

Secondary Metabolites Identification and Antioxidant Activity Determination of Crude Extract of *Barringtonia asiatica* L. (kurz) Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author AA performed the experiment and design the study. Author RP performed the experiment, design the study and write the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aims of this study were to elucidate the phytochemical compounds from the leaf of mangrove (*Barringtonia asiatica* L. (Kurz) collected from the coast of Pangandaran Regency, West Java, Indonesia as well as its antioxidant activity profile.

Study Design: The study was designed experimentally with two replication (duplo) for the extraction preparation and analyzed statistically using regression linear for the determination of Inhibition Concentration at 50% or IC50.

Place and Duration of Study: Sample were collected from the mangrove ecotourism site at Pangandaran Regency, West Java, Indonesia. The experimental study was performed at the Tropical Marine and Fisheries Laboratory, Fisheries Department, PSDKU Padjadjaran University, Indonesia.

Methodology: The identification of phytochemical contents of the leaf was carried out qualitatively

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with the principle of formation of precipitate, color and foam. The extraction was performed accordingly using three different types of solvent, n-hexane, ethyl acetate and methanol. Lastly, the antioxidant activity was tested using DPPH (1,-diphenyl-2-picrylhydrazyl) method and IC50 were analyzed subsequently.

Results: Based on the results of the research that has been carried out, it can be concluded that the leaves of *Barringtonia asiatica* L. (Kurz) contain bioactive compounds in the form of tannins and saponins. The methanol extract of *Barringtonia asiatica* L. (Kurz) was proven to have moderate strength antioxidant activity with IC50 value of 125.87 ppm.

Conclusion: this results concluded that different geographic region can influence the phytochemical constituent of the of *Barringtonia asiatica* L. (Kurz). Furthermore, this doesn't hindered its antioxidant activity potential as it still proven to have a relatively medium antioxidant activity.

Keywords: Antioxidant; *Barringtonia asiatica* L. (Kurz); DPPH; mangroves; secondary metabolites.

1. INTRODUCTION

Antioxidant is a chemical group that essentially needed by our body to prevent free radical and also promotes the longevity of our cells. It is required to boost our immune system so disease couldn't easily attack us. To date, many antioxidant has been introduced into our daily life. In particular, the synthetic antioxidant has been produced in numerous number for the past decades. However, the problems with synthetic antioxidant is that they have side effect which can lead to serious health effect [1,2]. For example, butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) which reported to be carcinogenic [3]. The same effect also goes to n-Propyl Gallate (PG), an antioxidant synthesized from the condensation of gallic acid and propanol [4,5]. This urge the finding of new natural antioxidant compound with little or no side effect.

Being situated in the tropical center of the globe, Indonesia is home for a diverse type of flora and fauna. It is a hot spot in terms of species diversity from the terrestrial as well as its aquatic biodiversity. It is known for the wallacea line which represent a huge number of endemic species in the series of islands located in central Indonesia [6]. It is also possessing a megabiodiversity of aquatic life including coral reef and fish in the coral triangle area [7]. This extraordinary biodiversity is the key to unlock a new potential chemical compounds that can benefit human health, including antioxidant.

As a tropical country, Indonesia is a home for almost all mangrove species in the world. Its representation has helped the coastal area protected and support the aquatic ecosystem. They live in relatively low-oxygen environment where slow current allows fine particle to sediment. Approximately there are 80 species of

mangrove in the world and most of them located in Indonesia [8]. One of its species is *Barringtonia asiatica* L. (Kurz), found ubiquitously in Indonesia coastal area. They were long utilized by the locals for various application including health and food. In Indonesia, the Philippines and Indo-China, the fruit or seeds are used to poison fish, while in the Bismarck Islands, the fresh seeds are grated and applied directly to sores [9]. The dried seeds are crushed, mixed with water and drunk for coughs and sore throat. It is applied externally to a sore or swollen spleen after malaria [9]. In Australia, Aboriginal people use it to poison fish and sometimes relieve headaches. In Indo-China the young fruit is eaten as a vegetable after long cooking [10]. Planted as shade trees along the main road along the sea. This species is often confused with *Terminalia catappa* or *Fagraea crenulata*. However, *Barringtonia asiatica* L. (Kurz) has leaves that are fleshier, shinier and have a more pointed tip than *T. catappa*. *F. crenulata* has leaves that grow in pairs and have spines along the stem.

