BIS HEALTH AND ENVIRONMENTAL SCIENCE

Volume 2 2025 | DOI: 10.31603/bishes.288
6th Borobudur International Symposium on Science and Technology (BIS-STE) 2024

UNIMMA PRESS
Universitas Muhammadiyah Magelang

Antioxidant activity of red pidada leaf extract (*Sonneratia caseolaris Engl.*) ABTS method (2,2 azinobis (3-ethylbenzothiazoline)-6-sulfonic acid)

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Abstract

Red pidada leaf (*Sonneratia Caseolaris Engl.*) contain chemical compounds including alkaloids, steroids, flavonoids and phenols. Flavonoids are compounds that can protect against UV rays. Antioxidants are compounds that are used to neutralize free radicals by donating electrons to free radical compounds. The extraction process used in this study was the maceration method using ethanol 96%. The qualitative analysis using the phytochemical screening test showed positive presence of phenol, flavonoid and tannin compounds, while the quantitative analysis used the antioxidant activity of red pidada leaf extract (*Sonneratia caseolaris Engl.*). Measurement of antioxidant activity was carried out using the ABTS method (2,2 azinobis (3-ethylbenzothiazoline)-6-sulfonic acid). The results showed that IC_{50} of ethanol extract of red pidada leaves (Sonneratia caseolaris Engl.) is 37.67 µg/mL and IC_{50} acidum ascorbicum is 28.42 µg/mL. So it can be concluded that the antioxidant activity of red pidada leaf extract (*Sonneratia caseolaris Engl.*) is strong category.

Keywords

Red pidada Leaf, Antioxidant, ABTS

Introduction

Indonesia is a country that has a lot of natural resources. Resources derived from mangrove forests which are places where they grow in coastal areas or river estuaries near the coast. One of the important mangrove plant products is as a raw material for medicines and cosmetic raw materials. Cosmetics is the Greek word that means 'decorate' (the addition of something decorative for people or something). Cosmetics are defined as substances that come in contact with various parts of the human body such as skin, hair, nails, lips, teeth, and lender membranes, etc. [1]. Cosmetics help in improving or changing the outside appearance of the body and covering the body's odor. Cosmetics can protect the skin and keep it in good condition. In general, cosmetics

Published:
May 26, 2025

Selection and Peer-review under the responsibility of the 6th BIS-STE 2024 Committee

are external preparations applied to the external parts of the body [1]. Cosmetics have many types, one of which is moisturizing cosmetics.

Mangrove plants which are important for cosmetic raw materials, namely the red pidada leaves (*Sonneratia caseolaris Engl.*). Red pidada leaves (*Sonneratia caseolaris Engl.*) contain many metabolites. The secondary metabolites contained are flavonoids, triterpenoids, saponins and tannins. Mangrove plants are used by the people of Kalimantan as traditional medicine as the leaves are often used as medicine, scars, and cold powder ingredients [2]. In addition, parts of the plant from the Sonneratia family are often used for traditional medicine such as treating hemorrhoid pain, bleeding, ulcers, sprains, hepatitis, heat-lowering drugs, and asthma [3]. The part of the red pidada plant that is often used is the leaf part.

Free radicals are molecules that have more than one free electron, have no pair and are unstable. So that it can bind a compound to get a partner to achieve a stability. Free radicals can form in the human body which will cause several diseases. Antioxidants are electron donor compounds. Antioxidant compounds have a small molecular weight, but are able to activate the development of oxidation reactions by restraining the formation of a radical.

Extraction is the separation of a simplex material from a certain solvent. The purpose of extraction is to withdraw all the active ingredients contained in a simplicia. The most commonly used solvents are ethanol, methanol, n-hexane, ethyl acetate, chloroform, acetone and benzene. Alcohol is one of the most widely used solvents for total dissolving. Extracts are liquid, thick or dry preparations derived from a process of extracting a simplex compound. Extracts are divided into three classes. Liquid extract is obtained from an extraction process where some of the simplicia still contains the liquid extract. The condensed extract itself comes from simplicia in which the extracting liquid has been evaporated, while the dry extract comes from simplicia which does not contain the extracting liquid [7]. Red pidada leaves contain 2 flavonoids namely luteolin glycoside and luteolin [4]. Based on Jubaidah's research, et al stated that red pidada leaves have antioxidant activity with secondary metabolites, namely flavonoid, tannin, phenolic, and saponin compounds. Flavonoids are one of the compounds that can protect against UV rays [5].

One of the methods used to test antioxidant activity is the ABTS method (2,2 azinobis (3-ethylbenzothiazoline)-6-sulfonic acid). This method has the principle of soaking the ABTS free radical compound, which initially has a blue color, derived from the ABTS free radical compound. and will be obtained colorless or disappear when tested with samples containing higher antioxidants. ABTS has a higher sensitivity than the DPPH method, can be used in a large pH range and has fast antioxidant activity in biological systems. Based on the description above, a study was conducted related to the antioxidant activity test of the ethanol extract of red pidada leaves (*Sonneratia caseolaris Engl.*) using the ABTS (2,2 azinobis (3-ethylbenzothiazoline)-6-sulfonic acid) method.

