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GC-MS ANALYSIS OF VOLATILE ACTIVE COMPOUNDS ISOLATED FROM *Aspergillus sp* OF *Rhizophora mucronata*

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ABSTRACT

This study aimed to identify anti-bacterial compounds consisted of the ethyl acetate fraction of Aspergillus sp extracted from the root of *Rhizophora mucronata*. The sample of *Aspergillus sp* cultivated from mangrove located in West Sumatera province. Each culture isolated was macerated in the ethyl acetate and analyzed using GC-MS instrumentation. The GC-MS spectra indicated fifteen compounds including isoindole derivative, organic acids (3 compounds), organic acid ester (4 compounds), acetate derivative, acetone derivative, alcohol derivatives (3 compounds) and naphthalene derivative. Among the identified compounds, 4-isopropyl-1,6-dimethyl-1,2,3,4,4A,7-hexahydronaphtalene, trifluoroacetyl-isopulegol, 2-ethyl-2,4,5-trimethyl-1,3-dioxolane and 4-isopropyl-1,6-dimethyl-1,2,3,4,4A,7-hexahydronaphthalene were reported as antibacterial and antimicrobial compounds.

Keywords: GC-MS, Biological Activities, *Aspergillus sp*, *Rhizophora mucronata*.

INTRODUCTION

Endophyte mould primarily resides in the tissues below the epidermal cell layers of the plants without harming the host[1]. Recent studies have shown that from over three hundred thousand species of plants that survive on the earth, nearly every single species is the host to at least one kind of endophytic fungi. The fungi can usually found in leaves, but it can also be manifested in the gasp of the root as the main entry door for all kinds of a microorganism to penetrate the plant. The colony can positively affect the mass plants by protecting the inner part of a plant from other microbial or bacterial attacks since the endophytic fungi exhibit sort of chemicals that actively defend the inner part of a plant from the incoming intruders like the pathogens. Furthermore, two stated that the endophytic fungi provides some benefits to the host plants including insect deterrence, mammalian herbivores deterrence, reduction of nematodes, increase the resistance of host disease, avoiding abiotic stress, increase the host biomass and protection from pathogens.

Aspergillus sp is the endophytic mould belongs to the group of *Filamentous Deuteromycetes*[2] which is highly aerobic growing on carbon-rich substrates contained either monosaccharide or polysaccharide[3]. *Aspergillus sp* is a fungus that forms long branched filaments, and in culture media, it forms mycelia and conidiophores. This species reproduces by forming hyphae or buds and produces spore-forming conidiophores.

Endophytic fungi have great potential as new sources of medicine since the microbes are easy to breed, have a short life cycle and can produce large amounts of bioactive compounds in a short time. Endophytic fungi are known as a source of abundant secondary metabolites and are very interesting[4] in terms of activity and chemical structure. In addition to heavy secondary metabolite molecules like alkaloids,

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flavonoids and peptides, the endophytic fungi also produce volatile compounds such as organic acids, organic ester and alcohols which can be determined using GC-MS instrumentation.
Since the secondary plant metabolites generated by a fungus can mutually benefit the host plant, the chemicals produced are expected to have a similar effect on protecting human body parts like tooth or nails. Therefore, this paper presents the volatile compounds generated by the endophytic fungi cultivated from the mangrove roots, and analyze the potential of the substances as anti-fungi, anti-bacterial and anti-microbial agents.

EXPERIMENTAL

Materials and Methods

The endophytic fungus cultivated from the district of Pantai Pasir Jambak, West Sumatera Province, Indonesia. The materials used in this research were ethyl acetate (Merck), ethanol (Sigma-Aldrich), filter paper and demineralized water. The cold macerated extraction was performed to collect the ethyl acetate fraction of the fungus. The volatile constituents were analyzed using GC-MS instrument.

