

An Evaluation of the Antidiabetic and Antimicrobial Activity of an Ethanolic Extract from *Rhizophora mucronata* Leaf

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Abstract

The aim of this research was to determine the antidiabetic and antimicrobial properties of ethanolic extract of *Rhizophora mucronata* leaves, as well as the phytochemical elements contained in the leaf extract. Extracts from *R. mucronata* leaf samples were obtained by maceration in ethanol-based solvents. The ethanolic extract of *R. mucronata* leaves is known to have an antidiabetic effect on mice, evidenced by its ability to reduce blood glucose levels. Giving ethanol extract at a dose of 600 mg/kg BW gives the greatest antidiabetic effect. The antibacterial activity of the extract against the tested bacteria and fungi was also limited due to the presence of contaminants in the ethanolic extract. The analysis of secondary metabolites using LC-MS revealed that the ethanolic extract of *R. mucronata* leaves contained 25 different secondary metabolite compounds. Furthermore research to purify and identify the compounds in the ethanolic extract of *R. mucronata* is required to develop this resource as an antimicrobial and antidiabetic drug based on natural materials.

Keywords: Rhizospora mucronata, Leaf extract, Antidiabetic and Antimicrobial activity

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1 Introduction

The traditional herbal medicinal treatment is nowadays widely used in the context of a more holistic approach to healthcare. Most of the population in developing countries, such as Indonesia, continue to rely on herbal remedies to help them fulfil their healthcare needs. The herbal medicines formulated based on natural products derived from plants are complex chemical mixtures of major and minor compounds with various pharmacological properties. Therefore, the hunt for natural bioactive chemicals in various plants has become a major goal of the herbal medicine industry [1-2].

In the past decade, herbal medicines have been widely used by diabetic patients to reduce their dependence on synthetic diabetes medications. The main factor driving the increased use of herbal medicines is the impression that they are natural and safe (less toxic). Many of the plants have strong pharmacological properties that can be used to treat diabetes without causing negative side effects [3-7]. Several plant phytochemicals, including alkaloids, flavonoids, tannins, saponins, terpenoids, carotenoids, polyphenol derivatives, and glycosides, have been shown to exhibit anti-diabetic activity [8-10]. The mangroves contain high concentrations of bioactive chemicals, and have antidiabetic effects, which have been demonstrated. Other research has also reported that mangrove plants are a rich source of steroids, triterpenes, tannins, alkaloids, polyphenols, as well as saponins, quinones and fatty acids, which have various medicinal properties [11-13]. *Rhizophora mucronata* and *Avicenna marina* are two examples of mangrove plants that fall into this category [14-15]. *Rhizophora apiculata*, *Lumnitzera racemosa*, *Sonneratia alba*,

Dalbergia spinos, and other mangrove species [16-19]. Apart from that, some species from the *Avicenniaceae*, *Rhizophorae*, *Combretaceae*, *Amaranthaceae*, and *Sonneratiaceae* families were found to have potent anti-microbial activity against a variety of pathogenic strains from the human population, as did some species from the *Rhizophorae* and *Combretaceae* families. Thus, in order to develop herbal medications, it is necessary to conduct research into the efficacy of the ethnomedical qualities attributed to local mangrove species [20 -22].

Rhizophora mucronata is one of the mangrove plants that is widely distributed along the delta of Mahakam River, East Kalimantan, Indonesia (117°10' - 117°30' E and 0°10' - 0°59' N). It is the largest mangrove environment in Indonesia, and it can be found in this region. Our previous research reported that the leaves extract of *R. mucronata* obtained from this area contained some phytochemical constituents, including alkaloids, flavonoids, steroids, triterpenoid, phenolic compounds, tannin, and saponin [23], in addition to other phytochemical constituents. The anti-diabetic and anti-bacterial properties of the ethanolic extract from *R. mucronata* have been studied in detail. as well as the phytochemical contents, were investigated in the present study using LC-MS. This information will help in upcoming traditional herbal medicines development using local indigenous plants as a natural product source.

The *R. mucronata* is a type of mangrove plant that has a rhizophora type root system or root support that grows downward from the stem to the soil surface, making the plant rooted deep into the soil. The mangrove plant of *R. mucronata* generally reaches a height of 3-4 m, characterised by dense leaves, creamy white flowers, and cigarshaped fruit (propagules) [24].



Figure 1. (A) plant mangrove *R. mucronata*, (B) flower, (C) cigar-shaped propagules (D) longitudinal section of root [24-25].

