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Essential Oils Etlingera Doliiformis A.D. Poulsen, An Endemic Ginger From Sulawesi, Indonesia

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Abstract

The study aimed to analyze Etlingera doliiformis essential oils, antioxidant capacity and review the potential bioactivity of the compound. The species is endemic in Sulawesi. The essential oils were obtained by hydrodistillation of the leaves, stems, flowers, and rhizomes of E. doliiformis. The plants were analyzed by GC-MS method using the Agilent Technologies 7890 Gas Chromatograph with Auto Sampler. Data analysis of GC-MS was determined based on a comparison of mass spectra from the NIST 2005 v.2.0 library and Wiley 7 library 2003. Antioxidant activity was determined using DPPH (1,1-difenil-1-pikrilhidrazil) assay. The five main compounds found in E. doliiformis were Hexadecanoic acid, methyl ester (50.72%), 2-Pyridinecarboxylic acid (49.06%), γ- Sitosterol (23.24%), 2-Hexadecanoyl glycerol (17.68%) and Ethyl linoleate (16.05%). The oils of E. doliiformis contain terpenoids, phenolic, steroids, and miscellaneous compounds. The terpenoids compound were dominant in flowers and leaves. Steroids were dominant in rhizomes and stems. Phenolics were dominant in leaves and rhizomes. Miscellaneous compounds were scattered in every organ analyzed. Compound review shows that E. doliiformis has the potential to be developed in the pharmaceutical and drug industry. The antioxidant activity showed a relatively high inhibition rate, 48.2% in flowers and 42.4% in leaves.

Keywords: Antioxidant capacity, Compound, DPPH, Essential oils, Etlingera

1. Introduction

Zingiberaceae is an aromatic plant that has the potential to produce essential oils. Zingiberaceae consists of 12 genera namely Zingiber, Alpinia, Ammomum, Boesenbergia, Curcuma, Elettaria, Etlingera, Globba, Hornstedtia, Kaempferia, Plagiostachys, and Vanoverberghia. One of the largest genera in the Zingiberaceae family is Etlingera. Poulsen (2012) reported 46 species and one new species (*Etlingera tjiasmantoi*) in Sulawesi [1]. Etlingera grows in forests, and some have been cultivated, such as Etlingera elatior [2]. The distribution of Etlingera is from India, Indo-China throughout Malesia to the Pacific Islands and consists of more than 100 species [3,4].

Etlingera doliiformis A.D. Poulsen is an endemic species to Sulawesi and reported to be found on Mt. Bawakaraeng, South Sulawesi [3]. The species is in the critically endangered category on the IUCN website due to the threat of forest fires. E. doliiformis grows at an altitude of 1800 m around the hiking trail of Mt. Bawakaraeng or Lembah Ramma'. The species grows in groups in areas close to water sources.

Essential oils of E. doliiformis have not been studied. Essential oils can be used for aromatherapy and biological activities such as antifungal, antibacterial, anti-inflammatory, anticancer, antioxidant, etc. Research on essential oils of the Zingiberaceae family in Indonesia has been carried out on the genera Curcuma and Zingiber. This genus has even been widely used in various industries such as medicine and aromatherapy. Therefore,

research on E. doliiformis essential oils is a preliminary study for future bioactivity potential analysis.

Several Etlingera species have been reported to have potential as antioxidants, such as E. sayapensis [5], E. pubescens [6], and E. elatior [7]. Therefore, *E. doliiformis* has the same potential as an antioxidant as other Etlingera species. The study also examines the antioxidant potential of E. doliiformis that can be used in the future.

The research potentially becomes a novel article that reports the essential oils and antioaxidant capacity of E. doliiformis. The study aimed to determine the content of E. doliiformis essential oils (leaves, stems, flowers, and rhizomes), antioxidant capacity (flowers and leaves), and review the potential its chemical component.

2. Methodology

Materials

Plant materials were collected from Mt. Bawakaraeng, Gowa Regency, South Sulawesi, Indonesia. Samples were collected along the hiking trail at an altitude of 1800 m. Fresh samples (leaves, stems, flowers, and rhizomes) were taken to the laboratory to analyze the essential oil components. Also, flowers and leaves were used for antioxidant analysis. Herbarium specimens were taken for further morphological character analysis. To ascertain species, morphological observations were made using living collections.

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis using Agilent Technologies 7890 Gas Chromatograph with Auto Sampler equipped with 5975 mass selective detectors and chemistation data system. The GC-MS analysis used a column in HP Ultra 2 with a capillary column length of 30 x 0.25 (mm) I.D x 0.25 (µm) film thickness.

