The Potential Active Chemical Compounds as Antioxidants and Antidiabetics from Rhizophora mucronata Derived from Sambera Beach, East Kalimantan

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ABSTRACT
This research aims to determine the content of active chemical compounds, antioxidant activity, and antidiabetes of methanol, dichloromethane, and ethyl acetate extracts of *Rhizophora mucronata* mangrove leaves. The *R. mucronata* leaf samples (1 Kg) were extracted using methanol solvent, then continued with liquid-liquid partition extraction using dichloromethane solvent, and finally ethyl acetate solvent. The extraction process was carried out for 1 x 24 hours (three repetitions). The third extract obtained was evaporated using a rotary evaporator so that three thick extracts of *R. mucronata* mangrove leaves were obtained. Furthermore, the three extracts were identified phytochemical content with color test method, followed by antioxidant activity test with DPPH (1,1-diphenyl-2-picrylhydrazyl) method, and oral glucose tolerance test (OGTT) to determine antidiabetic activity. The phytochemical contents of the extract of methanol, dichloromethane, and ethyl acetate of mangrove leaves of *R. mucronata* are alkaloids, flavonoids, phenolic compounds, triterpenoids, steroids, saponins, and tannins. The antioxidant activity of the ethyl acetate extract of *R. mucronata* leaves is classified as very strong with an IC_{50} value of 34.64 ppm. The ethyl acetate extract also showed the highest decrease in blood glucose levels at 24 hours of 57.64%. The mangrove plant *R. mucronata* from the coast of Sambaera has the potential to be developed as a natural antioxidant and alternative antidiabetic drug, especially ethyl acetate extract.
Keywords: *Mangrove plants, Antioxidant activity, and Andiabetic activity.*

Introduction

The mangrove forest in Indonesia is one of the largest mangrove forest areas in the world, so researchers in the field of natural organic chemistry and the herbal medicine industry are interested in conducting research on the utilization of mangrove forests as a source of antibiotics. In recent years, the demand for herbal medicine by diabetics has increased, along with the increasing number of diabetics in Indonesia. Data from the International Diabetes Federation (IDF) shows that the number of diabetics in Indonesia in 2019 is estimated to reach 10.7 million people and by 2045 it is expected to increase to 16.7 million people. [1] Therefore, exploration to find a cure for diabetes continues to develop.

The mangrove plant is a type of plant that is widely used by the community as a traditional medicine to cure various diseases, such as diarrhea, malaria, smallpox, asthma, diabetes, fever, swelling, rheumatism, skin diseases, hepatitis, diuretics, leprosy, antitumor, leukemia, anticancer, antiviral, and mumps. [2-4] Parts of mangrove plants that are utilized as medicinal materials are root tissue, stem wood, bark, leaves, twigs, flowers, and fruits. [5-6] Mangrove plants are rich in secondary metabolite compounds; alkaloids, polyphenols, flavonoids, tannins, saponins, triterpenes, anthroquinones, catechins, glucose, proteins, steroids, phenolic compounds, and glycosides that have antioxidant and antidiabetic activities. These mangrove plant species include *A. marina, A. ilicifolius, C. tagal, R. muronata, R. apiculata, S. alba, S. caseolaris, X. granatum,* and *N. fruticans*. [7-9] Therefore, research on the bioactivity of mangrove species *R. mucronata* from Sambera Beach, East Kalimantan, has the potential to find secondary metabolite compounds that have antioxidant and antidiabetic activities which can then be developed as natural herbal medicines.

Taxonomy of *R. mucronata*

The taxonomy of *R. mucronata* is as follows:

Kingdom : Plantae
Phylum : Magnoleophyta
Class : Magnoliopsida
Ordo : Malpighiales
Family : *Rhizophoraceae*
Genus : *Rhizophora*
Species : *Rhizophora mucronata*

Figure 1

Material and Methods.

Equipment and Reagents

This research uses several equipment, including glassware commonly used in laboratories, digital analytical, rotary evaporator, vortex, incubator, pH meter, and UV-Vis spectrophotometer. The materials used in this study were mangrove leaf of *R. mucronata*, aquades, 1,1-diphenyl-2-picrylhidrazyl (DPPH, Merck), methanol 70%, dichlorometane (Merck), Ethyl Acetate (Merck) H_2SO_4 (Merck), $FeCl_3$ 0.1%, several color reagents for phytochemical tests (alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins), CMC-Na, Glibenclamide, filter paper, and aluminum foil.

Sample collection and preparation

The mangrove leaf samples of *R. mucronata* were taken from Sambera Beach, East Kalimantan. Mangrove leaf samples were washed with running water to remove dirt attached to the leaves and then cut into small pieces. The dried samples were sorted and then pulverized into fine powder with a size of 90 mesh. Mangrove leaf powder is ready to be used for the subsequent procedures.

Extraction

About 1 kg of *R. mucronata* mangrove leaf powder was extracted by maceration method using 70% methanol solvent (powder and solvent ratio 1:10) and carried out for 1 x 24 hours (three repetitions). The methanol extract obtained was combined and then the solvent was evaporated with a rotary evaporator at 40 °C and 1 atm pressure, until a thick blackish green methanol extract was obtained. A portion of the methanol extract was extracted by liquid-liquid partition using dichloromethane solvent and then continued using ethyl acetate solvent to obtain dichloromethane, ethyl acetate, and methanol fractions. Then the three extracts obtained were continued with phytochemical, antioxidant and antidiabetic tests.

Phytochemical Test

Phytochemical tests of methanol, dichloromethane, and ethyl acetate extracts were carried out qualitatively using modified standard procedures. The phytochemical compounds to be determined are: alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins. [10-12]

Antioxidant Test

The antioxidant activity of the methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves of *R. muronata* and vitamin C (comparison) were evaluated in vitro using the DPPH (1,1-diphenyl-2-picrylhidrazyl) method following the procedure described by Senhaji B with modifications. 2 ml each of methanol, dichloromethane, and ethyl acetate extracts with a concentration of; 20, 40, 60 and 80 ppm, as well as vitamin C in concentration; 2, 4, 6, and 8 ppm mixed with 2 ml of DPPH (50 ppm) solution. After being incubated for 30 minutes in the dark at room temperature, the absorbance of the three extracts and vitamin C was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. [13] The percentage of antioxidant activity or the percentage of DPPH inhibition of the methanol, dichloromethane, and ethyl acetate extracts and vitamin C was calculated using the following formula:

$$\%\ \text{Inhibition DPPH} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100\ \%$$

Then a linear regression curve was made from the percentage data of DPPH inhibition on the concentrations of the three extracts and also the concentration of vitamin C. The IC50 value (as an indicator of antioxidant activity) of the methanol,

dichloromethane, and ethyl acetate extracts and vitamin C can be calculated through the regression equation of the linear regression curve obtained. [14]

Antidiabetic Test

Antidiabetic activity test using the oral glucose tolerance test (OGTT) method. The mice used as samples were male mice with a body weight of 150-200 grams, and were divided into 5 groups, each group consisting of 5 mice. Prior to treatment, all mice were fasted for 18 hours, then blood samples were taken from the vein in the tail of the mice using a glucometer to determine fasting/initial blood glucose levels. Then all groups were given 50% glucose monohydrate solution orally, except for the negative control group. After induction of glucose monohydrate for 180 minutes, the mice's blood glucose levels were measured again and mice that were already in a hyperglycemic condition (blood glucose level > 200 mg/dl) were selected as samples for treatment according to the group.

Group 1 (negative control) was the group of mice that were given 1% CMC-Na suspension. Group 2 (positive control) was the group of mice that were given glibenclamide suspension at a dose of 10 mg/kg BW. Group 3 (D1), mice were treated with methanol extract of mangrove leaves at a dose of 300 mg/kg BW. Group 4 (D2), mice were treated with methanol extract of mangrove leaves at a dose of 600 mg/kg body weight, and group 5 (D3), mice were treated with methanol extract of mangrove leaves at a dose of 1,200 mg/kg body weight. The same thing also applies to the treatment of dichloromethane and ethyl acetate extracts.

Measurement of blood glucose levels in mice was carried out after the treatment group (negative control, positive control, methanol extract, dichloromethane, and ethyl acetate of mangrove R. mucronata leaves) at 8, 16, and 24

hours, to determine the decrease in blood glucose levels in mice. Furthermore, the percentage of decrease in blood glucose levels of mice was calculated using the following formula. [15]

$$(\%DBGL) = \frac{\text{Glucose Level After (IGM)}}{\text{Final blood Glucose level}} \text{ X } 100\ \%$$

Description:
% DBGL: Percentage of Blood Glucose Level Decrease
IGM: Induction of Glucose Monohydrate

Results and Discusion

Phytochemical Analysis.

The results of the phytochemical screening of the methanol, dichloromethane, and ethyl acetate extracts of the mangrove leaves of *R. mucronata* showed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, steroids, triterpenoids, tannins, and saponins. Complete results can be seen in Table 1.

Table 1

The results of the phytochemical test as presented in Table 1 show that the methanol extract of mangrove leaves *R. muronata* contains a class of secondary metabolite compounds; alkaloids, flavonoids, triterpenoids and saponins. Meanwhile the dichloromethane extract is known to positively contain compounds; flavonoids, steroids, and tannins while the ethyl acetate extract is known to contain compounds; alkaloids, flavonoids, phenolic compounds, triterpenoids, tannins and saponins. Previous researchers have reported that *R. muronata* mangrove leaf extracts contain

phenolic compounds, flavonoids, tannins, saponins, terpenoids dihydroflavonols, caffeic acid, vanillic acid, p-hydroxybenzoic acid, alkaloids, coumarins, quinones, resins, phytosterols, xanthoprotins, pigments (chlorophyll, carotenoids), and glucose. [16-19]

The other research revealed that the mentanol extract of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* contains compounds; alkaloids, cardiac glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids. Meanwhile ethyl acetate extracts of the third mangrove leaf species contain compounds; alkaloids, cardiac glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids. [20] Phytochemical analysis of five mangrove leaf species, mainly *B. cylindrica*, *A. corniculatum*, *A. aureum*, *A. alba*, and *R. mucronata*, all contain secondary metabolite compounds of flavonoids, saponins, terpenoids, steroids, phenolics, tannins, and anthraquinones. [21-22]

Antioxidant Activity Test

In this study, the antioxidant activity of methanol, dichloromethane, ethyl acetate extracts of mangrove leaves of *R. mucronata* and vitamin C (as a comparison) with DPPH (1,1-diphenyl-2-picrylhydrazyl) method was tested. The results of the calculation of the percentage of inhibition against DPPH of the three mangrove leaf extracts and vitamin C are presented in Table 2.

Table 2

Then each graph was made of the relationship between the concentration of methanol, dichloromethane, ethyl acetate extracts of mangrove leaves of *R. muronata*

and vitamin C against the percentage of DPPH inhibition as shown in Figure 2.

Figure 2

The antioxidant activity of compounds or extracts is expressed by the IC_{50} value. Where IC_{50} is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%, which can be obtained from a linear regression equation, and which states the relationship between the concentration of extracts/compounds with the percentage of inhibition. The lower the IC_{50} value obtained, the stronger the antioxidant activity of the compound. [23] The results of the calculation of IC_{50} values for methanol, dichloromethane, ethyl acetate, mangrove extract of *R. muronata* and vitamin C can be seen in Table 3.

