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Preliminary Phytochemical Screening and GC-MS Analysis of Ethanol Extract of Bulbs of *Eleutherine* sp.

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ABSTRACT

The Dayak community is known to use *Eleutherine palmifolia* as a wound and ulcer treatment. However, according to published research, other species of *Eleutherine* possess wound healing properties. In addition, the active compound components of species *Eleutherine* induce antibacterial and antioxidant bioactivity which can aid the healing process. As a result, the aim of this research is to identify active components in an ethanol extract of *Eleutherine* sp bulb obtained through maceration with 70% ethanol as the solvent. Chemical compounds were analyzed using GC-MS, while the mass spectra of compounds found in extracts was compared to those contained in the National Institute of Standards and Technology (NIST) library. Preliminary phytochemical screening revealed the presence of bioactive compounds such as triterpenoids, steroids, and tannins. The mass spectral measurements revealed 13 peaks, indicating the existence of that number of active phytochemical components including 1H-Indene, 2-butyl-5-hexyloctahydro; Benzene, 1,2,3-trimethyl; and Phenol, 2,5-bis (1,1-dimethyl ethyl). It is intended that the findings of this study will provide more information about the active compound components of *Eleutherine* sp. which promote biological activity that aids wound healing.

Keywords: *Eleutherine* sp., GC-MS analysis, Phytochemical screening, Ethanol extract.

Introduction

Eleutherine is a common plant found in Borneo, particularly in East Kalimantan. The Dayak tribe of Central Kalimantan utilizes *Eleutherine palmifolia* (L) bulbs to treat high blood pressure, diabetes, cholesterol, wounds, and ulcers.[1,2] Scientific evidence of the use of *Eleutherine* bulbs in wound healing had already been published. A scientific review confirmed the use of *Eleutherine* plants in wound healing. Administered *in vitro* at a concentration of 100 mg/mL, *Eleutherine bulbosa* recorded a wound-breaking strength value of 23.6 percent in a study of various wound-healing medicinal plants grown in Peru.[3] Other research was performed on *Eleutherine* plants of numerous species, specifically *Eleutherine indica* which grows in India and China. The results showed that topical administration of 2.5% methanol extract ointment accelerated the cutaneous wound healing process *in vivo* by stimulating Smad-collagen production.[4]

Several studies have shown that *Eleutherine bulbosa*induces anti-inflammatory, antioxidant, and antibacterial activity which aids wound healing. According to studies on the activity of Dayak Onion as an antioxidant, *Eleutherine americana* (Merr) which is indigenous to South Kalimantan had an IC_{50} of 50.42 ppm, indicating significant antioxidant activity.[5] Based on *in vitro* test results showing inhibition of *Inducible Nitric Oxide Synthase* (iNOS) expression and excess production of Nitric Oxide (NO) by (-)-isoeleutherin, four naphthalene derivatives with anti-inflammatory activity were isolated from *Eleutherine americana* (Merr).

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In vivo tests showed that at a concentration of 30 µM carrageenan inhibited edema in carrageenan-induced rat paws.[6] Moreover, *Eleutherine americana* (Merr) extracts reduced the growth of *Staphylococcus aureus* ATCC 23235 and ATCC 27664, *Streptococcus mutant*, and *S. pyogens*. At dosages of 62.5 – 1000 g/mL in all isolates and 250 g/mL in ATCC 23235 and ATCC 27664, it also suppressed the growth of *Methicillin-resistant Staphylococcus aureus* (MRSA) by denaturing the cytoplasm and dissolving the membrane, causing leakage in the cell wall.[7] Another study on antibacterial action found that when chemical compounds from *Eleuthrine bulbosa* containing eleutherin and isoeleutherin were separated and applied to the surface of the rabbit skin for seven days the bacterial density/cm^2 decreased.[8] Alkaloids, flavonoids, triterpenoids, steroids, saponins, and tannins were found in *Eleutherine americana* (Merr) phytochemical screening.[9]*Eleutherine bulbosa*/*Eleutherine merr*/*Eleutherine palmifolia* originate from India and Indonesia (Kalimantan and Sulawesi). Based on previously conducted research, the results of which were compared with existing publications, it was clear that differences existed in the active compounds detected by GC-MS analysis. Certain new components, in addition to the same ones, were found at different concentrations, possibly influenced by factors such as where the plant grows which affect the amount, type, and concentration of active compounds detected by GCMS analysis.[10-13] *Gas chromatography* is a technique used to separate compounds that may be present in a sample. The time it takes for a particular compound to travel the length of the column is known as the retention time (RT) and represents the identifying characteristic.[11] Several analytical studies of the GC-MS of various *Eleutherine* species have been published. The GC-MS analysis results of ethanol extract *Eleutherine palmifolia* Linn bulbs indicated the presence of 17 compounds, including cyclohexane, which may be responsible for antimicrobial activity, while small concentrations of other compounds have a synergistic effect which increases the antimicrobial activity of cyclohexane.[11] The goal of this research was to identify active components in an ethanol extract from *Eleutherine* sp bulbs using the gas chromatography-mass spectrometry (GC-MS) analysis method.