According to Kong et. al [11] the main component of *Barringtonia asiatica* L. (Kurz) leaf are Polyphenols, terpenoids and phytosterols. These compounds, in particular polyphenol, give the antioxidant property. Another reported that the seed of *Barringtonia asiatica* L. (Kurz) generally has a higher diversity of secondary metabolites such as saponin, terpenoid, tannin, flavonoid, coumarine, cardiac glycoside and alkaloid [12]. Although the study of phytochemical analysis of *Barringtonia asiatica* L. (Kurz) is already established quiet well, but the same study from different geographical area is important to elucidate its potential locally. The presence of some compound maybe observed in some specific region, although they share the same species. Furthermore, it will also have

correlated with their antioxidant activities. For example, a study conducted by Kumar et. al [13] reported that Aloe vera grown under cold stress possess a relatively more phytochemical compounds than those in the warmer area. These are mostly true since secondary metabolites will only be produced when there is threat to the plants as it acts as defensive mechanism [14].

Here we try to characterized what secondary metabolites constituted the leaves of *Barringtonia asiatica* L. (Kurz) collected from the coast of Pangandaran Regency, southern part of West Java. It is an important part of west java and one of the most famous ecotourism site in Indonesia. Along with its phytochemical identification, its antioxidant activity using DPPH (1,1,-diphenyl-2-picrylhydrazyl) assay will also be performed to evaluate its potential application.

2. MATERIALS AND METHODS

2.1 Materials Used

The tools used in this study include Bunsen, measuring cup, filter paper, analytical balance, clamp, dropper, drip plate, test tube, stirring rod, filter paper, filter funnel, erlenmeyer, rotary evaporator, incubator, tube rack, vortex, micropipette and UV-Vis spectrophotometer. The materials used include 10% ammonia, 25% CHCl₃, 1% FeCl₃, 1N HCl, 2M HCl and concentrated HCl, NaCl, Meyer's reagent, Wagner's reagent, n-hexane, methanol, ethyl acetate and DPPH. The sample of *Barringtonia asiatica* L. (Kurz) used in the form of leaf parts was obtained from Pangandaran Regency, West Java Province, Indonesia.

2.2 Methods

2.2.1 Secondary metabolites identification

Identification of bioactive compounds contained in *Barringtonia asiatica* L. (Kurz) leaves was carried out qualitatively with the principle of formation of precipitate, color and foam [15]. Phytochemical content testing was carried out on samples of leaves that had been dried and mashed so that they had a larger surface area. The bioactive compounds in the form of secondary metabolites were tested qualitatively including alkaloids, terpenoids, saponins, tannins and flavonoids. Testing of alkaloid compounds used two reagents, namely Meyer's reagent and Wagner's reagent. A positive result for the

alkaloid test will show a reddish brown precipitate when Meyer's reagent is added and it forms a white precipitate when Wagner's reagent is added [16]. Steroid testing shows a blue or purple color if it is positive for steroid compounds [17]. Meanwhile, for testing for tannin compounds, a positive result will show a blackish green or dark blue color change [18]. A positive result for the saponin test will indicate the formation of a stable foam for approximately 10 minutes. The flavonoid test will show a purple orange color change if it is positive, while the phenolic test will produce a purple blue color if it is positive [17].

2.2.2 Solvent extraction

Extraction was carried out according to the method of Cujic et. al [19] which modified using three solvents with different polarity levels, namely methanol, ethyl acetate and n-hexane. The method used is the maceration method, namely soaking the sample in a solvent with a ratio of 1:20 (w/v) for three days. A volume of 100 mL of solvent was added to the five grams of mashed sample and then extracted by maceration for three days. After that, the filter from the maceration was evaporated using a rotary evaporator with a temperature according to the boiling point of each solvent. The crude extract obtained was then prepared in various concentrations (50 ppm, 100 ppm, 150 ppm and 200 ppm) for further testing.

2.2.3 Antioxidant activity assay

The antioxidant activity assay was carried out using a spectrophotometric method referring to Molyneux [20]. The extract solution with each concentration of 0 ppm (control), 50 ppm, 100 ppm, 150 ppm and 200 ppm was taken 4.5 mL and reacted with 500 l of 1 mM DPPH solution in a test tube. The blank solution was prepared by reacting 4.5 mL of methanol solvent with 500 l of 1 mM DPPH solution in a test tube. After that, the solution was homogenized using a vortex. The solution was incubated at 37°C for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

Data analysis was carried out descriptively and then compared with the published literature. The DPPH inhibition value was obtained using the calculation based on Siesler [21] as follows:

$$\%Inhibition = \frac{Blank\ Absorbance - Sample\ Absorbance}{Blank\ Absorbance}$$

Meanwhile, to get the IC50 value, analysis using linear regression was carried out using inhibition data at each concentration tested [22]. Calculations and construction of linear graphs are used using Microsoft Excel program.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Profile

Leaf samples from *Barringtonia asiatica* L. (Kurz) mangrove that have been dried and mashed were tested qualitatively for their bioactive content. Qualitative testing of bioactive compounds is carried out with various reagents that will produce a precipitate, color or foam as a marker for the detection of the compound being tested. The content of bioactive compounds contained in samples of *Barringtonia asiatica* L. (Kurz) leaf can be seen in Table 1.

