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Antidiabetic Activity of leaf extract from three types of Mangrove Originating from Sambera Coastal Region Indonesia

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ABSTRACT:

This study designed to determine the antidiabetic profile ethanol extracts of leaf extract from three types of mangroves; *Avicennia sp.*, *Rhizophora sp.*, and *Sonneratia sp.* Extraction process used maceration with ethanol solvent. The phytochemical assays carried out qualitatively including assay of alkaloids, flavonoids, steroids, terpenoids, phenolic compounds, tannins, and saponins. The method used for the antidiabetic test is the method of blood glucose tolerance. The results showed that ethanol extract of three types of mangrove leaves *Avicennia sp.*, *Rhizophora sp.*, and *Sonneratia sp.* containing secondary metabolite group alkaloids, flavonoids, steroids, terpenoids, phenolic compounds, tannins, and saponins. Ethanol extract of leaves of the three types of mangroves plants has antidiabetic activity. The ethanol extract of leaves of mangrove *Rhizophora sp.* with a dose of 200 mg/kg BB + glucose 2 g / kg (group P-2) showed the most excellent antidiabetic activity and lowering blood glucose levels by 31.27%. This study confirmed that three types of Mangrove from Sambera Coastal Region Indonesia showed the antidiabetic profile.

KEYWORDS: *Avicennia sp.*, *Rhizophora sp.*, *Sonneratia sp.*, Glucosetolerance, antidiabetic.

INTRODUCTION:

Diabetes mellitus (DM) is a non-infectious degenerative disease, caused by hormonal disorders characterized showed high blood sugar levels with carbohydrate, lipid, and protein metabolism disorders as a result of insulin function insufficiency. Based on the Diabetes Care journal report, people with diabetes in Indonesia in 2000 reached 8.4 million people and ranked 4th after India, China, and United States¹. An increasing number of diabetics in Indonesia will lead to an increase in the number of antidiabetic drugs². Therefore, exploration efforts to find new antidiabetic drugs from plants need to be done.

Types of plants that are widely used by the community as traditional medicine is a mangrove plant. This mangrove plant is efficacious to cure some of the disease: such as hepatitis, diuretic, leprosy, antimalarial, diarrhea, asthma, diabetes, fever, swelling, rheumatism, skin diseases, smallpox, antitumor, antiviral, leukemia anticancer, treat throat mumps, and beriberi^{3,4,5}

Several types of mangrove plants that are as antidiabetic drugs such as; *Acanthus illicifolius*, *Avicennia ebracteatus*, *Bruguiera conjugata*, *Bruguiera cylindrical*, *Bruguiera rumphii*, *Bruguiera sexangula*, *Dalbergia ecastophyllum*, *Excoecaria agallocha*, *Hertiera macrophylla*, *Kandelia candel*, *Kandelia rheedii*, *Rhizophora conjugata*, *Rhizophora gymnorrhiza*, *Rhizophora mangle*, *Rhizophora racemosa*, *Rhizophora stylosa*, *Salicornia brachiata*, *Sonneratia alba*, *Sonneratia ovata*, and *Xylocarpus granatum*, and *Xylocarpus moluccensis*.^{6,7}

Part of the mangrove plant tissue used as an antidiabetic drug is part of the root tissue, stem wood, bark, leaves, twigs, flowers, and fruit. Chemical content suspected to be antidiabetic in some types of mangrove plants are alkaloid compounds, steroids, triterpenes, phenolic compounds, flavonoids, stilbene, carotenoids, triterpenes, anthocyanins, anthocyanidins, inositol, saponins, long chain alcohols, tannins, amino acids, benzoquinone, coumarin, quinine, chalcone, lipid compounds, phorbol ester, rotenone, polyphenols, benzofuran, limonoid, sulfur alkaloids, procyanidin, gibberellin, and xiloccensins.^{4,7}

This means the exploration of secondary metabolite compounds in mangroves *Rhizophora sp.*, *Avicennia sp.*, and *Sonneratia sp.*, originating from Sambera Beach Marangkayu Sub-district, Kutai Kartanegara Regency, East Kalimantan, Indonesia has the potential to be developed as an alternative medicine for antidiabetes.