Method

Tools and Materials

The tools used in this study were analytical balance (OHAUS), stir bar, glass beaker, measuring cup, 40 mesh sieve, blender (ISOLAB), test tube, measuring flask, dropping pipette, volume pipette, measuring pipette, micropipette (MEMMERT), pH Universal, spatula, rotary evaporator (HEIDOLPH), maceration container, oven (MEMMERT), waterbath (FAITHFUL), mortar and tamfer, porcelain cup, centrifuge, incubator, moisture analyzer, spreadability tool, adhesion tool, viscometer (Brookfield LV), and a UV-Vis spectrophotometer (SHIMADZU).

The materials used were red pidada leaf extract (*Sonneratia caseolaris Engl.*), ethanol 96%, methanol, folin-ciocalteu, Mg powder, HCl concentrated, HCl 2N, mayer and dragendrof reagents, $FeCl_3$ 5%, chloroform, anhydrous stearic acid, H_2SO_4 concentrated, ABTS, Potassium Persulat, Vitamin C.

Preparation and Extraction

Red pidada leaves were obtained from Klidang Lor Village, Batang Regency. The samples were sorted, washed, drained and dried in the sun until simplicia was obtained. The simplicia was crushed using a blender, then each simplicia was macerated with ethanol 96% solvent. Each macerate was concentrated with a rotary evaporator.

Phytochemical Screening

1. Identification Phenols: 0.1 gram of red pidada leaf extract (*Sonneratia caseolaris Engl.*), add 1 mL of methanol and 5 drops of folin ciocalteu solution.
2. Identification of Flavonoids: 0.1 gram of red pidada leaf extract (*Sonneratia caseolaris Engl.*), added 3 mL of methanol and then heated over a water bath. Add 0.1 gram of Mg powder and 2 mL of concentrated HCl.
3. Identification of Saponins: 0.1 gram of red pidada leaf extract (*Sonneratia caseolaris Engl.*), add 10 mL of boiling water, shake for 10 seconds.
4. Identification of Alkaloids: 0.1 gram of red pidada leaf extract (*Sonneratia caseolaris Engl.*), add 2 mL of hydrochloric acid, then heat in a water bath, wait until it cools down. Filtered and the filtrate was divided into two to add Mayer's reagent and Dragendroft's reagent.
5. Identification of Tannins: 0.1 gram of red pidada leaf extract (*Sonneratia caseolaris Engl.*), dissolve in 5 mL of hot water and drop 2-3 drops of $FeCl_3$ 5% reagent.
6. Identification of Steroids & Terpenoids: 0.1 gram of red pidada leaf extract (*Sonneratia caseolaris Engl.*), dissolve it in 2 mL of chloroform, then add 10 drops of anhydrous stearic acid and 3 drops of H_2SO_4 concentrated.

Antioxidant Activity Test

1. Preparation of ABTS stock solution

a. Solution A: A total of 36 mg of ABTS was weighed, dissolved in 10 mL of distilled water.
b. Solution B: A total of 5.4 mg of Potassium persulate is weighed and dissolved in 10 mL of distilled water.
c. Incubated in a dark room at 22-24°C for 12 hours before use.
d. After incubation, the volume was made up with methanol pa to 50 mL [6]

2. ABTS testing
   a. Determination of the maximum wavelength of the ABTS solution. The ABTS solution which has been mixed with $K_2S_2O_8$ is pipetted as much as 1 mL and the volume is made up to 5 mL with methanol pa in a volumetric flask. then measured by spectrophotometry at a wavelength of 700-800 nm.
   b. ABTS solution operating time. 25 mg of vitamin C into a 25 mL volumetric flask. Dissolve with methanol up to the mark (1000 µg/mL), pipette 0.5 mL and dilute in a 10 mL volumetric flask. Take 1 mL of the solution, add 1 mL of ABTS solution and then make up the volume to 5 mL with methanol pa. Read the absorbance once at 1 minute until 30 minutes until constant.
   c. Vitamin C Testing. 2.5 mg of vitamin C was dissolved in 25 mL of methanol p.a to obtain a stock concentration of 1000 µg/mL. Dilution of the solution with concentrations of 10, 20, 30, 40 and 50 µg/mL was added to a 10 mL volumetric flask with methanol. Take 1 mL of each concentrated sample solution (Vitamin C) put in a test tube and add 1 mL of ABTS and add methanol to a 5 mL volumetric flask, then wait for the operating time.

3. Determination of antioxidant activity

As much as 25 mg of red pidada leaf extract was dissolved in 25 mL of methanol pro analysis ad, then a series of concentrations was made with concentrations of 10, 20, 30, 40 and 50 µg/mL. Each concentration was put in a 10 mL volumetric flask and diluted with methanol up to the mark, put into a test tube 1 mL each, then the test solution was added 1 mL of ABTS solution and then the volume was made up to 5 mL with methanol. Leave it for the operating time obtained and measure the absorbance at the maximum wavelength. The absorbance was read at each concentration by Uv-Vis spectrophotometry.

