FULLERENE Journal of Chemistry

Fullerene Journ. Of Chem Vol.6 No.1: 28-33, 2021
ISSN 2598-1269
doi 10.37033/fjc.v6i1.248

Phytochemical and Antioxidant Test of Binahong (*Anredera cordifolia* (Tenore) Steenis) Leaves Ethanol Extract

Fensia Analda Souhoka*, Imanuel Berly Delvis Kapelle, Elisabeth Sihasale

Jurusan Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Pattimura, Ambon, 97233, Indonesia

INFO ARTIKEL

Diterima 06 Maret 2021
Disetujui 26 April 2021

Key word:
Antioxidant
Phytochemical
Binahong
Anredera cordifolia
DPPH

Kata kunci:
Antioksidan
Fitokimia
Binahong
Anredera cordifolia
DPPH

**fensia@fmipa.unpatti.ac.id*

ABSTRACT

Binahong Anredera cordifolia (Tenore) Steenis leaves contain chemical compounds that can be used as antioxidants. This study aims to examine the phytochemical and antioxidant activity of the ethanol extract of binahong leaves. Binahong leaves were extracted using the maceration method with an ethanol solvent. The phytochemical test showed that the ethanol extract of binahong leaves contained phenolic compounds, flavonoids, alkaloids, and tannins to have potential antioxidants. The antioxidant activity test was carried out using the DPPH method and absorbance measurement with a UV-Vis spectrophotometer at a wavelength of 517 nm. The results showed that the ethanol extract of binahong leaves had strong antioxidant activity, as evidenced by the IC_{50} *value of* 87.423 *μg/mL.*

Introduction

Binahong is cultivated as herbal medicine, planted in pots, in the yard, or garden. Binahong leaves are often used as a traditional medicine to heal burns, rheumatism, gout, lack of appetite, nosebleeds, inflammation of the kidneys, colitis, and cancer. However, the binahong plant's benefits have not been widely known in Indonesian society [1]. Binahong leaves contain saponins, tannins, flavonoids, alkaloids, and polyphenols with antioxidant properties [2].

Antioxidants have an important role to play in maintaining health. It is due to the ability of antioxidants to scavenge free radicals. Free radicals are highly reactive because they have one or more unpaired electrons. Reactive oxygen compounds are produced continuously in the human body due to normal metabolic processes [3].

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have side effects on our health [4]. The side effect has spurred researchers to explore natural materials as a source of antioxidants. Generally, natural antioxidants are phenolic compounds scattered throughout the plant, both in the roots, stems, leaves, seeds, fruits, and flowers. Natural antioxidants from plants are polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbene), carotenoids (xanthophyll and carotene), and vitamins (vitamins E and C) [5].

Several natural-based antioxidants have been studied. The methanol extract of banyan fruit has an IC_{50} of 40.36 μg/mL against DPPH [6]. The IC_{50} of the methanol extract of Kesumba Keling seeds was 69.425 ppm [7]. Ethanolic extract of binahong possesses total antioxidants of 4.25 mmol/100 g (fresh) and 3.68 mmol/100 g (dry) [3]. Binahong leaf ethyl acetate extract had an IC_{50} 68.07 μg/mL [8]. EC_{50} of binahong tuber flavonoid extract was 178.60 mg/L, and 70% ethanol extract was 298.10 mg/L [9].

This study aimed to determine the chemical content and antioxidant power of binahong leaf ethanol extract. Binahong leaf

extract was obtained by maceration using ethanol. The antioxidant activity test was carried out in-vitro with the DPPH (1,1-diphenyl-2-picrylhydrazil) method. The DPPH method is often used for testing the antioxidant activity of several natural compounds because this method is relatively easy and simple compared to other methods [10].

Materials and Methods

Materials

The materials used in the study were binahong (*Anredera cordifolia* (Tenore) Steenis) leaves, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 96% of ethanol, $FeCl_3$, HCl, Mg powder, distilled water, Mayer's reagent, and quercetin. All the chemicals were purchased from Merck with pro analysis grade.

Sample preparation

Binahong leaves were taken, washed, sliced into small pieces, dried in an oven at 105 °C for 5 hours until a constant weight was calculated for the moisture content. The dry sample was mashed and sieved until a powder was obtained.

Procedure

Sample Extraction

The 10 g of binahong leaf powder was put into an Erlenmeyer and added 200 mL of 96% ethanol until the sample was immersed and then macerated for 24 hours. The filtrate is filtered and evaporated using a rotary evaporator. The binahong leaf ethanol extract obtained was weighed and performed a phytochemical test.

Phytochemical test

The phytochemical test is a preliminary test for the ethanol extract of binahong leaves, including phenolic, flavonoid, tannin, and alkaloid tests.

Preparation of test solutions

The ethanol extract of binahong leaves as much as 0.5 g was dissolved in ethanol 96% in a 10 mL volumetric flask.

Phenolic test

The 2 mL sample extract was put into a test tube added 5 mL of ethanol, and two drops of 2% $FeCl_3$ were added (positive if it produces a dark blue color) [11].