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Materials and Methods

Collection of plant materials

Fresh *Eleutherine* sp. bulbs were collected in April 2019 from Samarinda, East Kalimantan and authenticated by botanists attached to the Indonesian Institute of Sciences Research Center for Biology in Bogor, West Java with reference number 754/IPH.1.01/If.07/VII/2020.

Extraction of plant materials

The bulbs of the collected plants were separated from the leaves, cleaned, and dried in an oven at 50°C. Maceration using 1000 mL of 70% ethanol was undertaken to extract 100 g of dry Simplicia *Eleutherine* sp. bulb. This process yielded an average 70% ethanol extract of 10.66 g, resulting in a yield of 10.66%. Ethanol (70%) was used as a solvent in the maceration of Dayak Onion bulbs because its high polarity affects both the yield of the extract. In a previous study, maceration of *Eleutherine bulbosa* Urb. bulbs extract using 70% ethanol produced an extract yield of 10.78% which was greater than that of 96% ethanol (1.49%).[9,14]

Preliminary phytochemical screening

General tests for the presence of the following; alkaloids, flavonoid, Saponin, Triterpenoid, Steroid and tannins were carried out following methods previously described.[15,16]

GC-MS analysis

Aliquou 1 µL acetone solution 70% ethanol extract of *Eleutherine* sp. bulbs were analyzed using GC-MS (QP-2010S, Shimadzu, Japan). The VF5MS column used was 30 min length, 0.25mm in diameter, and 0.25 µm thick. The carrier gas used was helium. The programmed oven temperature was 70°C, maintained for three minutes, increased by 10°C/minute to 300°C, and then held at that figure for approximately nine minutes. The injector temperature was 240°C, the ionization potential -70ev, the ion source temperature 200°C and the scanning range 40-1000 m/z.[10]

Identification of components

The retention time contained in the spectral data was compared with the computer software data relating to various standards (NIST, REPLIB, and MAINLIB Libraries).

Results and Discussion

Table 1 contains the triterpenoid, steroids, and tannins chemicals found in the 70% ethanol extract of *Eleutherine* sp. bulbs. Table 2 lists the 13 compounds present as indicated by the peaks shown in the chromatog image which identifies compounds from the 70% ethanol extract derived from *Eleutherine* sp. bulbs using GC-MS. The three compounds with the largest surface area are 1H-Indene, 2-butyl-5-hexyloctahydro; Benzene, 1,2,3-trimethyl; and Phenol, 2,5-bis (1,1-dimethyl ethyl), while the ten compounds comparatively small in area, comprised Benzene, 1-ethyl-4-methyl; Isopropyl benzene; Benzenemethanol,.alpha.-phenyl; Caryophyllene; dibutyl phthalate; 1,2-Benzenedicarboxylic acid, diisooctyl ester; Nonaoic acid, [tetrahydro-2-furanyl]ethylester; Cyclohexanol, 1-methyl-4-(1-methyl ethyl)-; Propanedinitrile, [(3,4,5-trimethoxyphenyl)methylelene]-; and 1-methoxy-2-propyl acetate.

The results of the GC-MS analysis of the ethyl acetate extract of *Eleutherine bulbosa* bulbs identified 24 chemical compounds, the majority of whichindicated several bioactivities, including antibacterial and antitumor potential. The presence of 8-Naphthalenediol,7-diacetyl,6-dimethyl, Propanedinitrile, and 3, 4, 5-trimethoxyphenylmethylene in this plant has not previously been reported. The literature indicates that the family of 1,8 naphthalene diols and their derivatives act as potent antioxidants, while propane nitrile compounds exhibit antimicrobial, antitumor, and cytotoxic activities.[10] The results of the GC-MS analysis of *Eleutherine americana* Merr in fresh and dry forms indicated different active compounds.

Table 1: Preliminary phytochemical screening of ethanol 70% extract of *Eleutherine sp* bulbs

Secondary metabolite	Inference
Alkaloids	-
Flavonoids	-
Saponins	-
Triterpenoid	+
Steroids	+
Tannins	+

Table 2: phytochemical compounds found in 70% ethanol extract of *Eleutherine sp* bulbs