Results and Discussion

Preparation and Extraction

Obtained 10 kg of wet simplicia, after dry sorting, 1.5 kg of simplicia was obtained. Then the powder was made to obtain 1 kg of simplicia powder with a moisture content of 8.74%.

The yield of red pidada leaf extract (*Sonneratia caseolaris Engl.*) in this research was 19.66%. The yield of the extract is a comparison of the viscous extract sample to the initial weight of the simplicia. Extract yield can be used to show chemical compounds

extracted from extract samples. The results of phytochemical screening can be seen in Table 1.

Table 1. Phytochemical Screening

Phytochemical	Reagent	Result
Phenol	Folin Ciocalteu	+
Flavonoids	Mg + HCl	+
Alkaloids	Mayer	-
	Dragendroft	-
Saponin	HCl	+
Steroids & Terpenoids	Lieberman Burchad	+
Tannin	FeCl3	+

Table 1 showed that the leaf extract of red pidada (*Sonneratia caseolaris Engl.*) positively contained phenolic compounds, flavonoids, saponins, tannins and steroids. Phytochemical screening is an initial step that is used to determine the class of chemical compounds obtained in a plant that is being observed. Phytochemical testing includes tests for phenols, flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. From the results of the analysis of phenolic compounds with a test that turned black-green. The positive results of phenolic compounds are indicated by the presence of a black-green color change [7].

The results of the phytochemical screening showed that the leaf extract of red pidada (*Sonneratia caseolaris Engl.*) positively contained flavonoid compounds characterized by the formation of a reddish black color [8]. In the alkaloid test, it showed that it did not contain alkaloid compounds because no precipitate formed. The results of the phytochemical screening showed that the pidada leaf extract (*Sonneratia caseolaris Engl.*) contained saponins which formed a stable foam with the addition of HCl 2N to stabilize the foam [8]. The results of the positive phytochemical screening test indicated the presence of steroid/terpenoid compounds which formed a green-black color.

The results of the phytochemical screening test showed positive for containing tannin compounds which formed a bluish black color. The addition of $FeCl_3$ reacts with the hydroxyl groups found in tannins to hydrolyze the tannins so that they produce a bluish-black color change in tannins.

Determination of Antioxidant Activity

The results of the IC_{50} value of the antioxidant activity of vitamin C and red pidada leaf extract (*Sonneratia caseolaris Engl.*) can be seen in Table 2. In this study, the results of the IC_{50} value of red pidada leaf extract (*Sonneratia caseolaris Engl.*) were 37.67 µg/mL with the regression equation y = 1.1825x + 5.4529 with a correlation value (r) close to 1. In this study the results were obtained red pidada leaf extract (*Sonneratia caseolaris Engl.*) which has an antioxidant activity value with IC_{50} tilapia of 37.67 µg/mL, while the antioxidant activity value of vitamin C is 28.424 µg/mL. In comparison to vitamin C, the IC_{50} value was greater than the IC_{50} value of red panda leaf extract (*Sonneratia caseolaris Engl.*). The results of Antioxidant Activity Extract dan Vitamin C can be seen in Table 2.

Table 2. Antioxidant Activity of Vitamin C and Extracts

Concentration	%IC Vitamin C	%IC extract	IC_{50} Vitamin C (µg/mL)	IC_{50} extract (µg/mL)
10	22.629	16.411		
20	37.206	30.173		
30	50.407	42.405	28.42	37.67
40	65.647	50.050		
50	86.391	65.596		

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The wavelength determination was obtained at 745 nm, then the operating time was measured using a standard solution of vitamin C (1000 µg/mL). Pipette 0.5 mL diluted in a 10 mL volumetric flask, pipetted 1 mL of the solution and 1 mL of ABTS solution was made up to 5 mL with methanol pro analysis. Furthermore, the absorbance of the solution was read in the 1 minute to the 30 minute until the absorbance stabilized. The results of the operating time obtained stable absorbance at the 10 minute, the operating time was carried out with the aim of knowing the measurement time at a stable time [9].

Analysis of the value of antioxidant activity was carried out at a predetermined wavelength and the operating time was determined. The parameter used in this test is the value of antioxidant activity with IC_{50} value (50% inhibition) is a concentration that can counteract free radicals with 50% inhibition which is calculated from a linear regression equation that can be related to the extract concentration with %IC. Where the smaller the IC_{50} value, the greater the antioxidant activity [10].

The results obtained in the study showed that the average IC_{50} of the red panda leaf extract (*Sonneratia caseolaris Engl.*) was 37.67 ppm. So, it can be concluded that the antioxidant activity of red pidada leaf extract (*Sonneratia caseolaris Engl.*) is very strong.

Conclusion

From the results of the research that has been done, it can be concluded that the chemical compounds contained in the leaf extract of red pidada (*Sonneratia caseolaris Engl.*) leaves. Red pidada leaves (*Sonneratia caseolaris Engl.*) extracted using ethanol 96% solvent had antioxidant activity in the strong category with an IC50 value of 37.67 µg/mL.

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