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Research Article

The Essential Oils Constituent of *Etlingera flexuosa* (Zingiberaceae), An Endemic Plant from Central Sulawesi

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ABSTRACT

Introduction: The essential oils from rhizomes, pseudostems, and leaves of *Etlingera flexuosa,* an endemic ginger species of Sulawesi, were studied. **Methods:** The essential oils were extracted using solvent extraction and hydrodistillation methods and analysed by gas chromatography-mass spectrometry (GC-MS). **Results:** The percentage yield of volatile compounds obtained from solvent extraction method was higher than hydrodistillation. By solvent extraction, sesquiterpenes were found abundantly in rhizomes, pseudostems, and leaves with the percentage of 34.16%, 35.20% and 32.70%, respectively. The rhizome and pseudostems were found to contain spathulanol with the high percentage of 3.91% and 3.46%. Meanwhile, by hydrodistillation, the compounds were dominated by fatty acid compunds with the percentage of 82.26%, 82.79% and 76.1% on rhizome, pseudostems and leaves. Glycerol tricaprylate has the high percentage of 66.76% and 82.12% on rhizome and pseudostems, while in leaves, fatty acids 1,2,3-propanetriyl ester-decanoic acid was the highest with the percentage of 61.81%. Sesquiterpenes was the second with the percentage of 10.36%, 11.15%, and 11.61% in rhizomes, pseudostems, and leaves, respectively. **Conclusion:** The most essential oils of *E. flexuosa* was obtained from the solvent extraction method.

Key words: *Etlingera flexuosa,* Essential oils, GC-MS, Extraction, Hydrodistillation.

INTRODUCTION

The genus *Etlingera* Giseke (named after a German botanist Andreas Ernst Etlinger, 1730-1790), is a herbaceus flowering plant in the family *Zingiberaceae* with more than 150 species worldwide.[1,2] The genus is distributed from India, Indo-China throughout Malesia to the Pacific Islands.[1,3] There were 74 species of *Etlingera* in Malesia region, including 12 species in Malay Peninsula and 29 species in Borneo.[4] In 2012, Poulsen described 48 species *Etlingera* of Sulawesi based on his revision work but the number of the species have increased significantly in recent years, especially in Sulawesi, Indonesia.[2] So far, there are 52 species of *Etlingera* in Sulawesi, including several number new species such as; *Etlingera mamas arum*,[5] *Etlingera tjiasmantoi*[6] and *Etlingera comosa*.[2]

Like many other species of Zingiberaceae which have valued as ornamentals or employed as raw materials in the production of fibre, paper, dyes, foods, spices and perfumes,[7] *Etlingera flexuosa* AD Poulsen, locally known as "karondo" (*Topo Baria lang*. Poso region, central Sulawesi), is an endemic ginger species of Sulawesi that has been used traditionally by local community for different purposes in central Sulawesi.[8] The fruits are utilized for cooking fish dishes and as flavor enhancers, young leaves are eaten raw or boiled as vegetable whereas its rhizomes are being used as medicine while the leaves are utilized to roof huts.[8] In the prior study, it is reported that ethanol extract of *E. flexosa* rhizome has antimicrobial properties to inhibit the grow of *Candida albicans*,[8] *Staphylococcus aureus* and *Escherchia coli*.[9] Besides, the ethanol extracts of the leaves, pseudostems, and rhizomes parts of the species has antiviral activity of HIV-infected MT-4 cells.[10]

Essential oils were reported to be abundantly in Etlingera species. Studies from Wong *et al* (2010), showed that mono- and sesquiterpenoids were the major constituents from the leaf, rhizome, and root of *E. Elatior* such as myrcene, α-humulene, β-caryophyllene, camphene and β-pinene. Meanwhile, phenylpropanoids (E)-methyl isoeugenol was dominated in the leaf, rhizomes and roots of *E. Littoralis.*[11] Lechat-Vahirua *et al* (1993) reported that rhizome of *Etlingera cevuga* contain more oils of methyl eugenol and (*E*)-methyl isoeugenol.[12] Monoterpenes were the richest components in *E. sphaerocephala* var. *grandiflora* rhizomes, stem, leaves and whole plant.[13] *Etlingera punicea* was also reported to contain more phenolic compound of methyl chavicol.[14] Therefore, this study aims to examine the essential oils constituents of the leaf, stem and rhizome of *E. flexuosa* that has not been reported yet.

