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Antioxidant Activity Of Leaf Ethanol Extract And Balm Plant Sticks (*Polygala paniculata L.)*

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Abstract

This research aims to determine the antioxidant activity of the leaves and stems of the balsam plant (Polygala paniculata Linn). The balsam plant (Polygala paniculata Linn) is a small shrub that grows wild and is utilized by the community in Bonelemo Village, Bajo Barat Sub District, Luwu Regency, South Sulawesi, as a traditional medicine for removing black spots on the face. One of the compounds contained in the balsam plant is flavonoids, which have the potential as antioxidants. The procedures carried out in this research include sample preparation, sample extraction using 96% ethanol solvent, macerate concentration, and subsequently conducting antioxidant activity tests using the DPPH method. The results of antioxidant activity testing of the leaves and stems of the balsam plant (Polygala paniculata Linn) obtained an IC50 value of 44,987 µg/mL. Based on this research, the leaves and stems of the balsam plant (Polygala paniculata Linn) are categorized as containing very strong antioxidant components.

Keywords: *Antioxidant; DPPH; Polygala paniculata Linn*

Introduction

Indonesia has various kinds of medicinal plants spread throughout its regions. The use of medicinal plants has also been carried out traditionally since ancient times. The community uses medicinal plants because of previous parents' habits and because they are easy to find. Many medicinal plants around the community continue to be used for various diseases, from minor illnesses to severe illnesses like cancer. Medicinal plants are used for external wounds by placing them directly on the wound, while internal wounds are usually by consuming boiled water from medicinal plants (Azmin & Rahmawati, 2018).

The role of traditional medicine is vital in maintaining health, treating disease, and increasing stamina for the body (Nababan et al., 2020). One plant that can be used for health is the balsam plant (Polygala paniculata Linn). Another name for the balsam plant is vetiver because when the roots are removed, they emit a fragrant aroma. However, because the aroma emitted is like balsam, this plant is better known as the balsam plant. This plant is wild, so it is often considered a nuisance to plants in people's gardens. The size of a small bush makes this plant easier to eradicate.

Balsam plants have potential in the pharmaceutical field as cytotoxic, antimycotic, and antibacterial drugs (Rijai, 2013). Research by Simanjuntak

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(2020) explains that balsam plants contain secondary metabolite compounds: flavonoids, alkaloids, saponins, tannins, and triterpenoids. The balsam plant (Polygala paniculata L.) in Buntulemo Hamlet, Bonelemo Village, West Bajo District, Luwu Regency, is a traditional medicine to remove black spots by rubbing the leaves on the facial area when sweating. Black spots occur due to the presence of free radicals in the body. Free radicals can be overcome using antioxidant compounds. Simanjuntak (2012) stated that the flavonoid compounds in plants act as antioxidants. This indicates that balsam plants have the potential to act as antioxidants. However, so far, there has been no research regarding the antioxidant activity of this plant. Therefore, research was carried out to test the antioxidant activity of balsam plant leaves (Polygala paniculata L.) using the DPPH method. DPPH (1,1 diphenyl-2-picryhydrazyl) is a stable radical compound. DPPH is used to determine antioxidant activity through its ability to capture free radicals. The DPPH method is a simple, fast, easy, and sensitive analysis method for samples with small concentrations (Wulansari, 2018). In this study, the plant part used was a mixture of leaves and stems because the leaves of the balsam plant (Poligala paniculata L.) are tiny, making it difficult to separate them from the stem.

Method

Research Location and Time

This research was conducted at the Natural Materials Laboratory, Faculty of Science, Cokroaminoto Palopo University. The sampling location was Bonelemo Village, West Bajo District, Luwu Regency. This research was conducted from June to August 2023.

Tools and materials

The tools used in this research are a rotary evaporator, sieve, stirring rod, blender, glass funnel, Erlenmeyer, beaker, measuring cup, electric stove, cuvette, analytical balance, measuring pipette, test tube rack, test tube spatula, thermometer, jar, and UV-Vis spectrophotometer.

The materials used are aluminum foil, distilled water, leaves and stems of the balsam plant (Polygala paniculata L.), 96% ethanol, $FeCl_3$, concentrated HCl, label paper, filter paper, methanol, Libermann-Burchard reagent, Wagner reagent, plastic wrap, DPPH powder (1,1-diphenyl-2-picylhydrazyl), and Mg powder.

Making Powdered Leaves and Stems of Balsam Plants

The balsam plants obtained are cleaned of adhering dirt and then washed thoroughly in running water. Once clean, the leaves and stems are taken by cutting them into small pieces and mashing them.

Extraction of Leaves and Stems of Balsam Plants using the Maceration Method

The simplicia leaves and stems of the balsam plant were weighed as much as 150 g and then put into a maceration container. Then, add 750 mL of 96% ethanol and stir for 30 minutes. Next, macerate the sample for 3x24 hours. The filtrate is then concentrated to obtain a thick extract of the leaves and stems of the balsam plant.