From the results obtained that the leaf of *Barringtonia asiatica* L. (Kurz) only showed a positive result for Tannin and saponins while for other compound such as alkaloid, Flavanoid, phenolic compounds and triterpenoids or steroids were undetected. This results were a little bit different with previously reported study where Polyphenols, terpenoids and phytosterols were the main component found in the leaf [11]. However, here in our samples, three of those compounds were absence. This is probably due to the different geographic location. The samples collected from southern part of west java where the climate is warm all year. Warmer region may result in fewer chemical compound found in the plant [13].

Tannins are complex phenolic compounds having a molecular weight of 500-3000. Tannins are divided into two groups based on the type of structure and activity towards hydrolytic compounds, especially acids, *condensed tannins* and hydrolysable *tannins*. Polyphenols have a broad spectrum with solubility properties in different solvents. This is caused by the hydroxyl groups in these compounds which have different numbers and positions.

Saponins are glycosides that may be present in many types of plants. Saponins are present in all plants with high concentrations in certain parts, and are influenced by plant varieties and growth stages. its function in plants is unknown, perhaps as a form of carbohydrate storage, or as a waste product of plant metabolism. another possibility is as a protection against insect attack

3.2 Extract Preparation Result

The extraction results obtained from each solvent can be seen in Table 2. The extraction results show that the best solvent that is able to extract the bioactive compound content maximum in the *Barringtonia asiatica* L. (Kurz) mangrove leaf sample is methanol with a yield of 8.58%. Meanwhile, the solvent for ethyl acetate and n-hexane is less than 1%.

Extraction is the process of taking a single or multiple compound from a material using a certain solvent based on its distribution in two immiscible phases. There are various extraction methods that can be used. The exact method is determined based on the properties of the compound to be extracted. Solubility, heat resistance, and interaction with solvents are properties that must be considered. Therefore, the selection of the right method and solvent will give good results as well. The purpose of extraction is to extract all the chemical components contained in the sample. This extraction is based on mass transfer of solid components into the solvent where the transfer begins to occur at the interfacial layer, then diffuses into the solvent.

The process of extracting chemical components in plant cells, namely organic solvents will penetrate the cell wall and enter the cell cavity containing the active substance, the active substance will dissolve in organic solvents outside the cell, then the concentrated solution will diffuse out of the cell and this process will continue until There is a balance between the fluid concentration of the active substance inside and outside the cell.

The results showed that methanol extract has the highest rendement, this is due to the polar nature of the solvent methanol and in accordance with the content of bioactive compounds that have previously been tested positive in samples, namely tannins and saponins. Because the best yield was obtained from extraction using methanol solvent, then for the next testing process, namely antioxidant activity test, the crude extract used was the methanol extract of *Barringtonia asiatica* L. (Kurz) leaf which were made at different concentration series.

3.3. Antioxidant Activity

The resulting absorbance and inhibition for each tested concentration can be seen in Table 3. It

can be seen that the inhibition value is directly proportional to the extract concentration, forming a linear curve (Fig. 1).

The results of the antioxidant activity test with an extract concentration range from 0 ppm (control) to 200 ppm showed that the concentration of the methanol extract of the *Barringtonia asiatica* L. (Kurz) leaf affected the % DPPH inhibition with $R^2 = 0.9802$. According to Molyneux [20], a natural ingredient is said to have very strong antioxidant activity if it has an IC50 value of less than 50 ppm, strong if the IC50 is between 50-100 ppm, is if the IC50 value is between 100-150 ppm and weak if the IC50 is in the range 150-200 ppm. IC50 or 50% Inhibitory Concentration is a value that showing a concentration of a compound that can stop 50% of the oxidation process cause by free radical [23]. The result of IC50 calculation using a linear equation is 125.87 ppm, which means that the methanol extract of *Barringtonia asiatica* L. (Kurz) mangrove is in the medium range. This value is still relatively far when compared to the antioxidant activity of vitamin C which is in the very strong range with

an IC50 of 2.33 ppm, but is comparable to the IC50 value for organ extract in sea cucumbers (126.19 ppm) which is also in the moderate range [24].