Extraction of endophytic fungus

The extraction technique referred to the method modified by NY N (2018)[5]. Each cultured isolate and immersed in 300 mL of acetic acid for 96 hours. After four days, the concentrate was filtered using Whatman filter paper No. 1 until the ethyl acetate fraction obtained. The fraction was then evaporated using Rotary Evaporator (Perkin Elmer 6000), and the concentrated isolate stored for GCMS determination.

GC-MS Analysis

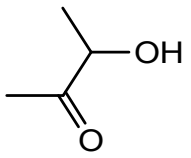
The GC-MS analysis of the ethyl acetate extract of *Aspergillus* conducted at the Provincial Health Laboratory of Special Capital of Jakarta using a Shimadzu QP2010PLUS system comprising an AOC-20i auto-sampler and the GC coupled to a Mass Spectrometer equipped with an Elite-5MS fused a capillary column of 30×0.25 µm ID×0.25 µm df. The electron ionization system operated in electron impact mode with ionization energy of 60 eV. The carrier gas set up at a constant flow rate of 1 mL/min with an injection volume of 2 µl.

RESULTS AND DISCUSSION

The GC spectrum of ethyl acetate extract of endophytic fungus (Fig.-1) confirmed the presence of at least 21 components with different retention times as concisely depicted by Table-1. The mass spectrometer analyses the compounds eluted at various times to identify the nature of the compounds by showing compound fragments at different m/z ratios. From 21 components found in the ethyl acetate extract, there are six significant compounds with the peak area of more than 4% as explained later in this article.

Major Components

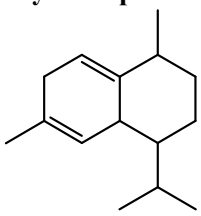
3-Hydroxybutan-2-one



3-Hydroxybutan-2-one

3-hydroxybutan-2-one or *acetoin* is a fermented product that generated by the butanediol life cycle of organism wherein mammals this compound turns to CO_2. The dimerization of acetoin resulted in the crystalline structure. In industries, this compound is used as a food additive and was among important chemicals prioritized by US Department of Technology for development and utilization[6]. *Acetoin* can be produced either through chemical synthesis or biological synthesis, but enzymatic synthesis is favorable due to cost efficiency[7]. Therefore, several research findings reported acetoin production using various yeasts, including *Saccharomyces cerevisiae*[8] *Lactococcus lactis*[9], *Bacillus myloliquefaciens*[10], and *Serratia marcescens*[11]. Generally, almost all fermentation process generates acetoin with different ratios.

4-Isopropyl-1,6-dimethyl-1,2,3,4,4A,7-hexahydronaphtalene



4-isopropyl-1,6-dimethyl-1,2,3,4,4A,7-hexahydronaphtalene

This compound is a derivative form of the naphthalene group with ten carbon member rings. Some reports revealed that this compound mainly found in *Syzygium aromaticum* oil[12], black pines[13], and cardamom oil. With a combination of other active components of the oil, this compound has been reported to have antioxidant activity, anti-inflammatory and antimutagenic[14]. The compound is relatively similar in terms of chemical structure to the eugenol family, a group of a compound that has a significant effect against bacteria[15].