Phytochemical Test

The leaf and stem extracts of the balsam plant (Polygala paniculata Linn) were tested for phytochemistry by adding a reagent to each compound. The secondary metabolite compounds that will be tested are as follows.

a. Flavonoid test

A total of 2 mL of the extract solution was pipetted into a test tube and then heated for 5 minutes. Then, add enough Mg powder and five drops of 2 N HCl and shake. A positive test for flavonoids is indicated by the formation of a red, yellow, or orange color (Octaviani et al., 2019)

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b. Alkaloid test

A total of 2 mL of the extract solution was pipetted into a test tube, and then 1 mL of 2 N HCl and 6 mL of distilled water were added. Next, it was heated for 2 minutes, and Wagner's reagent was added. A positive alkaloid test is indicated by forming a white or cream-colored precipitate (Kumalasari & Andiarna, 2020).

c. Saponin test

Two mL of the extract solution were pipetted into a test tube and heated for two minutes. Then, five drops of concentrated HCl were added. A positive saponin test is indicated by the formation of permanent foam for one to fifteen minutes.

d. Tannin Test

Two mL of the extract solution were pipetted into a test tube and reacted with iron (III) chloride. A positive test is indicated by dark blue or greenish black (Simanjuntak & Gurning, 2020).

e. Steroid and Terpenoid Test

A total of 2 mL of the extract solution was added with Libermann-Burchard reagent (sulfuric acid and acetic anhydride). A bluish-green color change characterizes a positive steroid test, while a positive terpenoid test will form a brownish ring (Tukiran et al., 2019).

Antioxidant Testing with the DPPH Method

a. Preparation of DPPH Mother Solution

A total of 10 mg of DPPH (1,1-diphenyl-2-picryhydrazyl) was dissolved in 100 mL of methanol and then homogenized until a concentration of 100 ppm was obtained. The DPPH solution must be made in a room that is not exposed to sunlight.

b. Determination of DPPH Maximum Wavelength

A total of 1 mL of 100 ppm DPPH solution was put into a test tube, and 4 mL of methanol was added. Homogenize, then incubate in a water bath at 37°C for 30 minutes. Next, measure the maximum wavelength and absorbance at a wavelength of 505-525 nm.

c. Preparation of Blank Solution

5 mL of 100 ppm DPPH solution was put into a volumetric flask, and 25 mL of methanol was added. Then, the absorbance was measured using UV-Vis.

d. Preparation of Test Solutions for Balsam Plant Leaf and Stem Extracts

10 mg of balsam plant (Polygala paniculata L.) leaf and stem extract was dissolved using 20 mL of ethanol to obtain a concentration of 500 ppm as a stock solution. The standard solution is made into several concentrations, namely 0 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm (0 mL; 0.5 mL; 1 mL; 1.5 mL; 2 mL; 2.5 mL). Next, the concentration of the standard solution was put into a 25 mL volumetric flask, and methanol was added to the mark. 4 mL of the standard solution from the concentration series was pipetted into a test tube, and 1 mL of 100 ppm DPPH was added (Kiromah et al., 2021). Homogenize the mixture, then leave it for 30 minutes so that the reaction between the sample and the DPPH solution occurs ideally. Measure the absorbance using UV-Vis.

Data Analysis

Data from the absorbance results of each sample will be used to find the % inhibition. The formula for finding inhibition is:

% inhibition: $\frac{Abs.\ Blanko - Abs.\ Sample}{Abs.\ Blanko}$ x 100% ……(1)

Details

Abs. Blanko = Absorbance on DPPH without sample

Abs. Sample = Absorbance of DPPH after adding sample

Then, create a curve of the relationship between sample concentration and % inhibition, determine the linear equation, and then obtain the linear regression equation y=ax+b

The resulting linear equation obtains the IC_{50} value where the y value is 50. The formula obtained is as follows.

50 = ax+b ……(2)

$x = \frac{50-b}{a}$ ……(3)

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Details:
y= % inhibition
x= Concentration (µg/mL)
a= Slope
b= Intersep

Results and Discussion

This research uses balsam plants (*Polygala paniculata L.*). The extraction method used is direct maceration with 96% ethanol. This method does not require heating, so there is little chance of damage to a compound. Apart from being cheap and easy to do, through maceration, there is a difference in pressure inside and outside the cell, such that the cell walls and membranes break, and the secondary metabolite compounds in the cytoplasm are dissolved in the organic solvent. The solvent used in the maceration process is 96% ethanol because it has a reasonably high polarity and easily dissolves the appropriate compounds. The higher the level of solvent polarity, the higher the yield obtained (Agustein & Susanti, 2021).

The results of the maceration of the leaves and stems of the Balsam Plant (Polygala paniculata Linn) are then filtered. The maserate obtained was then concentrated using a rotary evaporator. After the thick extract was obtained, a phytochemical test was carried out on the ethanol extract of the leaves and stems of the balsam plant (Polygala panicullata L.). The phytochemical test in this study aims to identify secondary metabolite compounds such as flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. The phytochemical test results of the leaf and stem extracts of balsam plants are in Table 1.