Flavonoid test

A total of 2 mL of binahong leaf extract was added with 1 mL of 1% HCl into a test tube, then added a little Mg powder (positive if a yellow color is formed in the presence of flavonoids) [12].

Tannin test

Ethanol extract 2 mL was dissolved in 1 mL of distilled water and brought to a boil. Then filtered and the filtrate is added 2 drops of $FeCl_3$ 1% and shaken (positive if it produces a brown color) [13].

Alkaloid test

2 mL of the sample extract was added with 5 mL of ethanol. Then added with Mayer's reagent drop by drop (positive for pink color) [14].

Preparation of 40 ppm DPPH solution

A total of 0.01 g of DPPH is put into a 250 mL measuring flask, then ethanol is added to the limit mark. The solution is used immediately and is kept at a low temperature and protected from light [8].

Determination of the maximum DPPH wavelength

A total of 5 mL of DPPH solution were observed for their absorption in the wavelength range of 400–600 nm using ethanol blanks.

Determination of DPPH free radical scavenging activity.

A total of 2 mg of ethanol extract of binahong leaf was made into a solution with 10, 20, 30, 40, 50, 60, 70, and 80 ppm. Quercetin comparison solution was made with a concentration of 2.5; 5.0; 10; 15; 20; and 25 ppm. Each test solution of 1 mL was put into a test tube, and 2 mL of 40 ppm DPPH solution were added, then left to stand for 30 minutes at room temperature. The absorbance measurement was carried out at a wavelength of 517 nm using a UV-Vis spectrophotometer [15]. The test was carried out with twice measurements. Furthermore, the IC_{50} value is calculated based

on the regression equation obtained.

Results and Discussions

Sample Preparation

The moisture content of the binahong leaf samples was 54.805%. The high and low water content will affect the maceration process. The lower the water content, the easier it is to withdraw the sample's active substance because the solvent easily penetrates the sample cell wall without interference from water molecules.

Sample Extraction

Binahong leaf antioxidant test begins with preparing a sample extract with 96% ethanol using the maceration method. The extraction results obtained dark green extract. The extract was filtered, then the filtrate was evaporated using a rotary evaporator to remove the solvent to obtain a thick extract weighing 0.94 g (1.063%). The amount of the active compound content of a sample is related to the yield value. If the yield obtained is large, the active compound content will also increase. The high bioactive compounds in a sample are indicated by the high yield value produced.

Phytochemical Test

Phenolic test

A positive result is indicated in phenol testing by forming a dark blue color when reacted with 1% $FeCl_3$. Binahong leaf ethanol extract showed positive results indicated by a change in color from yellow to dark blue. The test results showed that the ethanol extract of binahong leaves contained phenolic compounds. The hydroxyl groups in phenolic compounds can react with $FeCl_3$ to form dark blue complexes [11].

Flavonoid test

To determine the presence or absence of flavonoid compounds presence in the ethanol extract of binahong leaves, testing was carried out using the Shinoda test, namely by reacting the extract with 1% HCl powder and Mg powder. The formation of yellow color indicates a positive test [13]. The test results showed that the ethanol extract of binahong leaves contained flavonoids.

Tannin test

In the tannin test, the ethanol extract of binahong leaves reacted with 1% $FeCl_3$ showed positive results, marked by a change in color from green to brown and frothy. The test results showed that the ethanol extract of binahong leaves contained tannin compounds. Tannins will form complex compounds with the Fe^{3+} ion as the central atom. Tannins contain O atoms which have lone pairs that can coordinate with the central atom. The Fe^{3+} ion in the above reaction binds three tannins with two donor atoms, namely the O atoms in the 4' and 5' dihydroxy positions, so that six lone pairs can be coordinated to the central atom. Atoms O at 4' and 5' dihydroxy positions have the lowest energy in forming complex compounds [16].

Alkaloid test

Testing for the presence of alkaloid compounds in a sample was carried out using Mayer's reagent. The ethanol extract of binahong leaves showed positive results, indicated by a color change from white to pink. Mayer's reagent contains potassium iodide and mercuric chloride, producing alkaloid potassium, a white precipitate. The white precipitate is thought to be a complex alkaloid potassium compound. Potassium iodide, which is added excess, will form potassium tetraiodomercurate (II) [17]. The test results showed that the ethanol extract of binahong leaves contained alkaloid compounds.

Determination of the maximum DDPH wavelength

Determination of the maximum wavelength of DDPH is carried out to determine the wavelength required by the DPPH solution to achieve maximum absorption. The determination of wavelength was carried out using 96% ethanol blank. The maximum absorbance obtained was 0.736 in the 517 nm wavelength range. The wavelength corresponds to the maximum wavelength of the DPPH, which is 400–600 nm. Furthermore, the maximum wavelength of DPPH was used in determining the antioxidant activity of binahong leaf ethanol extract.

Determination of DPPH Free Radical Counter Activity

Calibration curve

Before determining the activity of the DPPH free radical scavenger of binahong leaf ethanol extract, a calibration curve was made to test the linearity of the sample. Based on the calculation results, the data presented in Table 1 and Figure 1 are obtained.

