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Research Article

DETERMINATION OF SECONDARY METABOLITES, TOXICITY AND ANTIOXIDANT ACTIVITIES OF BARK EXTRACTS OF *Artocarpus lanceifolius* ROXB

Hamsidar H [1], Nunuk H. Soekamto [2]*, Yana M. Syah [3], Firdaus [2]
[1]Department of Pharmacy, Faculty of Sports and Health, Gorontalo State University, Indonesia
[2]Department of Chemistry, Faculty of Mathematic and Natural Science, Hasanuddin University Makassar, Indonesia
[3]Division of Organic Chemistry, Faculty of Mathematics and Natural Science, Institute Technology Bandung, Indonesia
*Corresponding Author Email: nunukhariani@unhas.ac.id

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ABSTRACT

The purpose of study to determine the content of secondary metabolites, toxicity and antioxidant activity of bark extract from *Artocarpus lanceifolius* Roxb. The extracts were obtained by maceration method using hexane, chloroform, ethyl acetate and methanol solvents, respectively. The extract obtained was tested for phytochemicals, toxicity test using the shrimp Brine Lethality Test (BSLT), antioxidant test using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Phytochemical test results showed that n-hexane extract contained terpenoids and steroids while flavonoid and phenol compounds contained in extracts of chloroform, ethyl acetate, and methanol. The dominant alkaloid compounds were found in ethyl acetate and methanol extracts. Toxicity values (LC_{50}) for n-hexane, chloroform, ethyl acetate extract were 1.0853 µg/mL, 0.1635 µg/mL, 0.3615 µg/mL and 0.2609 µg/mL respectively. The IC_{50} value of free radical scevange activity of chloroform extract, ethyl acetate were 19.66 µg/mL, 15.85 µg/mL, and 19.01 µg/mL respectively.

Keywords: Secondary metabolites, toxicity, antioxidant, *Artocarpus lanceifolius* Roxb bark

INTRODUCTION

Research on medicinal plants is growing very rapidly throughout the world. This is due to a medicinal plant used as an alternative to new therapies. Currently herbal products and formulated medicines represent more than 60% of all clinically used medicines in the world and 25% are from plants. According to the World Health Organization (WHO), 80% of the world's population depends on plants for their primary health care. In addition they also use complementary and alternative medicine for several aspects of the main health care system and for the treatment of incurable diseases including cancer and various other infectious diseases.[1,2]

Various species of plants up to now been traced and assessed the potential of the active ingredient as a medicinal plant. One of the plants that has the potential as a source of bioactive chemicals is the Family Moraceae. Moraceae consists of 60 genera and 1400 species with three main genera being Artocarpus, Ficus and morus. Artocarpus consists of 50 species and spread from southern Asia, Southeast Asia to the Solomon Islands, Pacific islands, North Australia and Central America[3].

Based on the phytochemical test conducted, it was found that the genus Artocarpus has a large content of prenylated flavonoids, especially derivatives of the unique C-3 prenylated flavone compound. This unique structure of secondary metabolites can produce diverse physiological effects. The taxonomic literature review explains that the phylogeny of plants with the same family tends to have similar compounds contained or structural constituent characteristics. Some Artocarpus species generally have activities as antiviral, antidiabetic, antioxidant and antimicrobial[5,6,7]. Other studies also show that the Artocarpus plant has pharmacologically experimental biological activity. The biological activity of an extract depends on the chemical components contained in crude extracts of plants[7]. The more active the components of a compound contained in a material, the higher the potential of these ingredients can be used in treatment. Referring to this fact, research on the bioactivity of secondary metabolites from Artocarpus will provide benefits in finding new drugs from natural compounds and provide scientific explanations about the use of Artocarpus in traditional medicine.

One species of the Artocarpus plant is *Artocarpus lanceifolius* Roxb. A.lanceifolius Roxb is a Kalimantan endemic plant with a local name Kaledang. Acetone extract of A. lanceifolius Roxb showed antibacterial activity against Bacillus subtilis bacteria, Staphylococcus aureus, E. coli, and Pseudomonas aureginosa with inhibition diameter 8-15 mm[8]. This shows that Kaledang has biological activity experimentally. Based on the literature study that has been conducted until now there has been no research on toxicity tests and antioxidant tests that have been studied.