Table 3

Antioxidant Activity

Extracts with IC_{50} values < 50 ppm have antioxidant activity with a very strong category, if the IC_{50} value of 50 - 100 ppm then the antioxidant activity is categorized as strong, IC_{50} value of 100 - 150 ppm is categorized as moderate, IC_{50} value of 150 - 200 ppm is categorized as weak, and if the IC_{50} value > 200 ppm, then the antioxidant activity is categorized as inactive. [24] The results of the calculation of the IC_{50} value of methanol, dichloromethane, and ethylacetate extracts of mangrove leaves *R. mucronata* and vitamin C (comparison) can be seen in Table 3. Vitamin C and ethyl acetate extracts have antioxidant activity properties with a very strong category, with

IC_{50} values of 5.41 ppm and 34.64 ppm respectively, while the extract of *R. mucronata* mangrove leaf dichloromatana has antioxidant activity properties with a strong category, with an IC_{50} value of 93.25 ppm. While the antioxidant activity of methanol extract is included in the medium category with an IC_{50} value of 100.64 ppm. Ethyl acetate and methanol extracts of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* showed strong DPPH scavenging activity. The activity is in line with the presence of phytochemical compounds contained in the three mangrove plant species. Hence, the potential of the three Rhizophora species can be developed as natural antioxidants. [20]

The difference in antioxidant activity of the three *R. mucronata* mangrove leaf extracts is due to differences in the composition of chemically active compounds possessed by each extract. Differences in the composition of these active compounds can provide synergistic effects between compounds resulting in increased antioxidant activity. Active chemical compounds such as phenolics, flavonoids, anthocyanins, tannins, and other phenolic compounds contained in the extract are closely related to antioxidant activity. [25-26]

Secondary metabolite compounds contained in mangrove species *R. mucronata* include alkaloids, flavonoids, phenolics, steroids, tannins, and terpenoids that show strong antioxidant properties. [27-28] Then it has been reported that the methanol extract of mangrove leaves of *R. mucronata* showed strong antioxidant activity with an IC_{50} value of 47.39 ± 0.43 µg/mL. The presence of flavonoid compounds such as catechins in the methanol extract of *R. mucronata* mangrove leaves is thought to be responsible for cholinesterase inhibitory and antioxidant activity. [29] Chlorophyll a, chlorophyll b, beta-carotene, lutein, neoxanthin, pheophytin a, and violaxanthin are pigment profiles

in the leaves of mangrove plants *R. mucronata*. All identified pigments have strong antioxidant potential, especially as free radical scavengers and Nrf-2 stimulants. The mechanism of action of these pigments is by interacting with each other to inactivate antioxidant enzymes and inhibit the expression of oxidative stress proteins. [30]

Antidiabetic activity of *R. muronata*

Measurement of mice blood glucose levels using the oral glucose tolerance test (OGTT) method. The results of the calculation of the percentage reduction in blood glucose levels of mice in each treatment group, namely negative control, positive control, methanol extract, dichloromethane, and ethyl acetate of mangrove leaves of *R. muronata* are presented in Table 4.

Table 4

Data from Table 4 and Figure 3 show that the highest percentage reduction in blood glucose levels of mice is found in the positive control of glibenclamide (dose of 10 mg/kg BW), followed by ethyl acetate extract in treatment group D-3 (dose of 1,200 mg/kg BW). Furthermore, methanol extract in treatment group D-2 (dose of 600 mg/kg BW), and dichloromethane extract in treatment group D-3 (dose of 1,200 mg/kg BW). The highest decrease in glucose levels occurred in the positive control group/ glibenclamide. This is because glibenclamide can stimulate pancreatic beta cells to secrete insulin and increase the sensitivity of peripheral cells to increased insulin levels. [31]

The decrease in mice blood glucose levels shown by treatment groups D-1, D-2, and D-3 in methanol, dichloromethane, and ethyl acetate extracts of *R. mucronata*

mangrove leaves is due to the content of secondary metabolite compounds such as alkaloids, flavonoids, steroids, tannins, and saponins contained in the three mangrove leaf extracts. These secondary metabolite compounds are thought to have a role in reducing blood glucose levels in mice. Mangrove fruit extract *R. mucronata* with doses; 125, 250, and 500 mg/kg BW were able to reduce blood glucose levels of diabetic rats. The decrease in blood sugar levels in the group given glibenclamide at a dose of 5 mg/kg BW was more effective when compared to the treatment group given mangrove fruit extract of *R. mucronata* species. [32]

The content of secondary metabolite compounds of mangrove fruit extract *R. mucronata* which is thought to reduce blood sugar levels are flavonoids, steroids, saponins and tannins. [33] Alkaloids and saponins can have hypoglycemic effects because they can stimulate insulin secretion from pancreatic beta cells. [34] Flavonoids and tannins are able to reduce blood glucose levels by capturing free radicals and reducing the increase in oxidative stress that occurs in diabetics so as to control blood glucose. [35-36] Secondary metabolite compounds from ethanol extracts, chloroform, and mangrove root fractions of *R. mucronata* species showed antidiabetic activity. The mechanism of antidiabetic activity of mangrove bark extract of *R. mucronata* species is by increasing insulin secretion, and restraining the digestion and absorption of carbohydrates. [37]

Conlcusion

The content of secondary metabolite compounds from methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves of *R. mucronata* showed antioxidant activity and antidiabetic activity. Ethyl acetate extract of mangrove leaves from *R. mucronata* species has the potential to be developed as a

natural antioxidant and antidiabetic alternative medicinal material.

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Conflict of Interest

The authors declare no conflict of interest in writing this article.

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Tables:

Table 1. Phytochemical content of extracts of methanol, dichloromethane, and ethyl acetate of *R. mucronata* mangrove leaves.

No.	Phytochemical Test	Reagent	The Extract and Results of Observations		
			Methanol	Dichloromethane	Ethyl Acetate
1.	Alkaloids	Dragendorff	Positive (+) Brownish-red Precipitate	Negative (-) The color of the solution does not change	Positive (+) Brownish-redPrecipitate
		Mayer	Positive (+) White precipitate	Negative (-) The color of the solution does not change	Positive (+) White precipitate
		Wagner	Positive (+) Reddish-brownPrecipitate	Negative (-) The color of the solution does not change	Positive (+) Reddish-brownPrecipitate
2.	Flavanoids	Mg + Amyl alcohol (HCl 37 % & etanol 95 %	Positive (+) Pink-Orange Coloration	Positive (+) Pink-Orange Coloration	Positive (+) Pink-Orange Coloration
3.	Phenolics Compounds	$FeCl_3$ 5 %	Negative (-) The color of the solution does not change	Negative (-) The color of the solution does not Change	Positive (+) Bluish-black Color
4.	Steroids	Acetic anhydride + Sulfuric acid (concentrated)	Negative (-) The color of the solution does not change	Positive (+) Green color solution	Negative (-) The color of the solution does not change
5.	Triterpenoids	Acetic anhydride + Sulfuric acid (concentrated)	Positive (+)Red Color	Negative (-) The color of the solution does not Change	Positive (+)Red Color
6.	Tannins	$FeCl_3$ 1 %	Negative (-) The color of the solution does not change	Positive (+)Blue-blackPrecipitate	Positive (+) Blue-black Precipitate
7.	Saponins	HCl 2 N	Positive (+) Stable Foam Formed	Negative (-) Not Formed Foam Stable	Positive (+) Stable Foam Formed

Description : (-) = Absent (+) = Present

Table 2. The percentage of DPPH inhibition of methanol, dichloromethane, ethyl acetate extracts of *R. muronata* leaves and vitamin C in various concentrations.

Extract	Absorbance (517 nm)				Percentage of DPPH Inhibition			
	20 ppm	40 ppm	60 ppm	80 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Methanol	0,205	0,195	0,174	0,156	22,64	26,42	33,96	41,13
Dichloromethane	0,203	0,189	0,158	0,148	23,40	28,68	40,38	44,15

Ethyl Acetate	0,154	0,125	0,095	0,058	41,89	52,83	64,15	78,11
Sample	**Absorbance (517 nm)**				**Percentage of DPPH Inhibition**			
	2 ppm	**4 ppm**	**6 ppm**	**8 ppm**	**2 ppm**	**4 ppm**	**6 ppm**	**8 ppm**
Vitamin C	0,219	0,167	0,114	0,071	17,36	36,97	56,98	73,21

Table 3. Regression equations and IC_{50} values of methanol, dichloromethane, ethyl acetate extract of *R. muronata* mangrove leaves and vitamin C.

Extract	Regression Equation	IC_{50} Value (ppm)
Methanol	y = 0,345x + 15,28	100,64
Dichlorometane	y = 0,379x + 14,66	93,25
Ethyl Acetate	y = 0,599x + 29,25	34,64
Vitamin C	y = 9,378x - 0,76	5.41

Table 4. Percentage reduction in blood glucose levels of mice in treatment group on the 24th hour.

Treatment Group	Fasting Glucose Level (mg/dl)	Blood Glucose Level After (IGM) (mg/dl)	Blood Glucose Level After Treatment (mg/dl)	The Percentage of Blood Glucose Level Reduction (%)
Negative Control (CMC-Na 1 %)	85,7	-	-	-
Positive Control (Glibenclamide, Dose 10 mg/kg bw)	97,3	276	107	61,23
Methanol Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	95,7	285,7	170,3	40,39
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	97	276,3	158,3	42,71
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	92,7	269	162	39,78
Dichloromethane Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	99,3	276,3	224,4	18,78
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	93,7	306,3	229,7	25,01
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	99,3	259,7	178	21,81
Ethyl Acetate Extract				

D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	94,3	268	193	27,99
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	96,7	274	178	35,04
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	96	279,3	118,3	57,64

Figures:

Figure 1. The part of the tissue; fruit, leave and flower of the mangrove plant *R. mucronata*

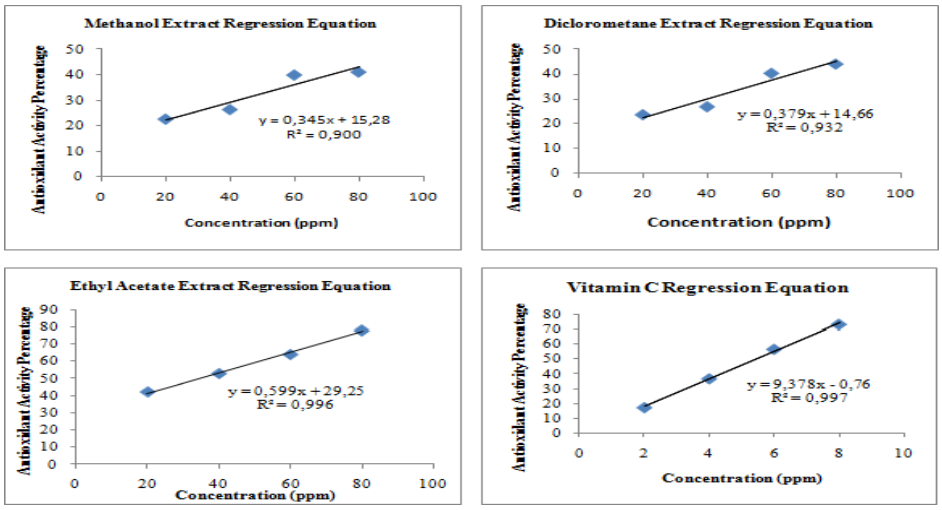

Figure 2. The graph of linear regression equation of methanol, dichloromethane, ethyl acetate extracts, and vitamin C.

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Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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A. MANUSCRIPT

Journal	**Tropical Journal of Natural Product Research**
Manuscript Number	TJNPR MY299AR
Type of paper	Research article
Title of paper	The Potential Active Chemical Compounds as Antioxidants and Antidiabetics from Rhizophora mucronata Derived from Sambera Beach, East Kalimantan, Indonesia
Name of Authors	Usman Usman, Muhammad A. Masruhim, Pintaka Kusumaningtiyas, Erwin Erwin, Dewi E. Bulan

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Title	Authors should include the country East Kalimantan is located
Abstract	The abstract word count is < 250. Abstract is unreadable due to grammatical errors • The abstract should be rewritten
Keywords	Keywords should be up to four
Introduction	Citation before period. The authors should follow the journal guideline in citations. All grammatical syntax, spelling and punctuation errors should be corrected. The aim and objectives of the research is lacking. The research novelty should be clarified. The relevance of the adopted methodologies to the research should be discussed in brief. The relevance of the antioxidant enzymes should be discussed in details. The medicinal relevance of the study plant should be discussed in brief comparative to other species elsewhere. Citations should be provided where needed • The discussion should be made explicit
Methodology	Method for the DPPH assay should be rewritten and made obvious. Equations should be mentioned and numbered serially. Equations should be edited using equation editor. All chemicals and reagents should be mentioned alongside their manufacturers, grade, % purity, concentrations and specificities. All equipment and instruments should be mentioned alongside their model, manufacturer and country. Author should include the GPS location of plant collection site. All grammatical syntax error should be corrected. The procedures used should be clarified for all experiments. Ascorbic acid should be ascribed "control drug" and not "comparison". Bogus sentences should be elucidated using citations while repetitive sentences should be expunged • The methods should be revised as recommended • The methods used is mostly appropriate for the research
Results	Commas should be used to designate decimals. • The results obtained are mostly appropriate for the methods used
Discussion	Only significant findings should be discovered in detail. The author should ensure that their discussion is comprehensive and easily comprehensible. The discussion should be comprehensive and purposeful. The presence of grammatical syntax errors made it difficult to easily comprehend the research. All repetitive sentences should be expunged. The discussion should be specific, authors

	should avoid lumping all the species of the studied plant together, it made the discussion somewhat ambiguous • The research discussion should only focus on the significant findings of the research
Conclusion	The future prospects of the research should be captured in the conclusion
References	The authors should adhere strictly to the journal guideline in referencing
Figures	All figures were captured and mentioned in the manuscript
Tables	All tables were captured and mentioned in the manuscript

C. REVIEWER'S GENERAL COMMENTS AND REMARKS

The authors studied "The Potential Active Chemical Compounds as Antioxidants and Antidiabetics from Rhizophora mucronata Derived from Sambera Beach, East Kalimantan". The abstract should be rewritten. The discussion should be made explicit. The methods should be revised as recommended. The methods used is mostly appropriate for the research. The results obtained are mostly appropriate for the methods used. The research discussion should only focus on the significant findings of the research. The conclusion didn't capture the future prospects of the research.

