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IDENTIFICATION AND ANTIOXIDANT ACTIVITY TEST FOR FRACTIONATED COMPOUND FROM ETHANOL EXTRACT OF THE SINGKUT RHIZOME (*Molineria latifolia* Dryand)

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ABSTRACT

Molineria latifolia (Singkut rhizome) is an endemic plant in tropical and humid climates, so it is widely distributed in several Asian countries. This research will determine the antioxidant activity from the rhizome ethanol extract of *Molineria latifolia* and the isolate compounds. The antioxidant activity of the extract is a powerful category with IC_{50} 26.033 ppm. The results of the isolation of the extract rhizome of *Molineria latifolia* is known to contain 2,6-dimethoxy-benzoic acid and 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid compounds.

Keywords: *Molineria latifolia*, antioxidant activity, extract rhizome, isolation

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INTRODUCTION

Molineria latifolia or known as Singkut belonging to the genus Molineria and family Hypoxidaceae is one of the little palms which is very common in protected areas from the sun[1,2,3,4]. Molineria latifolia contains secondary metabolite compounds in flavonoids and tannins, which the rhizome has the most flavonoids[5].

Crude extract of *Molineria latifolia* is reported to have anti-diabetic properties[6], ethyl acetate fraction, which is also known, can increase insulin sensitivity and glucose tolerance[7]. Previous research showed the total phenolic content of the ethyl acetate fraction of *Molineria latifolia* was 187.30 mg/g, and flavonoids from the ethyl acetate fraction of *Molineria latifolia* was 76.77 mg/g, which had an antioxidant activity with a value of IC_{50} up to 36.96 µg/mL[1]. The fruit of *Molineria latifolia* contains neoculin compounds which give a sweet taste of 430-2,070 times compared to sucrose at the same weight[8]. Moreover, quinine-ester gentisylquinonyl-2,6-dimethoxybenzoate has been isolated from *Molineria latifolia*[5].

Free radicals are a form of reactive oxygen compounds or reactive nitrogen, which are generally known as compounds that have unpaired electrons. The impact of free radical reactivity compounds in the body varies from damage to cells or tissues that cause degenerative diseases[9], autoimmune diseases to cancer[10].

Antioxidants are chemical compounds that can donate one or more electrons into free radicals. Antioxidants provide resistance to oxidative stress by inhibiting free radicals[11]. Sources of antioxidants from outside the body can be natural antioxidants and synthetic antioxidants. Many natural antioxidants are found in vegetables and fruits that contain vitamin C, vitamin E, carotenoids, and flavonoids. While synthetic antioxidants are BHA (butylated hydroxyanisole), BHT (butylhydroxytoluene), TBHQ (tert-butylhydroquinone), and PG (propyl gallate)[12], however, the use of synthetic antioxidants is starting to be limited because research results report that the use of synthetic antioxidants can poison experimental animals. It's carcinogenic, so the food and pharmaceutical industries have begun developing and seeking new sources of natural antioxidants. Based on the many benefits of *Molineria latifolia*, it is necessary to do further research of *Molineria latifolia* ethanol extract as a new sources of natural antioxidant and isolates the compound.

EXPERIMENTAL

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Preparation and Extraction

The rhizome of the fresh *Molineria latifolia* plant was washed and aired at room temperature, then mashed in a blender. A total of 1kg of *Molineria latifolia* powder was macerated using ethanol for 3 × 24 hours and filtered[13].

Antioxidant Activity Test

The antioxidant test was prescribed by the DPPH (*1,1 diphenyl-2-picrylhidrazil*) method using UV-Visible at 517 nm wavelength. The sample concentrations used were 2, 4, 6, 8, and 10 ppm with a comparison of vitamin C at a concentration of 1, 2, 3, 4, and 5 ppm and a DPPH concentration of 0.2 mM[13].

Fractionation and Identification

Initial fractionation was carried out by liquid vacuum chromatography with 11 eluent ratios, namely n-hexane: ethyl acetate sequentially (10:0), (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9), (0:10) and followed by column chromatography using suitable eluents[13]. GC-MS performed identification.

RESULTS AND DISCUSSION

Preparation and Extraction

Extraction of Singkut rhizome was carried out by the maceration method. The result of the concentrated extract was 81.42 grams (8.14%) with a blackish-brown color. Phytochemical test of ethanol extract *Molineria latifolia* rhizome showed alkaloid, flavonoid, saponin, and tannin compounds.