Based on this background, a study was conducted on the toxicity test with the brine shrimp test method and the antioxidant activity test on Artocarpus lanceifolius Roxb bark extract. The extracts used are derived from non-polar, semi-polar and polar fractions. This research is an effort to determine the potential baseline of biological activity of this plant based on toxicity and antioxidant effects as well as the initial screening of anticancer activities.

MATERIALS AND METHOD

Samples of the stem bark of *A. lanceifolius* Roxb were collected in January 2018 from Paramasan Atas village, Paramasan District, Southeast Kalimantan Province, Indonesia. The plant was identified by staff at herbarium Bogoriense Bogor, Indonesia

with voucher number IPH.1.01.IF.07 and the specimen has been deposited at the herbarium.

Extraction Process

Stem Bark powder of *Artocarpus lanceifolius* Roxb (4.8 Kg) was macerated with n-hexane, $CHCl_3$, ethyl acetate, and methanol solvents respectively for 72 hours. Every 24 hours the extract is filtered and replaced by a new solvent. The extraction results from each solvent are collected and concentrated using rotavapor at low temperature. Each crude extract obtained was carried out toxicity test by brine shirimp method with shrimp larvae as test animals and antioxidant activity test using DPPH (2,2-diphenyl 2-picrylhydrazyl) method.

Phytochemical screening

The crude extracts of stem bark were tested for the presence of flavonoid, steroid, triterpenoid, alkaloids, and phenol. These compound were screened in the laboratory as per the standard methods with little modification. Methods described by reference[12,13,14]

Determination of the Toxicity

Toxicity test in this study using BSLT method refers to reference[8]. In a two-insulated box, one side is covered with aluminum foil containing enough sea water. Next, the shrimp egg *Artemia salina* L. is put into it. The box is then placed under a UV lamp for 48 hours then the eggs hatch into larvae. Test solutions derived from crude extracts of n-hexane, chloroform, ethyl acetate and methanol from bark *A lanceifolius* Roxb bark were included in seawater with dilution concentrations of 100, 10 and 1 ppm respectively. Each is done using three repetitions. One vial bottle was used as a control containing 10-15 larvae without a test solution added. The main solution is made by weighing 20 mg of the test extract dissolved in 2 ml of sea water. If the sample is difficult to dissolve, DMSO (Dimethylsulfoxide) is added. After 48 hours 100 ml of sea water containing 10-15 shrimp larvae put in the test bottle and test solution until the concentration in each bottle is 50, 5 and 0.5 ppm. After being left for 24 hours the live and dead shrimp larvae are counted. BSLT testing data were analyzed based on the calculation of the number of dead and living larvae. The death rate or percent of mortality was obtained by comparing the number of dead larvae divided by the total number of larvae then analyzed using probit values. The LC50 value is obtained based on the linear regression value. Extracts are declared active if the LC50 value is smaller than 1000 µg/mL

Deterimination of the antioxidants activities

The methods described by reference [5,7,8,16]. A solution of DPPH (0,135 mmol/L) in methanol was prepared and 1 mL of the solution mixed with 1 ml of the extract with various concentration (1-5 mg/ml), The mixture was vortexed at room temperature for 30 min in the dark condition. The absorbance was measured at 517 nm using UV-Vis spectrophotometer. Blank sample (only solvent and DPPH - 2 mL of 500 µM in methanol) was also carried out to get the absorbance of DPPH before reacting with the compounds. The IC_{50} value is calculated by determining the decrease in sample concentration (mg / mL) which is used up to half of the initial concentration based on the absorbance value obtained at 517 nm. The whole activity of the compound activity test was carried out with a triplo system. Ability to capture DPPH (DAC) radicals is calculated using the following equation:

$$ACD\ (\%): [(A_1-A_2)/A_1)\ x100]$$

where A_1 is absorbance of the control (DPPH solution without test sample) and A_2 is absorbance of the test sample.

RESULTS AND DISCUSSION

Phytochemical tests on secondary metabolites obtained from each extract represent Table 1. Based on the results obtained was known that the n-hexane extract contains triterpenoid and steroid compounds, the ethyl acetate extract and chloroform containing flavonoids and phenols, while the methanol extract contains flavonoids, alkaloids, and phenols.