The abstract contained numerous grammatical syntax errors and should be rewritten

The manuscript should be formatted based on the journal guideline

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All supplementary documents should be supplied

The manuscript cannot be accepted the way it is

Accept with major corrections

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Include date (Month and Year) of sample collection

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All botanical and zoological names should be italicized

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The Potential Active Chemical Compounds as Antioxidants and Antidiabetics from Rhizophora mucronata Derived from Sambera Beach, East Kalimantan

ABSTRACT

This research aims to determine the content of active chemical compounds, antioxidant and antidiabetes activity of methanol, dichloromethane, and ethyl acetate extracts of *Rhizophora mucronata* mangrove leaves. The *R. mucronata* leaf samples (1 Kg) were extracted using methanol solvent, then continued with liquid-liquid partition extraction using dichloromethane solvent, and finally ethyl acetate solvent. The extraction process was carried out for 1 x 24 hours (three repetitions). The third extract obtained was evaporated using a rotary evaporator so that three thick extracts of *R. mucronata* mangrove leaves were obtained. Furthermore, the three extracts were identified phytochemical content with color test method, followed by antioxidant activity test with DPPH (1,1-diphenyl-2-picrylhydrazyl) method, and oral glucose tolerance test (OGTT) to determine antidiabetic activity. The phytochemical contents of the extract of methanol, dichloromethane, and ethyl acetate of mangrove leaves of *R. mucronata* are alkaloids, flavonoids, phenolic compounds, triterpenoids, steroids, saponins, and tannins. The antioxidant activity of the ethyl acetate extract of *R. mucronata* leaves is classified as very strong with an IC_{50} value of 34.64 ppm. The ethyl acetate extract also showed the highest decrease in blood glucose levels at 24 hours of 57.64%. The mangrove plant *R. mucronata* from the coast of Sambaera has the potential to be developed as a natural antioxidant and alternative antidiabetic drug, especially ethyl acetate extract.

Keywords: *Mangrove plants, Antioxidant activity, and Andiabetic activity.*

Introduction

The mangrove forest in Indonesia is one of the largest mangrove forest areas in the world, so researchers in the field of natural organic chemistry and the herbal medicine industry are interested in conducting research on the utilization of mangrove forests as a source of antibiotics. In recent years, the demand for herbal medicine by diabetics has increased, along with the increasing number of diabetics in Indonesia. Data from the International Diabetes Federation (IDF) shows that the number of diabetics in Indonesia in 2019 is estimated to reach 10.7 million people and by 2045 it is expected to increase to 16.7 million people. [1] Therefore, exploration to find a cure for diabetes continues to develop.

The mangrove plant is a type of plant that is widely used by the community as a traditional medicine to cure various diseases, such as diarrhea, malaria, smallpox, asthma, diabetes, fever, swelling, rheumatism, skin diseases, hepatitis, diuretics,

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leprosy, antitumor, leukemia, anticancer, antiviral, and mumps. [2-4] Parts of mangrove plants that are utilized as medicinal materials are root tissue, stem wood, bark, leaves, twigs, flowers, and fruits. [5-6] Mangrove plants are rich in secondary metabolite compounds such as alkaloids, polyphenols, flavonoids, tannins, saponins, triterpenes, anthroquinones, catechins, glucose, proteins, steroids, phenolic compounds, and glycosides that have antioxidant and antidiabetic activities. These mangrove plant species include *A. marina*, *A. ilicifolius*, *C. tagal*, *R. muronata*, *R. apiculata*, *S. alba*, *S. caseolaris*, *X. granatum*, and *N. fruticans*. [7-9] Therefore, research on the bioactivity of mangrove species *R. mucronata* from Sambera Beach, East Kalimantan, has the potential to find secondary metabolite compounds that have antioxidant and antidiabetic activities which can then be developed as natural herbal medicines.

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Taxonomy of *R. mucronata*

The taxonomy of *R. mucronata* is as follows:

Kingdom : Plantae
Phylum : Magnoleophyta
Class : Magnoliopsida
Ordo : Malpighiales
Family : *Rhizophoraceae*
Genus : *Rhizophora*
Species : *Rhizophora mucronata*

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Figure 1

Material and Methods.

Equipment and Reagents

This research used several equipment, including glassware commonly used in laboratories, digital analytical, rotary evaporator, vortex, incubator, pH meter, and UV-Vis spectrophotometer. The materials used in this study were mangrove leaf of *R.*

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mucronata, aquades, 1,1-diphenyl-2-picrylhidrazyl (DPPH, Merck), methanol 70%, dichlorometane (Merck), Ethyl Acetate (Merck) H_2SO_4 (Merck), $FeCl_3$ 0.1%, several color reagents for phytochemical tests (alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins), CMC-Na, Glibenclamide, filter paper, and aluminum foil.

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Sample collection and preparation

The mangrove leaf samples of *R. mucronata* were taken from Sambera Beach, East Kalimantan. Mangrove leaf samples were washed with running water to remove dirt attached to the leaves and then cut into small pieces. The dried samples were sorted and then pulverized into fine powder with a size of 90 mesh. Mangrove leaf powder is ready to be used for the subsequent procedures.

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Extraction

About 1 kg of *R. mucronata* mangrove leaf powder was extracted by maceration method using 70% methanol solvent (powder and solvent ratio 1:10) and carried out for 1 x 24 hours (three repetitions). The methanol extract obtained was combined and then the solvent was evaporated with a rotary evaporator at 40 °C and 1 atm pressure, until a thick blackish green methanol extract was obtained. A portion of the methanol extract was extracted by liquid-liquid partition using dichloromethane solvent and then continued using ethyl acetate solvent to obtain dichloromethane, ethyl acetate, and methanol fractions. Then the three extracts obtained were continued with phytochemical, antioxidant and antidiabetic tests.

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Phytochemical Test

Phytochemical tests of methanol, dichloromethane, and ethyl acetate extracts were carried out qualitatively using modified standard procedures. The phytochemical

compounds to be determined are: alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins. [10-12]

Antioxidant Test

The antioxidant activity of the methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves of *R. muronata* and vitamin C (standard drug) were evaluated in vitro using the DPPH (1,1-diphenyl-2-picrylhidrazyl) method following the procedure described by Senhaji B with modifications. 2 mL each of methanol, dichloromethane, and ethyl acetate extracts with a concentration of; 20, 40, 60 and 80 ppm, as well as vitamin C in concentration; 2, 4, 6, and 8 ppm mixed with 2 ml of DPPH (50 ppm) solution. After being incubated for 30 minutes in the dark at room temperature, the absorbance of the three extracts and vitamin C were measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. [13] The percentage of antioxidant activity or the percentage of DPPH inhibition of the methanol, dichloromethane, and ethyl acetate extracts and vitamin C were calculated using the following formula equation 1:

$$(\%)\ \text{Inhibition DPPH} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100\ \%$$

equation 1

Then a linear regression curve was made from the percentage data of DPPH inhibition on the concentrations of the three extracts and also the concentration of vitamin C. The IC_{50} value (as an indicator of antioxidant activity) of the methanol, dichloromethane, and ethyl acetate extracts and vitamin C was calculated using the regression equation of the linear regression curve obtained. [14]

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Antidiabetic Test

Antidiabetic activity test was carried out using the oral glucose tolerance test (OGTT) method. The mice used as samples were male mice with a body weight of 150-200 grams, and were divided into 5 groups, each group consisting of 5 mice. Prior to treatment, all mice were fasted for 18 hours, then blood samples were taken from the vein in the tail of the mice using a glucometer to determine fasting/initial blood glucose levels. Then all groups were given 50% glucose monohydrate solution orally, except for the negative control group. After induction of glucose monohydrate for 180 minutes, the mice's blood glucose levels were measured again and mice that were already in a hyperglycemic condition (blood glucose level > 200 mg/dl) were selected as samples for treatment. according to the group.

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Group 1 (negative control) was the group of mice that were given 1% CMC-Na suspension. Group 2 (positive control) was the group of mice that were given glibenclamide suspension at a dose of 10 mg/kg BW. Group 3 (D1), mice were treated with methanol extract of mangrove leaves at a dose of 300 mg/kg BW. Group 4 (D2), mice were treated with methanol extract of mangrove leaves at a dose of 600 mg/kg body weight, and group 5 (D3), mice were treated with methanol extract of mangrove leaves at a dose of 1,200 mg/kg body weight. The same thing also applies to treatments using dichloromethane and ethyl acetate extracts.

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Measurement of blood glucose levels in mice was carried out after the treatment group (negative control, positive control, methanol extract, dichloromethane, and ethyl acetate extracts of mangrove *R. mucronata* leaves) at 8, 16, and 24 hours, to determine the decrease in blood glucose levels in mice. Furthermore, the percentage of decrease in blood glucose levels of mice was calculated

using the following formula equation 2. [15]

$$(\%DBGL) = \frac{\text{Glucose Level After (IGM)}}{\text{Final blood Glucose level}} \text{ X } 100\ \%$$

equation 2

Description:
% DBGL: Percentage of Blood Glucose Level Decrease
IGM: Induction of Glucose Monohydrate

Results and Discusion

Phytochemical Analysis.

The results of the phytochemical screening of the methanol, dichloromethane, and ethyl acetate extracts of the mangrove leaves of *R. mucronata* showed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, steroids, triterpenoids, tannins, and saponins. Complete results can be seen in Table 1.

Table 1

The results of the phytochemical test as presented in Table 1 showed that the methanol extract of mangrove leaves of *R. muronata* contained alkaloids, flavonoids, triterpenoids and saponins. Meanwhile the dichloromethane extract contained flavonoids, steroids, and tannins while the ethyl acetate extract contained phytochemical compounds such as alkaloids, flavonoids, phenolic compounds, triterpenoids, tannins and saponins. Previous researchers have reported that *R. muronata* mangrove leaf extracts contain phenolic compounds, flavonoids, tannins, saponins, terpenoids dihydroflavonols, caffeic acid, vanillic acid, p-hydroxybenzoic acid, alkaloids, coumarins, quinones, resins, phytosterols, xanthoprotins, pigments

(chlorophyll, carotenoids), and glucose. [16-19]

The other research revealed that the mentanol extract of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* contains compounds; alkaloids, cardiac glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids. Meanwhile ethyl acetate extracts of the third mangrove leaf species contained compounds such as alkaloids, cardiac glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids. [20] Phytochemical analysis of five mangrove leaf species, mainly *B. cylindrica*, *A. corniculatum*, *A. aureum*, *A. alba*, and *R. mucronata*, all contain secondary metabolite compounds such as flavonoids, saponins, terpenoids, steroids, phenolics, tannins, and anthraquinones. [21-22]

Antioxidant Activity Test

In this study, the antioxidant activity of methanol, dichloromethane, ethyl acetate extracts of mangrove leaves of *R. mucronata* and vitamin C (standard drug) with DPPH (1,1-diphenyl-2-picrylhydrazyl) method was tested. The results of the calculation of the percentage of inhibition against DPPH radicals of the three mangrove leaf extracts and vitamin C are presented in Table 2.

Table 2

Then each graph was made of the relationship between the concentration of methanol, dichloromethane, ethyl acetate extracts of mangrove leaves of *R. muronata* and vitamin C against the percentage of DPPH inhibition as shown in Figure 2.

Figure 2

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The antioxidant activity of compounds or extracts is expressed by the IC_{50} value. Where IC_{50} is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%, which can be obtained from a linear regression equation, and which states the relationship between the concentration of extracts/compounds with the percentage of inhibition. The lower the IC_{50} value obtained, the stronger the antioxidant activity of the compound. [23] The results of the calculation of IC_{50} values for methanol, dichloromethane, ethyl acetate, mangrove extract of *R. muronata* and vitamin C can be seen in Table 3.

