ANTIOKSIDAN EKSTRAK ETANOL DAUN PEDADA (SONNERATIA OVATA BACKER)

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ABSTRACT

Free radicals are reactive oxygen compounds that are known to be compounds that have free or unpaired electrons. Free radicals will look for new partners so that they will easily bind to other substances such as proteins, fats, and DNA in the body, resulting in cell damage and causing various degenerative diseases. To overcome the negative effects of free radicals, a substance that acts as an antioxidant is needed. Sonneratia Ovata or often called pedada by coastal communities is often used to treat various diseases including diabetes, ulcers, analgesic diarrhea, inflammation, pain and accelerate wound healing. This study aims to examine the levels of flavonoid compounds in the ethanol extract of S. ovata leaves using the colorimetric method and antioxidant activity using the ABTS method (2,2 azinobis (3-ethylbenzothiazolin-6- sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) on the ethanol extract of S. ovata leaves. The research process began with the extraction of S. ovata leaves with 96% ethanol, followed by determination of total flavonoid levels and antioxidant testing. From the results of the determination of total flavonoid levels, it was found that the ethanol extract of S. ovata leaves had a flavonoid content of 38,248 ± 0.142 mgQE/gram. In measuring antioxidant activity using the DPPH and ABTS methods, the IC50 values were 31.785 ± 0.047 ppm and 28.303 ± 0.558 ppm, respectively. Based on these results, the ethanol extract of S. ovata provided very strong antioxidant activity with an IC50 value of less than 50 ppm.

KEYWORDS Sonneratia ovate Backer, Total flavonoid contents, Antioxidant, DPPH, ABTS

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INTRODUCTION

Free radicals can be produced by the body as a result of cellular metabolism products or external exposure to food, sunlight, cigarette smoke, or air pollution. Excessive amounts of free radicals can cause oxidative stress and excessive oxidation processes. This can lead to cell damage and diseases such as diabetes, atherosclerosis, cancer, heart disease, cataracts, premature aging, hypertension, inflammation, liver disease, Alzheimer's, Parkinson's, and other degenerative diseases (Flieger et al., 2021; Martemucci et al., 2022). Antioxidants are essential to prevent the destructive effects of

free radicals. Antioxidants are believed to play an important role in protecting cells from damage caused by free radicals (Binuni et al., 2020). Antioxidants are grouped into enzyme antioxidants and vitamins. Enzyme antioxidants include Superoxide Dismutase (SOD), Catalase and Glutathione Peroxidases (GSH.Prx). Carotenoids, phenolics, flavonoid groups (including flavones, anthocyanins, and their derivatives), unsaturated fatty acids, vitamins, enzymes, and cofactors are just some of the antioxidants found in plants that can scavenge free radicals. These compounds may be useful in maintaining health and treating or preventing diseases (Munteanu & Apetrei, 2021). Therefore, exploration of natural antioxidants is needed to clean free radicals. One of the antioxidant-producing plants is Pedada (Sonneratia ovata Backer) with its flavonoid content.

Extracts and raw materials from Sonneratia species have been widely used by coastal communities for natural medicine purposes. Many researchers have recently discovered chemical compounds such as phenolics and their derivatives, phenolic acids and their derivatives, steroids, triterpenoids, lignans, and cerebrosides from S. ovata fruits and leaves (Astuti et al., 2023; Nurmalasari et al., 2016). β-sitosterol & stigmasterol were isolated from the bark of S. ovata (Sachithanandam et al., 2019). In our previous study, phytochemical screening revealed that the roots, bark, leaves, and fruits of S. ovata Back contained saponins and flavonoids. In contrast, the roots, bark, and leaves contained alkaloids and tannins. Carotenoids have been found in the leaves, bark, and fruits (Astuti et al., 2021; Astuti & Rosyidah, 2020). From previous studies, three secondary metabolite compounds have been isolated from S. ovata fruits, namely (-)-(R)nyasol, (-)-(R)-4'-O-methylnyasol and maslinic acid. The three compounds showed cytotoxic activity against rat glioma cell line C-6 with IC50 values of 19.02; 20.21; and 31.77 µg/mL, respectively. Some of these compounds are considered to have antioxidant activity (Nguyen et al., 2024; Pudhom & Hansen, 2015). Flavonoid compounds play a role in antioxidant activity. Many studies have discussed the antioxidant activity and total flavonoids in Sonneratia plants, such as S. alba (Montolalu et al., 2021), S. apetala, and S. caseolaris (Munira et al., 2019; Raharjo & Haryoto, 2019; Rahman et al., 2021).

