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Analysis Phytochemical Compounds Of Ethyl Acetate Extract Garlic Tree, Scorodocarpus Borneensis Becc As A Source Of Bioactive Ingredients

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Abstract: This study aims to identify bioactive compounds contained in leaf extracts of garlic tree, Scorodocarpus borneensis as tropical forest trees that have the potential source of chemical natural ingredients. The GC-MS analysis of the plant extracts was performed using GC-MS a Shimadzu QP2010 GC / MS apparatus (Shimadzu Corp., Japan) and mass spectra of compounds found in the extracted data in the library of the National Institute of Standards and Technology (NIST). The results of the analysis were identified as much as the six compounds were presented. The results showed a major component with a maximum percentage (54.42%) of phytol, followed by Nonylphenol isomer (9.07%), Nonylphenol (5.93%, 6-octadecanoic acid, methyl ester (7.88%), 1.2 Benzene-dicarboxylic acid (5.47%) Based on this analysis it can be concluded from the present study that some of the identified phytochemicals could be responsible for the medicinal value or biological activity of the leaves of S.borneensis.

Index Terms: Phytochemical, Scorodocarpus borneensis. Ethyl acetate fraction, Gas chromatography-mass spectrometry

1 INTRODUCTION

Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since ancient times [1]. Plant extracts or their active constituents are used as folk medicine in traditional therapies of about 80% of the world's population and Over 50% of all modern clinical drugs are of natural product origin. Plant extracts or their active constituents are used as folk medicine in traditional therapies of about 80% of the world's population and Over 50% of all modern clinical drugs are of natural product origin[2]. Medicinal plants are directly analyzed by gas chromatography-mass spectrometry (GC-MS) for their existing phytochemicals. GC-MS is an advanced technology to determine the presence of phytochemicals in plant tissues, which is extensively being utilized to screen the phytochemicals in medicinal plant species. Using GC-MS, it is now possible to identify volatile compounds with ease. Garlic Tree S. borneensis Becc (Family Olacaceae) is one's of woody and wildlife plant in the lowland tropical forests of Kalimantan, Sumatera, Malay Peninsula, and Thailand, which has been named by natives as " wood garlic" due to its strong garlic-like smell. This garlic smell is present in the leaves, bark, wood, and fruit. The fallen fruit has a hard outer nutshell and is similar in shape and size to a walnut. The natives of Kutai in East Kalimantan occasionally use the sliced of the fruit as a seasoning just like garlic for cooking some types of soup. The leaves of Scorodocarpus borneensis are applied locally as one of the components in the folklore for diarrhea [3]. The Garlic Tree is large tree, 10-40 (rarely -60) m, 20-60 (-80, or more) cm ø, all parts reeking of garlic or onion especially after rain and from the cut or bruised parts; Branchlets smooth and glabrous at tips, older parts dark colored with elongate lenticels. Leaves generally elliptic, rarely lanceolate-oblong, apex rather abruptly acuminate for 1-2 cm, base cuneate to rounded, entire, subcoriaceous to coriaceous, shining green above, paler beneath when fresh, dull olive-green when dry, glabrous, often densely minutely tubercled mainly on upper surface by spicular cells or sclereids which show remarkably in dry leaves, 7-15 (-22, -32) by 3-5 (-7, -12) cm; Petals narrow-oblong, yellowish, pink or usually creamy white, 8-10 (-15) by 2 mm, brushed-woolly inside, finally reflexed. Ovary yellowish green, tapering to the thickish white style. Seed 1, subglobular [4]. This plant has been reported has content methyl disulfide, some megastigmanes and flavonoids and sulfur-containing compounds from the leaves and fruit [4,5,6], some sesquiterpenes and alkaloids from the fruit and bark [7] and scorodocarpin B and dehydroxyscorodocarpus B compounds in the fruits as anticancer[8]. Considering the importance of S.borneensis, this study was undertaken for the first time to analyze the bioactive compounds present in the ethyl acetate fraction of leaves from this plant.

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2 MATERIAL AND METHODS

2.1.Plant materials

Leaves of S.borneensis were collected from the Botanical Forest of Mulawarman University Samarinda, East Borneo. Only mature and healthy leaves were harvested and collected in the early morning, packed in polythene bags, and kept at room temperature for further processing. Leaves were washed with tap water and shade dried for 5-6 days. About 5 g of air-dried leaves were grounded to a fine powder

in a mortar pestle. The powdered were extracted with 100 ml ethanol. It was left for 24 hrs so that terpenoids and other constituents if present will get dissolved. The methanolic extract was then filtered through Whatman No. 1 filter paper. The filtrate then was concentrated to dryness by a rotary evaporator and dried in a fume hood until a dried crude paste formed.