Table 3

Antioxidant Activity

Extracts with IC_{50} values < 50 ppm have antioxidant activity categorized as very strong, if the IC_{50} value of 50 - 100 ppm then the antioxidant activity is categorized as strong, IC_{50} value of 100 - 150 ppm is categorized as moderate, IC_{50} value of 150 - 200 ppm is categorized as weak, and if the IC_{50} value > 200 ppm, then the antioxidant activity is categorized as inactive. [24] The results of the calculation of the IC_{50} value of methanol, dichloromethane, and ethylacetate extracts of mangrove leaves *R. mucronata* and vitamin C (standard drug) can be seen in Table 3. Vitamin C and ethyl acetate extracts have antioxidant activity properties with a very strong category, with IC_{50} values of 5.41 ppm and 34.64 ppm respectively, while the extract of *R. mucronata* mangrove leaf dichloromatana has antioxidant activity properties with a strong category, with an IC_{50} value of 93.25 ppm. While the antioxidant activity of

methanol extract is included in the medium category with an IC_{50} value of 100.64 ppm. Ethyl acetate and methanol extracts of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* showed strong DPPH scavenging activity (citation needed). The activity is in line with the presence of phytochemical compounds contained in the three mangrove plant species. Hence, the three Rhizophora species can be developed as natural antioxidants. [20]

The difference in antioxidant activity of the three *R. mucronata* mangrove leaf extracts is due to differences in the composition of chemically active compounds possessed by each extract. Differences in the composition of these active compounds can provide synergistic effects between compounds resulting in increased antioxidant activity. Active chemical compounds such as phenolics, flavonoids, anthocyanins, tannins, and other phenolic compounds contained in the extract are closely related to antioxidant activity. [25-26]

Secondary metabolite compounds contained in mangrove species of *R. mucronata* include alkaloids, flavonoids, phenolics, steroids, tannins, and terpenoids that show strong antioxidant properties. [27-28] Then it has been reported that the methanol extract of mangrove leaves of *R. mucronata* showed strong antioxidant activity with an IC_{50} value of 47.39 ± 0.43 µg/mL (citation needed). The presence of flavonoid compounds such as catechins in the methanol extract of *R. mucronata* mangrove leaves is thought to be responsible for cholinesterase inhibitory and antioxidant activity. [29] Chlorophyll a, chlorophyll b, beta-carotene, lutein, neoxanthin, pheophytin a, and violaxanthin are pigment profiles in the leaves of mangrove plants of *R. mucronata* (citation needed). All identified pigments have strong antioxidant potential, especially as free radical scavengers and Nrf-2 stimulants. The mechanism

of action of these pigments is by interacting with each other to inactivate antioxidant enzymes and inhibit the expression of oxidative stress proteins.[30]

Antidiabetic activity of *R. muronata*

Measurement of mice blood glucose levels was done using the oral glucose tolerance test (OGTT) method. The results of the calculation of the percentage reduction in blood glucose levels of mice in each treatment group, namely negative control, positive control, methanol extract, dichloromethane, and ethyl acetate of mangrove leaves of *R. muronata* are presented in Table 4.

Table 4

Data from Table 4 and Figure 3 show that the highest percentage reduction in blood glucose levels of mice is found in the positive control of glibenclamide (dose of 10 mg/kg BW), followed by ethyl acetate extract in treatment group D-3 (dose of 1,200 mg/kg BW). Furthermore, methanol extract in treatment group D-2 (dose of 600 mg/kg BW), and dichloromethane extract in treatment group D-3 (dose of 1,200 mg/kg BW). The highest decrease in glucose levels occurred in the positive control group/ glibenclamide. This is because glibenclamide can stimulate pancreatic beta cells to secrete insulin and increase the sensitivity of peripheral cells to increased insulin levels.[31]

The decrease in mice blood glucose levels shown by treatment groups D-1, D-2, and D-3 in methanol, dichloromethane, and ethyl acetate extracts of *R. mucronata* mangrove leaves is due to the content of secondary metabolite compounds such as alkaloids, flavonoids, steroids, tannins, and saponins contained in the three mangrove

leaf extracts (citation needed). These secondary metabolite compounds are thought to have a role in reducing blood glucose levels in mice (citation needed). Mangrove fruit extract *R. mucronata* with doses; 125, 250, and 500 mg/kg BW were able to reduce blood glucose levels of diabetic rats (citation needed). The decrease in blood sugar levels in the group given glibenclamide at a dose of 5 mg/kg BW was more effective when compared to the treatment group given mangrove fruit extract of *R. mucronata* species.[32]

The content of secondary metabolite compounds of mangrove fruit extract of *R. mucronata* which is thought to reduce blood sugar levels are flavonoids, steroids, saponins and tannins.[33] Alkaloids and saponins can have hypoglycemic effects because they can stimulate insulin secretion from pancreatic beta cells.[34] Flavonoids and tannins are able to reduce blood glucose levels by capturing free radicals and reducing the increase in oxidative stress that occurs in diabetics so as to control blood glucose.[35-36] Secondary metabolite compounds from ethanol extracts, chloroform, and mangrove root fractions of *R. mucronata* species showed antidiabetic activity (citation needed). The mechanism of antidiabetic activity of mangrove bark extract of *R. mucronata* species is by increasing insulin secretion, and restraining the digestion and absorption of carbohydrates.[37]

Conlcusion

The content of secondary metabolite compounds from methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves of *R. mucronata* showed antioxidant activity and antidiabetic activity. Ethyl acetate extract of mangrove leaves from *R. mucronata* species has the potential to be developed as a natural antioxidant and antidiabetic alternative medicinal material.

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Acknowledgments

The authors would like to thank PIU IsDB Mulawarman University Indonesia for funding this research through the Higher Education Research Grant Program and the Pharmacy Research Laboratory of Mulawarman University for the facilities provided to carry out this research.

Conflict of Interest

The authors declare no conflict of interest in writing this article.

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Tables:

Table 1. Phytochemical content of extracts of methanol, dichloromethane, and ethyl acetate of *R. mucronata* mangrove leaves.

No.	Phytochemical Test	Reagent	The Extract and Results of Observations		
			Methanol	Dichloromethane	Ethyl Acetate
1.	Alkaloids	Dragendorff	Positive (+) Brownish-red Precipitate	Negative (-) The color of the solution does not change	Positive (+) Brownish-redPrecipitate
		Mayer	Positive (+) White precipitate	Negative (-) The color of the solution does not change	Positive (+) White precipitate
		Wagner	Positive (+) Reddish-brownPrecipitate	Negative (-) The color of the solution does not change	Positive (+) Reddish-brownPrecipitate
2.	Flavanoids	Mg + Amyl alcohol (HCl 37 % & etanol 95 %	Positive (+) Pink-Orange Coloration	Positive (+) Pink-Orange Coloration	Positive (+) Pink-Orange Coloration
3.	Phenolics Compounds	$FeCl_3$ 5 %	Negative (-) The color of the solution does not change	Negative (-) The color of the solution does not Change	Positive (+) Bluish-black Color
4.	Steroids	Acetic anhydride + Sulfuric acid (concentrated)	Negative (-) The color of the solution does not change	Positive (+) Green color solution	Negative (-) The color of the solution does not change
5.	Triterpenoids	Acetic anhydride + Sulfuric acid (concentrated)	Positive (+)Red Color	Negative (-) The color of the solution does not Change	Positive (+)Red Color
6.	Tannins	$FeCl_3$ 1 %	Negative (-) The color of the solution does not change	Positive (+)Blue-blackPrecipitate	Positive (+) Blue-black Precipitate
7.	Saponins	HCl 2 N	Positive (+) Stable Foam Formed	Negative (-) Not Formed Foam Stable	Positive (+) Stable Foam Formed

Description : (-) = Absent (+) = Present

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Table 2. The percentage of DPPH inhibition of methanol, dichloromethane, ethyl acetate extracts of *R. muronata* leaves and vitamin C in various concentrations.

Extract	Absorbance (517 nm)				Percentage of DPPH Inhibition			
	20 ppm	40 ppm	60 ppm	80 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Methanol	0,205	0,195	0,174	0,156	22,64	26,42	33,96	41,13
Dichloromethane	0,203	0,189	0,158	0,148	23,40	28,68	40,38	44,15
Ethyl Acetate	0,154	0,125	0,095	0,058	41,89	52,83	64,15	78,11
	Absorbance (517 nm)				Percentage of DPPH Inhibition			

Sample	2 ppm	4 ppm	6 ppm	8 ppm	2 ppm	4 ppm	6 ppm	8 ppm
Vitamin C	0,219	0,167	0,114	0,071	17,36	36,97	56,98	73,21

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Table 3. Regression equations and IC_{50} values of methanol, dichloromethane, ethyl acetate extract of *R. muronata* mangrove leaves and vitamin C.

Extract	Regression Equation	IC_{50} Value (ppm)
Methanol	y = 0,345x + 15,28	100,64
Dichlorometane	y = 0,379x + 14,66	93,25
Ethyl Acetate	y = 0,599x + 29,25	34,64
Vitamin C	y = 9,378x - 0,76	5.41

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Table 4. Percentage reduction in blood glucose levels of mice in treatment group on the 24th hour.

Treatment Group	Fasting Glucose Level (mg/dl)	Blood Glucose Level After (IGM) (mg/dl)	Blood Glucose Level After Treatment (mg/dl)	The Percentage of Blood Glucose Level Reduction (%)
Negative Control (CMC-Na 1 %)	85,7	-	-	-
Positive Control (Glibenclamide, Dose 10 mg/kg bw)	97,3	276	107	61,23
Methanol Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	95,7	285,7	170,3	40,39
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	97	276,3	158,3	42,71
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	92,7	269	162	39,78
Dichloromethane Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	99,3	276,3	224,4	18,78
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	93,7	306,3	229,7	25,01
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	99,3	259,7	178	21,81
Ethyl Acetate Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg	94,3	268	193	27,99

bw)				
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	96,7	274	178	35,04
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	96	279,3	118,3	57,64

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Figures:

Figure 1. The part of the tissue; fruit, leave and flower of the mangrove plant *R. mucronata*

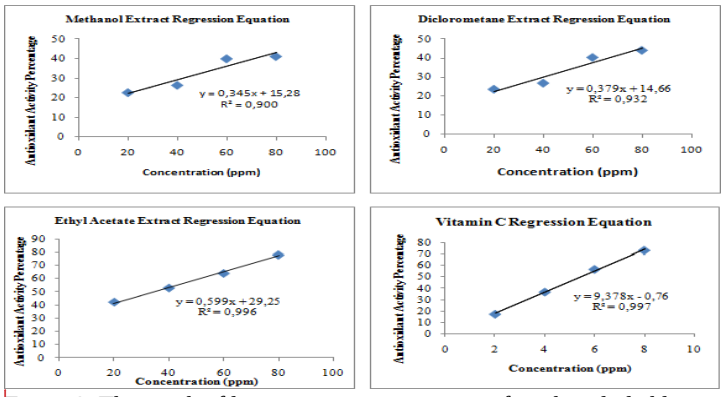

Figure 2. The graph of linear regression equation of methanol, dichloromethane, ethyl acetate extracts, and vitamin C.

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Dear Editor-in-Chief
Tropical Journal of Natural Product Research

Here are the responses for the reviewer's comments:

No	Reviewer comments	Author answer
Reviewer #1:		
1.	Authors should include the country East Kalimantan is located	We already included the country in the title
2.	The abstract word count is < 250. Abstract is unreadable due to grammatical errors. The abstract should be rewritten	We already rewrote the abstract and the word count is < 200.
3.	Keywords should be up to four	We already wrote four keywords
4.	Citation before period. The authors should follow the journal guideline in citations. All grammatical syntax, spelling and punctuation errors should be corrected. The aim and objectives of the research is lacking. The research novelty should be clarified. The relevance of the adopted methodologies to the research should be discussed in brief. The relevance of the antioxidant enzymes should be discussed in details. The medicinal relevance of the study plant should be discussed in brief comparative to other species elsewhere. Citations should be provided where needed. The discussion should be made explicit	We already revised the citation and follow the Vancouver citing and referencing style. The wrong grammatical syntax has been corrected. The novelty and aim of the research has been included.
5.	Method for the DPPH assay should be rewritten and made obvious. Equations should be mentioned and numbered serially. Equations should be edited using equation editor. All chemicals and reagents should be mentioned alongside their manufacturers, grade, % purity, concentrations and specificities. All equipment and instruments should be mentioned alongside their model, manufacturer and country. Author should include the GPS location of plant collection site. All grammatical syntax error should be corrected. The procedures used should be clarified for all experiments. Ascorbic acid should be ascribed "control drug" and not "comparison". Bogus sentences should be elucidated using citations while repetitive	The material and method section has been revised following the reviewer's suggestions. The GPS location of plant collection site has been included (Figure 1).

	sentences should be expunged. The methods should be revised as recommended. The methods used is mostly appropriate for the research	
6.	Commas should be used to designate decimals. The results obtained are mostly appropriate for the methods used	Table 2, Table 3, and Table 4 have been revised
7.	Only significant findings should be discovered in detail. The author should ensure that their discussion is comprehensive and easily comprehensible. The discussion should be comprehensive and purposeful. The presence of grammatical syntax errors made it difficult to easily comprehend the research. All repetitive sentences should be expunged. The discussion should be specific, authors should avoid lumping all the species of the studied plant together, it made the discussion somewhat ambiguous. The research discussion should only focus on the significant findings of the research	We have revised the discussion and added many references.
8.	The future prospects of the research should be captured in the conclusion	The potential of *R. mucronata* as a natural antioxidant and alternative anti-diabetic therapy has been written in the conclusion
9.	The authors should adhere strictly to the journal guideline in referencing	We already revised the references according to the journal guideline.
10.	All figures and tables were captured and mentioned in the manuscript	All figures and tables have been mentioned in the manuscript.
Reviewer #2:		
1.	Include date (Month and Year) of sample collection	Month and year of sample collection has been included in the material and method section.
2.	**For non-integers, use periods/ decimal point NOTcommas.**	Table 2, Table 3, and Table 4 have been revised
3.	**All botanical and zoological names should be italicized**	The names of all botanical and zoological have been italicized
4.	Paragraph 1 in introduction has only 2 references, which cannot represent the current progress on OPEFB treatment.	We already added many references in the introduction.
5.	**Conflict of interest** session should be included, and if there is no conflict of interest, this should be stated clearly as follows; **The authors declare no**	Conflict of interest has been revised accordingly

conflict of interest.	
6. **A declaration of the liability** of the authors for claims relating to the content of this article should also be included when submitting the revised manuscript. This should be stated as follows; **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.	We already included the author declaration accordingly
Reviewer #3: We already revised the manuscript according to the reviewer suggestions.	