MATERIAL AND METHODS:

Plants Material and Identification:

Three types of Mangrove leaf samples (*Avicennia sp.*, *Rhizophora sp.*, and *Sonneratia sp.*) were collected from Sambera Beach, Marangkayu Sub-district, Kutai

three types of mangrove leaf samples (*Avicennia sp.*, *Rhizophora sp.*, and *Sonneratia sp.*) were collected from Sambera Beach, Marangkayu sub-district, Kutai Kartanegara Regency, East Kalimantan, Indonesia and each type of mangroves was collected and identified at Dendrology Laboratory, Faculty of Forestry Universitas Mulawarman.

The tools used in this study are Scales (O'hauss), analytical balance (O'hauss), scissors, oral spoits, rotary evaporators (BUCHI), jars, filter paper, glass tool: commonly used in laboratories, UV lamps, stain sprayers, Buchner funnels, TLC chamber, column chromatography, evaporator, TLC plates, disposable syringe, and blood glucometer Nesco glucometer.

The chemicals used consisted of *n*-hexane (Merck), acetone (Merck), ethyl acetate (Merck) and methanol (Merck), chloroform (Merck), Silica Gel 60 GF254 (Merck) Silica Gel 60 (230 - 400 mesh)(Merck), Silica Gel Kiesegel 60 F254 0.25 mm (Merck) is used for column chromatography, 1.5% cerium sulfate solution in 2N sulfuric acid is used for stain removal, 1.5% cerium sulfate solution in 2N sulfuric acid is used for stain removal. Moreover, for glucose test used (diabetes); aquadest, 9% glucose solution, Na-CMC solution and glibenclamide.

Plants Material Extraction:

500 g plants material from the three species mangrove were macerated with methanol solvent and evaporated using a rotary evaporator at a temperature of 40° C to obtain a methanol extract. The methanol extract is further extracted by liquid-liquid partition using a solvent with an increased polarity level: *n*-hexane chloroform, and ethyl acetate solvents. Each of the extracts obtained was evaporated again using a rotary evaporator and then weighed and measured yield phytochemical assay and antidiabetic assay.

Phytochemical Assay:

Phytochemical assay of leaf extract from three types of mangrove was performed for secondary metabolite identification group of alkaloid using three types of reagents; Meyer, Wagner and Dragendorff. Group of flavonoid using concentrated HCl reagents with Mg, concentrated H₂SO₄, and 10% NaOH solution. Group of Steroid and Triterpenoid using Lieberman-Burchard reagent. Group of phenolic using FeCl₃ reagent and for saponin group using hot water and 2 N HC solution^{8,9,10}.

Glucose Tolerance Test:

Preparation of 1% CMC-Na solution.

1 gram of methylcellulose sodium carboxyose (CMC-Na) was developed by using 10 mL of hot water inside the mortar then crushed until homogeny was then poured into a 100 mL measuring flask, and added distilled water to 100 mL.

Preparation of glucose solution

50% glucose solution, prepared by dissolving 50 mg D-glucose into 100 mL measuring flask and added aquadest. The D-glucose dose used for the mice is 5 g / kg BW of the test animal.

Preparation of glibenclamide solution:

0.05% glibenclamide comparison solution was prepared by weighing as much as 0.05 mg of glibenclamide and put into a 100 mL measuring flask and then adding a 1% CMC-Na suspension to 100 mL of precise volume. The dose of glibenclamide used was 10 mg/kg bb of test animals¹¹.