2.2.Extraction

Powdered leaf (200 g, dry weight basis) were weighed and then macerated using 75% ethanol in a glass container with a ratio of 1:1 overnight and then filtered using filter paper No 1. The maceration was repeated thrice until filtrate clear. The extract solution obtained was concentrated by rotary evaporator and put in a vacuum oven to give a solid paste. The ethanol extract was fractionated with ethyl acetate to give the respective soluble fractions. The next phase of ethyl acetate was collected and evaporated extract of ethyl acetate. Further, the extract has analyzed the content of bioactive compounds have prior isolation using column chromatography. Fractionated using chromatographic columns techniques with a fixed phase Silica gel. A total of 0.5 g of the sample has evaporated the solvent was stirred with celite until homogeneous. Then the compacted silica gel 60 columns using a mixture of chloroform: methanol: water = 7: 3: 1. After the solid coated filter paper as a cover and put the mixture celite and the sample was then eluted. Eluent accommodated with a test tube and test fractions by thin layer chromatography to unify the factions have the same Rf. Purification is done to the fraction of ethanol and the results of column chromatography against potential fraction that has the same stain pattern combined and coded separately. Isolates obtained is then washed continuously with pure methanol pa to obtain colorless filtra and analyzed by GC/MS Shimadzu QP2010S.

2.3.GC–MS Method

The ethyl acetate fractions obtained from TLC was subjected to GC-MS analysis. This fractions were dissolved in HPLC grade solution and subjected to GC-MS a Shimadzu QP2010 GC/MS apparatus (Shimadzu Corp., Japan) equipped with Ri-5MS column Rastex capillary (30 m, 0.25 mm, ionization: EI 70 Ev), heated at a temperature of 80°C, pressure of 16.5 kPa, total flow of 20.0 ml/min, column flow of 0.50 ml/min, linear velocity 26.1 cm/sec, purge flow 3.0 ml/min, injector and detector temperature was 310°C, using helium as the carrier gas, at a flow rate 3.0 ml/min. Total running time of GC-MS is 40 minutes, the relative percentage of each extract constituent was expressed as a percentage with peak area normalization. Interpretation of the mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08 and Wiley08 library. The name, molecular, and structure of the components of the test materials were ascertained.

3 RESULTS AND DISCUSSION

3.1.Phytocomponents

In the present study, a total of 6 different phytocomponents have been found in the ethyl acetate extract of the leaves of S. borneensis. The gas chromatography profile of this extract was displayed in **Figure 1.**The identified compounds of garlic tree, their retention indices (RT), compounds and percentage composition (area %) are given in Table 1.

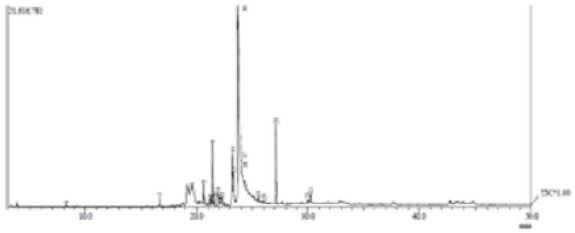

Fig. 1: *Gas chromatography-mass spectrometry chromatogram of the ethyl acetate leaf extract of Scorodocarpus borneensis*

Table 1: *Phytocomponents detected in the ethyl acetate leaf extract of Scorodocarpus borneensis*

No peak	Retention Time (min)	Compounds	Area(%)
3	18.203	Phenol,4-nonyl	5.93
4	19.597	Nonylphenol isomer	9.07
9	21.45	Eicosanoic acid, methyl ester	4.48
14	23.240	6-octadecenoic acid methyl ester	7.88
16	23.654	Phytol	54.42
21	27.017	1,2-benzene dicarboxylic acid bis (2-ethylhexyl)ester (a dioctyl phthalate ester).	5.47

The results showed the presence of six major components with maximum percentage (54.42%) of phytol, followed by Nonylphenol isomer (9.07%), Nonylphenol (5.93%, 6-octadecanoic acid, methyl ester (7.88%), 1,2 Benzene-dicarboxylic acid(5.47%).On comparison of the mass spectra of each phytochemical with the NIST and Wiley library, six phytoconstituents were characterized and identified. The individual fragmentation of six major components is illustrated in **Fig.2**.