2 Methods

2.1 Materials

In order to conduct phytochemical screening and an antidiabetic assay, Merck provided analytical grade solvents (ethanol and acetonitrile) and chemicals for the experiment (Darmstadt, Germany). Agricultural Faculty, Mulawarman University provided male Wistar rats for use in the anti-diabetic assay, which were used in this study. Positive controls, such as glibenclamide, chloramphenicol, and nystatin, were obtained from a local pharmacy in Samarinda, East Kalimantan, Indonesia, and used in the study. The Microbiology Laboratory of the Pharmacy Faculty of Mulawarman University provided the pure cultures of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231, and *Aspergillus niger* used in this study. Mueller Hinton Broth (MHB), Potato Dextrose Broth (PDB), and bacteriological agar as media for culturing microorganisms were purchased from Oxoid (Thermo Scientific, UK).

2.2 Plants Materials

Rhizophora mucronata leaves were collected from a mangrove forest in the coastal area of Muara district, Kutai Kartanegara Regency, East Kalimantan. The plant is native to Southeast Asia. The leaf samples were cut into little pieces and dried in the open air, away from direct sunlight, to ensure that they were as fresh as possible. Following drying, the mangrove leaf samples were ground until they were smooth and had a mesh size of 90. Then, the sample was ready to be macerated.

2.3 Preparation of Extract

The ethanol extract was prepared from dried mangrove leaves were extracted by maceration using ethanol for 24 h in three repetitions. Whatmann No.1 filter paper (Whatmann, England) was used for filtration, and the filtrate obtained was evaporated in a rotary evaporator at 40 °C and 1 atm pressure to yield the final product, so that a thick green ethanol extract was obtained. The extract was stored at 40 °C until used [23].

2.4 Antidiabetic Activity Test

The antidiabetic activities of the compound was assessed using an oral glucose tolerance test (OGTT) methode in mice (*Mus musculus*). Healthy mice weighing 20–30 grams, aged 2–3 months, were procured from the Faculty of Pharmacy at Mulawarman University in Samarinda and used in this study. They were adapted for seven days and provided with food and drink in a controlled manner every day. The mice were fasted for 18 hours before to treatment, and their blood glucose levels were subsequently tested. The mice were separated into five groups (n = 4) after being fed, and each group received a single intraperitoneal (i.p.) injection of a 50 percent glucose solution at a dose of 5 g/kg BW to induce diabetes. The blood glucose levels of mice were tested using a glucometer after 30 minutes. Then, each group was given the following treatment: Group I, normal mice administrated with CMC-Na (Carboxymethyl Cellulose-Sodium) 1% (b/b) (negative control); Animals were divided into five groups: Group II received the conventional medicine (glibenclamide at a dose of 10 mg/kg BW);

Group III received ethanolic extract (300 mg/kg BW); Group IV received ethanolic extract (600 mg/kg BW); and Group V received 1,200 mg/kg BW of the ethanolic extract. After 30 minutes, blood glucose levels were measured on a glucometer. Subsequent glucose levels measurements were carried out at time intervals the day: 60, 120, and 180 minutes after the glucose loading and measured by glucometer [23-24], [26].

2.5 Antimicrobial Activity Test

The antimicrobial activities of the ethanolic extract were evaluated against two pathogenic fungi, *C. albicans* ATCC 10231 and *A. niger*, as well as two Gram-positive bacteria, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, and two Gram-negative bacteria, *Escherichia coli* ATCC 25922. MHB was used to pre-culture the bacteria, which was done overnight in a rotating shaker at 37 °C, and PDB was used to prepare the fungal inoculum, which took 48 hours in a rotary shaker at 37 °C. Following that, the bacterial culture was adjusted to a concentration of 10⁸ cells/mL, and the fungal culture was adjusted to a spore density of 10⁶ spores/mL, and the results were recorded. Agar well diffusion was utilized to evaluate the antibacterial activity of the compounds in the laboratory setting. On a Petri plate, Mueller Hinton Agar (MHA) for bacteria and Potato Dextrose Agar (PDA) for fungi were placed in separate compartments. containing 1 ml of the fresh bacterial or fungal inoculum and then thoroughly stirred to incorporate all of the ingredients. Following solidification, the agar wells were created on agar plates containing inoculums with a sterilized cork borer with a 6 mm diameter, which was used to bore holes in the agar. Four distinct quantities of the ethanolic extract (0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, and 1.0 mg/ml) were carefully applied to the wells, with each concentration being properly measured. While the extracts were allowed to diffuse into the agar during the course of the one-hour incubation period, After that, the plates were incubated at 37 °C for 24 hours (for bacterial growth) and 72 hours (for fungal growth) before being removed from the oven (for fungi). Following the incubation time, the presence of anti-microbial activity was assessed by measuring the diameter of the inhibition

zones (which included the well) that appeared. Positive controls for bacteria were performed using chloramphenicol (0.03 mg/mL), while positive controls for fungi were performed using nystatin (0.03 mg/mL).