Preparation of mangrove leaf extract solution:

The concentration of methanol extract from the three types of mangrove leaves (*Avicennia sp.*, *Rhizophora sp.*, and *Sonneratia sp.*) used for the assay of antidiabetic activity in this study was 2% (500 mg mangrove leaf extract dissolved in 25 mL 1% CMC-Na solution).

Assay for the effect of methanol extract from the leaves of the three types of mangrove plants tested its bioactivity against the decrease in blood glucose levels of mice (*Mus musculus*) by the method of glucose tolerance.

Glucose Tolerance Assay:

Glucose tolerance method is carried out by mice adaptation process for seven days of daily feeding and drinking. Mice fasted for 18 hours, but still given drink. Fasted mice were divided into six groups, each group consisting of three mice, the normal group (KN), the negative control group (K-), the positive control group (K +), the P-1 (*Avicenna Sp*) treatment group, the P- 2 (*Rhizophora sp.*), And the P-3 treatment group (*Soneratia Sp*). Oral glucose administration with a dose of 5 g / kg bw of oral test animals in mice except for normal group mice. After 60 minutes the positive control group (K +) was given glibenclamide at a dose of 10 mg/kgBW orally; P-1, P-2 and P-3 groups were given a mangrove leaf extract of methanol at a dose of 0.5 g / kg bw orally. Blood samples were obtained from taking the tail of the mice by cutting the tail before and after 60 minutes oral glucose administration and after extract induced in 60, 120, and 180 minutes [12]. The outer blood is collected in an Eppendorf tube, then from this tube the blood is taken with a capillary tube (1 drop) to be measured using a gluco test device. Blood collection continued at 120 min, and 180 (t-120, and t-180). Blood glucose level calculated by the formula of relative blood glucose (Cr) with the formula:

Description:

Ct: blood glucose level at time t (t30, t60, t90 and t120).

Co: initial blood glucose level (t = 0)

Decrease Percentage (% P) Relative blood glucose level calculated by the formula:

Description:

Cr (p): relative blood glucose levels of the test group

Cr (-): blood glucose level is a relatively negative group

RESULTS AND DISCUSSION:

Plant Extraction:

The extraction of the samples from the leaves of the three mangrove species was carried out using the maceration method, since this method is easier than the other extraction methods. The extraction process in this way accelerates the process of withdrawing the chemical compounds contained in the sample. The result of ethanol extract from the leaves of the three mangrove species can be seen in Table 1.

Table 1. Extraction data from ethanol extract leaf of three species of mangrove

SW (gram)	VS (mL)	Type of Mangroves	Temp. Evap	VE (mL)	EW (gram)	Color Extract
500	1.500	<i>Avicenna Sp</i>	40 °C	3.758	114.68	Dark Green
		<i>Rhizophora sp.</i>	40 °C	3.785	147.40	Dark Green
		<i>Sonneratia sp.</i>	40 °C	3.572	106.22	Dark Green

Description: SW=sample weight; VS=volume of solvent; LW=Extract weight; VE=volume Extract

Based on data from Table 1 the weight of extracts can be seen that the extract of ethanol leaves *Rhizophora sp.* obtained as much as 147.40 grams and higher than methanol extract from mangrove leaves *Avicenna sp.* and *Sonneratia sp.* with the weight of each extract was 114.68 grams and 106.22 grams. This is in accordance with the results of Suciati research (2012), the active compound of *Rhizophora mucronata* leaves are higher in semi-polar and polar solvents, since most of the active compounds of the *Rhizophora mucronata* leaf dissolve in the semipolar and polar solvents resulting in the high yield value produced¹³.

Phytochemical Assay:

The phytochemical assay is intended to know the class of secondary metabolite compounds contained in the ethanol extract of the leaf tissue of the mangrove plant species; *Avicenna Sp.*, *Rhizophora sp.*, and *Sonneratia sp.* Phytochemical tests include; test alkaloids, flavonoids, steroids, terpenoids, phenolics, tannins, and saponins. Phytochemical test results of ethanol extract of leaf from three species of mangrove plants can be seen in Table 2.