No	Name of compound	Retention time (min)	Chemical formula	Molecular weight (g/mol)	%Area	CAS no
1	1-methoxy-2-propyl acetate	3.45	$C_6H_{12}O_3$	132	7.33	108-65-6
2	Benzene, 1-ethyl-4-methyl	5.11	C_9H_{12}	120	4.53	622-96-8
3	Benzene, 1,2,3-trimethyl-	5.71	C_9H_{12}	120	16.24	526-73-8
4	Isoprophylbenzene	6.21	C_9H_{12}	120	4.88	98-82-8
5	Cyclohexanol, 1-methyl-4-(1-methylethyl)-	8.86	$C_{10}H_{20}O$	156	6.65	21129-27-1
6	Caryophyllene	12.41	$C_{15}H_{24}$	204	0.88	87-44-5
7	Nonaoic acid, [tetrahydro-2-furanyl]ethyl ester	12.86	$C_{14}H_{26}O_3$	242	8.32	None
8	Phenol, 2,4-bis (1,1-dimethylethyl)-	13.38	$C_{14}H_{22}O$	256	12.33	5875-45-8
9	Benzenemethanol,alpha-phenyl-	15.08	$C_{13}H_{12}O$	184	10.53	91-1-0
10	1H-Indene, 2-butyl-5-hexyloctahydro-	15.61	$C_{19}H_{36}$	264	17.42	55044-33-2
11	Dibutyl phthalate	18.35	$C_{16}H_{22}O_4$	278	5.08	84-74-2
12	Propanedinitrile, [(3,4,5-trimethoxyphenyl)methylelene]-	22.61	$C_{13}H_{12}N_2O_3$	244	1.54	5688-82-4
13	1,2-Benzenecicarboxylic acid, diisooctyl ester	23.30	$C_{24}H_{38}O_4$	390	4.26	27554-26-3

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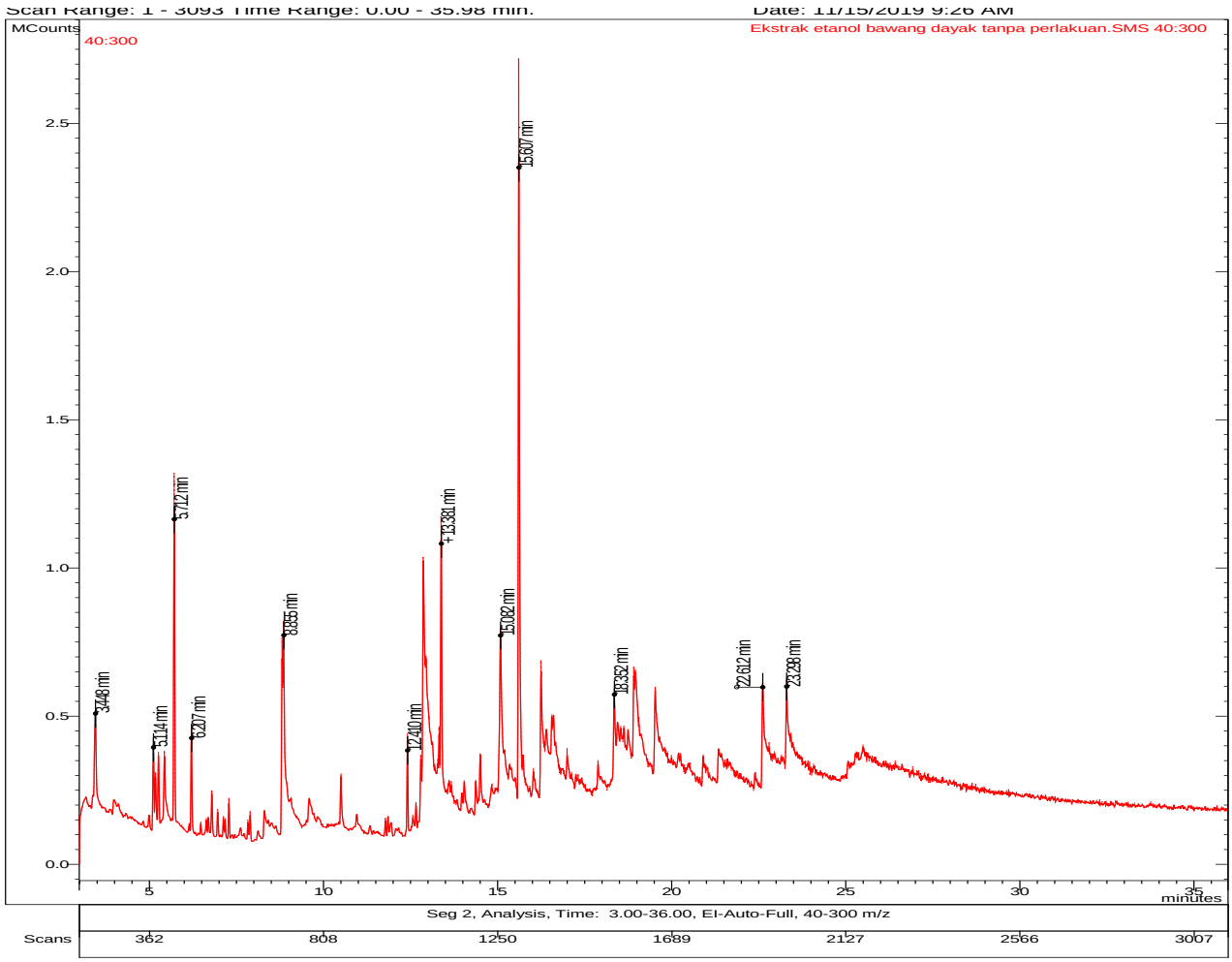

Figure 1: A typical GC-MS chromatog of ethanol extract constituents of *Eleutherine* sp bulbs.