E. doliiformis oil was injected into the column using a GC-MS syringe as much as 0.1 µl and carried by helium gas. The column temperature was increased from 80 °C to 150 °C (1 min) and ended at 280 °C (26 min). The mobile phase flow rate was set to 1.2 /min, the injector temperature was 250 °C, the pressure was 12 kPa, and the injector split ratio was set to 8:1.

The components of essential oils were shown as a percentage of the total area of the chromatogram peak. Chromatogram results of GC-MS analysis include peak number, retention time (R), initial time (I), end time (F), curve area (area), curve height (height), curve (m/z), and the area of each curve (%T). The area of each curve represents the percentage of essential oils.

The chromatogram peak that appears from the GC-MS results represents the essential oil compounds. It is identified by comparing them with the Essential oils compound library (library). Identification of essential oils components was carried out based on the comparison of the mass spectra from the NIST 2005 v.2.0 library and the Wiley 7 library 2003 [8]. After being matched with the library, the essential oil component is then showed its identity, including the retention time and compound name.

The main compound was determined based on the percentage from each part. Compound grouping was based on information on Pubchem NCBI. Then, a potential review was compiled on several components that have been studied and have the potential for bioactivity and industry.

Antioxidant Capacity Analysis

Antioxidant capacity analysis was carried out using DPPH (1,1-difenil-1-pikrilhidrazil) method. Preparation of 0.1 mM DPPH was carried out by dissolving 4 mg of DPPH in 1000 ml of methanol. The extract of 1000 ppm was made by weighing 12.5 mg of extract, dissolved in 1250 l of dimethyl sulfoxide, heated until dissolved, and vortexed. The antioxidant activity test was carried out by taking a 50 µl and placing it in a test tube, adding 450 µl of methanol, then adding 3 ml of DPPH solution, vortexed until homogeneous, allowed to stand for 30 minutes in a closed and darkroom. The absorbance was measured on UV-vis spectrophotometry with a wavelength of 517 nm.

The antioxidant test of vitamin C was carried out by dissolving 20 mg vitamin C in 100 ml of 96% ethanol. The solution was made in several concentrations, namely 500 µl, 400 µl, 300 µl, 200 µl, 100 µl of methanol were added to each concentration. Then 3 ml of DPPH solution was added, and the absorbance was measured using UV-Vis spectrophotometry with a wavelength of 517 nm.

The percent inhibition of the sample was calculated using the formula:

Inhibition rate (%) = "Ablank - Asample" /"Ablank" x 100%

3. Result and Discussion

Morphological Character of *Etlingera doliiformis*

The morphological character was describe by Poulsen (2012). *E. doliiformis* belongs to the Acanthodes group of Sulawesian Etlingera. *E. doliiformis* has characteristics rhizome 2.5 cm diameter, subterranean, pubescent, scales 3.5 cm long; stilt roots absent. The rhizome is an organ that has a sharp aroma typical of the Zingiberaceae family. Leafy shoots to 2.5 m to long, base to 3.5 cm diameter (when dry), sheath yellowish to reddish brown, with scattered hairs, margin glabrous or ciliate near ligule; ligule to 16-18 mm long, reddish brown, rounded; petiole 5-6 mm long, orange-brown, with scattered hairs. The stems of *E. doliiformis* have clear ligules. Flowering shoot 13 cm long, arising from rhizome, erect, receptacle 2-7 cm long, with 120-125 flowers, and 2-4 open at time. Flower 3.9 – 4.9 cm long; calix 2.5 cm long, reaching 5.5 – 16 mm short of apex of stamen and 7 – 13 mm short of apex of corolla lobes, and pinkish (figure 1) (Poulsen 2012). Young inflorescence *E. doliiformis* is reddish-orange and is conical in shape with a larger diameter base

