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Research Article

TOXICITY AND FREE RADICAL SCAVENGING ACTIVITY OF *RHIZOPHORA MUCRONATA* LAMK. BARK EXTRACTS FROM KENDARI BAY OF SOUTHEAST SULAWESI PROVINCE INDONESIA

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ABSTRACT

The aim of this study was to evaluate the toxicity and free radical scavenging activity of *Rhizophora mucronata* Lamk. bark extracts from the mangrove forest of Kendari Bay, Southeast Sulawesi Province Indonesia. The extracts were obtained by maceration of *R. mucronata* Lamk. bark with n-hexane, ethyl acetate, and methanol, respectively. Toxicity test was conducted by using Brine Shrimp Lethal Test (BSLT) method with *Artemia salina* Leach as the animal test, while free radical scavenging activity test was carried out by using DPPH (1,1-diphenyl 2-picryl-hydrazyl) free radical. The test results showed n-hexane, ethyl acetate and methanol extracts had LC_{50} values of 136.40 µg/mL, 82.43 µg/mL and 109.38 µg/mL, respectively. The IC_{50} values for the free radical scavenging activity of the n-hexane, ethyl acetate, and methanol extracts were 122.19 µg/mL, 37.84 µg/mL, 29.32µg/mL, respectively. Result of phytochemical test showed n-hexane extract was dominated by terpenoid and steroid group compounds, while flavonoids, alkaloids, and tannins were predominantly found in both ethyl acetate and methanol extracts. Extracts of *R. mucronata* bark, especially ethyl acetate extracts had toxic properties for *A. salina* larvae, therefore it has potential to be developed as an anti-cancer drug. Metahanol extract, on the other hand, indicated strong anti-oxidant activity, then it is potential to develop it as cosmetics agent.

Keywords: Free radical scavenging, *Rhizophora mucronata*, bark, toxicity

INTRODUCTION

Because of its ability to grow and develop in harsh environmental conditions, such as relatively high salt levels, changing water salinity, living among diverse predator organisms make the mangrove as a unique plant. This harsh habitat will initiate the mangrove plants to produce a typical secondary metabolite to survive in life[1]. Due to the diversity of secondary metabolites of mangrove plants, it makes them as potential source of nutritious chemicals. Moreover, the secondary metabolites of the mangroves plant can be used as raw materials of drugs[2].

Literature study shows that the mangrove plant *R. mucronata* Lamk. has been used traditionally by communities in several nations to treat various diseases. In India, for example, the plant extracts are used to treat diseases such as ulcers, typhoid, hepatitis, elephantiasis, diabetes, analgesics, anti-inflammation and as an insecticide[3,4,5]. Bark extracts are also used to treat inflammatory diseases and diarrhea[6,7]. The bark extracts are used by local communities in Thailand to treat diarrhea, nausea, vomiting, and to stop bleeding in new wounds[8]. In Bangladesh bark extract has been used traditionally to treat diseases: hyperglycemic, diarrhea, nausea, haematuria, hemorrhages and angina[9]. In some countries such as South Africa, Southeast Asia, North America and Australia, extracts of the plant were used to treat leprosies, elephantiasis, tuberculosis, malaria, dysentery, ulcers and some other skin diseases[10].

It is reported that root extract of *R. mucronata* Lamk. has an anti-oxidant capacity and can repair liver damage in rats due to CCl_4 hepatotoxin administration [11]. The extracts of some mangrove plants show an antibacterial activity of *Mycobacterium tuberculosis* that has been resistant to a variety of previously administered drugs[12]. The test results performed by[13,14] showed the *R. mucronata* leaf extract had antihyperglycemic and anti-oxidant activity. The ethanol extracts of bark of *R. mucronata* Lamk. has antibacterial, cytotoxic, analgesic, and diuretic properties[15].

The main objective of this paper is to inform the toxicity dan free radical scavenger evaluation as well as secondary metabolites screening of *R. mucronata* Lamk. bark extract from Bay of Kendari Southeast Sulawesi Province, Indonesia as an indication of active potential compound in extract against cancer cells.

MATERIALS AND METHODS

General

The extraction process and toxicity and free radical scavenging tests were done in the Organic Chemistry Laboratory Faculty of Mathematics and Natural Sciences and Natural Product Organic Chemistry Laboratory Faculty of Pharmacy Halu Oleo University, Kendari – Indonesia.

Plant material

Plant samples of *R. mucronata* Lamk. were taken from the mangrove forest in Kendari Bay, Southeast Sulawesi Province. These samples have been identified and collected by Research

Center for Biology of Indonesian Institute of Sciences (LIPI) with voucher number FR.7.5.1.PU.01-02

Extraction of *R. mucronata* bark

Three kilograms of bark powder (230-270 mesh) of *R. mucronata* was extracted by maceration method successively with n-hexane, ethyl acetate, and methanol solvents for 3x24 hours. Extract filtering and replacement of new solvents were done every 24 hours. The results of extraction from each solvent used were combined and concentrated using a vacuum rotavapor at a low temperature. Each crude extract obtained was tested for the toxicity using *A. salina* as animals test and free radical scavenging activity using DPPH. Moreover, the phytochemical test procedure was done according to a known method[16], particularly to know the chemical content of extracts.