Antioxidant Activity Test

The principle of this method is to reduce the intensity of the DPPH color, which reacts with the sample to form DPPH-H. The difference in absorbance of the samples reduced by DPPH with absorban control was the remaining DPPH radicals read on a UV-Vis spectrophotometer. The percent yield of antioxidant activity from the ethanol extract of *Molineria latifolia* rhizome, fraction I-2, and vitamin C can be seen in figure 1.

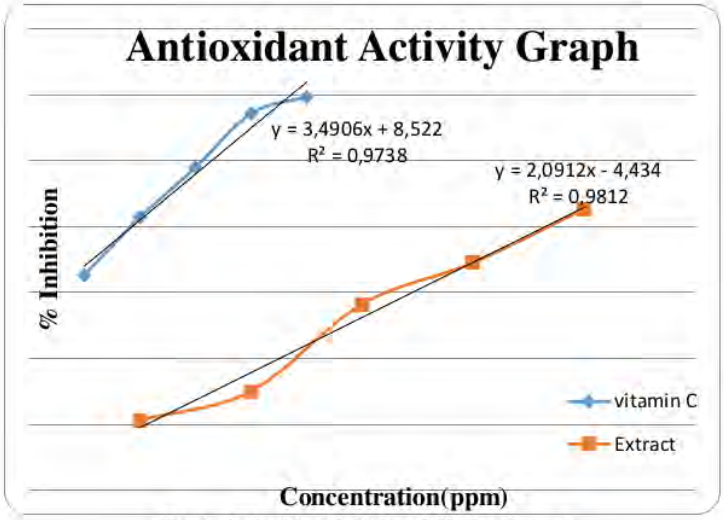

Fig.-1: Antioxidant Activity of Singkut Extract

Data on percent (%) of antioxidant activity from the ethanol extract of Singkut rhizome can be used to determine the antioxidant potential of the sample indicated by the IC_{50} value. The IC_{50} value is the parameter used to indicate the concentration of the test extract, which can ward off DPPH free radicals as much as 50%[14]. The antioxidant activity of a sample is classified into five types based on the magnitude of the IC_{50} value, namely very strong (<50 ppm), strong (50-100 ppm), moderate (100-150 ppm), weak (150-200 ppm), and very weak[15].

Table-1: IC_{50} Value from Vitamin C, Extract and Fraction of *Molineria latifolia*

Sample	IC_{50} value	Category
Vitamin C	12,246	Very strong
Ethanol extract	26,033	Very strong

Based on the table, it can be seen the magnitude of the IC_{50} value from each sample. In the ethanol extract of the Singkut rhizome, the IC_{50} value is 26.033 ppm, and the vitamin C value is 12.246 ppm.

Fractionations and Identification

Fractionation of Singkut rhizome extract was carried out by liquid vacuum chromatography based on eluent polarity. From 11 fractions, n-hexane: ethyl acetate (2:8) was taken to be separated using column chromatography. The column chromatography results were identified by thin-layer chromatography and obtained isolate with similar separation pattern, then isolate with the single spot was determined by GC-MS.

Based on the spectrum analysis results, there is an incomplete separation because there is more than one peak in it, which indicates that there is still more than 1 compound from the result fraction. However, the analysis shows one prominent peak with the most abundance and similarity above 90%, shown in figure 2.

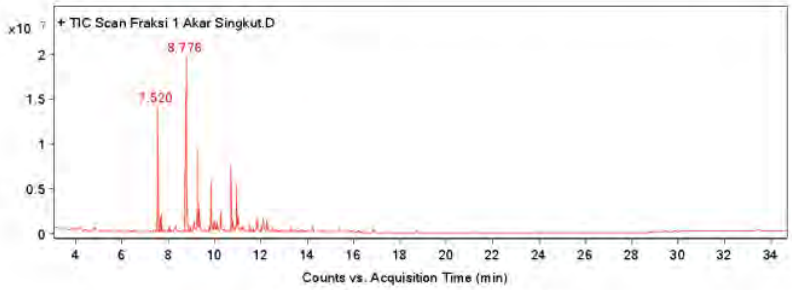

Fig.-2: Peak in GC-MS Analysis

The peak that appeared at the retention time of 8,776 is the most abundant compound in ethanol extract of Singkut rhizome isolate with a value of 70,49% (table 2). The peak with RT = 8,776, believed as 2,6-dimethoxybenzoic acid compound and similarity percentage equal to 98%, also amplified from the GC-MS fragmentation peaks in Figure 2. The fragmentation spectra show a base peak with m/z 182.1 and the molecular weight of the 2,6-dimethoxybenzoic acid compound.

