylidenebicyclo	15.133	83	0.45
6	Myrtenyl Acetate	16.038	97	0.65
	Total of Monoterpene			**93.50**
	Sesquiterpene			
7	β-elemene	16.704	91	**1.18**
	Fenil Propanoid			
8	Eugenol	16.411	97	0.45
	Other Compounds			
9	Trans-Anethole	15.725	98	3.51
10	1-para-menthen-8-yl acetate	16.242	94	0.55
11	Isopiperitenone	15.654	79	0.62
	Total of other compounds			**5.13**
	Total compounds			**99.81**

Note: R.Time: Retention Time, Conc. %: Concentration of each detected compounds of a total of essential oil, SI: Similarity Index

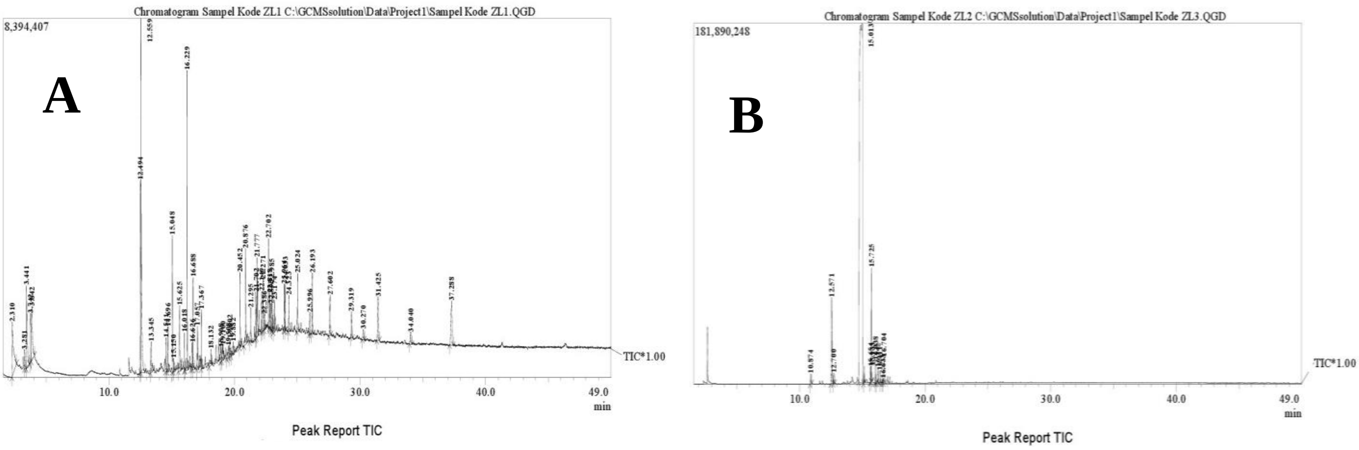

Figure 2. GC-MS chromatogram of *Zanthoxylum avicennae* [Karangean] *from Batulanteh Mountain*, West Sumbawa Regency, West Nusa Tenggara, Indonesia, A. fruit essential oil, B. Leaf essential oil

Discussions

The phytochemical compositions of the *Z. avicennae* [Karangean] essential oils *from Batulanteh Mountain*, Sumbawa Regency,West Nusa Tenggara, Indonesia were shown in Table 1 and 2. When comparing these results with chemical compounds of essential oil of *Z. avicennae*, [Karangean] from different area and condition, completely various compositions were obtained. In Vietnam, it had been reported that the highest contens of the lead essential oil of *Z. avicennae* [Karangean] were β-caryophyllene (17.01%), α-pinene (10.07%), β-phellandrene (9.42%), γ-terpinene (4.53%), (E)-β-ocimene (3.87%), α-terpinene [Karangean] (3.09%) and α-humulene [Karangean] (10.38%) (Dai et al., 2012). In China, a complicated results were found. In this country, the essential oil of *Z. avicennae* [Karangean] have been studied by several researchers because of its biological properties potential and varying chemical composition.

