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Short Communications

Essential Oils of *Etlingera acanthodes* A.D. Poulsen, An Endemic Ginger from Sulawesi Island

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ABSTRACT

Etlingera acanthodes A.D. Poulsen is a ginger endemic to Sulawesi, and there is no research on its essential oils. The Essential oil information of *E. acanthodes* is the first reported and has a high novelty. The objective of the study is to analyze the component of *E. acanthodes* essential oils, which are endemic to Sulawesi. The essential oils obtained by hydrodistillation of the leaves, stems, flowers, and rhizomes of *E. acanthodes* A.D. Poulsen. The samples were analyzed by GC-MS method using the Agilent Technologies 7890 Gas Chromatograph with Auto Sampler. Data analysis of essential oils of GC-MS results were determined based on comparing mass spectra from the NIST 2005 v.2.0 library and Wiley 7 library 2003. The oils of *E. acanthodes* contain terpenoids, phenolic, steroids, and other compounds. Phenolic compounds (39.56%) predominate in leaves, other compounds (41.25%) on stems, diterpenes hydrocarbons (77.3%) in flowers, and other compounds (43.5%) and steroids (40.4%) on rhizomes. The five main compounds of *E. acanthodes* are Neophytadiene; (+)-De-O-Methylcentrolobine; Cholest-5-En-3-Ol,23-Ethyl-,(3. Beta.23S)-; 9,12-Octadecadienoic Acid, Phenol, 2-ethyl-. The analysis found several compounds that can be used for industry and medicine in the future.

Etlingera acanthodes A.D. Poulsen, a family of Zingiberaceae, is endemic ginger from Sulawesi. The species is commonly known as "Tikala" in the local community of Central Sulawesi (Kulawi Language) (Poulsen 2012; Pitopang et al. 2019). The fruit of the species is also edible and used in fish dishes and as a flavor enhancer (Pitopang et al. 2019). The distribution of *E. acanthodes* is only known in the mountains at Lore Lindu National Park, around 1000 - 2025 m. Ecology and habitat of *E. acanthodes* in primary lower montane forest, mixed several plants, tend to grow in humid areas and clustered in one population. The genus *Etlingera* is one of the aromatic medicinal plants (Ud-Daula & Basher 2019), which contains essential oils.

The genus *Etlingera* is distributed from India, Indo-China throughout Malesia to the Pacific Islands and consists of more than 100 species (Poulsen 2012; Poulsen & Docot 2018). In Indonesia, research on essential oils in the Zingiberaceae family has mostly been carried out in the genus *Curcuma* and *Zingiber*. In contrast, the genus *Etlingera* has not been widely studied. Only *Etlingera elatior* (Torch Ginger, Kecombrang, or Patikala) essential oils have been analyzed by the GC-MS method (Wong et al. 2010). Also, this species has potential medicine-like antioxidants (Juwita et al. 2018), antibacterial properties (Chan et al. 2011), and anticancer (Krajarng et al. 2017).

Studies on essential oils in other *Etlingera* species from Sulawesi have not been carried out, one of which is *E. acanthodes*. In Sulawesi, Poulsen (2012) reported ± 46 *Etlingera* species and new species (*Etlingera tjiasmantoi*) was published in 2020 (Ardiyani et al. 2020). Therefore, the Essential oil information of *E. acanthodes* is the first reported and has a high novelty. The information can also get potential chemical substances such as antimicrobials, antioxidants, and other potencies like other Etlingera species (Ud-Daula & Basher 2019).

Essential oils from plants are commonly used in several industries such as perfume, medicine, food enhancements, and flavors. Essential oils can also be used as aromatherapy, antiseptics, animal feed supplements, and other industrial products (Ribeiro-santos et al. 2017). Zingiberaceae is a family that produces essential oils that have benefits for human life.

The research aims to determine the content of *E. acanthodes* essential oils in leaves, stems, flowers, and rhizomes. Information on essential oils content in each part of the plant has a different composition. Apart from that, the research also observed the morphological characteristics of *E. acanthodes*.

Plant materials collected from Mt. Nokilalaki, Lore Lindu National Park, Central Sulawesi. Samples were collected along the climbing route at 1000-2025 m. Fresh samples (leaves, stems, flowers, and rhizomes) were taken to the laboratory to analyze the essential oil components using Gas Chromatography-Mass Spectrometry (GC-MS) method. Also, herbarium specimens were taken for further morphological character analysis. To a certain species, morphological observations were made using living collections in the wild and herbarium specimens (ANG_S_007) and described by Muh. Fajri Ramadhan in Herbarium Celebense. Measurements were made using a ruler and microscope.