Table 2. Phytochemical Assay Result from Ethanol Leaf Extract of Three Mangrove Type.

No.	Phytochemical Test	Ethanol Extract		
		<i>Avicenna Sp</i>	<i>Rhizophora sp.</i>	<i>Sonneratia sp.</i>
	Alkaloids Test			
1	· Meyer	(-)	(-)	(-)
	· Dragendorff	(+)	(+)	(+)
	· Wagner	(+)	(+)	(+)
2	Flavonoids Test Mg + HCl(Conc.)	(+)	(+)	(+)
3	Steroids Test (acetic anhydride + conc. sulfuric acid)	(-)	(+)	(+)
4	Triterpenoids Test (acetic anhydride + conc. sulfuric acid)	(+)	(+)	(+)
5	Phenolic Test FeCl ₃ 1 %	(+)	(+)	(-)
6	Tannin Test Gelatin 2%	(+)	(+)	(-)
7	Saponin Test Hot Water+HCl(conc.)	(+)	(+)	(+)

Description:

(+)=positive test (exists)

(-)=negative test (not exist)

Based on the qualitative phytochemical test (Table 2) known that the extract of ethanol *Avicenna Sp* leaves containing secondary metabolite alkaloid, flavonoids, terpenoids, phenolic, tannins, and saponins group. The ethanol extract in a leaf of *Rhizophora sp.* containing alkaloids, flavonoids, steroids, terpenoids, phenolic, tannins, and saponins groups. The ethanol extract of the leaf tissue of *Sonneratia sp.* containing secondary metabolite group compounds; alkaloids, flavonoids, steroids, terpenoids, and saponins. Previous phytochemical results have been reported that the chemical content of bioactive compounds of methanol extracts of the leaves and stems of *Avicenna marina* derived from Sudan containing a group of alkaloids, steroids, flavonoids, triterpenes, tannins, and saponins¹⁴. Wibowo et al., 2009 reported that *Avicenna marina* contains a class of compounds; alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides¹⁵.

The results of phytochemical studies of methanolic extract of *Rhizophora mucronata* leaves contain phenolic compounds, saponins, terpenoids, and flavonoids¹⁶ Usman (2017) reported that methanolic extract of *Rhizophora apiculata* mangrove root contained secondary compound metabolites; alkaloids, steroids and flavonoids¹⁷. Other research suggests that the content of secondary metabolite compounds in mangrove species of *Sonneratia sp* type is triterpenoid, steroid flavonoid^{18,19}. The results of phytochemical screening of methanol extract from skin tissue of *Sonneratia* stem and leaf are known to contain class compounds alkaloids, carbohydrates, flavonoids, glycosides, phenolic, tannins, and saponins²⁰.

Glucose Tolerance assay:

The methanol extract test was performed to prove the pharmacological effects of the leaves of the three types of mangrove plants (*Avicenna sp.*, *Rhizophora sp.*, and *Sonneratia sp.*) As medicinal ingredients that can treat the condition of diabetes and determine the type of mangrove that has potential as anti-diabetes. This study used methanol extract from the leaves of the three mangrove plants, With 1% Na-CMC (b/v) suspension solution used as normal control and glibenclamide was used as a positive control.

The animal model used in this study was male mice, in healthy condition and the food given at the time of adaptation must be the same. Before treatment, the test animals (mice) are first empowered for ± 16 hours which aims to determine the actual blood glucose levels of mice on examination of baseline blood glucose level: and not blood glucose levels caused by food consumed.

