Bioactive Compounds and Antioxidant Activity of Mangrove Fruit Extract *Bruguiera gymnorrhiza* from Pengudang Village, Indonesia

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Abstract. In some parts of Indonesia, people have consumed mangrove fruit as a traditional food. *Bruguiera gymnorrhiza* or lindur plant, is one of the mangroves that have bioactive compounds as antioxidant. Antioxidant compounds found in *Brugueira gymnorrizha* fruit are thought to be able to reduce free radicals. However, the information on the benefits and potential of mangrove fruit as a source of antioxidants is still limited. Not many studies have shown that lindur plant fruit can be used as antioxidants, Therefore, further research on this topic is needed. This research aimed to identify the content of bioactive compounds and the activity of *Brugueira gymnorrhiza* fruit extract obtained from the Pengudang waters of Bintan Island. This research includes several analyses, including analysis of bioactive compound content, total phenolics and flavonoid content, pigment content, and antioxidant activity analysis. The method used in this research was a laboratory experimental and the DPPH (1,1-diphenyl-2-picrylhydrazyl) method was used to test the antioxidant activity of *Bruguiera gymnorrhiza* fruit extract. The results showed that the bioactive compounds contained in *Bruguiera gymnorrhiza* fruit extract are flavonoid and tannin compounds. The total phenolics and flavonoids amounted to 4.05 mg GAE/g sample and 9.36 mg QE/g sample. The content of chlorophyll a, chlorophyll b, and carotenoid pigments were 0.47 mg/g, 1.03 mg/g, and 2.72 µmol/g. *Bruguiera gymnorrhiza* extract has potent antioxidant activity (IC_{50} 60.77 ppm).

1 Introduction

Free radicals are unstable molecules or atoms because they contain unpaired electrons and are reactive [17]. These atoms are hydrogen atoms, transition metals, and oxygen molecules. Free radicals can be formed through metabolic processes in the body and external exposure, including vehicle pollution, cigarette smoke, and ultraviolet radiation. One of the compounds that can reduce free radicals is antioxidant compounds by inhibit, delay, prevent, or slow down the oxidation reaction process [9]. Antioxidants are interpreted by the IC_{50} (Inhibition Concentration), which is the concentration that required to inhibit 50% of free radicals [11]. One plant that has the potential to act as an antioxidant is the *Bruguiera gymnorrhiza* type of mangrove plant.

Bruguiera gymnorrhiza or lindur plant is a mangrove plant known as extensive leaf mangrove, with plank and knee roots and a height of up to 30 m. *Bruguiera gymnorrhiza* is a mangrove plant that has bioactive or antioxidant compounds [4]. This is proven by several studies which show that *Bruguiera gymnorrhiza* fruit has good antioxidant activity with

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IC_{50} values of 9.42 ppm, 26.69 ppm, 70.19 ppm, and 70.45 ppm in methanol extract treatment [10; 23; 1; 19] and showed the presence of tannin, steroid and flavonoid [10].

From observations, *Bruguiera gymnorrhiza* fruit has been used in the medical field, however, information about the potential use of *Bruguiera gymnorrhiza* fruit as a source of antioxidants is still limited, and not much research has shown that *Bruguiera gymnorrhiza* fruit can be used as a source of antioxidants. To explain this scientifically, the information needed is still insufficient, so further research is needed to determine the content of bioactive compounds and antioxidant activity of *Bruguiera gymnorrhiza* fruit for wider exploration of the existing potential. Therefore, this study aims to compare the potential bioactivity of *Bruguiera gymnorrhiza* fruit from Pengudang waters of Bintan Island by conducting further research regarding antioxidant activity and the content of bioactive compounds in *Bruguiera gymnorrhiza* fruit.

2 Material and Methods

2.1 Study areas

The study was carried out from March to June 2023. Samples were taken in Pengudang Village, Bintan Regency. The study areas are presented in Figure 1.