MATERIALS AND METHODS

Plant materials

The plant sample was collected in July 2022 from the montane forest of Lore Lindu National Park (LLNP), at the altitude 1500 – 1700 m asl., near Sedoa village, Poso regency Indonesia. Plant material (Figure 1) was identified by the author (Ramadanil Pitopang). Voucher specimen (RP. 10041) was deposited at Herbarium Celebense (CEB) and the Laboratory of Plant Biosystematics, Department of Biology, Tadulako University.

The natural habitat of *E. flexuosa* is a protected area that usually receives an annual rainfall of about

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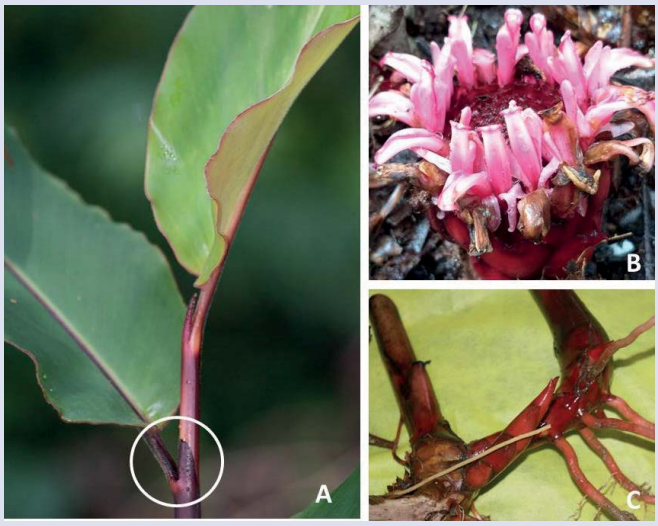

Figure 1: *Etlingera flexuosa* Poulsen, A. Leaves with ligule (in white circle), B. (flower) and C. Rhizome.

1500-2000 mm, with mean maximum temperature of 30-32°C, while the mean minimum is usually around 18-21°C, and average of relative humidity of about 59.62 - 81.74%.[15] LLNP is a protected area with about 220,000 ha of land and an habitat to a number of Sulawesi's endemic plants and animals,[8] macrofungus[16,17]) and it plays important roles in various functions such as; education and ecotourism destination[18] (and as watershed protection.[19]

Hydrodistillation

The plants were washed thoroughly under tap water in order to remove insects etc., and placed in boxes for transportation. In the laboratory, the leaves, pseudo stem and rhizome were washed in running tap water cut into small pieces and again washed, soaked in running tap water for five minutes. The essential oils were extracted by hydrodistillation using a vertical hydrodistillation unit. A round-bottomed flask containing the homogenate was heated during 24 h and the vapor condensed and separated throughout an auto-oil/water separator. Each essential oil extraction was running in triplicate. Yield percentages were recorded as dry basis material.

Extraction

Each part of the plant (leaves, pseudostems, and rhizomes) was washed in running tap water, then cut into small pieces and dried at room temperature with no direct sunlight. After drying, about 2.0 kg of each part of the plant was extracted by maceration using 5 L of 96% ethanol for 3 × 24 h. The maceration was repeated three times, and then the filtrates were filtered and evaporated in a rotary evaporator to obtain the viscous extract. The oil was stored in dark vials at 4°C before analyzing. The waste or residue after extracted by petroleum ether of plant materials was repeated once with ethanol, called secondary extraction, similar to the above condition.

GC-MS analysis

The GC-MS analysis of sample extracts was carried out on a Shimadzu QP-2010 Gas Chromatograph Mass Spectrometer (GC-MS) Ultra, which is equipped with Autosampler AOC-20i and SH-Rxi-5Sil MS capillary column (30 m - 0.25 mm x 0.25 µm) using Helium as carrier gas (1.0 mL/ min). The column temperature program was set as follows: an injection temperature of 250^0 C, splitless mode, a column oven temperature of 70^0 C at the beginning and held for 2 min, then ramped to 200^0 C at the rate of 10^0 C/min and end temperature 280^0 C and held for 9 min at the rate 5^0 C/min, an MS ion source temperature of 200^0 C, and an interface temperature of 280^0 C. The spectra for each of the chromatogram peaks were compared with the database library in the NIST and Willey.