For dried *Eleutherine americana* Merr onions, the dominant active compounds were phenol, Eicosane, 1-Amino-3-4-dihydro-4-4-dimethoxy-3-methyl-2 naphthalene carbonitrile. Meanwhile, fresh *Eleutherine americana* Merr onions produced coumarin, nickel, cyclopentadienyl, campesterol, stigmasterol, and gamma-sitosterol. However, there are similarities in the two treatments of raw materials, namely the hexadecanoic acid, octadecanoic acid, and linoleic acid content.[12] The results of GC-MS analysis of 96% ethanol extract of *Eleutherine bulbosa* bulbs indicated that securixanthone E (7-hydroxy-1,2-dimethoxyxanthone) constituted the compound with the most significant area. Furthermore, a group of compounds; fatty acid esters, isoquinolines, naphthalenes, and phenolics was identified.[13]

Aromatic hydrocarbon compounds with an area of 25.65% represented the largest compounds identified in this study. Aromatic hydrocarbon compounds consist of Benzene, 1-ethyl-4-methyl (4.53%), Benzene, 1,2,3-trimethyl- (16.24%), and Isopropyl benzene (4.88%). It is suspected that aromatic hydrocarbon compounds have antioxidant and antimicrobial activity.[17-19]According to the published results of GCMS analysis,1H-Indene, 2-butyl-5-hexyloctahydro- at a concentration of 17.42%, has not been reported as present in small concentrations in other species of eleutherine. However, it is found in Agarwood, called Chen-Xiang in Chinese, which is a valuable resinous wood derived from *Aquilaria spp.* or *Gyrinops spp.* Trees, and *Ligularia narynensis* root.[20,21]

Phenol, 2,4-bis (1,1-dimethyl ethyl)-, with an area of 12.33%, was the main phenol compound detected in this study. Phenol, 2, 4-bis (1, 1-dimethyl ethyl) was expected to develop the green fungicides providing protection against the fungal plant diseases affecting agriculture. These have been reported to have antioxidant, anticancer, antifungal, antibacterial, and protective properties against trimethyltin-induced cognitive dysfunction (TMT).[22] Phenolic compounds exhibit anti-inflammatory properties and their mechanism of action is reported to be similar to that of non-steroidal anti-inflammatory drugs. In addition to the inhibition of COX enzymes, phenolic compounds also inhibit other pro-inflammatory mediators and their activities through gene suppression.[23]

Other compounds present, including minority groups, can synergise both the main and minor compounds to increase their bioactivity. Propanedinitrile, [(3,4,5-trimethoxyphenyl)methylene]- (1.54%), 1,2-Benzenecicarboxylic acid, and diisooctyl ester (4.26%) have all been identified in previous studies.[10] Propanenitrile compounds demonstrated antimicrobial activity against g-positive and g-negative bacteria.[24] 1,2-Benzenecicarboxylic acid, diisooctyl ester, caused by environmental pollution, release the compound from plastic waste. Bulbs of *Eleutherine* sp. are plant parts that have a greater ability to absorb compounds, thus affecting the area value of the compound at the time of identification.[10,25] Caryophyllene (0.88%) is an odorous bicyclic sesquiterpene found in various herbs and spices. Recently, it was discovered that beta-caryophyllene is a ligand of the cannabinoid receptor 2 (CB2). CB2 activation reduces pain, a key signal for the inflammatory response. It is suspected that beta-caryophyllene may affect wound healing by reducing inflammation. This belief is supported by further *in vivo* research, where -caryophyllene is made in the form of nanoemulsion, showing that treatment with -caryophyllene nanoemulsion is more efficient than the control group in accelerating wound healing and reducing skin irritation.[26,27] To strengthen the analysis of the activity of the active compounds found in the GC-MS test, in future studies it would be necessary to utilize a 70% ethanol extract in the form of developmental preparations and proceed with in vivo testing on experimental subjects.

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Conclusion

The active chemicals found in *Eleutherine sp* bulbs are considered to have antioxidant, antibacterial, and anti-inflammatory properties which promote wound healing. Further research into the pharmacological activity of the compounds in *Eleutherine sp* currently under progress is expected to confirm that the wound healing process can be accelerated.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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