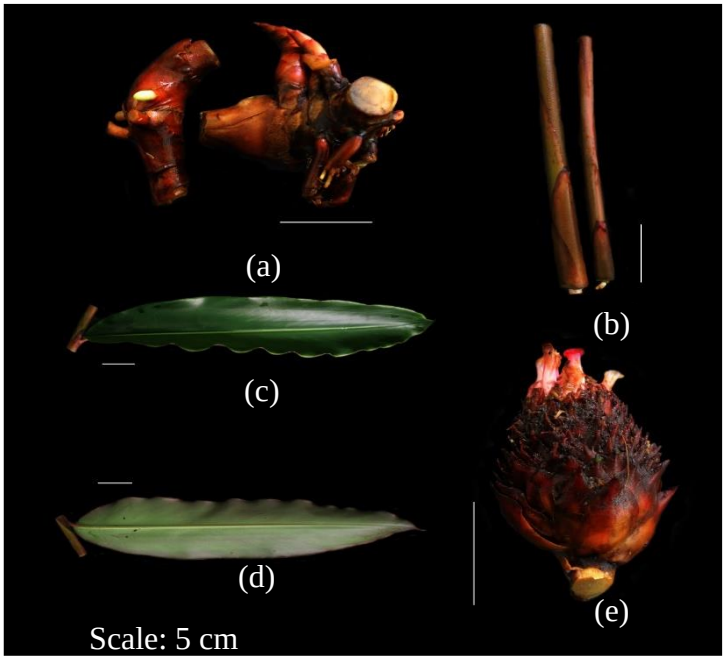

Figure 1. Morphology of *E. doliiformis*. (a) rhizome, (b) stem (c) upper surface of leaf, (d) lower surface of leaf, (e) inflorescence

Essential oils Component

The hydro distillated 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 Hexadecanoic acid, methyl ester (50.72%), 2-Pyridinecarboxylic acid (49.06%), γ-Sitosterol (23.24%), 2-Hexadecanoyl glycerol (17.68%) and Ethyl linoleate (16.05%).

The main compound in rhizomes are 2-Pyridinecarboxylic acid (201.12%), 9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester (14.6%) and γ-Sitosterol (9.52%). The main compound in flowers are Ethyl linoleate (16.05%), Glycerol 1-monolinolat (12.42%) and 9-Tricosene, (Z) – (11.13%). Hexadecanoic acid, methyl ester (35.84%), 2-Pyridinecarboxylic acid (14.67%) and γ- Sitosterol (8.89%) are the main compound in stems. The main compound in leaves are Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-,2-(hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z) (13.70%), Phytol (11.37%) and 2-Pyridinecarboxylic acid (9.91%).

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

Chemical Compounds	Retention Times	Percentage (%)			
		Rhizomes	Flowers	Stems	Leaves
Monoterpene					
Cineole	4.483	2.27			
Diterpene					
9-Tricosene, (Z) -	30.396		11.13		
Neophytadiene	27.513				7.43
Acetylcysteine	27.313				1.93
Phytol	29.972			1.68	11.37
Triterpene					
Squalene	33.333		1.22		
Sesquiterpene					
Caryophyllene	15.440		1.73		
α-caryophyllene	16.681		1.16		
Nerolidol	20.970	4.97			
.+/-.-trans-Nerolidol	21.039		3.66		1.36
(-)-Germacrene d	17.764	3.84	5.06		1.70
Steroid					
Stigmasterol	38.519		1.70	1.46	
Stigmast-4-en-3-one	42.325		1.32		
γ-Sitosterol	39.539	9.52		8.89	4.83
Ethyl 9,12,15-octadecatrienoate	29.906				1.78
5,6,6,-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro [2.5]octan 4-one	28.486				2.29
Phenolic					
γ-Tocopherol	35.512	1.61	1.04	1.73	
Vitamin E	36.636			1.76	8.89
2-Methoxy-4-vinylphenol	12.103		1.24	2.30	
n-Tetracosanol-1	32.327		4.84		
6-Isopropenyl-4,8a-dimethyl-12345678,8a-octahydro-naphthalen-2-ol	27.445	6.65		1.74	
1,2-Trans-2,3-trans-plinol	27.872	3.44			
(1RS,2RS,6RS,7SR,1'RS)-6-(1'-Hydroxyethyl)-7-isopropenyl-2,5,5-trimethylbicyclo[4.1.0]heptan-2-ol	26.831				2.49
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	27.927				6.06
1,6-Germacradien-5-ol	28.699				1.22
Miscellaneous Compounds					
2-Pyridinecarboxylic acid	8.372	20.12	4.36	14.67	9.91
Hexadecanoic acid, methyl ester	28.851	5.46	7.95	35.84	1.47
Heptadecanoic acid, ethyl ester	29.458		5.56		
Ethyl linolenata	29.906		16.05		
2-Aminoethanethiol hydrogen sulfate (ester)	30.679		2.02		
Methyl 19-methyl-eicosanoate	30.679		7.13		