Fig. 1. Study areas

2.2 Research Methods and Procedures

In this research, the experimental method was laboratory by testing antioxidant activity using the DPPH on *Brugueira gymnorrizha* fruit extract from Pengudang Village. Then, the data obtained will be presented in descriptive form. The research started by taking samples of *Brugueira gymnorrizha* fruit from Pengudang Village as research test material.

2.2.1 Collection and Preparation of Sample

Brugueira gymnorrizha fruit samples were taken by climbing a mangrove tree and selecting a little old fruit. The fruit collected is assumed to be homogeneous, and the samples are accumulated into one. *Brugueira gymnorrizha* fruit samples were rinsed using fresh water to remove dirt and then peeled. Next, the *Brugueira gymnorrizha* fruit is cut into small pieces and blended until the sample becomes a rough extract.

2.2.2 Sample Extraction

Brugueira gymnorrizha extraction was carried out using a single maceration method. *Brugueira gymnorrizha*, which has been blended (50 g), is ground and macerated using methanol for 24 hours, and then the solution is filtered. The filtrate is collected using a glass beaker. Meanwhile, the residue was re-macerated with new methanol for 24 hours and filtered again. The results of the second filtrate are combined with the first filtrate to be concentrated using a rotatory evaporator to obtain a paste-like extract.

2.2.3 Determination of DPPH Maximum Wavelength

Preparation of 0.1 mM DPPH solution carried out by weighing 1 mg DPPH powder and then dissolving it using 25 mL methanol solvent [14]. Then the absorbance of DPPH was observed at a wavelength of 400-800 nm using spectrophotometer (Shimadzu UV-1800).

2.2.4 Antioxidant Activity Test

1000 ppm extract solution are produced by weighing 100 mg of *Bruguiera gymnorhiza* fruit extract. They were then dissolved using 100 ml methanol solvent. Next, the 1000 ppm stock solution was diluted into several concentrations, namely 50, 100, 150, 200, and 250 ppm. While, The BHT with several concentrations, including 2, 4, 6, 8, and 10 ppm [24].

Three ml of extract or BHT was put into a test tube, and 1 ml of DPPH solution was added. The solution was incubated (30 minutes) and the absorbance at the maximum DPPH wavelength obtained was measured [12]. The percent inhibition at various concentration was regressed to obtain linear regression values and IC50 values.

2.2.5 Bioactive Compound Analysis

Bioactive compound analysis is carried out to identify whether there are active compounds in the sample. Analysis of bioactive compounds was carried out using the crude extracts. The crude extract was soaked using methanol for 2 hours, after which the solution was filtered using filter paper. The filtrate results are used to test bioactive compounds in flavonoids [5][7], steroids, triterpenoids [7], saponins [7][15], and tannins [5].

2.2.6 Total Phenolic and Flavonoid Test

Total phenolic and flavonoid testing uses a standard solution of gallic acid and quercetin [13] [21]. The standard solution regression equation obtained was used to calculate total phenolics and total flavonoids. Gallic acid is made in several concentrations, namely 5,10, 15, 20, and 25 ppm. Then, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent are added. One mL of 5% Natrium carbonate solution was added and it was incubated at room temperature for one hour under closed (dark) conditions. Absorbance was measured using a spectrophotometer with a wavelength range of 725 nm [6]. The same steps were used for *Brugueira gymnorrhiza* extract at a concentration of 1000 ppm.

Quercetin solution with 5, 10, 15, 20, and 25 ppm concentrations was taken as 1 mL. Then, 1 mL of 10% $AlCl_3$ and 8 mL of 5% acetic acid is added. The solution mixture is left for 16

minutes. Next, it is measured at a wavelength of 415 nm [3]. The same procedure was used for the *Brugueira gymnorrhiza* extract with a concentration of 1000 ppm.

2.2.7 Chlorophyll and Carotenoid Content Test

Brugueira gymnorrhiza extract with concentration of 1000 ppm was put into a cuvette, and then the absorbance was measured at wavelengths of 480 nm, 645 nm and 663 nm [18].