Table 1: Phytochemical Screening Tests result of each extract

Phytochemical Test	Reagent	Extracts			
		n-hexane	Chloroform	Ethyl Acetate	Methanol
Flavonoid	• Mg + amyl alcohol	-	++	++	++
	• NaOH 10%	-	+	++	++
Triterpenoid	• L-B	+	-	-	-
	• Salkowski	+			
Steroid	• L-B	+	-	-	-
		-			
Alkaloid	• Dragendroff's	-	-	+	+
	• Wagner		-	-	+
	• Meyer	-	-	+	+
Phenol	• $FeCl_3$ + Ethanol 70%	-	++	+	++

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

Furthermore, the extract was also tested for toxicity using the Lethal Concentration values (LC_{50}). Lethal Concentration values (LC_{50}) are obtained from the linear regression equation which is determined by the probit value and the concentration log. The result of BSLT test from each extract is presented in Table 2. The highest LC_{50} value is in chloroform extract and the lowest is in n-hexane extract. But all extracts showed very high toxicity. According to reference[17], the extracts with LC_{50} values less than 100 µg/mL considered highly toxic, LC_{50} values between 100 µg/mL to 500 µg/mL is quite toxic, LC_{50} value of 500 µg/mL to 1000 µg/mL are slightly toxic, and LC_{50} values above 1000 µg/ml considered non-toxic.

Based on the results using the BSLT method known that the secondary metabolites derived from the bark of *Artocarpus lanceifolius* Roxb proved to significantly affect the breeding rate of *Artemia salina* L shrimp larvae after a 24-hour incubation period with very high toxicity. These results can be used as a screening method for selecting compounds as anticancer agents from plants. This assumption is based on the fact that the higher the level of toxicity of the secondary metabolites of a plant by the BSLT method (the smaller the LC50 value) the more potential the plant will be used in anticancer treatment

Table 2: BSLT result of *Artocarpus lanceifolius* Roxb Bark extract

Extract	Concentration (µg/mL)	LC_{50} (µg/mL)
n-hexane	1	1.0853
	10	
	100	
Chloroform	1	0.1635
	10	
	100	
Ethyl acetate	1	0.3615
	10	
	100	
Methanol	1	0.2609
	10	
	100	

The toxic properties of all extracts are considered to be related to secondary metabolites contained in the extract. Flavonoid compounds are the most abundant compounds found in the genus Artocarpus, in chloroform extract, ethyl acetate extract and methanol positively contain flavonoids. Flavonoid compounds in the Artocarpus genus generally are prenylated flavonoids[3]. The most dominant factor that determines cytotoxic activity is the isoprenyl group substituted on the C-3 flavone framework and molecular planarities[18]. The n-hexane extract contains triterpenoid compounds and steroid secondary metabolites. Various reports have reported that triterpenoids can inhibit various intracellular signals and transcription factors[19].

Table 3: Percentage Inhibition Scavenging Extracts towards DPPH-free radical

Crude extracts	Inhibitor Scavenging (%)					IC50 (µg/mL)
	2 (µg/mL)	4 (µg/mL)	8(µg/mL)	16 (µg/mL)	32 (µg/mL)	
Chloroform	0.65	4.87	20.78	44.81	80.84	19.66
Ethyl acetate	26.80	63.92	86.86	87.63	87.63	15.85
Methanol	1.39	10.86	28.13	50.97	77.18	19.01

Note: The data are expressed as means ± SD (n = 3)

The antioxidant activity test for four fractions, namely n-hexane fraction, chloroform fraction, ethyl acetate fraction, and methanol fraction were carried out on DPPH radicals. This antioxidant test was carried out to determine the activity of each fraction of the extract of *Artocarpus lanceifolius* Roxb in reducing radical DPPH. In this study IC_{50} values for chloroform fraction is 21.33 µg/mL, ethyl acetate fractions is 16.05 µg/mL, and methanol fraction is 19.57 µg/mL. For extracts derived from hexane fraction the IC_{50} value cannot be determined because it is insoluble in methanol solvents. The complete percentage of DPPH free radical inhibition is presented in Table 3.

CONCLUSION

Artocarpus lanceifolius Roxb bark extract showed very strong toxicity characterized by a very small LC50 value, this material has the potential to be developed as an anticancer drug. Antioxidant tests on chloroform, ethyl acetate and methanol extract showed very strong activity, this material is also very potential to be developed as a raw material for the treatment of degenerative diseases.

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