Tetradecanoic acid, ethyl ester	31.320		2.13		
Glycerol 1-monolinolat	32.671		12.42		
Octadecanoic acid, ethyl ester	33.071		1.40		
4H-Pyran-4-one-,2,3-dihydro-3,5-dihydroxy-6-methyl-	7.131	1.72			2.48
7-Acetyl-2-hydroxy-2-metyl-5-isopropylbicyclo [4.3.0] nonane	26.272	4.58			
Oxireno [g] benzofuran, 1a,2,3,4,5,6a,6b-hexahydro-3,3,6a-trimethyl-5-(1-methylethenyl)-,(1a.alpha., 5.beta.,6a.alpha., 6b.alpha.)-	26.769	3.02		1.28	
Vinbarbital					
Ethyl 9,12,15-octadecatrienoate	29.148	4.30			
1,3,2-Dioxaphosphorinane-4,4,6,6-d4,2,5,5-trimethyl-,2 oxide	29.872	4.64			
2-Hexadecanoyl glycerol	31.292	1.24			
9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester	31.589	6.33		5.00	6.35
(-)-β.-caryophyllene epoxide	32.602	14.56			
Benzenamine, 2,4-dimethoxy-					
Dipentene diepoxide	21.452			2.13	1.12
2-Penten-1-one, 1-bicyclo[6.1.0]non-9-yl-4-methyl-	26.341				1.94
Spiro[2.5]octane, 5,5-dimethyl-4-(3-oxobutyl)-	27.010				1.35
3-Buten-2-one, 4-(5-hydroxy-2,2,6-trimethyl-7-oxabicyclo [4.1.0]hept-1-yl-	27.141				1.57
	28.300				1.50
O-hydroxybenzaldehyde thiosemicarbazone	29.010				1.49
Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-,2-(hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)	29.272				2.14
(6Z)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	32.788				13.70
1-(4-Hydroxy-7-isopropyl-4-methyloctahydro-1h-inden-1-yl)ethanone	20.956			3.78	
10,11,dihydro-5h-dibenz (b,f) azepin	26.259			2.88	
9,12,15,-Octadecatrienoic acid, ethyl ester					
S[2-[N,N-Dimethylamino]ethyl]N,N-dimethylcarbamoyl thicarbohydroximate	29.168			3.12	
	29.885			6.96	
(1SR,3RS,4RS)-3-exo-methoxy-7-oxabicyclo[2.2.1]heptan-2-one	30.506			1.14	
	31.299			3.26	

The compounds found are divided into four groups: terpenoids (monoterpenes, diterpenes, triterpenes, and sesquiterpenes), phenolic, steroids, and miscellaneous compounds (Figure 2). Terpenes are dominant in flowers and leaves. Steroids are dominant in rhizomes and stems. Phenolic are dominant in leaves and rhizomes. Miscellaneous compounds are dispersed in every organ analyzed. Flowers have the most different compounds compared to other organs. Terpenes, steroids, and phenolic have many potential bioactivities such as antibacterial, antioxidant, anti-inflammatory, anticancer, antidiabetic, etc. Isolation of this group of compounds from *E. doliiformis* can be used for the potential bioactivity. GC-MS

analysis has not been carried out on *E. doliiformis* fruit because no fruit was found in all populations at the time of sampling.

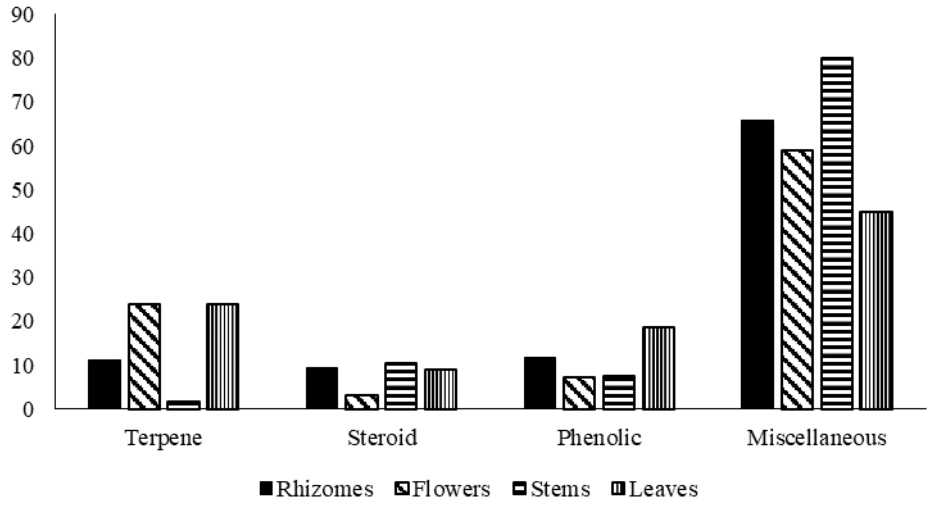

Figure 2. Chemical Composition of the Essential Oils of *E. doliiformis* A.D. Poulsen

4. Conclusion

The main compounds of E. doliiformis are hexadecanoic acid, methyl ester (50.72%), 2-pyridinecarboxylic acid (49.06%), γ-sitosterol (23.24%), 2-hexadecanoyl glycerol (17.68%), and ethyl linoleate (16.05%). The review of essential oil compounds in E. doliiformis has much potential in bioactivity and industry that can be used in the future.

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