However, to the best of our knowledge, no studies have been conducted on the antioxidant activity and flavonoid content of S. ovata Back. Therefore, this study aims to determine the antioxidant activity and total flavonoid content of ethanol extract of S. ovata Back.

RESEARCH METHOD

Materials

Maceration vessel, separating funnel (pyrex), rotary evaporator (D-LAB RE 100 pro), water bath (Faitful), uv vis spectrophotometry (Genesys 10s), S. ovata leaf stem powder, 96% Ethanol (technical), DPPH (2,2-Diphenyl-1-Picrylhydrazil) (Sigma aldrich), quercetin (Sigma aldrich), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma aldrich), methanol (Sigma aldrich), kalium persulfat (Sigma aldrich)

Extraction

A total of 500 grams of S. ovata leaf frond powder was taken from Bugel Beach, Panjatan District, Kulon Progo Regency, Special Region of Yogyakarta (-7.5833032, 110.8173056) and determined at the Biology Laboratory of Ahmad Dahlan University with the number 190 / Lab.Bio / B / III / 2023 was extracted using 96% ethanol in a maceration vessel. The solvent was replaced every 24 hours, while the extraction process was repeated three times. The maceration filtrate was concentrated using a rotary

evaporator and then thickened using a water bath at a temperature of 60oC (Haryoto et al, 2023).

Total Flavonoids Content

The total flavonoid content was measured by a colorimetric assay (Shraim et al., 2021). One hundred micro liters of extract was added to 4 ml of distilled water. Then, 0.3 ml 5% sodium nitrite was added. After 5 min, 0.3 ml of 10% aluminium chloride was added. In 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the mixture was diluted by the addition of 3.3 ml distilled water and mixed thoroughly. The absorbance was determined at 510 nm versus a blank. Quercetin was used as standard for the calibration curve. Total flavonoids content of the extract was expressed as mg catechin equivalents per gram of sample (mgQE/g).

DPPH Antioxidant Assay

An antioxidant activity assay was carried out based on scavenging free radicals of DPPH (2,2-diphenylpicrylhidrazyl) according to the modified method described Baliyan et al., (2022). Briefly, 0.1 mL of sample solution (Quercetin, and ethanol extract) with concentrations of 1, 5, 10, 25, and 50 ppm was added with 1 mL of 0.004% DPPH. The solution was shaken until homogeneous and left for 30 minutes at room temperature without light. Absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The scavenging of free radical DPPH was calculated based on the following equation:

$$\% \, Inhibition = \frac{Abs\ Control - Abs\ Sample}{Abs\ Control} \ x \ 100\%$$

The IC50 value is calculated using the linear regression equation formula between concentration versus % inhibition. The IC50 value is obtained from the x value after replacing y = 50

ABTS Antioxidant Assay

Antioxidant ABTS Method ABTS solution of 5 mL was added with 5 mL of potassium persulfate solution, incubated in a dark room at a temperature of 22-24ºC for 12-16 hours before use, producing ABTS with a dark blue color. This solution was then measured by UV-Vis spectrophotometry at a wavelength of 750 nm. A total of 0.1 mL of sample solution (Quercetin, and ethanol extract) with concentrations of 1, 5, 10, 25, and 50 ppm was added with 2 mL of ABTS radical solution, the solution was then incubated for 6 minutes and the absorbance was measured by UV-Vis spectrophotometry at a wavelength of 750 nm. The scavenging of free radical ABTS was calculated based on the following equation:

$$\% \, Inhibition = \frac{Abs\ Control - Abs\ Sample}{Abs\ Control} \ x \ 100\%$$