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted to obtain data on essential oils component in *E. acanthodes* using the Agilent Technologies 7890 Gas Chromatograph with Auto Sampler. The specifications for the tools used consist of columns in the form of HP Ultra; column length 30 x 0.25 (mm) I.D x 0.25 (μm) film thickness. *E. acanthodes* oil was injected into the column using a 0.1 μl GC-MS syringe and carried by helium gas. The column temperature is increased from 80°C (held for 0 minutes) to up to 150°C (held for 1 minute) and ends at 280°C (held for 26 minutes). The mobile phase flow rate is set at 1.2 μ/min, the injector temperature is 250°C, the pressure is 12 kPa, and the injector split ratio is set at 8:1.

Data analysis of essential oil components of GC-MS results is determined based on a comparison of mass spectra from the NIST 2005 v.2.0 library and Wiley 7 library 2003 (Wenqiang et al. 2007; Hossain et al. 2012). The GC-MS analysis result component data were analyzed with the R Stat 3.1.0 software to get the main compounds from each part. Compound grouping based on information on Pubchem NCBI.

The results of the morphological observation indicated that *E. acanthodes* has characteristics stem up to 2 m. Rhizome 1.5-2 cm diameter, 10-20 cm above ground, green-maroon. Leafly shoots up to 2 m- long, with 18-22 leaves. Lingule bilobed, 5 mm long. Lamina sessile, green upper leaf, red lower leaf. Inflorescent arising from rhizome, up to 20 cm long, many flowers. Staminal tube 10-11 mm long, white; labellum ovate, 10-11 x 9-10 mm, pink. lateral lobes erect, covering the stamen, margin undulating; stamen 6 mm long; filament 0.5-1 x 2-2.5 mm, white; anther pink-white; style 2.5 cm long, glabrous; stigma 1.5-1.8 mm wide (Figure 1)

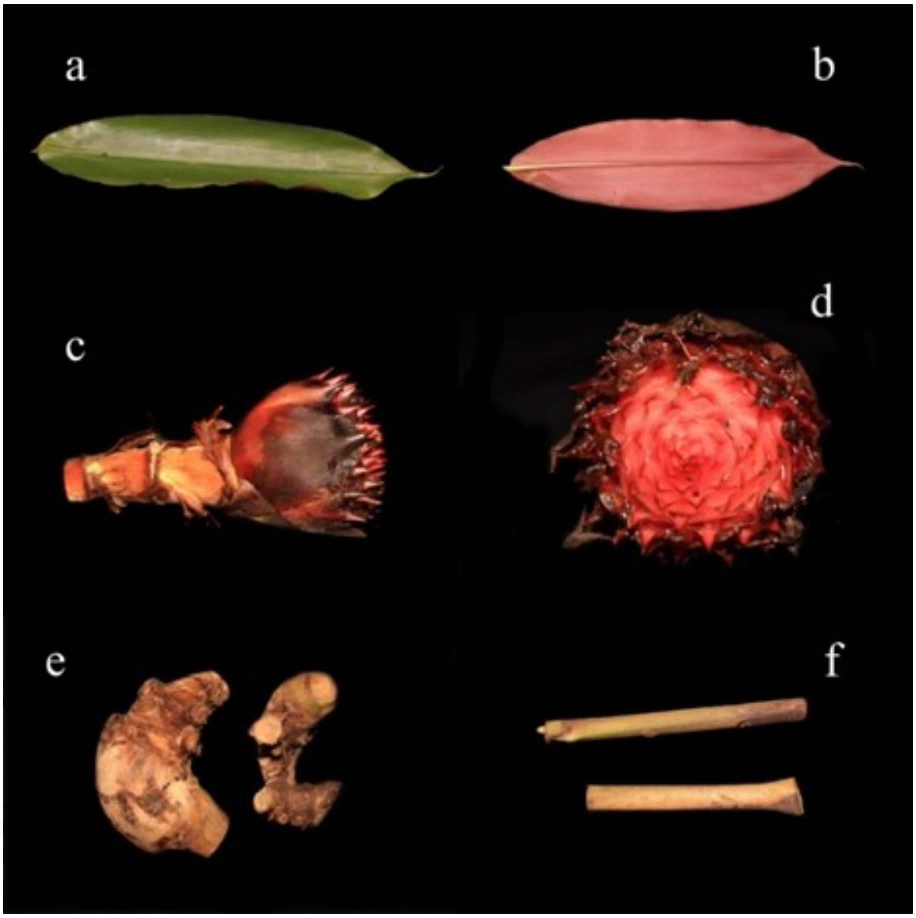

Figure 1. Morphology of *E. acanthodes.* (a) upper surface of leaf, (b) lower surface of leaf, (c) inflorescence, (d) upper view of inflorescence, (e) rhizome, (f) stem