RESULTS AND DISCUSSIONS

Etlingera flexuosa is one of endemic Zinger plant of Sulawesi Indonesia. It has used traditionally as medicine,[20] because its high concentration of phytochemical constituent such as Flavonoid, Tannin, Saponin and Terpenoid.[20] The essential oils of this plants, that might be responsible for the biological activity, was not reported yet. However, the other species such as *E. elatior* was reported by many studies to contain essential oils. β-pinene, caryophyllene, myrcene, α-humulene, and (E)- β-farnesene were reported from the leaves. 1,1-dodecanediol diacetate and (E)-5-dodecane were the major compounds from the stem, 1,1-dodecanediol diacetate and cyclododecane were the major compounds on rhizome and flowers.[11,21]

In this study, the results and characteristics (appearance and olfactory) of *Etlingera flexuosa* essential oil are presented (Table 1). The yield of essential oils obtained by solvent extraction were 4.1% (rhizome), 8.6% (pseudostems) and 19.9% (leaves). Meanwhile, by using hydrodistillation method was 1.763% for the rhizome, 0.381% for pseudostem and 0.290% for leaves. The specific gravity for the three types of plant organs was 0.813 g/ml, 0.975 g/ml and 0.713 g/ml respectively. There are 39 difference kind of compounds that are classified as essential oils from the total 69 compounds that were analyzed using the hydro distillation method for rhizome organs. 24 out of 40 total of compounds on pseudostem organs and 58 out of 67 total on leaves organs. While the essential compound as the result of extraction using 96% ethanol solvent there are as many as 59 types of the total 77 compounds contained in the leaf organ, 54 out of total 103 on pseudostem and 76 types out of 120 total compounds on rhizome organs.

Essential oils compounds of *Etlingera flexuosa* Poulsen

Essential oils of *E. flexuosa* was obtained from two extraction methods: hydrodistillation and 96% ethanol solvent extraction. The essential oils were abundantly consisting of monoterpenes and sesquiterpenes. By solvent extraction, sesquiterpens was the first with the high percentage of 34.16%, 35.2% and 32.7% on rhizome, pseudostems and leaves, respectively. Then, monoterpenes were the second with the percentage of 21.73%, 12.23% and 24.32%. Diterpenes and triterpenes were also detected with low percentage. Non-terpenes and fatty acid compounds were also found from the rhizome, pseudostems and leaves with the percentage of 16.32%, 30.28%, and 17.41%, respectively (non-terpenes) and 21.3%, 12.32% and 4.74%, respectively (fatty acids). By hydrodistillation, volatile compounds on rhizome, pseudostems and leaves part of *E. flexuosa* were dominated by fatty acid compounds with the high percentage of 82.26%, 82.79% and 76.1% and continued by sesquiterpenes with the percentage of 10.36%, 11.15%, and 11.61%, respectively. Monoterpene, diterpene and non-terpenes compounds were detected in low percentage (Figure 2).

Monoterpenes and sesquiterpenes compounds that identified by GC-MS in rhizomes, pseudostems and leaves by solvent extraction and named according to database of NIST and Wiley were 11 compounds. The compounds are pinocarveol or bicycle [3.1.1] heptan-3-ol, 6,6-dimethyl-2-methylene- (1), d-verbenol (2), 7-oxabicyclo[4.1.0] heptan-2-one, 6-methyl-3-(1-methylethylidene (3), selina-6-en-4-ol (4), trans-nerolidol (5), 1,4,4,7a-tetramethyl-2,4,5,6,7,7a-hexahydro-1h-indene-1,7-diol (6), beta-copaen-4.alpha.-ol (7), δ-Guaijene or 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-azulene (8), cubenol (9), δ-Cadinol or 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-1-naphthalenol (10), and 10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienyl acetate (11)

Table 1: The yield and characteristics (Appearance and olfactory) of essential oils from *Etlingera flexuosa* Poulsen.

Methods / Plant organ	No. of EO Compound identified	Others	Total	Yield (%)	Specific gravity (g/ml)	Appearance and olfactory characteristics
Solvent extraction						
Rhizomes	76	44	120	4.1	ND	brown, pungen odor
Pseudostems	54	49	103	8.6	ND	brown, pungen odor
Leaves	59	18	77	19.9	ND	brown, pungen odor
Hydrodistilation						
Rhizomes	39	30	69	1.763	0.813	Dark brown, sharp pungen odor
Pseudostems	24	16	40	0.381	0.795	Light brownish, pungen odor
Leaves	58	9	67	0.290	0.713	Light yellow, sweet pungen odor