3 RESULT AND DISCUSSION

3.1 Extract of *Brugueira gymnorrhiza* fruit

The characteristics and yield of *Brugueira gymnorrhiza* extract produced a yield value of 14.92% from 200 grams of extracted crude samples. Visually, *Brugueira gymnorrhiza* extract is yellowish brown and liquid paste (Table 1).

Table 1. Extract of Brugueira gymnorrhiza fruit

Solvent	Weight of samples (gram)	Weight of Extract (gram)	Yield Percentage (%)	Extract colour	Extract Form
Methanol	200	13.4	14.92	yellowish brown	Liquid

3.2 Bioactive Compound Content

Brugueira gymnorrhiza extract was analyzed for bioactive compound content using phytochemical methods. The only bioactive compounds detected in *Brugueira gymnorrhiza* extract were flavonoid compounds (Table 2).

Table 2. Phytochemical test results of *Brugueira gymnorrhiza* fruit extract

Extract	Bioactive compounds	Result	Visualitation
Brugueira gymnorrhiza fruit	Flavonoid	+	Showed a yellow color
	Steroid	-	Doesn't show a blue or green color
	Triterpenoid	-	Doesn't show a red or purple color
	Saponin	-	Doesn't form a foam
	Tanin	+	Formed a white deposited at the bottom

The bioactive compounds extracted in this research tend to be less compared to other studies. In this study, *Brugueira gymnorrhiza* extract extracted with methanol solvent only had bioactive compounds in the form of flavonoids. Flavonoid compounds have a strong role as compounds with antioxidant activity in *Brugueira gymnorrhiza* fruit. Flavonol compounds, glycosylfalvones, and flavones are a group of flavonoids that are thought to be found in *Brugueira gymnorrhiza* fruit [10]. Flavonoids are classified as phytochemical compounds that have protective properties and are often found in plants. Flavonoids, also known as bioflavonoids, function as antioxidants [23].

3.3 Phenolic and Flavonoid Compounds

Phenolic compounds have a positive relationship with antioxidant activity. The class of bioactive compounds which include derivatives of phenolic compounds are flavonoids. The results showed that Brugueira gymnorrhiza extract had a total phenolic content of 4.05 mg/GAE g and flavonoids of 9.36 mg/QE g.

3.4 Pigments Content

Another bioactive component that has the potential to act as an antioxidant is pigment. The pigments contained in *Brugueira gymnorrhiza* extract are chlorophyll and carotenoids. The pigment content of chlorophyll a, chlorophyll b, and carotenoids of *Brugueira gymnorrhiza* extract, whose absorbance was measured at wavelengths of 480, 663, and 645 nm, is presented in Table 3.

Table 3. Pigments content of *Brugueira gymnorrhiza* fruit extract

Extract	Solvent	Absorbance			Pigment	Result
		663	**645**	**480**	Chlorophyll a (mg/g)	0.50
Brugueira gymnorrhiza fruit	Acetone	0.056	0.052	0.089	Chlorophyll b (mg/g)	1.03
					Carotenoid (µmol/g)	2.63

The results obtained from calculating the pigment content show relatively small values. The use of polar solvents causes this because, generally, chlorophyll and carotenoids are semi-polar compounds, so they dissolve more easily in solvents that have the same polarity. In accordance with the statement that chlorophyll and carotenoids are more easily dissolved in semi-polar solvents [8]. Pigment is an antioxidant because it contains chlorophyll, which can reduce free radicals [16]. Apart from chlorophyll, there are carotenoids that have antioxidant capabilities as single oxygen reducers through conjugate bonds in the carbon chain [8].

3.5 Antioxidant Activity

3.5.1 The DPPH maximum wavelength

The maximum absorbance of DPPH solution (0.1 mM) in methanol was obtained at wavelength 515 nm (Figure 2). This is in accordance with the statement that free radicals that have unpaired electronic electrons have a purple complementary color with maximum absorbance at a wavelength of 515-520 nm [20].