Cheng et al. (1990) found and published that the major compounds of fruit essential oil of *Z. avicennae* [Karangean] harvested from Yunnan Province, were α-pinene (16%), sylvestrene (50%) and octanal (8.7%) and this result was rather discrepant with our result. While Liu et al (2014) reported that that the fresh aerial parts of *Z. avicennae* [Karangean] from Qujing City, Yunnan Province contained β-elemene (6.13%), 1,8-cineol (53.05%), α-caryophyllene (5.96%), β-caryophyllene (5.96%), caryophyllene oxide(4.59%), Humulene oxide II (3.42%), α-terpineol (3.20%), linalool (3.28%) as major compounds. Zhang et al. (2012) reported that in Hainan Province, linalool (24.36%), β-elemene (12.03%), (E)-2-hexen-1-ol (11.73%), caryophyllene oxide (10.84%) were the main compounds in the leaf essential oil of *Z. avicennae* [Karangean]. By comparison, this result was significant difference with the subsequent study which showed that the major components of the leaf essential oil of *Z. avicennae* [Karangean] grown in Sanya City, Hainan Island, Hainan Province, were caryophyllene oxide (1.58%), β-phellandrene (23.33%), 2-methox1-3-(2-propenyl)-phenol (42.94%), 1,2-dimethoxy-4-(2-propenyl)-benzena (1.40%), 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl-[is-(1a,2a, 4a)-Cyclohexane(6.20%),1,1,4,8-tetramethyl-cis,cis,cis-4, 7,10-cyvloundecatriene (8.98%) (Lin et al., 2014). These results confirmed that the qualitative and quantitative chemical compounds of essential oil could be effects of some factors, such as geographical location, the climate conditions, nature of the soil, harvest season, the parts of plant, the age of plant organs, and time of collecting (Ben Marzoug et al., 2011; El-Akhal et al., 2014; Pirbalouti et al., 2015).

However, it has been known that the chemical component of plants essential oils were affected by various factors, among others: diversity of species (Purushothman & Ravi, 2013), differences of geographical location, season, and climate (Mahzooni-Kachapi et al., 2014; Shams et al., 2016; Sanli et al., 2017), various parts of plant (stem, flower, fruit) (Faidi et al., 2014), and differences stage of plant growth (Flamini et al., 2013; Faidi et al., 2014) and other factors.

Several researches have shown that environmental and genetic parameters significantly affect the biosynthesis of plants secondary metabolites, including the essential oils contents. The previous study found that salinity changed the chemical composition of leaves essential oil of coriander grown in hydroponic and water stress caused the reduction of its essential oil content (Shams et al., 2016). While Mahzooni-Kachapi et al. (2014) and Sanli et al. (2017) reported that the change of altitude seems to be an important factor for yielding the chemical profile of K. anatolica essential oils and Stachys lavandulifolia Vahl by affecting the terpenoid biosynthesis.

An analysis of essential oil chemical component from *Z. avicennae* [Karangean] revealed that the highest content of those is monoterpene and sesquiterpene. This research showed that *Z. avicennae* [Karangean] harvested from West Nusa Tenggara, Indonesia contained monoterpene and sesquiterpene as the highest compound. The fruit oil comprised 26.01% monoterpene and 4.48% sesquiterpene, while leaf oil contained 93.54% monoterpene and 1.18% sesquiterpene. By comparison, the result is similar to the previous reports in which monoterpene and sesquiterpene were the most abundant contents. The stem and leaf oil of *Z. avicennae* [Karangean] from Yunnan Province comprised 65.70% monoterpene and 33.45% sesquiterpene (Liu et al., 2014), while the leaf oil *Z. avicennae* [Karangean] from Nghean Province, Vietnam contained 44.3% monoterpene and 40.75% sesquiterpene (Dai et al., 2012).

In conclusion, the fruit and leaf essential oil of *Zanthoxylum avicennae* (Lam.) DC [Karangean], collected from West Nusa Tenggara, contained the various chemical components. The dominant contents of those are monoterpene and sesquiterpene. The fruit essential oil contained 26.01% monoterpene and 4.48% sesquiterpene, while the leaf essential oil comprised 93.50% monoterpene and 1.18% sesquiterpene. The data showed the existence of different composition for this species compared to previous reports.

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