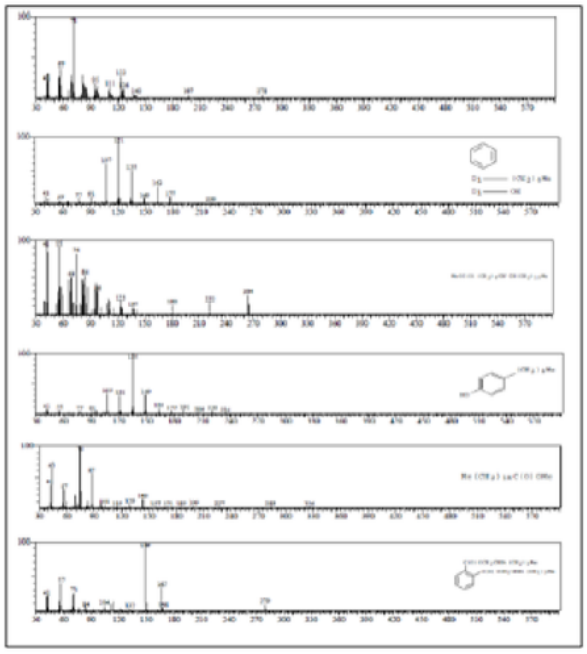

***Fig.2**: Mass spectrum of (A) phytol,(B) nonyl phenol isomer,(C) 6-octadecenoic acid,methyl ester,(D) phenol,4-nonyl, (E)1,2-benzenedicarboxylic acid,bis(2-ethylhexyl)ester and (E) eicosanoic acid, methyl ester.*

3.2.Discussion

GC-MS chromatogram of the ethyl acetate fraction extract of the leaves of S. borneensis showed the presence of six different phytocomponents. From observations and comparison with the mass spectrum of each constituent with NIST and Wiley library, it was found that phytol, nonylphenol isomer, 6-octadecenoic acid methyl ester(D) phenol, 4-nonyl, 2-benzene dicarboxylic acid, bis(2-ethylhexyl)ester and eicosanoic acid, methyl ester and phytol are the major components (Fig. 2). Phytol is the product of chlorophyll metabolism in plants. It is chemically called acrylic diterpene alcohol which is used in the manufacture of Vitamin E[9] and K[10] which are important in the many functions of the human body. It is used in shampoos, cosmetics, toilet soaps, fragrance industry, household cleanser, and detergents [11]. Biological activity this compound as hypocholesterolemic, anti-microbial, cancer preventive, diuretic, anti-inflammatory[12,13], induces apoptosis in human gastric adenocarcinoma AGS cells[14], antinociceptive and antioxidant activities [15] and immunostimulatory[16]. Nonylphenol isomer(9.07%) and phenol,4-nonyl (5.93%), were plant phenolic compounds can act as antioxidants, structural polymers (lignin), attractants (flavonoids and carotenoids), UV screens (flavonoids), signal compounds (salicylic acid and flavonoids) and defense response chemicals (tannins and phytoalexins). From a human physiological standpoint, phenolic compounds are vital in defense responses, such as anti-aging, anti-inflammatory, antioxidant and antiproliferative activities[17]. The linoleic acid ester, 9-octadecenoic acid (Z) methyl ester having anti-inflammatory, antiandrogenic, and anemiagenic properties [18].However, 1,2-benzene dicarboxylic acid bis(2-ethylhexyl) phthalate (a dioctyl phthalate), which constitutes about 5.47% in the tested extract, is a fatty acid ester has plastics similar and when added to thermoplastics they increase their flexibility, transparency, and durability[19]. This is the first report on identification of this phthalate from the leaves of S.borneensis. Eicosanoic acid, methyl ester derivate arachidic acid importance as alpha-glucosidase inhibitors[20], as an anti-inflammatory, antioxidant[21]. Our investigation through the present study revealed that the species S. borneensis is a reliable source of bioactive compounds that justify the traditional usage of this species by the local healers in East Kalimantan, Indonesia, for various ailments. As GC-MS is the first step towards understanding the nature of active principles, further investigation in this species is suggested for the development of novel drugs.

4.Conclusion

The GC-MS studies of ethyl acetate fraction of Scorodocarpus borneensis leaves clearly indicate that the major compounds are Phytol, Nonylphenol isomer, Nonylphenol, 6-octadecanoic acid methyl ester,1,2 Benzene-dicarboxylic acid are identified.

5.CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

6.Acknowledgment

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