ND: Not determined

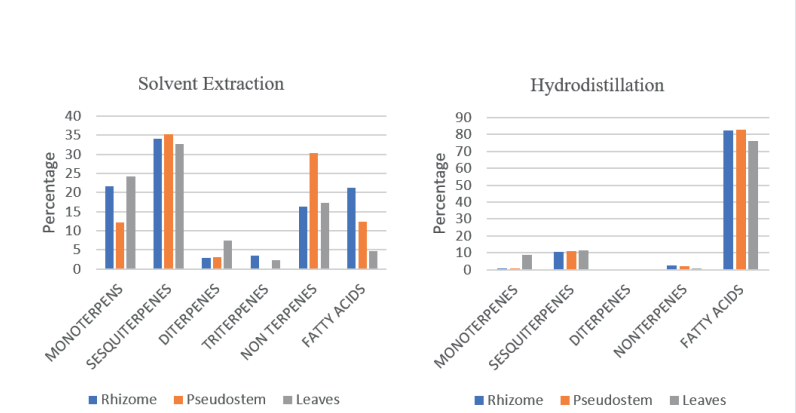

Figure 2: Diversity of volatile oils on *E. flexuosa* obtained from solvent extraction (left) and hydrodistillation (right).

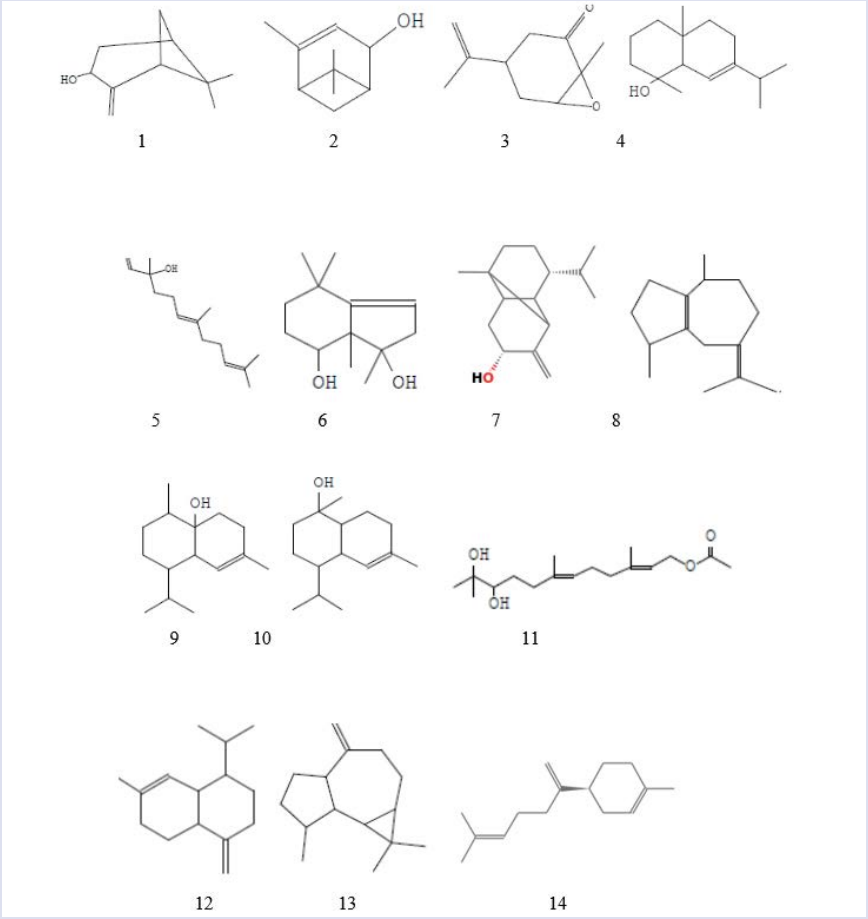

Figure 3: Sesquiterpenes and monoterpenes found in rhizome, pseudostems and leaves on *E. flexuosa*.

(Figure 3). Trans-nerolidol was found in high percentage of 5.29% on pseudostems. Sesquiterpenes spathulanol were also found in high percentage of 3.91% and 3.46% on rhizome and pseudostems, respectively.

The sesquiterpenes type essential oils was about 10-11% in *E. flexuosa* by hydrodistillation. γ-Cadinene (1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene) (12), Spatulenol (decahydro-1,1,7-trimethyl-4-methylene-1h-cycloprop[e]azulene) (13), and β-Bisabolene (1-methyl-4-(5-methyl-1-methylene-4-hexenyl)- cyclohexene) (14), were found in rhizome, pseudostems and leaves. 5-(1,5-dimethyl-4-hexenyl)-2-methyl- 1,3-cyclohexadiene or α-Zingiberene was sesquiterpenes with the high percentage of 5.66% in pseudostems and 2,6-dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1] hept-2-ene or 2-Norpinene was the high in rhizome with the percentage of 4.91%.