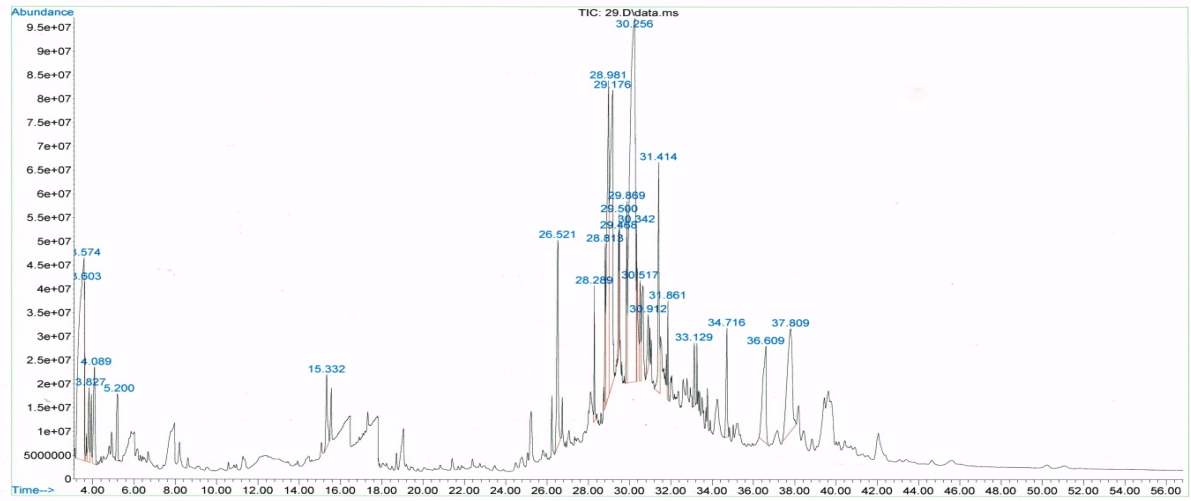
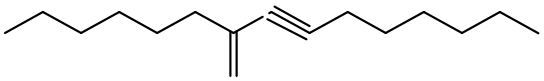


Fig.-1: Typical GC Spectrum of Endophytic Fungus Extract

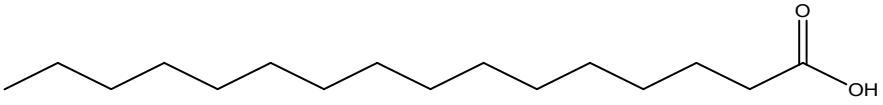
7-Pentadecyne,-9-methylene



7-Pentadecyne,-9-methylene

7-pentadecyne,-9-methylene is the member of alkyne that Giacomelli in 1979 synthesized it by introducing the synthesis of dialkyl-butyne through the reaction of acetylene diisobutyl zinc with a nickel catalyst. Naturally, the fungus metabolism process generates alkyne group compounds. Most of the alkyne group compound has been reported to have antimicrobial and antifungal activities[16]. Furthermore, the structural analysis of the compound shown that the compound has a positive Milog p indicated the bioactivity of the compound[17].

Hexadecanoic acid



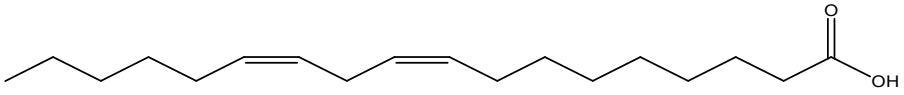
Hexadecanoic Acid

Hexadecanoic acid is trivially known as palmitic acid and is ubiquitous organic acid found in various sources like coconut oil, palm oil, and nuts. In nature, this compound is available as triglyceride and used as a food additive and preservative agent. Besides, this organic acid also gives a mitotic effect to test mice[18]. However, another report revealed that the excess of palmitic acid in the human body could promote the dyslipidemia dan hyperglycemia[19].

Table-1: Volatile Components Identified in Ethyl Acetate Fraction of Endophytic Fungus

RT (min)	IUPAC Name	MW (g mol^{-1})	Peak area (%)
3.577	3-hydroxybutan-2-one	88.11	13.59
3.604	2-[2-(2-ethoxyethoxy)ethoxyethanol	178.23	0.87
3.825	2-ethylbutanoic acid	116.16	1.16
4.087	2-ethyl-2,4,5-trimethyl-1,3-dioxolane	144.21	2.22
5.197	2,3-butanedioldiacetate	174.20	1.30
26.264	4-isopropyl-1,6-dimethyl-1,2,3,4,4A,7-hexahydronaphtalene	204.36	4.34
28.289	Hexadecanoic acid methyl ester	284.48	1.00
28.813	Hexadecanoic acid ethyl ester	284.48	1.53
28.979	7-pentadecyne,-9-methylene	221.41	8.53
29.179	Hexadecanoic acid	270.46	11.64
29.468	Methyl linoleat	308.51	1.08
29.868	9-octadecanoic acid (Z)-ethyl ester	310.52	1.96
30.255	9,12-octadecanoic acid	294.48	26.40
30.344	Hexadecanamide	255.44	0.96
30.517	Trifluoroacetyl-isopulegol	250.26	1.51
30.910	9-chloro-1-azaphenoxathiin	235.69	0.99
31.413	Octadeca-9,12-dien-1-ol	280.50	3.55
31.861	1-Methyl-2-pyrrolidinethione	115.19	0.80
33.130	Cis-11-octadecanal	268.47	0.86
36.612	2-Methyl-2H-isoindole-1-carbonitrile	156.19	3.46
37.812	(22E)-Ergosta-5,7,22-trien-3-ol	396.65	7.17