The IC50 value is calculated using the linear regression equation formula between concentration versus % inhibition. The IC50 value is obtained from the x value after replacing y = 50

RESULT AND DISCUSSION

The S.ovata leaves samples used in the study were obtained from Bugel Beach, Kulon Progo, Special Region of Yogyakarta (-7.5833032, 110.8173056) which were then determined at the Biology Learning Laboratory of Ahmad Dahlan University. Sample

extraction was carried out using the maceration method with 96% ethanol as a solvent. The maceration method was chosen to avoid heat which is feared to damage the active compounds in the sample. The extraction results obtained a yield of S. ovata leaf extract of 9.8%. The initial stage in this study was phytochemical screening. Phytochemical screening was carried out to identify groups of compounds found in plant samples `. Table 1 shows the results of phytochemical screening of ethanol extract of S. ovata leaves.

Table 1. Phytochemical Screening of Ethanol Extract of S. ovata Leaves

Compound group	Reagent	Result	Interpretation
Alkaloid	Mayer	White	+
	Dragendorf	Brick red	+
	Wagner	Yellow	+
Steroid	Acetic acid anhydride + Concentrated sulfuric acid	Dark green	+
Terpenoid	Acetic acid anhydride + Concentrated sulfuric acid	Brown	+
Flavonoid	Mg powder + Concentrated hydrochloric acid	Orange	++
Fenolik	1% $FeCl_3$	Dark green	++
Saponin	Aquadest	Foam	+
Tanin	1% gelatin solution	Black Green	+

Phytochemical screening was carried out using a test tube with the addition of several specific reagents to identify compounds of the alkaloid, steroid, terpenoid, flavonoid, phenolic, saponin and tannin groups. From the results of the phytochemical screening presented in table 3, the ethanol extract of S. ovata leaves positively contained compounds of the alkaloid, steroid, terpenoid, flavonoid, phenolic, saponin and tannin groups. Flavonoid compounds showed the most striking color changes, indicating substantial concentrations of flavonoid compounds. Pharmacological activities associated with the flavonoid compound group include a variety of effects, especially antioxidant, anti-inflammatory, and antidiabetic properties (Maryam et al., 2020).

Determination of total flavonoid levels in ethanol extract of S. ovata leaves was carried out using the colorimetric method. The principle of this method is the formation of a stable acid complex between AlCl3 with the C-4 ketone group and the hydroxyl group at C-3 or C-5 in flavones and flavonols. While AlCl3 forms a labile bond with the ortho dihydroxy group in ring A or B in flavonoids so that a color is formed that can be measured with a UV/VIS spectrophotometer. Quercetin is used as a standard because quercetin is a flavonoid in the flavonol group (Sapiun et al., 2020). In addition, quercetin is used as a standard because quercetin is a flavonoid that has high reactivity compared to rutin, daflon, diosmin and morin (Rana & Gulliya, 2019). The addition of sodium acetate to stabilize the formation of the complex between AlCl3 and flavonoids (Ngoumen et al., 2023). In determining the levels of flavonoids contained in the ethanol extract of S. ovata leaves, it is necessary to determine the maximum wavelength, operating time and make a standard curve of quercetin to be used in the test.

Based on the results of determining the maximum wavelength and operating time, it can be seen that the maximum wavelength of quercetin obtained is 440 nm with an operating time of between 10 - 14 minutes. This is in accordance with the research of Aryal et al., (2019), a study was conducted on the total flavonoid content in ethanol extract of wild vegetables from Western Nepal vetiver herb using quercetin standards. In determining the standard curve of quercetin, the equation $y = 0.0077x + 0.2825$ was obtained which was used to calculate the total flavonoid content in the ethanol extract sample of waru stems, (y) represents the absorbance value of the sample and (x)

represents the flavonoid content in the sample. The correlation coefficient value (r) = 0.9972. The R2 value approaching 1 indicates a linear calibration curve and there is a relationship between the concentration of quercetin solution and the absorption value.

