Evaluation of Antioxidant Activity of Botto-Botto Leaf Fraction (Chromolaena Odorata L.) Using DPPH and ABTS Methods

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Abstract

Background : Antioxidants are substances that can inhibit the oxidation process, so that they have a protective effect on cells from the dangers of free radicals. One of the plants that has activity as natural antioxidants is Botto-Botto leaves (Chromolaena odorata L.). **Aim:** This study aims to determine the antioxidant activity of the Botto-Botto leaf fraction using the DPPH and ABTS methods. **Method:** The leaves of Botto-Botto (Chromolaena odorata L.) obtained from Takalar District, South Sulawesi are sorted, dried and pollinated. The powder was immersed in 70% ethanol solvent and sonicated. Furthermore, the extraction was carried out by maceration method with 70% ethanol solvent and fractionated using water, n-hexane, and n-butanol as solvents and dried. The fractionation results were continued for antioxidant testing using the DPPH and ABTS methods. **Result:** The n-butanol fraction of Botto-Botto leaves had the highest antioxidant activity, namely the strong active category. In the DPPH and ABTS methods, the n-butanol fraction has IC50 values of 33.535 μg / mL and 60.885 μg / mL, respectively. **Conclusion:** The DPPH and ABTS methods, the n-butanol fraction of botto-botto leaves have strong antioxidant activity compared to other fractions, namely the water fraction and the n-hexane fraction..

Keywords: Antioxidant, Botto-Botto (*Chromolaena odorata* L.), DPPH, ABTS,

INTRODUCTION

The medicinal properties of plants are caused by the active chemical elements present in various plant parts. This chemical element has a physiological effect on the human body. The most important bioactive compounds from plants are alkaloids, flavonoids, tannins, and phenolics. Phytochemical research based on ethnopharmacological information is generally considered a practical approach to discovering new antioxidants from higher plants [1]–[3].

Antioxidants are broadly defined as substances that inhibit the oxidation process from protecting cells from the dangers of free radicals that arise from body metabolism or other external factors. Chemically, natural antioxidants found in plants and foodstuffs are mainly derived from phenol-derived compounds such as flavonoids (quercetin) [4]–[6]. *Chromolaena odorata* (Family: Asteraceae) is synonymous with *Eupatorium odoratum*, a traditional medicinal plant widely used for its wound healing properties. Some parts of this plant have been used to treat wounds, burns, and skin infections [7]. Also, this plant has been shown to have an anticancer, antidiabetic, antihepatotoxic, anti-inflammatory, antimicrobial, and antioxidant profile [8]. The phytochemical components are alkaloids, flavonoids, flavanones, essential oils, phenolics, saponins, tannins, and terpenoids. Other important constituents of this plant are Eupolin, chromomoric acid, quercetin, and quercetin. The efficiency of wound healing comes from the drug or plant's antioxidant properties, which increase the preservation of fibroblasts and the proliferation of keratinocytes in the wound [9]–[11].

Oxidation is an essential process in living things. Free radicals are produced from metabolic pathways or environmental sources that interact continuously with biological systems. Reactive oxygen species (ROS) are the most significant primary catalysts that initiate the oxidation process in vivo and in vitro and generate oxidative stress [12]. Oxidative stress products when a reactive form of oxygen is produced more rapidly and can be neutralized with antioxidants [13], [14]. The potential antioxidant effects of these plants are significant. It is shown that evidence suggests that oxidative stress is induced by reactive oxygen species (ROS). ROS plays a vital role in the etiology and development of several human diseases. Oxidative stress is one of the reasons that is thought to be involved in almost every disease process. Free radicals are the leading cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus, and cancer [15].

The need for antioxidants is increasing due to the development of various diseases that require appropriate therapy and do not cause dangerous side effects. The discovery of the antioxidant profile can be scientific evidence for developing natural ingredients into a solution for a disease or metabolic disorder in humans. This study aims to determine the antioxidant activity of the Botto-Botto leaf fraction using the DPPH and ABTS methods.

METHODS

Design, Place, and Time

This research was laboratory research with qualitative and quantitative approaches. This research was designed as pure experimental research (True Experimental). Post-test only control design was carried out from May 2019 to September 2019 in the Laboratory, the Botanical Field Herbarium Center for Botany Biological Research Center of the Indonesian Institute of Sciences (LIPI) Bogor, and Biopharmaca Laboratory (Research Activity Center) Hasanuddin University Makassar.