I have also attached the revised manuscript for resubmission. Thank you very much.

On behalf of authors,
Usman usman

The Potential Active Chemical Compounds as Antioxidants and Antidiabetics from *Rhizophora mucronata* Derived from Sambera Beach, East Kalimantan, Indonesia

ABSTRACT

This study aims to evaluate the bioactive compounds, antioxidant, and antidiabetic properties of methanol, dichloromethane, and ethyl acetate extracts of *Rhizophora mucronata* mangrove leaves. The *R. mucronata* leaf samples (1 kg) were extracted with methanol, followed by liquid-liquid partition extraction with dichloromethane, and finally ethyl acetate solvent. The extraction procedure was repeated three times for one 24-hour period. The phytochemical content of the three extracts was determined using the color test method, followed by an antioxidant activity test using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, and an Oral Glucose Tolerance Test (OGTT) to detect antidiabetic activity. The phytochemical contents of methanol, dichloromethane, and ethyl acetate extracts of *R. mucronata* mangrove leaves are alkaloids, flavonoids, phenolic compounds, triterpenoids, steroids, saponins, and tannins. The antioxidant activity of the ethyl acetate extract is categorized as extremely strong, with an IC_{50} value of 34.64 ppm. In addition, the ethyl acetate extracts reduced blood glucose levels in mice by 57.64% after 24 hours. The mangrove plant *R. mucronata* from the Sambera beach, particularly the ethyl acetate extract, has the potential to be developed as a natural antioxidant and alternative anti-diabetic medicine.

Keywords: *DPPH-scavenging activity, mangrove plants, oral glucose tolerance test, and phytochemical screening*

Introduction

The mangrove forest in Indonesia is one of the largest mangrove forest areas in the world,[1] so researchers in the field of natural organic chemistry and the herbal medicine industry are interested in researching on the utilization of mangrove forests as a source of antibiotics. In recent years, the demand for herbal medicine by people with diabetes has increased, along with the increasing number of diabetics in Indonesia. Data from the International Diabetes Federation (IDF) shows that the number of diabetics in Indonesia in 2019 is estimated to reach 10.7 million people. By 2045, it is expected to increase to 16.7 million people.[2] Therefore, exploration to find a cure for diabetes continues to develop.

The mangrove plant is a type of plant that is widely used by the community as a traditional medicine to cure various diseases, such as diarrhea, malaria, smallpox, asthma, diabetes, fever, swelling, rheumatism, skin diseases, hepatitis, diuretics,

leprosy, antitumor, leukemia, anticancer, antiviral, and mumps.[3–5] Parts of mangrove plants utilized as medicinal materials are root tissue, stem wood, bark, leaves, twigs, flowers, and fruits.[6,7] Mangrove plants are rich in secondary metabolite compounds such as alkaloids, polyphenols, flavonoids, tannins, saponins, triterpenes, anthraquinones, catechins, glucose, proteins, steroids, phenolic compounds, and glycosides that have antioxidant and antidiabetic activities. These mangrove plant species include *Avicennia marina*, *Acanthus ilicifolius*, *Ceriops tagal*, *Rhizophora mucronata*, *Rhizophora apiculata*, *Sonneratia alba*, *Sonneratia caseolaris*, *Xylocarpus granatum*, and *Nypa fruticans*.[8–10] Research on the bioactivity of mangrove species *R. mucronata* from Sambera Beach, East Kalimantan, Indonesia is very limited. Therefore, it is important to investigate the potential of this species in order to identify the secondary metabolite compounds with antioxidant and antidiabetic activities, which can subsequently be developed as natural herbal medicines.

Material and Methods.

Equipment and Reagents

This research used equipment, including glassware commonly used in laboratories, digital analytical balance (XPR106DUHQ), rotary evaporator (RE301A-W, Yamato Scientific Co.Ltd.Japan), vortex (Labnet Vortex Mixer VX-200), incubator (Memmert Incubator I IN 55 PLUS), pH meter (Lutron PH-208), and UV-Vis spectrophotometer (Shimadzu UV-Vis UV-1280), aquadest, 1,1-diphenyl-2-picrylhidrazyl (Sigma Aldrich, USA), methanol 70%, dichloromethane (Merck, Germany), Ethyl Acetate (Riedel-deHaen, Germany), H_2SO_4 (Merck, Germany),

$FeCl_3$ 0.1% (Merck, Germany), chemicals for color reagents to test the phytochemicals (alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins) purchased from Merck (Germany), CMC-Na (Sigma-Aldrich, USA)), Glibenclamide (First Medifarma), filter paper, and aluminum foil.

Sample collection and preparation

The mangrove leaf samples of *R. mucronata* were taken in February 2022 from Sambera Beach (0°14'44.8"S, 117°25'00.6"E), East Kalimantan, Indonesia (Fig. 1). Mangrove leaf samples were washed with running water to remove dirt attached to the leaves and then cut into small pieces. The dried samples were sorted and then pulverized into fine powder with a size of 90 mesh. Mangrove leaf powder is ready to be used for the subsequent procedures.

Extraction

About 1 kg of *R. mucronata* mangrove leaf powder was extracted by maceration method and filtered every 24 hours three times using 70% methanol solvent (powder and solvent ratio 1:10). The methanol extract obtained was combined, and then the solvent was evaporated with a rotary evaporator at 40 °C at 1 atm pressure until a thick blackish-green methanol extract was obtained. A portion of the methanol extract was extracted by liquid-liquid partition using dichloromethane and continued using ethyl acetate solvent. Furthermore, the methanol, dichloromethane, and ethyl acetate extracts were prepared for the phytochemical, antioxidant, and antidiabetic analysis.

Phytochemical Test

Phytochemical tests of methanol, dichloromethane, and ethyl acetate extracts were carried out qualitatively using modified standard procedures. The phytochemical

compounds to be determined namely alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins.[11–13]

Antioxidant Test

The antioxidant activity of the methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves of *R. mucronata* and vitamin C (standard drug) were evaluated in vitro using the DPPH (1,1-diphenyl-2-picrylhidrazyl) method following the procedure described by Senhaji et al. with minor modifications.[14] Two milliliters of each methanol, dichloromethane, and ethyl acetate extract were created in four concentrations (20, 40, 60, and 80 ppm), whereas Vitamin C was prepared in concentrations of 2, 4, 6, and 8 ppm. All samples were mixed continuously with 2 ml of DPPH (50 ppm) solution. After being incubated for 30 minutes in the dark at room temperature, the absorbance of the three extracts and vitamin C were measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.[14] The percentage of antioxidant activity or the percentage of DPPH inhibition of the methanol, dichloromethane, and ethyl acetate extracts and vitamin C were calculated using the following formula equation 1:

$$\%\ \text{Inhibition DPPH} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \text{ x } 100\% \qquad (1)$$

Then, a linear regression curve was made from the percentage data of DPPH inhibition on the concentrations of the three extracts and the concentration of vitamin C. The IC_{50} value (as an indicator of antioxidant activity) of the methanol, dichloromethane, and ethyl acetate extracts and vitamin C was calculated using the regression equation of the linear regression curve obtained.[15]

Antidiabetic Test

Antidiabetic activity test was carried out using the oral glucose tolerance test (OGTT) method. The mice used as samples were male mice with a body weight of 150-200 grams and were divided into five groups, each consisting of 5 mice. Before treatment, all mice were fasted for 18 hours, and then blood samples were taken from the vein in the tail of the mice using a glucometer to determine fasting/initial blood glucose levels. Then all groups were given 50% glucose monohydrate solution orally, except for the negative control group. After induction of glucose monohydrate for 180 minutes, the mice's blood glucose levels were measured again, and mice already in a hyperglycemic condition (blood glucose level > 200 mg/dl) were selected as samples for treatment.

Group 1 (negative control) was the group of mice that were given 1% CMC-Na (Sodium-Carboxymethyl Cellulose) suspension. Group 2 (positive control) was the group of mice given glibenclamide suspension at 10 mg/kg BW. Group 3 (D1), mice were treated with methanol extract of mangrove leaves at a dose of 300 mg/kg BW. Group 4 (D2), mice were treated with methanol extract of mangrove leaves at a dose of 600 mg/kg body weight, and group 5 (D3), mice were treated with methanol extract of mangrove leaves at a dose of 1,200 mg/kg body weight. The same thing also applies to treatments using dichloromethane and ethyl acetate extracts.

Measurement of blood glucose levels in mice was carried out after the treatment group (negative control, positive control, methanol extract, dichloromethane, and ethyl acetate extracts of mangrove *R. mucronata* leaves) at 8, 16, and 24 hours to determine the decrease in blood glucose levels in mice. Furthermore, the percentage of decrease in blood glucose levels of mice was calculated

using the following formula equation 2:[16]

$$\%\text{DBGL} = \frac{\text{Glucose level after (IGM)}}{\text{Final blood glucose level}} \text{ x } 100\% \qquad (2)$$

Whereas % DBGL is the percentage of blood glucose level decrease and IGM is the induction of Glucose Monohydrate.

Results and Discussion

Phytochemical Analysis

The results of the phytochemical screening of the methanol, dichloromethane, and ethyl acetate extracts of the mangrove leaves of *R. mucronata* showed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, steroids, triterpenoids, tannins, and saponins. Complete results can be seen in Table 1.

The results of the phytochemical test, as presented in Table 1, showed that the methanol extract of mangrove leaves of *R. mucronata* contained alkaloids, flavonoids, triterpenoids, and saponins. Meanwhile, the dichloromethane extract contained flavonoids, steroids, and tannins, whereas the ethyl acetate extract contained phytochemical compounds such as alkaloids, flavonoids, phenolic compounds, triterpenoids, tannins, and saponins. Previous researchers have reported that *R. mucronata* mangrove leaf extracts contain phenolic compounds, flavonoids, tannins, saponins, terpenoids dihydroflavonols, caffeic acid, vanillic acid, p-hydroxybenzoic acid, alkaloids, coumarins, quinones, resins, phytosterols, xanthoprotins, pigments (chlorophyll, carotenoids), and glucose.[17–20]

The other research by Bulan et al. revealed that the mentanol extract of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* contains compounds; alkaloids,

cardiac glycosides, saponins, phenolic compounds, tannins, steroids, and terpenoids. Meanwhile ethyl acetate extracts of the third mangrove leaf species (*R. mucronata, R. stylosa, and R. apiculata*) contained compounds such as alkaloids, cardiac glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids.[21] Phytochemical analysis of five mangrove leaf species, mainly *Bruguiera cylindrica*, *Aegiceras corniculatum*, *Acrostichum aureum*, *Avicennia alba*, and *R. mucronata*, all contain secondary metabolite compounds such as flavonoids, saponins, terpenoids, steroids, phenolics, tannins, and anthraquinones.[22,23]

Antioxidant Activity Test

In this study, the antioxidant activity of methanol, dichloromethane, ethyl acetate extracts of mangrove leaves of *R. mucronata*, and vitamin C (standard drug) with DPPH (1,1-diphenyl-2-picrylhydrazyl) method was tested. The results of the calculation of the percentage of inhibition against DPPH radicals of the three mangrove leaf extracts and vitamin C are presented in Table 2. The concentrations of methanol, dichloromethane, and ethyl acetate extracts of *R. muronata* mangrove leaves and vitamin C were then graphed against the percentage of DPPH inhibition, as shown in Figure 2.