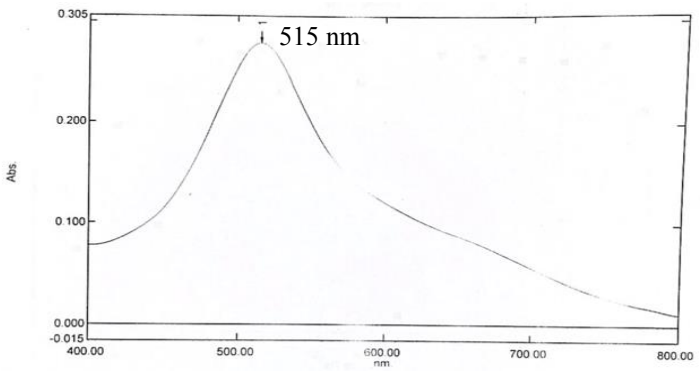

Fig. 2. DPPH maximum wavelength

3.5.2 Positive Control and Antioxidants of Brugueira gymnorrhiza Fruit Extract

The positive control for antioxidants used *Butyl Hydroxyl Toluene* (BHT) as an antioxidant standard. Antioxidant activity is determined by IC_{50}. The IC_{50} value is calculated based on the linear regression equation obtained from the correlation graph between concentration and inhibition percentage. Based on the graph above, it can be seen that the concentration of BHT as a positive control and *Brugueira gymnorrhiza* extract are directly correlated to the percentage of inhibition (Table 3 and 4).

Table 1. Antioxidant activity of BHT

Solvent	Concentration (ppm)	Abs. DPPH	Abs. BHT + DPPH	% Inhibition	IC_{50} (ppm)	Regression Equation
Methanol	2	0.281	0.256	8.889	17.800	y = 2,669x + 2,4911
	4		0.248	11.749		
	6		0.229	18.505		
	8		0.214	23.843		
	10		0.198	29.537		

Table 5. Antioxidant activity of *Brugueira gymnorrhiza* fruit extract

Solvent	Concentration (ppm)	Abs. DPPH	Abs. extract + DPPH	% Inhibition	IC_{50} (ppm)	Regression Equation
Methanol	50	0.275	0,169	42,905	60.77	y = 0.2068x + 37.432
	100		0,117	60,472		
	150		0,077	73,986		
	200		0,059	80,067		
	250		0,045	84,797		

The presentation inhibitoin of the BHT solution and *Brugueira gymnorrhiza* fruit extract increased with increasing the concentration (Table 3 and 4). An antioxidant compound can be said to have high activity if its IC_{50} value is smaller [25]. Antioxidant compounds will react with DPPH radicals through a hydrogen atom donation mechanism, which causes the color of DPPH to decay from purple to yellow as measured at the maximum DPPH wavelength.

Brugueira gymnorrhiza fruit extract had potent antioxidant activity, considering that the IC_{50} value of this extract is 60.77 ppm. In comparison, the positive control using BHT solution had an IC_{50} value of 17.800 ppm and is classified as a strong antioxidant. The research results show *that Brugueira gymnorrhiza* fruit extract has antioxidant activity that is almost comparable to synthetic antioxidants (BHT solution). *Brugueira gymnorrhiza* fruit extract has the potential to be a source of antioxidants because of its bioactive compounds. Apart from that, *Brugueira gymnorrizha* extract can be developed in the fields of health, science and pharmaceuticals seen from the IC_{50} value obtained.

4 CONCLUSIONS

Brugueira gymnorrhiza fruit extract contains flavonoid and tannin compounds. The phenolic and flavonoid content was 4.05 mg/GAE g and 9.36 mg/QE g. The contents of chlorophyll a, chlorophyll b, and carotenoids were 0.50 mg/g, 1.03 mg/g, and 2.63 μmol/g, respectively. Brugueira gymnorrhiza extract has potent or strong antioxidant activity with an IC_{50} value of 60.77 ppm. Antioxidant activity almost comparable to synthetic antioxidants (BHT solution).

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