2.6 LC-MS Profiling of Extract

Using an ACQUITY UPLC® BEH C18 column (2.1 mm 100 mm) for extraction and profiling of extracts, the experiments were carried out 1.7 m) and the Waters Xevo Q-ToF-MS (Waters Corporation, Massachusetts, US). To construct the solvent gradient, water (solvent A) and acetonitrile (solvent B) were employed in conjunction with each other (solvent B). A flow rate of 0.2 milliliters per minute with a step gradient of 10–90 percent B was used (30 min) isocratic at 10 percent B (2 min), and 90 percent B (30 min), the compounds were eluted into an ES+ ion source with a gradient of 10–90 percent B. (15 min). After that, the column was re-equilibrated for 1 minute at 10% for a total of 1 minute and 30 seconds. In order to keep the temperature of the column constant during the run, a temperature of 100 °C was used. In positive mode, the MS was designed to perform a full scan over the m/z 100–2000 range in a single pass. The desolvation temperature was maintained at 350 °C with a flow rate of 13.3 L/min of nebulizer gas, and the presence of desolvation was detected with a detector voltage of 2 kV. Spectra of the substances were evaluated using mass spectrometry, and they were compared to those already published in the literature.

3 Results and Discussions

3.1 Antidiabetic Activity

Table 1 contains the findings of measurements of mice's blood glucose levels before and after treatment, as well as their final glucose levels, and Figure 1 depicts the proportion of their blood glucose levels that decreased as a result of therapy. In the positive control group (Glibenklamide 10 mg/kg BW), there is a drop in the maximum glucose level measured at the end of the study. A drop in mice's blood glucose levels was observed after administration with an ethanolic extract at doses of 300, 600, and 1,200 mg/kg BW, There were 38.09 percent, 46.81 percent, and 39.45

percent declines in percentage, respectively. The treatment of ethanolic extract with a 600 mg/kg BW dosage, on the other hand, resulted

in the greatest reduction in blood glucose levels [27].

Table 1. The blood glucose levels of mice in several treatment groups were measured, and the results were presented.

Treatment Groups	Initial glucose level (mg/dl)	Glucose levels after glucose induction (mg/dl)	Final glucose level after 180 min (mg/dl)	Decrease in blood glucose level (%)
Group I: CMC 1 % (negative control)	99.6	266.8	251.2	0,0
Group II: Glibenclamide 10 mg/kg bb (positive control)	98.8	271.4	127.8	52,91
Group III: ethanolic extract 300 mg/kg BW	92.2	273.0	169.8	38,09
Group IV: ethanolic extract 600 mg/kg BW	98.8	276.2	146.6	46,81
Group V: ethanolic extract 1.200 mg/kg BW	102.6	268.2	162.4	39,45

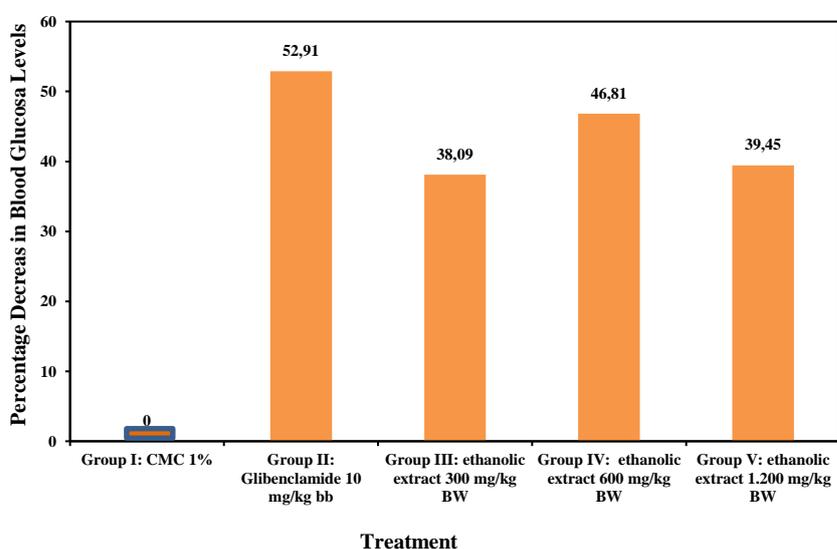


Figure 1. The percentage drop in blood glucose levels in mice is depicted in this diagram.