The IC_{50} value expresses the antioxidant activity of compounds or extracts. IC_{50} is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%, which can be obtained from a linear regression equation and states the relationship between the concentration of extracts/compounds with the percentage of inhibition. The lower the IC_{50} value gained, the stronger compound's antioxidant activity.[24] The results of the calculation of IC_{50} values for methanol, dichloromethane, ethyl acetate, mangrove extract of *R. mucronata*, and vitamin C can be seen in Table

3.

Antioxidant Activity

Extracts with IC_{50} values < 50 ppm have antioxidant activity categorized as very strong; if the IC_{50} value of 50 - 100 ppm, then the antioxidant activity is categorized as strong; IC_{50} value of 100 - 150 ppm is classified as moderate, IC_{50} value of 150 - 200 ppm is categorized as weak, and if the IC_{50} value > 200 ppm, then the antioxidant activity is tagged as inactive.[25] The results of the calculation of the IC_{50} value of methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves *R. mucronata* and vitamin C (standard drug) can be seen in Table 3. Vitamin C and ethyl acetate extracts have antioxidant activity properties with a robust category, with IC_{50} values of 5.41 ppm and 34.64 ppm, respectively. In contrast, the extract of *R. mucronata* mangrove leaf dichloromethane has antioxidant activity properties with a strong category, with an IC_{50} value of 93.25 ppm. The antioxidant activity of methanol extract is included in the medium category with an IC_{50} value of 100.64 ppm. Ethyl acetate and methanol extracts of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* showed vigorous DPPH scavenging activity.[25] The activity is in line with the phytochemical compounds in the three mangrove plant species. Hence, the three Rhizophora species can be developed as natural antioxidants.[21]

The difference in antioxidant activity of the three *R. mucronata* mangrove leaf extracts is due to differences in the composition of chemically active compounds each extract possesses. Differences in the composition of these active compounds can provide synergistic effects between compounds resulting in increased antioxidant activity. Active chemical compounds such as phenolics, flavonoids, anthocyanins, tannins, and other phenolic compounds contained in the extract are closely related to

antioxidant activity.[16,26]

Secondary metabolite compounds in mangrove species of *R. mucronata* include alkaloids, flavonoids, phenolics, steroids, tannins, and terpenoids with strong antioxidant properties.[27,28] Then, it has been reported that the methanol extract of mangrove leaves of *R. mucronata* showed strong antioxidant activity with an IC_{50} value of 47.39 ± 0.43 µg/mL. The presence of flavonoid compounds such as catechins in the methanol extract of *R. mucronata* mangrove leaves is thought to be responsible for cholinesterase inhibitory and antioxidant activity.[29] Chlorophyll a, chlorophyll b, beta-carotene, lutein, neoxanthin, pheophytin a, and violaxanthin are pigment profiles in the leaves of mangrove plants of *R. mucronata*.[30] All identified pigments have strong antioxidant potential, especially as free radical scavengers and Nrf-2 stimulants. The mechanism of action of these pigments is by interacting with each other to inactivate antioxidant enzymes and inhibit the expression of oxidative stress proteins.[30]

Antidiabetic activity of *R. muronata*

Measurement of mice blood glucose levels was done using the oral glucose tolerance test (OGTT) method. The results of the calculation of the percentage reduction in blood glucose levels of mice in each treatment group, namely negative control, positive control, methanol extract, dichloromethane, and ethyl acetate of mangrove leaves of *R. mucronata* are presented in Table 4.

In accordance with the data in Table 4 and Figure 3, the positive control glibenclamide (dosage 10 mg/kg BW) had the greatest percentage of decrease in blood glucose levels in mice, followed by ethyl acetate extract in the D-3 treatment group (dose 1,200 mg/kg BW). Furthermore, methanol extract in treatment group D-2 (dose of 600 mg/kg BW), and dichloromethane extract in treatment group D-3 (dose of 1,200

mg/kg BW). The highest decrease in glucose levels occurred in the positive control group/ glibenclamide. This is because glibenclamide can stimulate pancreatic beta cells to secrete insulin and increase the sensitivity of peripheral cells to increased insulin levels.[31]

The decrease in mice blood glucose levels shown by treatment groups D-1, D-2, and D-3 in methanol, dichloromethane, and ethyl acetate extracts of *R. mucronata* mangrove leaves is due to the content of secondary metabolite compounds such as alkaloids, flavonoids, steroids, tannins, and saponins contained in the three mangrove leaf extracts.[32] These secondary metabolite compounds are thought to have a role in reducing blood glucose levels in mice.[33] Mangrove fruit extract *R. mucronata* with doses 125, 250, and 500 mg/kg BW were able to reduce blood glucose levels of diabetic rats.[32,33] The decrease in blood sugar levels in the group given glibenclamide at a dose of 5 mg/kg BW was more effective when compared to the treatment group given mangrove fruit extract of *R. mucronata* species.[32]

The content of secondary metabolite compounds of mangrove fruit extract of *R. mucronata* which is thought to reduce blood sugar levels are flavonoids, steroids, saponins and tannins.[33] Alkaloids and saponins can have hypoglycemic effects because they can stimulate insulin secretion from pancreatic beta cells.[34] Flavonoids and tannins can reduce blood glucose levels by capturing free radicals and reducing the increase in oxidative stress that occurs in people with diabetes to control blood glucose.[35,36] Secondary metabolite compounds from ethanol extracts, chloroform, and mangrove root fractions of *R. mucronata* species showed antidiabetic activity.[37] The mechanism of antidiabetic activity of mangrove bark extract of *R. mucronata* species is by increasing insulin secretion and restraining the digestion and absorption of

carbohydrates.[38]

Conclusion

Secondary metabolites isolated from *R. mucronata* mangrove leaves in methanol, dichloromethane, and ethyl acetate extracts indicated antioxidant and anti-diabetic activity. With an IC_{50} value of 34.64 ppm, the antioxidant activity of the ethyl acetate extract is classified as extremely robust. Furthermore, after 24 hours, the ethyl acetate extracts decreased blood glucose levels in mice by 57.64%. The ethyl acetate extract of the mangrove plant *R. mucronata* from Sambera beach, East Kalimantan has the potential to be developed as a natural antioxidant and alternative anti-diabetic therapy.

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Conflict of Interest

The authors declare no conflict of interest in writing this article.

Authors' Declaration

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

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Table 1. Phytochemical content of extracts of methanol, dichloromethane, and ethyl acetate of *R. mucronata* mangrove leaves.

No.	Phytochemical Test	Reagent	The extract and results of observations		
			Methanol	Dichloromethane	Ethyl Acetate
1.	Alkaloids	Dragendorff	positive (+) brownish-red precipitate	negative (-) the color of the solution does not change	positive (+) brownish-red precipitate
		Mayer	positive (+) white precipitate	negative (-) the color of the solution does not change	positive (+) white precipitate
		Wagner	positive (+) reddish-brown precipitate	negative (-) the color of the solution does not change	positive (+) reddish-brown precipitate
2.	Flavanoids	Mg + Amyl alcohol (HCl 37% & etanol 95%)	positive (+) pink-orange coloration	positive (+) pink-orange coloration	positive (+) pink-orange coloration

3.	Phenolics Compounds	$FeCl_3$ 5 %	negative (-) the color of the solution does not change	negative (-) the color of the solution does not change	positive (+) bluish-black color
4.	Steroids	Acetic anhydride + Sulfuric acid (concentrated)	negative (-) the color of the solution does not change	positive (+) green color solution	negative (-) the color of the solution does not change
5.	Triterpenoids	Acetic anhydride + Sulfuric acid (concentrated)	positive (+) red color	negative (-) the color of the solution does not change	positive (+) red color
6.	Tannins	$FeCl_3$ 1 %	negative (-) the color of the solution does not change	positive (+)blue-blackprecipitate	positive (+) blue-black precipitate
7.	Saponins	HCl 2 N	positive (+) stable foam formed	negative (-) not formed foam stable	positive (+) stable foam formed

- = Absent + = Present

Table 2. The percentage of DPPH inhibition of methanol, dichloromethane, ethyl acetate extracts of *R. muronata* leaves and vitamin C in various concentrations.

Extract	Absorbance (517 nm)				Percentage of DPPH inhibition			
	20 ppm	40 ppm	60 ppm	80 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Methanol	0.205	0.195	0.174	0.156	22.64	26.42	33.96	41.13
Dichloromethane	0.203	0.189	0.158	0.148	23.40	28.68	40.38	44.15
Ethyl Acetate	0.154	0.125	0.095	0.058	41.89	52.83	64.15	78.11
	Absorbance (517 nm)				Percentage of DPPH inhibition			
	2 ppm	4 ppm	6 ppm	8 ppm	2 ppm	4 ppm	6 ppm	8 ppm
Vitamin C	0.219	0.167	0.114	0.071	17.36	36.97	56.98	73.21

Table 3. Regression equations and IC_{50} values of methanol. dichloromethane. ethyl acetate extract of *R. muronata* mangrove leaves and vitamin C.

Extract	Regression Equation	IC_{50} Value (ppm)
Methanol	y = 0.345x + 15.28	100.64
Dichloromethane	y = 0.379x + 14.66	93.25
Ethyl Acetate	y = 0.599x + 29.25	34.64

Vitamin C	y = 9.378x - 0.76	5.41

Table 4. Percentage reduction in blood glucose levels of mice in treatment group on the 24th hour.

Treatment Group	Fasting Glucose Level (mg/dl)	Blood Glucose Level After (IGM) (mg/dl)	Blood Glucose Level After Treatment (mg/dl)	The Percentage of Blood Glucose Level Reduction (%)
Negative Control (CMC-Na 1 %)	85.7	-	-	-
Positive Control (Glibenclamide. Dose 10 mg/kg bw)	97.3	276	107	61.23
Methanol Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	95.7	285.7	170.3	40.39
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	97	276.3	158.3	42.71
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	92.7	269	162	39.78
Dichloromethane Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	99.3	276.3	224.4	18.78
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	93.7	306.3	229.7	25.01
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	99.3	259.7	178	21.81
Ethyl Acetate Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	94.3	268	193	27.99
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	96.7	274	178	35.04
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	96	279.3	118.3	57.64

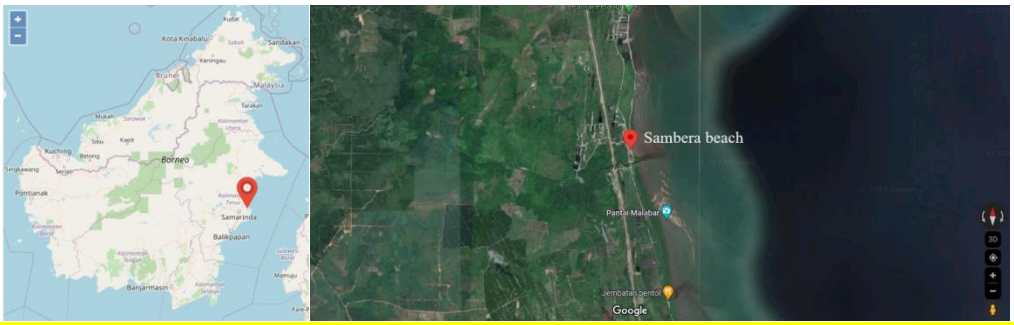

Figure 1. Location of *R. mucronata* sampling in Sambera beach, East Kalimantan, Indonesia

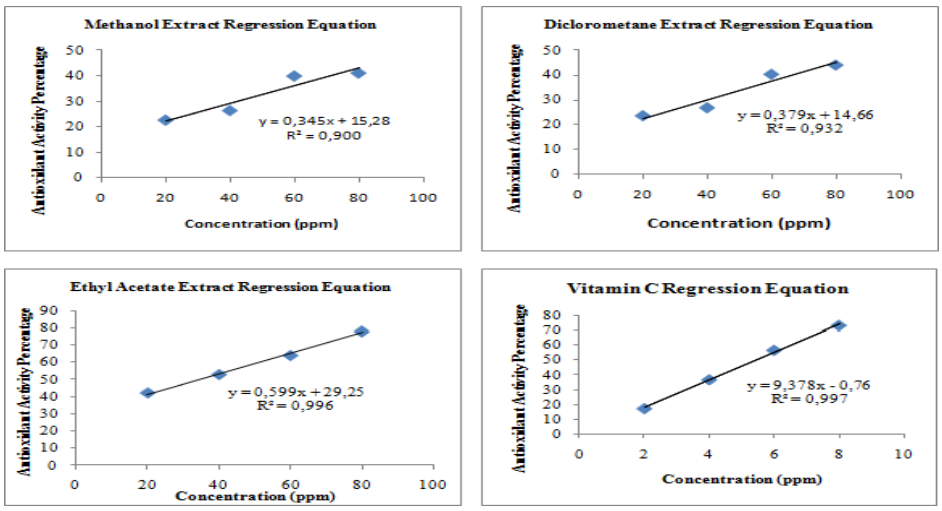

Figure 2. The graph of linear regression equation of methanol, dichloromethane, ethyl acetate extracts, and vitamin C.