(z,z)-9,12-Octadecadienoic Acid



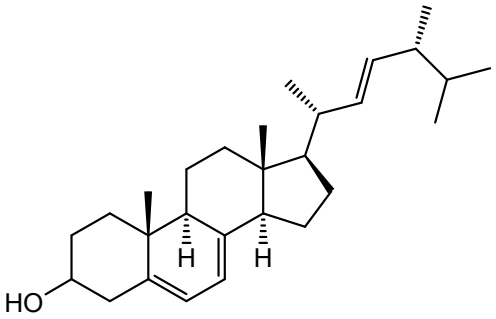
(z,z)-9,12-Octadecadienoic Acid

Linoleic acid (LA) is an essential nutrient that cannot be synthesised by the human body. Therefore, the nutrient must be taken from foods because humans cannot incorporate a double bond beyond the ninth carbon of a fatty acid[20]. LA; 18:2n-6 plays a significant role in cell physiology, immunity, and reproduction, and is an essential nutrient in diverse organisms[21]. LA is synthesized in plants, bacteria, and protozoa. As a necessary component of ceramides, linoleic acid involves the maintenance of the transdermal water barrier of the epidermis, LA also a precursor for other prominent PUFA members including arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid which are essential nutrients for human brain development[22]. Biosynthesis of this compound in plant occurs either in two enzymatic processes (1) plasmodial enzyme (FAD6) where methyl station is utilized as a critical point and classified as ω-6 desaturase enzyme by encouraging ω-6 carbon. (2) extra-plastidial system and also known as oleate Δ12 desaturase (FAD2) which is very selective and specific to C12 and C13[9].

According to the recent reports, LA associates with the degradation of melanin production of human skin through dynamically accelerate the deterioration of tyrosinase, the enzyme responsible for the production of melanin. The clinical test revealed that topical application of 0.1% of LA could effectively treat melasma and brighten the darken skin due to UVB exposure [23]. Moreover, LA also plays a significant role in many biochemical pathways, thus possess useful medicinal properties such as cardio-protective and

anti-inflammatory[24,25]. In addition to the biosynthesis of several necessary compounds in the human body, the essential oils are also commonly used as aromatic therapeutic agents[26] and plasticisers[27].

(22E)-Ergosta-5,7,22-triene-3-ol



(22E)-Ergosta-5,7,22-triene-3-ol

One of the interesting compounds found in the ethyl acetate extract of *Aspergillus sp* from mangrove root is the (22E)-Ergosta-5,7,22-trien-3-ol as above depicted structure. The compound is known as a precursor for the production of estradiol, the compound that has an antirachitic activity and known as provitamin D_2[28]. A recent study showed that ergosterol extracted from edible mushrooms is proactive against leukaemia, and 25 µg of the extract was able to inhibit the growth and induced apoptosis of HL60 cells completely[29].

CONCLUSION

The presence of various bio-active compounds of the ethyl acetate extract of *Aspergillus sp* cultivated from the root of *Rhizophora mucronata* was confirmed using GC-MS analysis. There were up to 21 volatile compounds detected, and about six significant components were biologically active. However, phytochemical screening and isolation of individual compounds and proper biological activity tests will undoubtedly provide more meaningful results and will open a new area of investigation of different components and their potential pharmacological use.

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