Table-2: Retention Time (RT), Area, Concentration, and Name of Chemical Compounds

No	Retention Time (min)	Area	Concentration %	Name of Compound
1.	7,520	33326057.47	29.51	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-
2.	8,776	19671529.51	70,49	Benzoic acid, 2,6-dimethoxy-acid

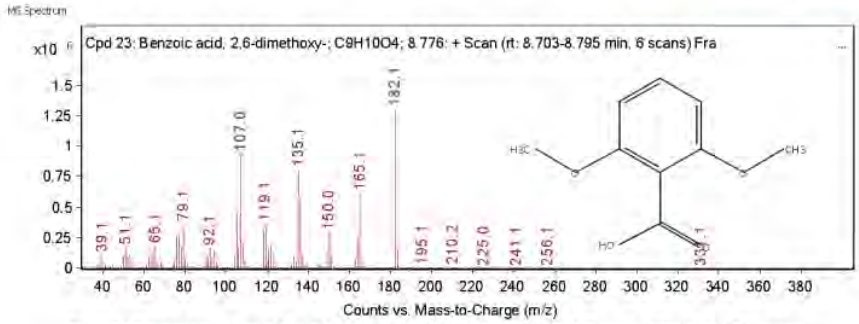

Fig.-3: Fragmentation of Isolate Compounds from Ethanol Extract Singkut Rhizome

The release of the hydroxyl group (-OH) is shown in fragment a with m / z = 165.1. The peak of fragment b with m / z = 150 formed after the C2 methoxy group from fragment one releases the CH_3 atom and is accompanied by rearrangement and removal of the methoxy group on C6 produce c fragment with m / z = 119. Furthermore, fragment c releases the CCO group to produce fragment 6 with m / z = 79. The fragment also releases the OCH_3 group followed by rearrangement of the H atom to produce fragment d with m / z = 135. The release of the COOH group from the base peak and accompanied by rearrangement of the H atom results in fragment f with m / z = 135.1, which is then the result followed by the release of the CO group yielding a fragment f with m / z = 107 (figure 3).

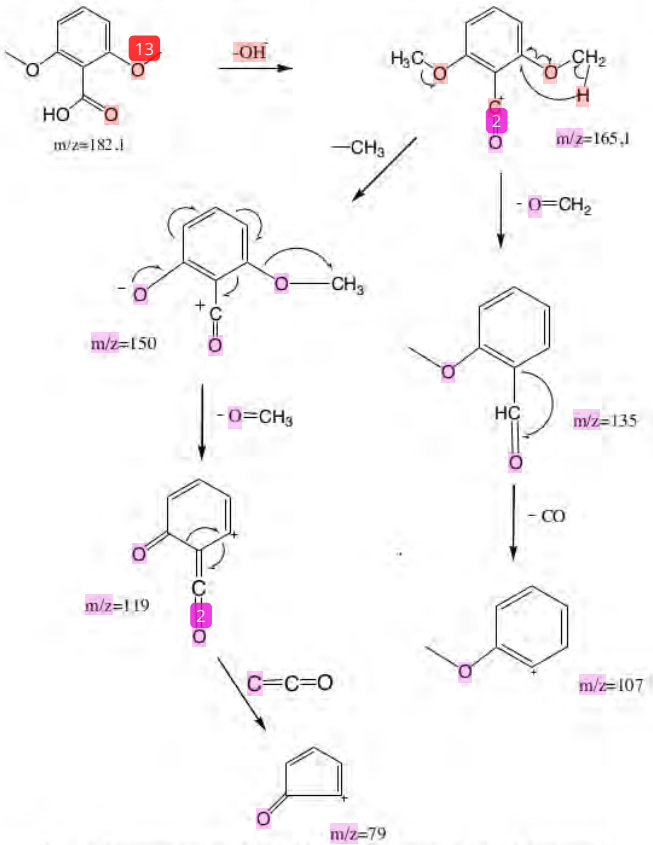

Fig.-4: Fragmentation of 2,6-Dimethoxybenzoic Acid Compound

The compound 2,6-dimethoxybenzoic acid (CAS Number 1466-79-8) is used in the synthesis of indoles as well as the coumarins associated with Novobiocin. Novobiocin is used for Infections due to staphylococci and other susceptible organisms and other conditions used as a potent inhibitor of heat-shock protein 90 in the treatment of breast cancer[14,15].

CONCLUSION

The antioxidant activity of the ethanol extract of Singkut rhizome was categorized as powerful with an IC_{50} value of 26.033 ppm. The isolation of secondary metabolites from the Singkut rhizome has succeeded in separating two compounds, namely 2,6-dimethoxy-benzoic acid with a concentration of 70, 49%, and acetic acid 3-Furan, 4-hexyl-2,5-dihydro-2,5-dioxo with a concentration of 29.51%.

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