The animal group divided into six groups aimed to determine the blood glucose level based on the different treatment given. Normal control group (KN), test animals were only given a 1% Na-CMC suspension used to determine normal blood glucose levels from animals model. The negative control group (K-), test animals were loaded with 50% glucose solution, used to increase blood glucose levels beyond normal (hyperglycemic) levels in test animals. The positive control group (K+), glucose-induced animal test and then given a comparative drug that is glibenclamide. This group is used to see a decrease in glucose levels caused by glibenclamide. Test group; treatment of P-1, P-2 treatment, and treatment-3, glucose-induced animal test and given leaf methanol extract from three types of mangrove with a concentration of 2% (w/v). This group is used to see the decrease in glucose levels caused by the extract of leaf methanol from the three types of mangrove for 3 hours.

Measurement of glucose level was done 5 times Test, first, test of glucose level before induced with glucose solution and animal fasted for ± 16 hours; secondly blood glucose test of animal test after 60 minutes induced 50% glucose solution, glucose of animal blood test after induced glucose and given treatment of methanol extract of three species of mangrove leaves that is at minute 60, 120, and 180. The results of the measurement of blood glucose levels of mice are presented in Table 3.

Table 3. Average measurements of blood glucose levels

Treatment	Glucose Level (mg/dl)		Glucose levels Giving Every Hour After Treatment Test Preparations (mg/dl)			The rate of decline Glucose, K (mg/dl. Hours)
	Before Induced	After Induced	1	2	3	

	Glucose	Glucose				
Na-CMC 1 % (NCTR)	< 95	235.25	226.26	220.86	213.54	30.19
Glibenclamide 10 mg/kg bb (PC)		236.00	178.20	162.91	157.35	52.87
Methanol Extract (P1) <i>Avicenna Sp.</i> 2 % (b/v)		236.84	217.22	202.64	186.68	36.73
Methanol Extract (P2) <i>Rhizophora sp.</i> 2 % (b/v)		237.32	205.65	178.90	163.10	51.16
Methanol Extract (P3) <i>Sonneratia sp.</i> 2 % (b/v)		236.51	212.96	196.19	176.46	47.08

Description: NCTR = Normal Control (Na-CMC 1%); NC = Negative Control (glukosa); PC = Positive Control (glibenclamide); P-1 (EMD) = Treatment 1 (Leaf Methanol Extract) *Avicenna Sp.*; P-2 (EMD) = Treatment 2 (Leaf Methanol Extract) *Rhizophora sp.*; P-3 (EMD) = Treatment 3 (Leaf Methanol Extract) *Sonneratia sp.*

Based on the data in Table 3 it is known that all samples of test animals that have been given a load of glucose solution (50%) and left for 60 minutes have elevated blood glucose (hyperglycemia) > 230 mg/dl. Increased blood glucose levels (hyperglycemia) are caused by excessive absorption of glucose so that pancreatic β cells not working optimally to producing insulin hormones that respond to high blood glucose levels [21]. After the treatment from the 60th minute until the 180th minute all the test group had decreased the blood glucose level is high enough, especially the positive control group (glibenclamide) with the decrease of blood glucose level 78,65 mg/dl, after 3 hours of treatment. Next, the P-2 test group (*Rhizophora sp.* Leaf extract of methanol) with decreased blood glucose level was 74,22 mg / dl after 3 hours of treatment, then P-3 test group (methanolic extract of *Sonneratia sp.* Leaf) with changes of blood glucose level at 60,05 mg/dl after 3 hours of treatment, then P-1 test group (leaf methanol extract (*Avicenna Sp.*)) with change of blood glucose level of test animal 50,16 mg / dl after 3 hours treatment

Figure 1. Graph of decrease rate from blood glucose level of animal test result induced by leaf methanol extract from three species of a mangrove plant, a comparator (glibenclamide) and negative control (Na-CMC 1%).