Instruments and Materials

The tools used are maceration tools, test kits for chemical content, glass tools, UV-Vis spectrophotometer (Agilent®), ELISA reader (Biotek®), analytical scales. The materials used were the leaves of Botto-Botto (*Chromolaena odorata* L.), ethanol (Merck), N-hexane (Merck), Aquadest (Merck), ascorbic acid (Merck), 1-diphenyl-2-picryl-hydrazyl (DPPH)

(Sigma-Aldrich), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid diammonium salt) (ABTS) (Sigma-Aldrich),

Research Procedure

Plant determination

Plant determination was carried out at the Botanical Field Herbarium Bogoriense Center, Center for Biological Research, the Indonesian Institute of Sciences (LIPI) Bogor. All parts of the Botto-Botto plant (*Chromolaena odorata* L.) were used in the determination process.

Sample processing

Extraction and Fractionation

The leaves of Botto-Botto (*Chromolaena odorata* L.) obtained from Takalar District, South Sulawesi, are cleaned, dried, and pollinated. The powder was immersed in 70% ethanol solvent and sonicated using a sonicator (Elma®) for 15 minutes. Extraction was carried out with 70% ethanol solvent three times until the filtrate became clear. The concentrated filtrate was evaporated at a rotary evaporator and dried using desiccator. The extract was then fractionated using water, n-hexane, and n-butanol solvents and dried using a freeze dryer Lyophilizer (Büchi L200, Germany).

Phytochemical Screening of Botto-Botto Leaf Extract Fraction (Chromolaena odorata L.)

Screening using the TLC method with 10% AlCl3 spray reagent for identification of flavonoid compounds and 10% FeCl3 for identification of phenolic compounds. The eluent used was ethyl acetate: methanol with a ratio of 5: 1. The spots were observed using UV 254 nm and 366 nm.

Quantitative test of chemical content of Botto-Botto (*Chromolaena odorata* l.) Leaf extract fraction

Total flavonoid levels were tested using quercetin standards and total polyphenol levels using gallic acid standards.

Antioxidant Activity Test of Botto-Botto Leaf Extract Fraction

DPPH Methods

Preparation of DPPH solution

Weighed 8 mg DPPH put in a brown bottle then dissolved in 50 ml ethanol p.a. After DPPH dissolves, it was wrapped in aluminum foil and stored in a dark place away from sunlight.

Preparation of Ascorbic Acid Solution (Positive Control)

Ascorbic acid dilution was made of 1, 2, 3, 4, 5 μg / mL and put in the vial, then dissolved p.a ethanol gradually until the quercetin dissolves. Wrapped in aluminum foil and stored in a dark place away from sunlight.

Antioxidant Activity Test

Determination of the ability of free radical scavenging from leaf extract was carried out by using the DPPH α-diphenyl-β-picryl-hydrazine test. This method is based on reducing DPPH in a methanol solution in the presence of the donation of antioxidant hydrogen to form non-radical DPPH-H. The reduction of free radicals results in color changes as measured by spectroscopy. The reaction mixture (3.0 mL) consisted of 1.0 mL of DPPH in methanol (0.3 mM), 1.0 mL of extract and 1.0 mL of methanol. The reaction mixture was incubated for 10 minutes in the dark before absorbance was measured at a wavelength of 516 nm using ascorbic acid as a comparison. Five concentrations were made for each fraction and measured in triplo.

Antioxidant activity was expressed by the percentage of free radical binding with the formula:

free radical binding =((blank absorption - sample absorption)/(blank absorption)) * 100%

ABTS Methods

The samples were diluted to yield five concentrations suitable for calculating IC50. Into the wellplate was inserted into the sample (five concentrations), added with ABTS 100 μL solution and the volume was sufficient to 200 μL with ethanol p.a so that the concentration of each sample fraction μg / mL was obtained, then incubated for 30 minutes at room temperature. The absorbance is read at a maximum wavelength of 650 nm and the percentage of inhibition is calculated. Testing of each sample concentration was carried out triplo. The inhibition is calculated according to the equation:

% ABTS radical binding = ((control absorption – sample absorption) / (control absorption)) * 100%