The hydrodistillated oils through GC-MS Analysis obtained data on chemical compound, retention time (RT) and percentage of the essential oils shown in table 1. Retention times were used for preliminary information for the identification of the peak. Primary data at this retention time was used to identify the compound. The GC-MS method is powerful method to identify component of essential oils at the plant. The main compounds are Neophytadiene (10.78% - 33.73%), (+) - De -O-Methylcentrolobine (7.67% - 13.08%), Cholest-5-En-3-Ol, 23-Ethyl-, (3.Beta.23S) - (14.73% - 26.86%), 9,12-Octadecadienoic Acid (1.75% - 21.59%), and Phenol, 2-ethyl- (3.35% - 21.76%), Hexadeconoic Acid (13.03% - 17.77%), (9Z)-9-Tricosene (21.71%), 9-Tricosene (21.32%), and Z-5-Nonadecene (16.9%).

The main compounds in each part of the plant also vary. The main compound in leaves are Neophytadiene (33.73%), Phenol,2-ethyl- (21.76%), and (+)-De-O-Methylcentrolobine (12.96%). The main compounds in stem are 9,12-Octadecadienoic Acid (21.59%), Hexadeconoic Acid (17.77%), and Cholest-5-En-3-Ol, 23-Ethyl-,(3.Beta.23S)- (14.73%). The main compounds in flowers are (9Z)-9-Tricosene (21.71%), 9-Tricosene (21.32%), and Z-5-Nonadecene (16.9%). Cholest-5-En-3-Ol, 23 -Ethyl-, (3.Beta.23S)- (26.86%), Hexadeconoic Acid (13.03%), and 9,12-Octadecadienoic Acid (11.34%) are the main component in rhizomes.