Determination of flavonoid levels contained in ethanol extract of S. ovata leaves by entering the resulting absorbance into the quercetin curve equation as the y-axis. The measurement results showed that the ethanol extract of S. ovata leaves contained flavonoids of 38,803 ± 0.198 mgQE/g. The results of the measurement of flavonoid levels can be seen in table 2.

Table 2. Flavonoid Content of Ethanol Extract of S.ovata Leaves.

Replication	TPC (mg QE/g)	Average ± SD
1	38,381	
2	38,267	38,248 ±0,142
3	38,097	

Antioxidants are defined as substances that can prevent the occurrence of free radical autooxidation reactions in lipid oxidation (Gulcin, 2020). The ability of the extract to inhibit antioxidants is determined based on the IC50 value.

The working principle of the DPPH method is the presence of hydrogen atoms from antioxidant compounds that bind to free electrons in radical compounds, causing a change from free radicals (diphenylpicrylhydrazyl) to non-radical compounds (diphenylpicrylhydrazine). This is indicated by a color change from purple to yellow (free radical compounds are reduced by the presence of antioxidants (Romulo, 2020). The principle of measuring antioxidant activity using the ABTS method is the reduction of ABTS+ free radicals so that the blue color of ABTS+ free radicals disappears. ABTS+ free radicals occur from the reaction of ABTS diammonium salt with potassium persulfate which produces a blue color (Cano et al., 2023). The antioxidant activity value in S. alba mangrove leaves can be presented in Table 3 and Table 4.

Table 3. IC50 Value of Antioxidant Ethanol Extract of S. ovata Leaves DPPH

Group	Consentration (ppm)	% Inhibition	IC_{50}	SD	Category
Quersetin	1 ppm	40,651	8,695	0,047	Very Strong
	5 ppm	48,640			
	10 ppm	50,405			
	25 ppm	52,455			
	50 ppm	54,125			
Extract	1 ppm	35,673	31,785	0,900	Very Strong
	5 ppm	40,772			
	10 ppm	42,584			
	25 ppm	47,538			
	50 ppm	56,256			

Table 4. IC50 Value of Antioxidant Ethanol Extract of S. ovata Leaves ABTS

Group	Consentration (ppm)	% Inhibition	IC_{50}	SD	Category
Quersetin	1 ppm	49,962	4,921	0,149	Very Strong
	5 ppm	53,180			
	10 ppm	58,927			
	25 ppm	61,839			
	50 ppm	64,904			
Extract	1 ppm	33,356	28,303	0,558	Very Strong
	5 ppm	38,254			
	10 ppm	42,698			
	25 ppm	48,816			
	50 ppm	57,664			

Based on the results of antioxidant measurements in Table 3 and Table 4, the ethanol extract of S. ovata leaves provided very strong antioxidant activity with the DPPH and ABTS methods with IC50 values of 31.785 ± 0.047 ppm and 28.303 ± 0.558 ppm, respectively. Quercetin as a comparison gave IC50 values of 8.695 ± 0.047 ppm and 4.921 ± 0.149 ppm. The antioxidant activity of the ethanol extract of S. ovata leaves is thought to be due to the flavonoid content. Flavonoids act as exogenous antioxidants and are directly oxidized by radicals to form less reactive species through four mechanisms, namely (1) inhibition of nitric oxide synthase activity, (2) inhibition of xanthine oxidase activity, (3) modulation of the channel pathway, or by (4) interacting with other enzyme systems (Ullah et al., 2020). The mechanism of flavonoids as antioxidants directly occurs by donating hydrogen ions so that they can neutralize the toxic effects of free radicals, while the indirect mechanism is by increasing the expression of endogenous antioxidant genes through several mechanisms (Adwas et al., 2019).

CONCLUSION

The results showed that the ethanol extract of S. ovata leaves provided very strong antioxidant activity with IC50 values of 31,785 ± 0.047 ppm and 28,303 ± 0.558 ppm tested using DPPH and ABTS. From the results of the determination of total flavonoid levels, it was found that the ethanol extract of S. ovata leaves had a flavonoid content of 38.248 ± 0.142 mgQE/gram.

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