DPPH compound is one of the free radical compounds that are relatively stable and can react with hydrogen from antioxidants and then change in a reduced form. Based on the results obtained, the higher the concentration of the tested mangrove leaf methanol extract, the lower the absorbance of DPPH produced. This proves that there is free radical scavenging activity from the methanol extract of *Barringtonia asiatica* L. (Kurz) leaf which is measured colorimetrically through absorbance at a wavelength of 517 nm [25]. Quantitative measurements of the antioxidant activity of a material can be known based on the decay of the purple color [26]. If a DPPH solution is added to a material containing antioxidants, the intensity of the color of the DPPH solution will decrease according to the concentration and inhibition of the material containing antioxidants [27].

Table 1. Phytochemical compound from *Barringtonia asiatica* L. (Kurz) leaves

No	Phytochemical	<i>Barringtonia asiatica</i> L. (Kurz)		
			Result (+/-)	Remarks
1.	Alkaloids	Meyer	-	No precipitate formed.
		Wagner	-	
2.	Flavanoid I		-	No color change.
3.	Flavanoid II		-	No color change, becomes cloudy.
4.	Phenolic Compounds		-	No color change.
5.	Triterpenoids/ Steroids		-	There is no color change due to poor flavonoid test.
6.	Tannins	Tanin 1	+	There is a color change to dark blue.
		Tanin 2	+	There is a color change to blackish green (hydrolyzed).
7.	Saponins		+	There is a 3 cm high foam that does not disappear when 1 drop of 2N HCl is added.

Table 2. Extraction result of *Barringtonia asiatica* L. (Kurz) leaf using three different solvents

Sample	Solvent	Rep	Volume Filtrate (ml)	Extrcat Weight (gr)	Rendement (%)	Color
<i>Barringtonia asiatica</i> L. (Kurz)	N-hexane	1	38	0.0256	0.256	Clear
		2	38			
	Ethyl Acetate	1	35	-	0	Clear
		2	38			
	Methanol	1	42	0.8585	8.58	Yellow
		2	38			

Table 3. Absorbance and % inhibition of *Barringtonia asiatica* L. (Kurz) leaf extract towards DPPH

Absorbance	Sample (ppm)	% inhibition
0.675	0	0
0.621	50	8.00
0.432	100	37.63
0.292	150	56.74
0.152	200	77.48

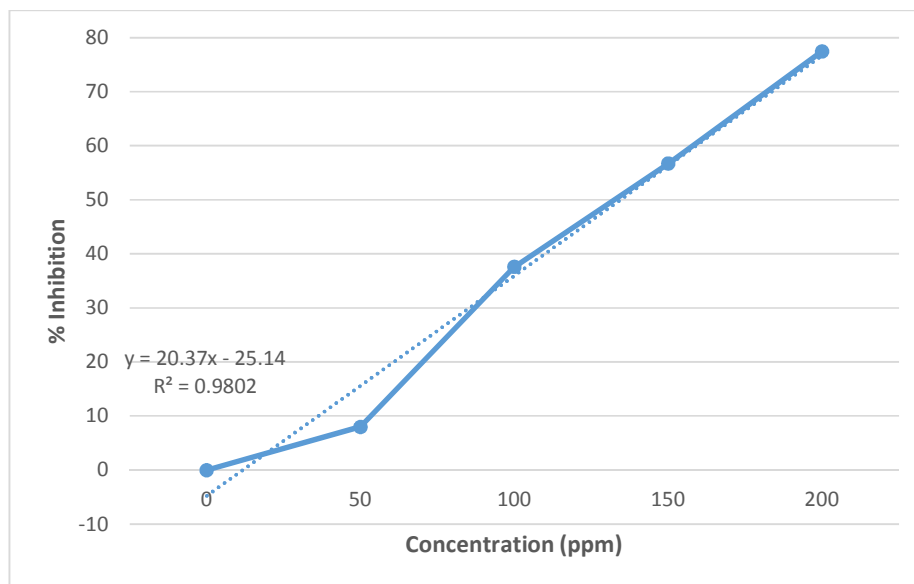


Fig. 1. Linear graph between extract concentration (ppm) and % inhibition

4. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the leaves of *Barringtonia asiatica* L. (Kurz) contain bioactive compounds in the form of tannins and saponins. The methanol extract of *Barringtonia asiatica* L. (Kurz) was proven to have moderate strength antioxidant activity with IC50 value of 125.87 ppm. *Barringtonia asiatica* L. (Kurz) Mangrove leaves have the potential to be further developed as a source of active compounds, given their abundance in Indonesian waters. Research to optimize the extraction process is still needed to get maximum results. In addition to measuring the content of bioactive compounds, it is also necessary to analyze quantitatively using good measurement methods and analytical instruments to get a more detailed picture of the profile of bioactive compounds in the leaves of *Barringtonia asiatica* L. (Kurz).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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