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The Potential Active Chemical Compounds as Antioxidants and Antidiabetics from *Rhizophora mucronata* Derived from Sambera Beach, East Kalimantan, Indonesia

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ABSTRACT

This study aims to evaluate the bioactive compounds, antioxidant, and antidiabetic properties of methanol, dichloromethane, and ethyl acetate extracts of *Rhizophora mucronata* mangrove leaves. The *R. mucronata* leaf samples (1 kg) were extracted with methanol, followed by liquid-liquid partition extraction with dichloromethane, and finally ethyl acetate solvent. The extraction procedure was repeated three times for one 24-hour period. The phytochemical content of the three extracts was determined using the color test method, followed by an antioxidant activity test using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, and an Oral Glucose Tolerance Test (OGTT) to detect antidiabetic activity. The phytochemical contents of methanol, dichloromethane, and ethyl acetate extracts of *R. mucronata* mangrove leaves are alkaloids, flavonoids, phenolic compounds, triterpenoids, steroids, saponins, and tannins. The antioxidant activity of the ethyl acetate extract is categorized as extremely strong, with an IC_{50} value of 34.64 ppm. In addition, the ethyl acetate extracts reduced blood glucose levels in mice by 57.64% after 24 hours. The mangrove plant *R. mucronata* from the Sambera beach, particularly the ethyl acetate extract, has the potential to be developed as a natural antioxidant and alternative anti-diabetic medicine.

Keywords: *DPPH-scavenging activity, mangrove plants, oral glucose tolerance test, and phytochemical screening*

Introduction

The mangrove forest in Indonesia is one of the largest mangrove forest areas in the world,[1] so researchers in the field of natural organic chemistry and the herbal medicine industry are interested in researching on the utilization of mangrove forests as a source of antibiotics. In recent years, the demand for herbal medicine by people with diabetes has increased, along with the increasing number of diabetics in Indonesia. Data from the International Diabetes Federation (IDF) shows that the number of diabetics in Indonesia in 2019 is estimated to reach 10.7 million people. By 2045, it is expected to increase to 16.7 million people.[2] Therefore, exploration to find a cure for diabetes continues to develop.

The mangrove plant is a type of plant that is widely used by the community as a traditional medicine to cure various diseases, such as diarrhea, malaria, smallpox, asthma, diabetes, fever, swelling, rheumatism, skin diseases, hepatitis, diuretics,

leprosy, antitumor, leukemia, anticancer, antiviral, and mumps.[3–5] Parts of mangrove plants utilized as medicinal materials are root tissue, stem wood, bark, leaves, twigs, flowers, and fruits.[6,7] Mangrove plants are rich in secondary metabolite compounds such as alkaloids, polyphenols, flavonoids, tannins, saponins, triterpenes, anthraquinones, catechins, glucose, proteins, steroids, phenolic compounds, and glycosides that have antioxidant and antidiabetic activities. These mangrove plant species include *Avicennia marina*, *Acanthus ilicifolius*, *Ceriops tagal*, *Rhizophora mucronata*, *Rhizophora apiculata*, *Sonneratia alba*, *Sonneratia caseolaris*, *Xylocarpus granatum*, and *Nypa fruticans*.[8–10] Research on the bioactivity of mangrove species *R. mucronata* from Sambera Beach, East Kalimantan, Indonesia is very limited. Therefore, it is important to investigate the potential of this species in order to identify the secondary metabolite compounds with antioxidant and antidiabetic activities, which can subsequently be developed as natural herbal medicines.

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Material and Methods.

Equipment and Reagents

This research used equipment, including glassware commonly used in laboratories, digital analytical balance (XPR106DUHQ), rotary evaporator (RE301A-W, Yamato Scientific Co.Ltd.Japan), vortex (Labnet Vortex Mixer VX-200), incubator (Memmert Incubator I IN 55 PLUS), pH meter (Lutron PH-208), and UV-Vis spectrophotometer (Shimadzu UV-Vis UV-1280), aquadest, 1,1-diphenyl-2-picrylhidrazyl (Sigma Aldrich, USA), methanol 70%, dichloromethane (Merck, Germany), Ethyl Acetate (Riedel-deHaen, Germany), H_2SO_4 (Merck, Germany),

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$FeCl_3$ 0.1% (Merck, Germany), chemicals for color reagents to test the phytochemicals (alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins) purchased from Merck (Germany), CMC-Na (Sigma-Aldrich, USA)), Glibenclamide (First Medifarma), filter paper, and aluminum foil.

Sample collection and preparation

The mangrove leaf samples of *R. mucronata* were taken in February 2022 from Sambera Beach (0°14'44.8"S, 117°25'00.6"E), East Kalimantan, Indonesia (Fig. 1). Mangrove leaf samples were washed with running water to remove dirt attached to the leaves and then cut into small pieces. The dried samples were sorted and then pulverized into fine powder with a size of 90 mesh. Mangrove leaf powder is ready to be used for the subsequent procedures.

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Extraction

About 1 kg of *R. mucronata* mangrove leaf powder was extracted by maceration method and filtered every 24 hours three times using 70% methanol solvent (powder and solvent ratio 1:10). The methanol extract obtained was combined, and then the solvent was evaporated with a rotary evaporator at 40 °C at 1 atm pressure until a thick blackish-green methanol extract was obtained. A portion of the methanol extract was extracted by liquid-liquid partition using dichloromethane and continued using ethyl acetate solvent. Furthermore, the methanol, dichloromethane, and ethyl acetate extracts were prepared for the phytochemical, antioxidant, and antidiabetic analysis.

Phytochemical Test

Phytochemical tests of methanol, dichloromethane, and ethyl acetate extracts were carried out qualitatively using modified standard procedures. The phytochemical

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compounds to be determined namely alkaloids, flavonoids, phenolic compounds, steroids, triterpenoids, tannins, and saponins.[11–13]

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Antioxidant Test

The antioxidant activity of the methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves of *R. mucronata* and vitamin C (standard drug) were evaluated in vitro using the DPPH (1,1-diphenyl-2-picrylhidrazyl) method following the procedure described by Senhaji et al. with minor modifications.[14] Two milliliters of each methanol, dichloromethane, and ethyl acetate extract were created in four concentrations (20, 40, 60, and 80 ppm), whereas Vitamin C was prepared in concentrations of 2, 4, 6, and 8 ppm. All samples were mixed continuously with 2 ml of DPPH (50 ppm) solution. After being incubated for 30 minutes in the dark at room temperature, the absorbance of the three extracts and vitamin C were measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.[14] The percentage of antioxidant activity or the percentage of DPPH inhibition of the methanol, dichloromethane, and ethyl acetate extracts and vitamin C were calculated using the following formula equation 1:

$$\%\ \text{Inhibition DPPH} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100\% \quad (1)$$

Then, a linear regression curve was made from the percentage data of DPPH inhibition on the concentrations of the three extracts and the concentration of vitamin C. The IC_{50} value (as an indicator of antioxidant activity) of the methanol, dichloromethane, and ethyl acetate extracts and vitamin C was calculated using the regression equation of the linear regression curve obtained.[15]

Antidiabetic Test

Antidiabetic activity test was carried out using the oral glucose tolerance test (OGTT) method. The mice used as samples were male mice with a body weight of 150-200 grams and were divided into five groups, each consisting of 5 mice. Before treatment, all mice were fasted for 18 hours, and then blood samples were taken from the vein in the tail of the mice using a glucometer to determine fasting/initial blood glucose levels. Then all groups were given 50% glucose monohydrate solution orally, except for the negative control group. After induction of glucose monohydrate for 180 minutes, the mice's blood glucose levels were measured again, and mice already in a hyperglycemic condition (blood glucose level > 200 mg/dl) were selected as samples for treatment.

Group 1 (negative control) was the group of mice that were given 1% CMC-Na (Sodium-Carboxymethyl Cellulose) suspension. Group 2 (positive control) was the group of mice given glibenclamide suspension at 10 mg/kg BW. Group 3 (D1), mice were treated with methanol extract of mangrove leaves at a dose of 300 mg/kg BW. Group 4 (D2), mice were treated with methanol extract of mangrove leaves at a dose of 600 mg/kg body weight, and group 5 (D3), mice were treated with methanol extract of mangrove leaves at a dose of 1,200 mg/kg body weight. The same thing also applies to treatments using dichloromethane and ethyl acetate extracts.

Measurement of blood glucose levels in mice was carried out after the treatment group (negative control, positive control, methanol extract, dichloromethane, and ethyl acetate extracts of mangrove *R. mucronata* leaves) at 8, 16, and 24 hours to determine the decrease in blood glucose levels in mice. Furthermore, the percentage of decrease in blood glucose levels of mice was calculated

using the following formula equation 2:[16]

$$\%\text{DBGL} = \frac{\text{Glucose level after (IGM)}}{\text{Final blood glucose level}} \text{ x } 100\% \qquad (2)$$

Whereas % DBGL is the percentage of blood glucose level decrease and IGM is the induction of Glucose Monohydrate.

Results and Discussion

Phytochemical Analysis

The results of the phytochemical screening of the methanol, dichloromethane, and ethyl acetate extracts of the mangrove leaves of *R. mucronata* showed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, steroids, triterpenoids, tannins, and saponins. Complete results can be seen in Table 1.

The results of the phytochemical test, as presented in Table 1, showed that the methanol extract of mangrove leaves of *R. mucronata* contained alkaloids, flavonoids, triterpenoids, and saponins. Meanwhile, the dichloromethane extract contained flavonoids, steroids, and tannins, whereas the ethyl acetate extract contained phytochemical compounds such as alkaloids, flavonoids, phenolic compounds, triterpenoids, tannins, and saponins. Previous researchers have reported that *R. mucronata* mangrove leaf extracts contain phenolic compounds, flavonoids, tannins, saponins, terpenoids dihydroflavonols, caffeic acid, vanillic acid, p-hydroxybenzoic acid, alkaloids, coumarins, quinones, resins, phytosterols, xanthoprotins, pigments (chlorophyll, carotenoids), and glucose.[17–20]

The other research by Bulan et al. revealed that the mentanol extract of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* contains compounds; alkaloids,

cardiac glycosides, saponins, phenolic compounds, tannins, steroids, and terpenoids. Meanwhile ethyl acetate extracts of the third mangrove leaf species (*R. mucronata, R. stylosa, and R. apiculata*) contained compounds such as alkaloids, cardiac glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids.[21] Phytochemical analysis of five mangrove leaf species, mainly *Bruguiera cylindrica*, *Aegiceras corniculatum*, *Acrostichum aureum*, *Avicennia alba*, and *R. mucronata*, all contain secondary metabolite compounds such as flavonoids, saponins, terpenoids, steroids, phenolics, tannins, and anthraquinones.[22,23]

Antioxidant Activity Test

In this study, the antioxidant activity of methanol, dichloromethane, ethyl acetate extracts of mangrove leaves of *R. mucronata*, and vitamin C (standard drug) with DPPH (1,1-diphenyl-2-picrylhydrazyl) method was tested. The results of the calculation of the percentage of inhibition against DPPH radicals of the three mangrove leaf extracts and vitamin C are presented in Table 2. The concentrations of methanol, dichloromethane, and ethyl acetate extracts of *R. muronata* mangrove leaves and vitamin C were then graphed against the percentage of DPPH inhibition, as shown in Figure 2.

The IC_{50} value expresses the antioxidant activity of compounds or extracts. IC_{50} is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%, which can be obtained from a linear regression equation and states the relationship between the concentration of extracts/compounds with the percentage of inhibition. The lower the IC_{50} value gained, the stronger compound's antioxidant activity.[24] The results of the calculation of IC_{50} values for methanol, dichloromethane, ethyl acetate, mangrove extract of *R. mucronata*, and vitamin C can be seen in Table

3.