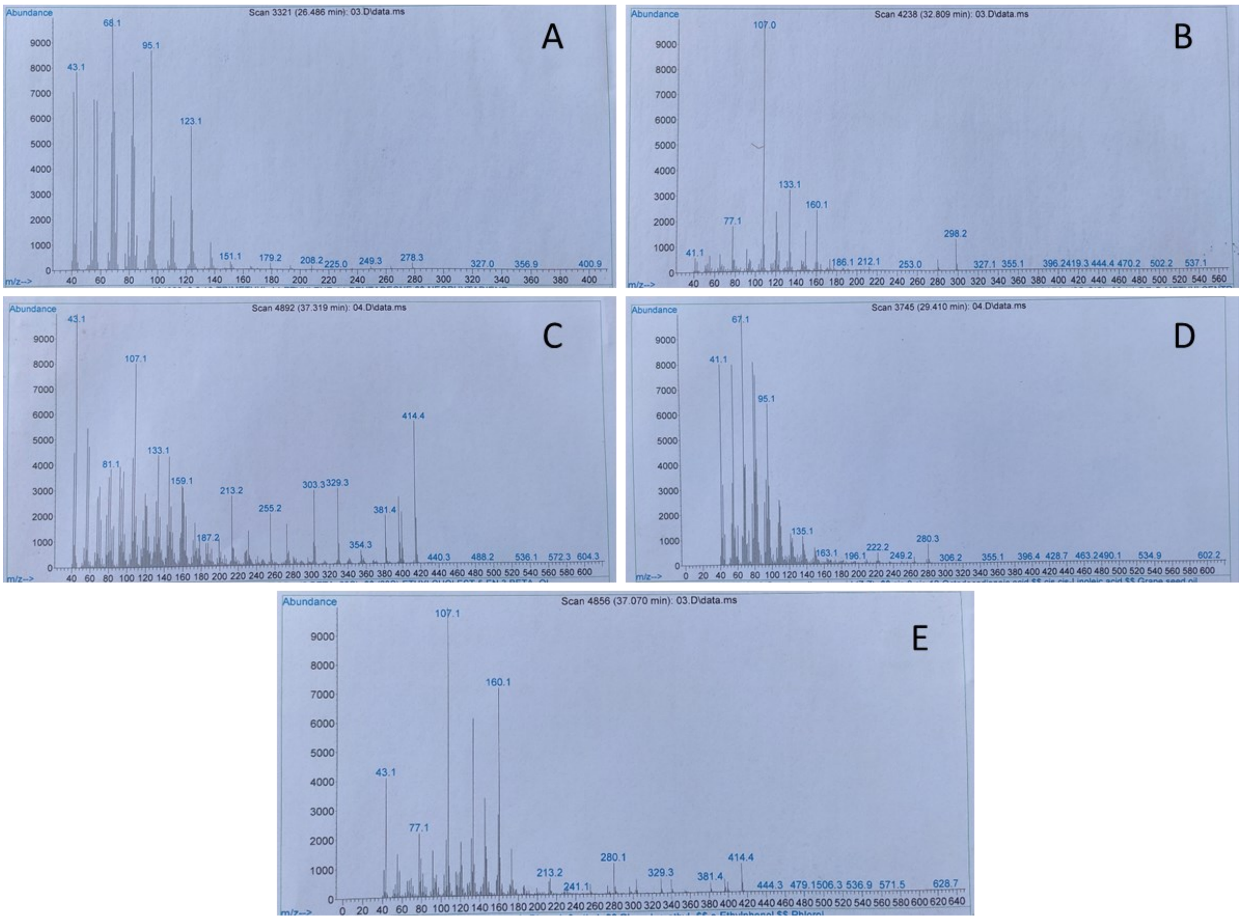

Figure 2. The chromatogram for main components of *E. acanthodes*. (A) Neophytadiene; (B) (+)-De-O-Methylcentrolobine; (C) Cholest-5-En-3-Ol,23-Ethyl-,(3. Beta.23S)-; (D) 9,12-Octadecadienoic Acid, (E) Phenol, 2-ethyl-

The compounds found are grouped into four parts: terpenoids (monoterpene, sesquiterpenes, and diterpenes), phenolic, steroids, and other compounds (Figure 2). Phenolic compounds (39.56%) predominate in leaves, other compounds (41.25%) in stems, diterpene hydrocarbons (77.3%) in flowers, and Other compounds (43.5%) and steroids (40.4%) in rhizomes. The compounds in rhizomes, stems, and leaves are grouped and tend to have similarities. It is due to the growth of stems and leaves that comes from rhizomes (Poulsen 2012). In comparison, flowers have compound characters that are very different from the others. Analysis of the essential oils in the fruit has not been carried out because no fruit was found at sampling in the field. The chemical composition is very different from the Elingera elatior species, with terpenoids as the predominant compound (Ud-Daula & Basher 2019).

There are several exciting compounds to develop their potential in the future. (+)- Alpha-Tocopherol is part of vitamin E (Jilani & Iqbal 2018). *E. acanthodes* contains Gamma-Sitosterol, which has high antioxidant activity and anti-cancer potency (Ambarwati et al. 2019). (Z)-9-tricosene can be used as an effective strategy for housefly management (Kannan et al. 2020). The rhizome of *E. acanthodes* also contains Stigmasterol which has been documented as immunomodulatory with huge therapeutic potential, immune response, and proposed as candidate for anticancer agents (Antwi et al. 2017; Kangsamaksin et al. 2017). (Z)-9-tricosene has been used in several studies as a pheromone for house flies in domo traps (Kannan et al. 2020). Information on the essential oils of *E. acanthodes* can be used as an initial guide in developing potential in industry and medicine.