Table 1 contains the findings of measurements of mice's blood glucose levels before and after treatment, as well as their final glucose levels, and Figure 2 depicts the proportion of their blood glucose levels that decreased as a result of therapy. In the positive control group (Glibenclamide 10 mg/kg BW) highest decrease the blood glucose level until reaching 52.91%. This is because glibenclamide can stimulate pancreatic beta cells to secrete insulin and increase the sensitivity of peripheral cells to increased insulin levels. Due to glibenclamide, it is an oral diabetic drug that inhibits the ATP-sensitive K⁺ channels in pancreatic beta cells [28]. A drop in mice's blood glucose levels was observed after administration with an ethanolic extract at

doses of 300, 600, and 1,200 mg/kg BW, respectively, with 38.09 percent, 46.81 percent, and 39.45 percent reductions in percentage terms. The treatment of ethanolic extract with a 600 mg/kg BW dosage, on the other hand, resulted in the greatest reduction in blood glucose levels [29-30].

Decreased glucose levels in all treatment of ethanolic extract of *R. mucronata* leaves were caused by the content of secondary metabolites compounds such as alkaloids, flavonoids, steroids, tannins, and saponins, as reported previously [23], [31]. These secondary metabolites compounds are thought to play a role in decrease blood glucose levels in mice. Several studies have reported that bioactive compounds in the ethanolic extract can play a

role as inhibitors of alpha-glucosidase enzyme and alpha-amylase enzyme [32-33]. Alkaloids and saponins can provide hypoglycemic effects because they can stimulate insulin secretion from pancreatic beta cells [28], [34]. Whereas, flavonoids and tannins can reduce blood glucose levels by capturing free radicals and reducing the increase in oxidative stress in people with diabetes so the compounds can control blood glucose [29], [35].

3.2 Antimicrobial Activity

The antimicrobial activities of ethanolic extract of *R. mucronata* leaves against

pathogenic bacteria and fungi are given in Table 2. The results revealed that the ethanolic extract exhibited the zone of inhibition against all microorganisms tested at the concentration of 0.8 and 1.0 mg/mL, and no inhibition zone was observed in the concentration at < 0.8 mg/ml. However, the inhibition activities of the extract are weak with zone inhibition of ≤ 10 mm. Compared to the positive controls, chloramphenicol and nystatin at the concentration of 0.03 mg/ml exhibited higher inhibitory effect with the inhibition criteria were intermediate (14 – 17 mm).

Table 2. The result of the anti-microbial activity of ethanolic extract from *R. mucronata* leaves.

Concentration of ethanolic extract (mg/mL)	Inhibition Zone Diameter (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
0.40	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.60	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.80	1.0 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.4 ± 0.2
1.00	1.2 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	0.9 ± 0.1
Chloramphenicol (0.03 mg/mL)	14.8 ± 0.4	16.3 ± 0.2	-	-
Nystatin (0.03 mg/mL)	-	-	16.40 ± 0.2	16.24 ± 0.2

The present investigation showed that the antibacterial and antifungal activities of the ethanol extract against pathogenic microorganisms (*S. aureus*, *E. coli*, *C. albicans*, and *A. niger*) in humans were found to be significantly weaker when compared to the positive controls, with a zone inhibition of only 12 mm. This was due to many contaminants with a larger quantity than bioactive substances were found in the crude extract during the testing, which was most likely related to the extract utilized in the testing [36-37]. Several study demonstrating the antibacterial and antifungal activities of mangrove showed that ethanol extract of *R. mucronata* contained several phytochemicals, such as alkaloids, flavonoids, steroid, tannins, saponins, and some unknown phytochemicals, making it more active against pathogenic microorganisms [38-39].

Phytochemical screening of mangrove plants *R. mucronata*, *R. apiculata*, and *R. annamalayana* showed the presence of phytochemical compounds such as proteins, phenols and flavonoids that exhibited antioxidant and antimicrobial activity, whereas *R. mucronata* showed relatively much higher

antioxidant and antimicrobial activity than *R. apiculata* and *R. annamalayana* at a concentration of 100 g/mL. The content of polyphenolic compounds and other bioactive compounds from these three mangrove extracts can be used for the formulation of herbal and nutraceutical drugs [40].

3.3 Characterized Compounds from Ethanolic Extract

The compounds included in an ethanol extract of *R. mucronata* were examined using liquid chromatography-top of the light-mass spectrometer to determine whether or not they were present in the extract (LC-TOF-MS). Chemometric screening of *R. mucronata* secondary metabolite substituents using LC-MS reveals the profile of the compounds and the prediction of molecular formulas based on the species' molecular weight. Positive ion mode was used to detect all of the chemicals, which were identified as $[M + H]^+$.