The difference of types and amounts of obtained essential oils might be because the different methods of extractions. This study highlighted the most essential oils of *E. flexuosa* was obtained from the solvent extraction method.

CONCLUSION

The percentage yield of volatile compounds from solvent extraction was higher than hydrodistillation. By solvent extraction, sesquiterpenes were found abundantly in rhizomes, pseudostems, and leaves. Meanwhile, by hydrodistillation, the compounds were dominated by fatty acid compunds and then continued by sesquiterpenes compounds.

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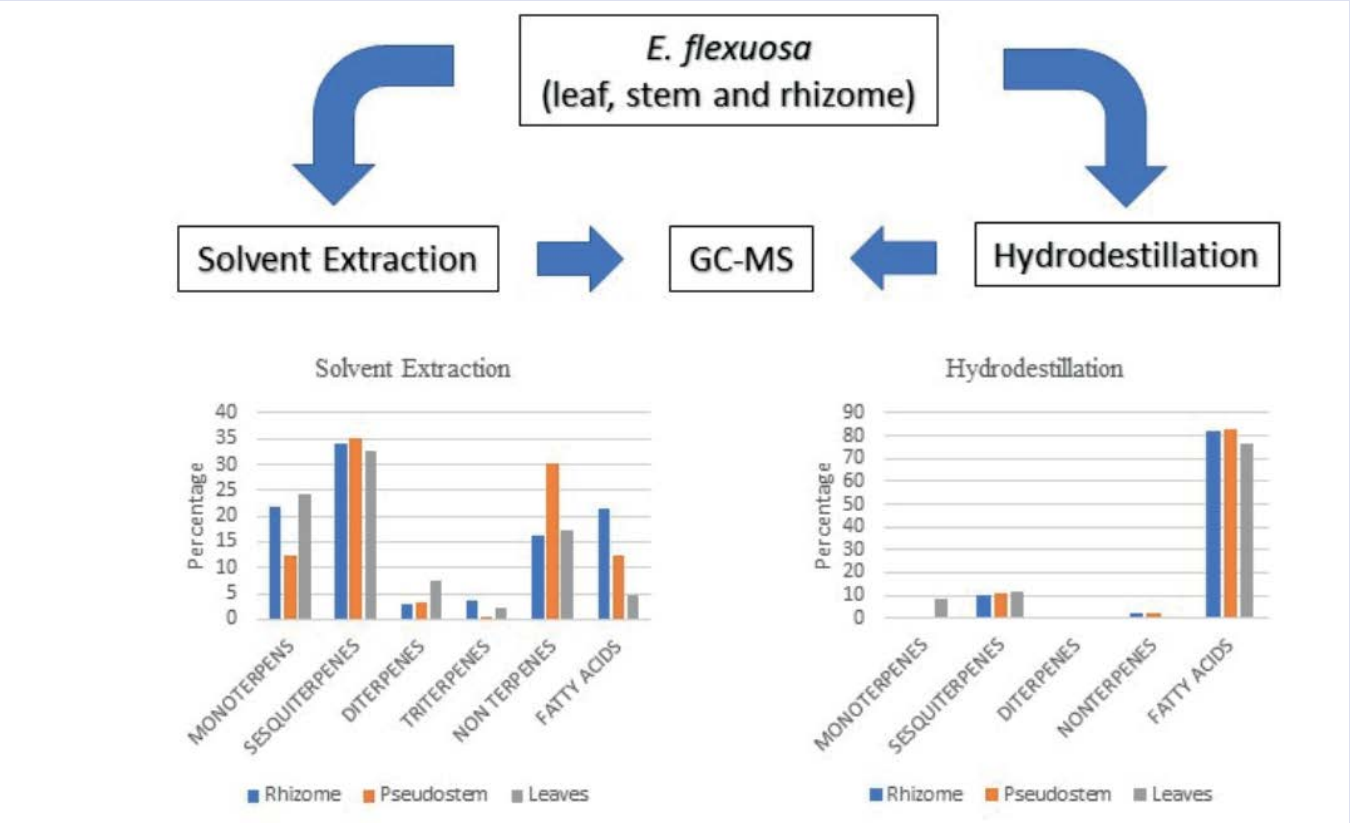
GRAPHICAL ABSTRACT



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