Table 1. The activity of DPPH free radical scavenger of quercetin

No	Quercetin concentration (ppm)	Absorbance	% Inhibition
1	2.5	0.451	27.84
2	5	0.342	45.28
3	10	0.256	59.04
4	15	0.122	80.48
5	20	0.025	96.00
6	25	0.020	96.80
	DPPH + Ethanol	**0.625**	

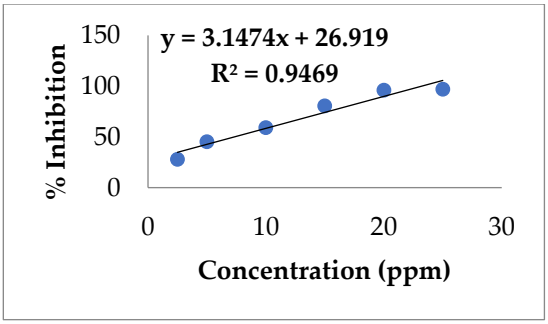

Figure 1. Standard curve of quercetin DPPH free radical scavenging activity

Determination of DPPH free radical scavenging activity was expressed by IC_{50} value using quercetin control. The measurements using UV-Vis spectroscopy at a wavelength of 517 nm obtained each concentration's absorbance value. The absorbance value was used to calculate % inhibition and IC_{50} quercetin. Based on Table 1 and Figure 1, it can be seen that the increase in quercetin concentration means the higher the ability to ward off DPPH free radicals. From the calculation, the IC_{50} quercetin value was 46.88 µg/mL.

Results of determining DPPH free radical scavenging activity

The antioxidant activity of binahong leaf ethanol extract was tested using the DPPH method. DPPH radical is an organic compound containing unstable nitrogen with strong absorbance at a wavelength of 517 nm. The presence of antioxidant activity from the sample results in a color change from purple to yellow. This change occurs when DPPH free radicals are captured by antioxidants and release hydrogen atoms to capture stable DPPH-H. Qualitatively, the ethanol extract of binahong leaves contains phenolic compounds, flavonoids, alkaloids, and tannins which can donate electrons to ward off free radicals.

The quantitative antioxidant activity test was carried out by measuring the free radical scavenger of a compound with an antioxidant activity using UV-Vis spectrophotometry. Thus will be known the activity of reducing free radicals is expressed by the value of IC_{50}. The IC_{50} value is defined as the test compound's concentration that can reduce free radicals by 50%. The smaller the IC_{50} value, the higher the free radical scavenging activity. Specifically, a compound is a powerful antioxidant if the IC_{50} value is <50 ppm, 50-100 ppm strong, 100-150 ppm medium, 150-200 ppm weak, and very weak >200 ppm.

Binahong leaf ethanol extract showed an increase in % inhibition per concentration. The percentage of inhibition was obtained from the difference in the absorbance of the control and the sample's absorbance. The activity of the DPPH free radical scavenger of binahong leaf ethanol extract is presented in Table 2.

Table 2. The activity of DPPH free radical scavenger of binahong leaf ethanol extract

No	Sampel Concentration (ppm)	Average Absorbance	Average % Inhibition
1	10	0.615	1.75
2	20	0.606	3.27
3	30	0.588	6.06
4	40	0.574	8.38
5	50	0.562	10.29
6	60	0.530	15.20
7	70	0.487	23.52
8	80	0.371	40.78

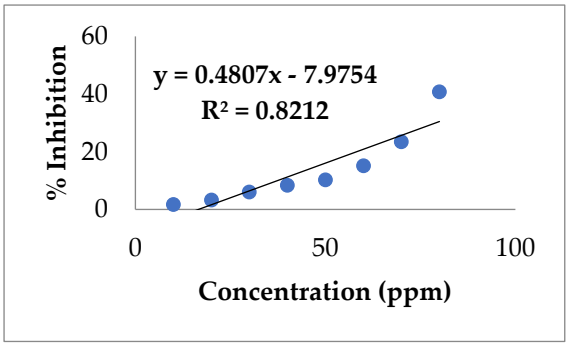

Figure 2. Determination of DPPH free radical scavenging activity of ethanol extract of binahong leaves

Based on Figure 2, the regression equation Y=0.4807x–7.9754 and the IC_{50} value is 87.423 µg/mL, which is classified as a strong antioxidant. Compared with the results of a study regarding the antioxidant activity test of the ether fraction from the hydrolysis of binahong leaf infusion, an IC_{50} was obtained of 249.31 ppm, which means that the antioxidants are fragile [18]. Meanwhile, the antioxidant activity test of binahong leaf ethanol extract with the maceration method obtained an IC_{50} of 40.27 ppm, which is classified as a powerful antioxidant [14].

Conclusions

Based on the research results, it can be concluded that the phytochemical test showed that the ethanol extract of binahong leaves contained phenolic compounds, flavonoids, alkaloids, and tannins. The ethanol extract of binahong leaves has a strong antioxidant activity with an IC_{50} value of 87.423 µg/mL.

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