Table 1. Essential oils component of *E. acanthodes* A.D. Poulsen

Chemical Compounds	Retention Times	Percentage (%)			
		Leaves	Stems	Flowers	Rhizomes
Monoterpenes hydrocarbons					
1,2-Dimethyl-3,5-Divinylcyclohexane.	34.264	1.12	-	-	-
Sesquiterpens hydrocarbons					
Tridecene.	28.865	-	-	9.24	-
Ethyl (9Z)-9-Octadecenoate.	29.106	-	-	-	1.58
Pentadecene.	29.975	-	-	2.54	-
Diterpens hydrocarbons					
Neophytadiene.	26.486	33.73	10.78	-	-
Nonadecene.	26.893	-	-	1.78	-
Z-5-Nonadecene.	28.665	-	-	16.9	-
9-Tricosene.	29.782	-	-	21.32	-
(9Z)-9-Tricosene.	29.851	-	-	21.71	-
Pentacosane.	30.713	-	-	1.88	-
Docosene.	31.410	-	-	2.41	-
Bicyclo [10.9.0] eicosane, cis-.	31.582	-	-	9.84	-
Cyclotetracosane.	31.644	-	-	1.49	-
Octacosane.	32.271	-	-	-	1.89
Phenolic					
(+)-D-O-Methylcentrolobine.	33.120	12.96	13.08	0	7.67
(+) - Alpha-Tocopherol.	34.485	2.9	-	-	-
Phenol,2-ethyl.	37.070	21.76	0	0	3.35
(2RS,3RS)-2-T-Butyl-6-Methoxy-4 Phenylsulfonyl-1,2,3,4 Tetrahhydrocyclopent[B]Indol-3-OL	36.043	1.94	0	0	0
Steroid					
Cholestan-7-one,8,14-epoxy-(5.alpha., 8.alpha).	35.788	2.18	-	-	-
Gamma-Sitosterol.	37.450	0.27	2.23	-	-
Stigmasterol.	36.326	-	-	-	2.41
Sitosterol.	37.401	-	-	-	11.13
Cholest-5-En-3-Ol,23-Ethyl-,(3.Beta.23S)-.	37.319	-	14.73	-	26.86
Other compounds					
Hexadecanoic Acid, Ethyl Ester.	27.989	-	1.89	-	1.99
Hexadeconoic Acid.	28.382	-	17.77	-	13.03
9,12-Octadecadienoic Acid.	29.410	-	21.59	1.75	11.34
Hydroxypentadecanoic Acid.	30.865	2.64	-	-	-
p-toluenesulfony-tyrosyl-S-carbo xymethylcysteine.	34.257	-	-	-	8.91
1-(1,5-Dimethylhexyl)-10A,12A-Dimethyltetradecahydro-5-H-Cyclopenta [1,2] Phenanthro [1,10A-B] Oxire-5-One.	35.402	1.64	-	-	-
N-(4-Bromophenyl)-2-(4-Chlorophenoxy) Acetamide.	35.567	-	-	-	8.27
Butane, 1,4-Bis [3-(4-Tolyl).					
3-Butyl-5-Methyl-1,2,3,8A-	36.690	11.83	-	-	-
Tetrahydroindolizine.	37.416	3.33	-	-	-
Total		96.03	91.58	93.09	98.43

Several *Etlingera* species have been studied and have potential in medicine and bioactivity. *Etlingera wingensis* has the potential as antioxidant and antimicrobial activity on its rhizomes, stems, and leaves (Mahdavi et al. 2017). *Etlingera punicea* rhizome has antimicrobial activity tested on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella Albany*, and fungus *Candida albicans* (Tadtong et al. 2014). *Etlingera brevilabrum* also has bioactivity as antioxidants and antibacterials (Mahdawi et al. 2016). *Etlingera coccinea* and *Etlingera sessilanthera*, endemic to Borneo, have antimicrobial activity against *Bacillus cereus*, *Bacillus subtilis*, and

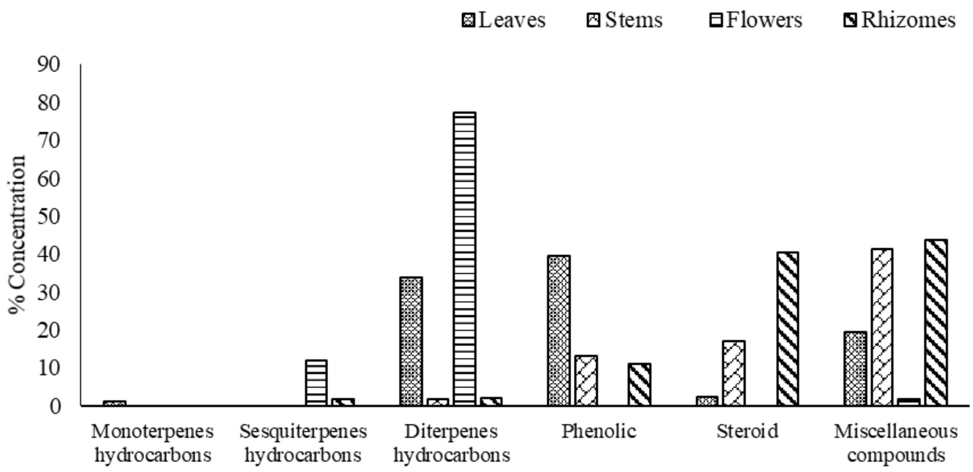

Figure 2. Chemical Composition of the Essential Oils of *E.acnthodes* A.D. Poulsen

Staphylococcus aureus (Daniel-Jambun et al. 2019). *Etlingera pavieana* has potential as an anti-inflammatory effect from rhizome extract and its phenolic compounds (Srisook et al. 2017). *Etlingera pubescens*, endemic to Borneo, can potentially be a bactericidal and cytotoxic activity of a diarylheptanoid (etlingerin) (Daniel-Jambun et al. 2019).

AUTHOR CONTRIBUTION

H., A.A., and M.M., conducted research of essential oils and wrote the manuscript. M.F.R., and W. provide sample from the forest. A.S.M., and H.D. conducted data analysis and supervised the manuscript.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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