Description: NC = Normal Control (Na-CMC 1%); PC = Positive Control (glibenclamide); P-1 (EMD) = Treatment 1 (Leaf Methanol Extract) *Avicenna Sp.*; P-2 (EMD) = Treatment 2 (Leaf Methanol Extract) *Rhizophora sp.*; P-3 (EMD) = Treatment 3 (Leaf Methanol Extract) *Sonneratia sp.*

Figure 2. Diagram of the percentage reduction in blood glucose levels relative (%)

Description: K+ = Positive Control (glibenclamide); P-1 (EMD): Treatment 1 (Leaf Methanol Extract) *Avicenna Sp.*; P-2 (EMD): Treatment 2 (Leaf Methanol Extract) *Rhizophora sp.*; P-3 (EMD): Treatment 3 (Leaf Methanol Extract) *Sonneratia sp.*

Based on the graph in figure 1, show the rate of decreased blood glucose levels highest until 180 minutes (3 hours) occurred in the positive control group (glibenclamide) with a rate of decreased blood glucose level of 52.87 mg/dl hours. P-2 treatment group with the rate of decrease of blood glucose level 51,15 mg/d hour. P-3 treatment group with the rate of decrease of blood glucose level 47,08 mg/dl hour, and last one is P-1 treatment group with a rate of decrease of glucose level equal to 36.73 mg/dl hour.

Table 4. Percentage Reduction of Relative Blood Glucose Levels (%)

Time (minutes)	Mean Decreased Relative Glucose Blood Levels (%)			
	PC	P-1	P-2	P-3
0	0	0	0	0
60	24.49	8.28	13.34	9.96
120	30.97	14.44	24.62	17.05
180	33.27	21.19	31.27	25.39

Description: PC = Positive Control (glibenclamide); P-1 (EMD) = Treatment T-1 (Leaf Methanol Extract) *Avicenna Sp.*; P-2 (EMD) = Treatment T-2 (Leaf Methanol Extract) *Rhizophora sp.*; P-3 (EMD) = Treatment T-3 (Leaf Methanol Extract) *Sonneratia sp.*

From table 4 and figure 2 shows the largest percentage of relative decrease in blood glucose level was a positive control group (glibenclamide) in the 60th minute until 180 minutes with a decreasing percentage of 33.27%. Moreover, the highest decrease of glucose level from the treatment group at minute 60 until minute 180 was shown by treatment group P-2 (methanol extract from *Rhizophora sp.*) with percentage decrease 31,27%.

Based on data of percentage of blood glucose level relative at minute 60, 120, and 180, P-2 test group (giving of *Rhizophora sp.* methanol extract) gave the best result of antidiabetic activity compared to P-3 test group (giving of *Sonneratia sp.* methanol extract) and P-3 test group (giving *Avicenna Sp.* methanol extract). The decrease of blood glucose level of tested animal is determined by the content of secondary metabolite compounds contained in *Rhizophora sp.* Mangrove leaves. The content of secondary metabolite compounds in *Rhizophora sp.* yaitu alkaloid, flavonoids, steroids, terpenoid, phenolic, tannin, and saponin, where secondary metabolite compounds that have hypoglycemic effects are phenolic compounds. The mechanism of action of flavonoid compounds is to improve the function of pancreatic cells.

CONCLUSION:

The phytochemical assay showed a group of a secondary metabolite from ethanol leaf extract of *Avicenna sp.* leaves was alkaloids, flavonoids, steroids, terpenoids phenolic compounds, tannins, and saponins. *Rhizophora sp.* showed alkaloids, flavonoids, steroids, terpenoids, phenolic compounds, tannins, and saponins. *Sonneratia sp.* showed alkaloids, flavonoids, steroids, terpenoids, phenolic compounds, tannins, and saponins. The Leaf ethanol extracts of three types of mangrove plants (*Avicenna sp.*, *Rhizophora sp.*, and *Sonneratia sp.*) are known to have antidiabetic activity with glucose tolerance assay used mice. Ethanol extract of mangrove leaves from *Rhizophora sp.* with a dose of 200 mg/kg BB+glucose 2 g/kg BW (group P-2) showed antidiabetic activity result and can decrease blood glucose level by 31,27%.

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