RESULTS

Table 1. Identification Results of Botto-Botto Leaf Fraction Compound Components

Compound	**Information**		
	Water fraction	**n-butanol fraction**	**n-hexane fraction**
Phenolic	+	+	+
Flavonoids	+	+	+

Note: Present (+), Absent (-)

Identification of Botto-Botto leaves contained Phenolic and Flavonoids in 3 samples which were checked with positive results (Table 1). The quantitative test of the total flavonoid content used the standard quercetin standard using five concentrations, namely 2, 4, 6, 8 and 10 μg / mL (table 2) with a correlation R2 of 0.9963 (Figure 1). The polyphenol content test used the standard gallic acid standard using six concentrations, namely 2, 4, 6, 8, 10, 12 μg / mL (table 4) with a correlation of R2 of 0.996 (Figure 2)

Table 2. Flavonoid Testing Standards

Samples	**Concentration (μg/mL)**	**Absorbances**
Blank	0.0	0.000
Quercetin 1	2.0	0.251
Quercetin 2	4.0	0.408
Quercetin 3	6	0.606
Quercetin 4	8	0.831
Quercetin 5	10	1.071

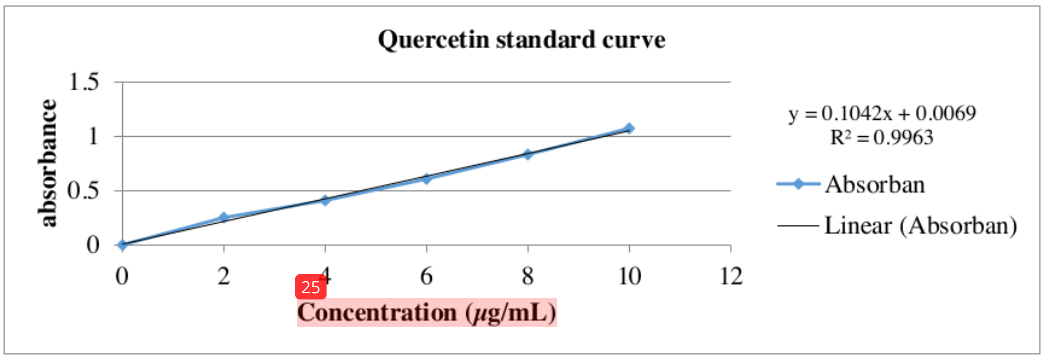

Figure 1. Quercetin Standard Curve for Testing of Flavonoids

Table 3 Levels of Total Sample Flavonoids

Samples	**Absorbances**	**Flavonoid concentration (μg/mL)**	**Sample weight (mg)**	**Dilution factor**	**Flavonoid levels (%)**	**Average Flavonoid levels (%)**
Ethanol Extract	0,280	2,618	10,1	10,00	2,59	2,70
Ethanol Extract	0,307	2,877	10,3	10,00	2,79	
Ethanol Extract	0,290	2,713	10,0	10,00	2,71	
n- Hexan fraction	0,102	0,915	10,1	10,00	0,91	0,92
n- Hexan fraction	0,103	0,922	10,2	10,00	0,90	
n- Hexan fraction	0,108	0,967	10,2	10,00	0,95	
n-Butanol fraction	0,785	7,470	10,1	10,00	7,40	

n-Butanol fraction	0,742	7,051	10,3	10,00	6,85	7,06
n-Butanol fraction	0,745	7,081	10,2	10,00	6,94	
Water fraction	0,104	0,931	10,1	10,00	0,92	1,00
Water fraction	0,116	1,043	10,3	10,00	1,01	
Water fraction	0,118	1,065	10,0	10,00	1,07	

Table 3 shows that the total flavonoid level in the sample shows that the highest level is in the n-butanol fraction with an average level of 7.06%. In the ethanol extract of 2.70%, followed by the water fraction of 1% and the smallest in the n-hexane fraction of 0.92%. This test is carried out in triplo.