Toxicity test

The toxicity test was performed by using BSLT methods, as reference[17]. The procedure can be outlined as follows:

A. salina eggs were hatched in a shallow rectangular dish (22x32 cm), then filled it with the seawater while lighted and aerated for 48 hours. Substances/extracts to be tested were prepared in various concentrations, i.e. 10, 100, and 1000 ppm which is loaded into each test container (vial) then topped it up with the seawater to 5 mL. Another vial containing seawater only, without extracts, was used as the negative controls. At each tested substance concentration, 5 replications were performed. Furthermore, 3 mg of yeast was added into each vial. Selected healthy larvae (10 tails) were inserted into each vial, then all vials containing both test and larvae substances include those containing only larvae were left at room temperature for 24 hours while lighted. After 24 hours, observations were made by counting the number of larvae still alive within each vial, to determine the number of dead larvae at each treatment of the test substance concentration. The value of LC_{50} (µg/mL) was determined by probit analysis[18]. Compounds that gave LC_{50} values less than 1000 µg/mL and ≤ 30 µg/mL for the extract were categorized as active and toxic to *A. salina* [17].

Free Radical Scavenging Activity Test

The quantitative analysis procedure was adopted from[19,20,21] with minor modifications. One mL of 500 µM (0.2 mg/ mL) DPPH in methanol was mixed with the same volumes of the tested compounds at various concentrations. They were mixed well and kept in the dark for 30 minutes. The absorbance at 517 nm was monitored in the presence of different concentrations of the samples. The blank experiment, i.e., with only solvent and DPPH (i.e. 2 mL of 500 µM in methanol), was also carried out to determine the absorbance of DPPH before interacting with the compounds. The amount of sample in mg/mL at which the absorbance at 517 nm decreased to half of its initial value was used as the IC_{50} value of compounds. The analysis was done in triplicate for standard and tested compounds. The capability to scavenging the DPPH free radical was calculated using the following equation[22]

DPPH free radical scavenging effect (%) : [(A1-A2)/A1) x100]

where A1 is the absorbance of the control (DPPH solution without test sample) and A2 is the absorbance in the presence of the test sample. And ascorbic acid was used as reference compounds.

RESULTS AND DISCUSSION

The yield of extract obtained from the maceration of the bark of *R. mucronata* Lamk. from Kendari Bay of Southeast Sulawesi were reported in table 1. The results of phytochemical test of the compound class from each extract of maceration results are presented in Table 2. And Toxicity and free radical scavenging activity of extracts are presented in Table 3. Table 1 shows the amount of methanol extract is higher than the ethyl acetate and hexane extract, it indicates that the stem bark of *R. mucronata* is dominated by polar compounds, while non-polar compounds content are very small.

Table 2 showed n-hexane extract dominated by terpenoids and steroids. Ethyl acetate and methanol extracts contain flavonoids, alkaloids, and tannins. The two compounds from n-hexane extract that had been isolated and purified were α-amyrin and β-sitosterol[23].

Table 3 showed that ethyl acetate extract had LC_{50} (82,43 µg/mL), it was more toxic than others extracts, although all extract possessed toxic properties for *A. salina* larvae. The data indicating that chemical compound in the extract of ethyl acetate had a potency to be developed as anti cancer cells. The IC_{50} of methanol extract was 29.32 µg/mL, indicating antioxidant properties stronger than other extracts, but lower than ascorbic acid. From this result, it is quite wisely to say that the methanol extract is potential to develop it as cosmetics agent.

Table 1: **The Yield of Extracts of *R. mucronata* Lamk.**

No	Extracts	Weight (g)	Yield (%)
1	n-Hexane	5	0.16%
2	Ethyl acetate	205	6.83%
3	Methanol	450	24.30%

Table 2: Secondary Metabolites of *R. mucronata* Bark Extracts

Secondary metabolites	Reagent	Extracts		
		n-Hexane	Ethyl acetate	Methanol
Terpenoids	L-B	++	-	-
Steroids	L-B	++	+	
Flavonoids	$FeCl_3$	-	+	++
	Mg + amyl alcohol	-	+	++
Alkaloids	Wagner	-	+	++
	Meyer	-	+	++
	Dragendorf	-	+	++
Tannins	$FeCl_3$	-	+	+++

L-B: Liebermann-Burchard,
(-)=Negative; (+) = Weak positive; (++) = Positive; (+++) = strong positive

Table 3: The Toxicity and Free Radical Scavenging Activity of all Extracts

No.	Extracts	LC_{50} of toxicity (µg/mL)	IC_{50} of antioxidant (µg/mL)
1.	n-hexane	136,4	122.19
2.	Ethyl acetate	82,43	37.84
3.	Methanol	109,38	29.32
4.	Ascorbic Acid (reference)	-	10.26

CONCLUSION

Extract of *R. mucronata* stem bark espcially their ethyl acetate extracts showed toxic properties for *A. salina* larvae, it also had potential to be developed as an anti-cancer drug. And its methanol extracts displayed a strong anti-oxidant activity, thus it is very potential to develop it as a cosmetics agent.

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