Antioxidant Activity

Extracts with IC_{50} values < 50 ppm have antioxidant activity categorized as very strong; if the IC_{50} value of 50 - 100 ppm, then the antioxidant activity is categorized as strong; IC_{50} value of 100 - 150 ppm is classified as moderate, IC_{50} value of 150 - 200 ppm is categorized as weak, and if the IC_{50} value > 200 ppm, then the antioxidant activity is tagged as inactive.[25] The results of the calculation of the IC_{50} value of methanol, dichloromethane, and ethyl acetate extracts of mangrove leaves *R. mucronata* and vitamin C (standard drug) can be seen in Table 3. Vitamin C and ethyl acetate extracts have antioxidant activity properties with a robust category, with IC_{50} values of 5.41 ppm and 34.64 ppm, respectively. In contrast, the extract of *R. mucronata* mangrove leaf dichloromethane has antioxidant activity properties with a strong category, with an IC_{50} value of 93.25 ppm. The antioxidant activity of methanol extract is included in the medium category with an IC_{50} value of 100.64 ppm. Ethyl acetate and methanol extracts of mangrove leaves of *R. mucronata*, *R. stylosa*, and *R. apiculata* showed vigorous DPPH scavenging activity.[25] The activity is in line with the phytochemical compounds in the three mangrove plant species. Hence, the three Rhizophora species can be developed as natural antioxidants.[21]

The difference in antioxidant activity of the three *R. mucronata* mangrove leaf extracts is due to differences in the composition of chemically active compounds each extract possesses. Differences in the composition of these active compounds can provide synergistic effects between compounds resulting in increased antioxidant activity. Active chemical compounds such as phenolics, flavonoids, anthocyanins, tannins, and other phenolic compounds contained in the extract are closely related to

antioxidant activity.[16,26]

Secondary metabolite compounds in mangrove species of *R. mucronata* include alkaloids, flavonoids, phenolics, steroids, tannins, and terpenoids with strong antioxidant properties.[27,28] Then, it has been reported that the methanol extract of mangrove leaves of *R. mucronata* showed strong antioxidant activity with an IC_{50} value of 47.39 ± 0.43 µg/mL. The presence of flavonoid compounds such as catechins in the methanol extract of *R. mucronata* mangrove leaves is thought to be responsible for cholinesterase inhibitory and antioxidant activity.[29] Chlorophyll a, chlorophyll b, beta-carotene, lutein, neoxanthin, pheophytin a, and violaxanthin are pigment profiles in the leaves of mangrove plants of *R. mucronata*.[30] All identified pigments have strong antioxidant potential, especially as free radical scavengers and Nrf-2 stimulants. The mechanism of action of these pigments is by interacting with each other to inactivate antioxidant enzymes and inhibit the expression of oxidative stress proteins.[30]

Antidiabetic activity of *R. muronata*

Measurement of mice blood glucose levels was done using the oral glucose tolerance test (OGTT) method. The results of the calculation of the percentage reduction in blood glucose levels of mice in each treatment group, namely negative control, positive control, methanol extract, dichloromethane, and ethyl acetate of mangrove leaves of *R. mucronata* are presented in Table 4.

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In accordance with the data in Table 4 and Figure 3, the positive control glibenclamide (dosage 10 mg/kg BW) had the greatest percentage of decrease in blood glucose levels in mice, followed by ethyl acetate extract in the D-3 treatment group (dose 1,200 mg/kg BW). Furthermore, methanol extract in treatment group D-2 (dose of 600 mg/kg BW), and dichloromethane extract in treatment group D-3 (dose of 1,200

mg/kg BW). The highest decrease in glucose levels occurred in the positive control group/ glibenclamide. This is because glibenclamide can stimulate pancreatic beta cells to secrete insulin and increase the sensitivity of peripheral cells to increased insulin levels.[31]

The decrease in mice blood glucose levels shown by treatment groups D-1, D-2, and D-3 in methanol, dichloromethane, and ethyl acetate extracts of *R. mucronata* mangrove leaves is due to the content of secondary metabolite compounds such as alkaloids, flavonoids, steroids, tannins, and saponins contained in the three mangrove leaf extracts.[32] These secondary metabolite compounds are thought to have a role in reducing blood glucose levels in mice.[33] Mangrove fruit extract *R. mucronata* with doses 125, 250, and 500 mg/kg BW were able to reduce blood glucose levels of diabetic rats.[32,33] The decrease in blood sugar levels in the group given glibenclamide at a dose of 5 mg/kg BW was more effective when compared to the treatment group given mangrove fruit extract of *R. mucronata* species.[32]

The content of secondary metabolite compounds of mangrove fruit extract of *R. mucronata* which is thought to reduce blood sugar levels are flavonoids, steroids, saponins and tannins.[33] Alkaloids and saponins can have hypoglycemic effects because they can stimulate insulin secretion from pancreatic beta cells.[34] Flavonoids and tannins can reduce blood glucose levels by capturing free radicals and reducing the increase in oxidative stress that occurs in people with diabetes to control blood glucose.[35,36] Secondary metabolite compounds from ethanol extracts, chloroform, and mangrove root fractions of *R. mucronata* species showed antidiabetic activity.[37] The mechanism of antidiabetic activity of mangrove bark extract of *R. mucronata* species is by increasing insulin secretion and restraining the digestion and absorption of

carbohydrates.[38]

Conclusion

Secondary metabolites isolated from *R. mucronata* mangrove leaves in methanol, dichloromethane, and ethyl acetate extracts indicated antioxidant and anti-diabetic activity. With an IC_{50} value of 34.64 ppm, the antioxidant activity of the ethyl acetate extract is classified as extremely robust. Furthermore, after 24 hours, the ethyl acetate extracts decreased blood glucose levels in mice by 57.64%. The ethyl acetate extract of the mangrove plant *R. mucronata* from Sambera beach, East Kalimantan has the potential to be developed as a natural antioxidant and alternative anti-diabetic therapy.

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Conflict of Interest

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Table 1. Phytochemical content of extracts of methanol, dichloromethane, and ethyl acetate of *R. mucronata* mangrove leaves.

No.	Phytochemical Test	Reagent	The extract and results of observations		
			Methanol	Dichloromethane	Ethyl Acetate
1.	Alkaloids	Dragendorff	positive (+) brownish-red precipitate	negative (-) the color of the solution does not change	positive (+) brownish-red precipitate
		Mayer	positive (+) white precipitate	negative (-) the color of the solution does not change	positive (+) white precipitate
		Wagner	positive (+) reddish-brown precipitate	negative (-) the color of the solution does not change	positive (+) reddish-brown precipitate
2.	Flavanoids	Mg + Amyl alcohol (HCl 37% & etanol 95%)	positive (+) pink-orange coloration	positive (+) pink-orange coloration	positive (+) pink-orange coloration

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3.	Phenolics Compounds	$FeCl_3$ 5 %	negative (-) the color of the solution does not change	negative (-) the color of the solution does not change	positive (+) bluish-black color
4.	Steroids	Acetic anhydride + Sulfuric acid (concentrated)	negative (-) the color of the solution does not change	positive (+) green color solution	negative (-) the color of the solution does not change
5.	Triterpenoids	Acetic anhydride + Sulfuric acid (concentrated)	positive (+) red color	negative (-) the color of the solution does not change	positive (+) red color
6.	Tannins	$FeCl_3$ 1 %	negative (-) the color of the solution does not change	positive (+)blue-blackprecipitate	positive (+) blue-black precipitate
7.	Saponins	HCl 2 N	positive (+) stable foam formed	negative (-) not formed foam stable	positive (+) stable foam formed

- = Absent + = Present

Table 2. The percentage of DPPH inhibition of methanol, dichloromethane, ethyl acetate extracts of *R. muronata* leaves and vitamin C in various concentrations.

Extract	Absorbance (517 nm)				Percentage of DPPH inhibition			
	20 ppm	40 ppm	60 ppm	80 ppm	20 ppm	40 ppm	60 ppm	80 ppm
Methanol	0.205	0.195	0.174	0.156	22.64	26.42	33.96	41.13
Dichloromethane	0.203	0.189	0.158	0.148	23.40	28.68	40.38	44.15
Ethyl Acetate	0.154	0.125	0.095	0.058	41.89	52.83	64.15	78.11
	Absorbance (517 nm)				Percentage of DPPH inhibition			
	2 ppm	4 ppm	6 ppm	8 ppm	2 ppm	4 ppm	6 ppm	8 ppm
Vitamin C	0.219	0.167	0.114	0.071	17.36	36.97	56.98	73.21

Table 3. Regression equations and IC_{50} values of methanol. dichloromethane. ethyl acetate extract of *R. muronata* mangrove leaves and vitamin C.

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Extract	Regression Equation	IC50 Value (ppm)
Methanol	y = 0.345x + 15.28	100.64
Dichloromethane	y = 0.379x + 14.66	93.25
Ethyl Acetate	y = 0.599x + 29.25	34.64

Vitamin C	y = 9.378x - 0.76	5.41

Table 4. Percentage reduction in blood glucose levels of mice in treatment group on the 24th hour.

Treatment Group	Fasting Glucose Level (mg/dl)	Blood Glucose Level After (IGM) (mg/dl)	Blood Glucose Level After Treatment (mg/dl)	The Percentage of Blood Glucose Level Reduction (%)
Negative Control (CMC-Na 1 %)	85.7	-	-	-
Positive Control (Glibenclamide. Dose 10 mg/kg bw)	97.3	276	107	61.23
Methanol Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	95.7	285.7	170.3	40.39
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	97	276.3	158.3	42.71
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	92.7	269	162	39.78
Dichloromethane Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	99.3	276.3	224.4	18.78
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	93.7	306.3	229.7	25.01
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	99.3	259.7	178	21.81
Ethyl Acetate Extract				
D-1 (*R. mucronata* Leaf Extract Dose 300 mg/kg bw)	94.3	268	193	27.99
D-2 (*R. mucronata* Leaf Extract Dose 600 mg/kg bw)	96.7	274	178	35.04
D-3 (*R. mucronata* Leaf Extract Dose 1.200 mg/kg bw)	96	279.3	118.3	57.64

Figure 1. Location of *R. mucronata* sampling in Sambera beach, East Kalimantan, Indonesia

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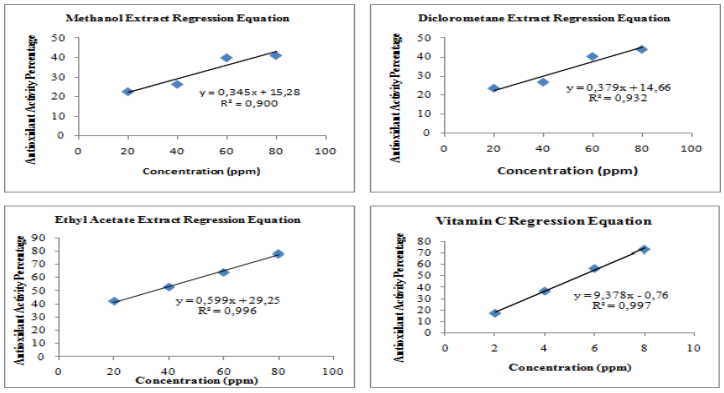

Figure 2. The graph of linear regression equation of methanol, dichloromethane, ethyl acetate extracts, and vitamin C.

Dear Editor-in-Chief
Tropical Journal of Natural Product Research

Here are the responses for the reviewer's comments:

No.	Reviewer comments	Author answer
1.	In Title; The author must delete the words "The Potential Active Chemical Compounds"	We have removed the word in the title
2.	Paragraph 2 in Introduction, line 14, Poor expression	We have paraphrased and changed the sentence.
3.	Paragraf 1 in Material and Methods, line 1, Poor expression	We have revised the sentence in material and methods section.
4.	Paragraf 2 in Material and Methods, line 5, Poor grammatical expression	We have revised the sentence in material and methods section.
5.	Paragraf 4 in Material and Methods, line 2, The author must delete the word procedures and delete the sentence	We have revised the sentences according to reviewer suggestion
6.	Paragraf 8-9 in Material and Methods dan paragraf 1-9 in Results and Discussion, The Author should be re-written the manuscript with the support of a science editor, and a native English speaker.	The manuscript has been edited for correct English grammar, punctuation, spelling and formatting style by Solution Biothecnology Laboratory (SBL) English Proofreading Service
7.	In Conclusion, line 1, The author did not isolate secondary metabolite compounds	We have revised the sentence in conclusion section.
8.	In references, The authors must rewrite references using journal style (www.tjnpr.org), and italicize and complete a bibliography.	We have rewritten the references according to the journal style suggested by www.tjnpr.org, and completed the references.
9.	In the table 1, The authors should be removed unimportant reagents and only indicate + or - signs in the extract and results of observations	We have changed table 1 according to the reviewer's direction
10.	In the table 3, The author must change the title of the table	We have changed the sentence in the title of table 3
11.	In the figure 1, The author must provide a reference for the location where Rhizophora mucronata leaves were	We have provided reference sampling locations in the location

	collected	

I have also attached the revised manuscript for resubmission. Thank you very much.

On behalf of authors,

Usman usman

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