Table 4 Polyphenol Testing Standards

Samples	**Concentration (μg/mL)**	**Absorbance**
Blank	0.0	0.000
Gallic acid 1	2.0	0.179
Gallic acid 2	4.0	0.409
Gallic acid 3	6.0	0.558
Gallic acid 4	8.0	0.686
Gallic acid 5	10.0	0.887
Gallic acid 6	12.0	1.074

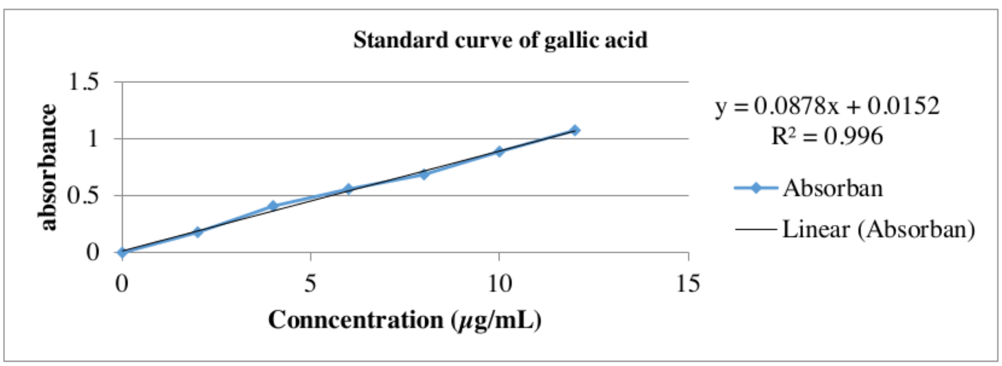

Figure 2. Standard Curves of Gallic Acid for Polyphenols

Table 5 Total Polyphenol Levels in Samples

Samples	**Absorbances**	**Polyphenol concentration (μg/mL)**	**Sample weight (mg)**	**Dilution factor**	**Polyphenols levels (%)**	**Average polyphenol levels (%)**
Ethanol Extract	0,571	6,334	10,1	16,67	10,45	10,56
Ethanol Extract	0,591	6,558	10,3	16,67	10,61	
Ethanol Extract	0,574	6,363	10,0	16,67	10,61	

n- Hexan fraction	0,748	8,353	10,1	16,67	13,78	14,30
n- Hexan fraction	0,818	9,153	10,2	16,67	14,96	
n- Hexan fraction	0,776	8,672	10,2	16,67	14,17	
n-Butanol fraction	0.97	10.882	10.1	16.67	17.96	18,08
n-Butanol fraction	0.991	11.119	10.3	16.67	17.99	
n-Butanol fraction	0,998	11.200	10.2	16.67	18.30	
Water fraction	0,634	7,055	10,1	16,67	11,64	11,87
Water fraction	0,661	7,353	10,3	16,67	11,90	
Water fraction	0,651	7,248	10,0	16,67	12,08	

The highest Polyphenol content was found in n-butanol fraction with an average of 18.08 %, followed by n-hexane fraction of 14.30%, water fraction of 11.87% and the lowest was ethanol extract of 10.56%. The results of this study were expressed in antioxidant activity in the form of inhibitor concentration (IC50). The antioxidant activity of Botto-Botto leaves based on the DPPH and ABTS methods are described in the following table 6 and 7:

Table 6. IC_{50} value on the DPPH Method

Samples	**IC_{50} (μg/mL)**
Water fraction	47.232
N-Heksan fraction	80.249
N-Butanol fraction	33.535
Ascorbic acid	3.905

The results of the Antioxidant activity (IC_{50}) with the DPPH method were the highest in n-butanol fraction (33,535 μg/mL) (Table 6).

Table 7 IC_{50} value on the ABTS Method

Samples	**IC_{50} (μg/mL)**
Water fraction	114.233
N-Heksan fraction	156.037
N-Butanol fraction	60.885
Ascorbic acid	4.869

The results of the Antioxidant activity (IC_{50}) with the ABTS method were the highest in n-butanol fraction (60,885 μg/mL) (Table 7).