Table 3 contains a summary of the mass spectrum data obtained for the described substances. A total of 25 compounds with their MS are detected according to their elution order.

Table 3. Shows the mass spectral data for the chemicals identified in the ethanolic extract of *R. mucronata* leaves.

Code	Retention time (min)	MS (m/z)	Molecule Formula Prediction
D-01	1.17	411.1451	C ₂₃ H ₂₃ O ₇ ⁺
D-02	4.59	519.2435	C ₃₇ H ₃₁ N ₂ O ⁺
D-03	4.79	303.0539	C ₁₅ H ₁₁ O ₇ ⁺
D-04	5.00	317.0688	C ₂₀ H ₁₀ N ₂₀ Na
D-05	5.15	303.0428	C ₂₀ H ₈ O ₂ Na
D-06	5.41	625.1844	C ₃₉ H ₂₉ O ₈ ⁺
D-07	9.20	353.2777	C ₂₀ H ₃₇ N ₂ O ₃ ⁺
D-08	9.88	701.3723	C ₃₃ H ₅₈ O ₁₄ Na
D-09	10.17	283.2650	C ₁₅ H ₃₆ N ₂ O ₃ Na
D-10	10.52	331.2852	C ₁₉ H ₃₉ O ₄ ⁺
D-11	10.80	496.3531	C ₃₀ H ₄₆ N ₃ O ₃ ⁺
D-12	11.99	628.4688	C ₃₇ H ₆₂ N ₃ O ₅ ⁺
D-13	12.16	545.3548	C ₃₂ H ₄₉ O ₇ ⁺
D-14	12.38	457.3228	C ₃₁ H ₄₁ N ₂ O ⁺
D-15	12.51	227.1996	C ₁₄ H ₂₇ O ₂ ⁺
D-16	12.82	255.2291	C ₁₆ H ₃₁ O ₂ ⁺
D-17	13.62	609.2673	C ₂₉ H ₄₁ N ₂ O ₁₂ ⁺
D-18	15.22	813.5115	C ₄₂ H ₇₃ N ₂ O ₁₃ ⁺
D-19	15.71	623.2844	C ₃₅ H ₄₃ O ₁₀ ⁺
D-20	16.92	696.5302	C ₃₆ H ₇₄ NO ₁₁ ⁺
D-21	18.28	413.2552	C ₂₂ H ₃₇ O ₇ ⁺
D-22	19.33	397.3842	C ₂₄ H ₄₉ N ₂ O ₂ ⁺
D-23	21.13	613.4667	C ₃₅ H ₆₅ O ₈ ⁺
D-24	25.57	599.4368	C ₃₀ H ₆₃ O ₁₁ ⁺
D-25	26.64	663.4490	C ₃₈ H ₆₃ O ₉ ⁺

This is evidenced by the LC-MS profile which shows a total of 25 compounds contained in the ethanolic extract. While the LC-MS profile cannot analyse the molecular structure of the compounds, we can detect the number of compounds contained in it and predict their molecular formula. To improve the inhibitory activity of plant extracts against pathogenic microorganisms, we can purify the crude extract and increase the concentration of the extract. To improve the activity of plant extracts against pathogenic bacteria, we can purify the crude extract to improve its inhibitory activity against pathogenic bacteria, and we can increase the concentration of the extract to improve the activity of plant extracts against pathogenic microorganisms. Hence, as a result of these findings, we have gained new insights into the potential of ethanolic extracts of *R. mucronata* leaves as anti-bacterial and anti-diabetic agents in future herbal medicine development [41].

4 Conclusions

The ethanolic extract of the leaves of *R. mucronata* has significant anti-diabetic activity, as evidenced by the fact that it can significantly lower blood glucose levels in rats by a significant percentage. The ethanolic extract, at

the other hand, showed only mild anti-microbial action against the tested bacteria and fungi. Furthermore, based on LC-MS spectroscopy analysis, it was indicated that the ethanol extract of *R. mucronata* leaves contained 25 secondary metabolites. More studies are needed on the purification and isolation of the components found in the ethanolic extract of *R. mucronata*, especially in the search for natural ingredients that can be used to prepare alternative anti-diabetic therapies.

5 Declarations

5.1 Authors Contributions

The names of the authors listed in this journal contributed to this research.

5.2 Funding

This research was not supported by any funding sources.

5.3 Conflict of Interest

The authors have confirmed that they do not have any conflicts of interest to disclose.

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