DISCUSSION

The sample used was Botto-Botto's (Chromolaena odorata (L)) leaves, which were identified scientifically at LIPI Bogor. The type of *Chromolaena odorata* (L) R. M. King and H. Rob were included in this study. The existence of this termination was to clarify further the type of sample used in the study. The measurement of antioxidant activity was carried out on the Botto-Botto leaf extract fraction. The antioxidant activity was determined using the

DPPH (1,1-diphenyl-2-picrylhydrazine) method. DPPH acts as a free radical that will react with antioxidants to form DPPH-H (1,1-diphenyl-2-picrylhydrazine). Antioxidants will donate their hydrogen atoms to DPPH radicals to complement the electron deficiency and form more stable antioxidant radicals. Apart from DPPH, the method used in this study was ABTS [16], [17]. It is a method of determining antioxidant activity obtained from potassium oxidation persulfate with diammonium ABTS salt. In this method, the antioxidant mechanism occurs by donating proton radicals. The presence of the sample's antioxidant activity is indicated by the loss of blue color in the ABTS reagent [18]. These two analysis methods are common tests for antioxidant measurements. The advantage of ABTS over DPPH is the higher sensitivity to ABTS and can be applied for testing food antioxidants.

Flavonoids contain a conjugated aromatic system that will show a strong absorption band in UV and visible light. In the TLC analysis and the appearance with AlCl3 10% flavonoid reagent will appear yellow spots and depending on the structure. For phenolic compounds, a bluish black spot will appear at 366 nm UV which indicates a positive result. Phytochemical screening of samples of n-hexane fraction, n-butanol fraction and water fraction showed positive results on the flavonoid compound test. and phenolic. Meanwhile, the total flavonoid and total polyphenol content test showed that the highest levels were in the n-butanol fraction, respectively, namely 7.06% and 18.08%. Flavonoids and polyphenols are closely related to the antioxidant effect shown by the leaves of Botto-Botto. The antioxidant capacity of these compounds is closely related to electron donation. The electron donation activity reflects the reducing power of bioactive compounds, which means that it is related to antioxidant activity. These results indicate the potential of the n-butanol fraction in its role as an antioxidant that can reduce free radicals.

Measurement of antioxidant activity was carried out on n-hexane fraction, n-butanol fraction and water fraction from botto-botto leaves. In the DPPH method, the measurement of the antioxidant activity of the botto-botto leaf extract fraction which is included in the strong active antioxidant group (IC50 value range 10-50 μg / mL) is an IC50 value of 47,232 μg / mL in the water fraction and 33,535 μg / mL in fraction n -butanol while the n-hexane fraction is moderately active (range of IC50 values 50 - 100 μg / mL) with an IC50 value of 80.249 μg / mL.

The test using the ABTS method, the IC50 value with an IC50 value range of 50-100 μg / mL was included in the strong antioxidant category. The n-butanol fraction includes strong antioxidants with an IC50 value of 60.885 μg / mL, the water fraction IC50 value of 114.233 μg / mL is included in the moderate antioxidant activity group (IC50 value range

101-150 μg / mL), and the h-hexane fraction IC50 value is 156.037 μg / mL including the group of weak antioxidant activity (range IC50 values 150-200 μg / mL). The test results from DPPH and ABTS show that botto-botto leaves have the potential as a source of natural antioxidant compounds and the n-butanol fraction has stronger antioxidant activity than other fractions, namely the n-hexane fraction and the water fraction because it has an IC50 value that is in the range. strong category. Furthermore, phytochemical screening was carried out to screen Botto-Botto leaves' content. From this process, alkaloids, flavonoids, polyphenols, coumarins, and organic compounds were obtained. Alkaloids, flavonoids, and polyphenols are closely related to the antioxidant effect shown by Botto-Botto leaves. The antioxidant capacity of these compounds is closely related to electron donation. The presence of electron donation activity reflects the reduction power of bioactive compounds, which means that it is related to antioxidant activity. It has been found in many previous studies [19], [20] . With the results shown, the Botto-Botto leaves have the potential as antioxidants. The limitation of this research is that in this study the isolation of pure compounds has not been carried out so that it is not known what flavonoid compounds act as antioxidants and their antioxidant ability is tested to be compared with other fractions..

CONCLUSION

The results showed that the n-butanol fraction from the leaf extract of Botto-Botto (Chromolaena odorata L) had the highest antioxidant activity compared to the n-hexane fraction and water fraction, where in the DPPH method the IC50 value of the n-butanol fraction was 33.535 μg / mL and in the ABTS method the IC50 value was 60.885 μg / mL. This antioxidant property is influenced by the presence of flavonoid and phenolic compounds in the leaves of the botto-botto. Therefore, this n-butanol fraction has the potential as a source of natural antioxidants used in pharmaceutical preparation formulations

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Conflict of Interest

There is no conflict of interest in this study.

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