Table 1. Results of phytochemical tests on leaves and stems of balsam plants

Compound	Result
Flavonoid	**+++**
Alkaloid	**+**
Saponin	**-**
Tanin	**+++**
Steroid	**++**

The antioxidant activity test of the leaves and stems of the Balsam Plant (*Polygala paniculata L.*) was carried out using the DPPH method with UV-Vis spectrophotometry. The leaves and stems of Polygala paniculata Linn contain flavonoid compounds. The presence of flavonoid compounds in plants can provide an electron donor or a hydrogen atom to free radicals, thereby stopping the initial stage of the reaction (Sukarti & Hasanah, 2018). Flavonoids can inhibit lipid peroxidation, inhibit several enzymes' activity and tissue damage caused by free radicals (Sukarti, 2016).

The test sample was diluted to several concentrations, and its absorbance was measured at a wavelength of 510 nm. The absorbance of the blank was obtained at 0.206, while the absorbance of each sample concentration is in Table 2.

Table 2. Results of absorbance measurements for each sample concentration

Sample Concentration (ppm)	Absorbance
0	0,206
10	0,166
20	0,147
30	0,132
40	0,116
50	0,096

The absorbance of the test solution is measured to calculate the % inhibition value. The inhibition percentage is the extent of the sample's ability to reduce free radicals (Hartini, 2020). These values indicate a decrease because some samples have reacted with DPPH. The relationship between sample concentration and absorbance is in Figure 1.

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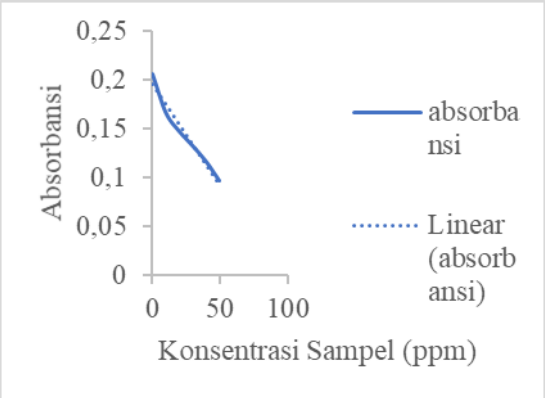

Figure 1. Relationship curve between sample concentration and absorbance

Based on equation (1), Table 3 shows the per cent inhibition value for each concentration obtained from the calculation between the blank's absorbance and the sample's absorbance.

Table 3. per cent inhibition value for each sample concentration

Sample Concentration (ppm)	Inhibition (%)
0	0
20	19,417
20	28,640
30	35,922
40	43,689
50	53,398

The data obtained shows an increase in the per cent inhibition value. The higher the sample concentration, the greater the per cent inhibition. This happens because the active substances from the leaf and stem extract of Polygala paniculata Linn increasingly oxidise free radicals (Indrawati et al., 2022). Kamoda et al. (2021) also stated that the inhibitory power against DPPH increases as the sample concentration increases.

Each sample concentration's per cent inhibition value was then made into a linear regression curve to obtain the line equation y=ax+b. This equation is used to calculate the IC_{50} value. The curve of the relationship between concentration and per cent inhibition can be seen in Figure 2.

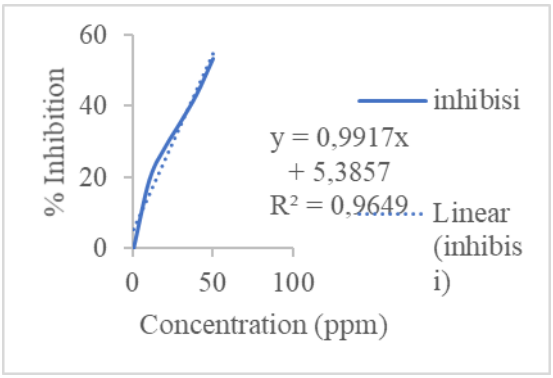

Figure 2. Relationship curve between sample concentration and per cent inhibition

Determining the IC_{50} value is used to determine the amount of extract that can inhibit free radicals by 50% (Souhoka et al., 2019). Anwar and Triyasmono (2016) stated that the smaller the IC50 value, the stronger the antioxidant activity. From the curve shown in Figure 2, the equation y=0.9917x + 5.3857 is obtained so that the IC50 value is obtained through equations (2) and (3). Based on the calculation results, the IC_{50} value of Polygala paniculata Linn leaf and stem extract was 44.987 µg/mL. This value indicates that the leaf and stem extract of Polygala paniculata Linn is a powerful antioxidant. The leaves and stems of the balsam plant have better antioxidant capacity than ganitri leaves in research by Kiromah et al. (2021), with an IC_{50} value of 54.12 µg/mL, classified as a potent antioxidant. The antioxidant capacity of each plant can be different because the content of secondary metabolite compounds contained in plants is different, especially in the content of phenolic compounds, flavonoids and tannins, which are responsible for supporting antioxidant compounds (Supriatna et al., 2019). Antioxidant compounds with an IC_{50} value of less than 50 µg/mL are classified as very strong, an IC_{50} with a value of 50-100 µg/mL is classified as vital, an IC_{50} with a value of 100-150 µg/mL is classified as moderate, and an IC_{50} with a

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value of 151-200 µg/mL is classified as weak. (Salim, 2018).

extract of *Semambu rattan* also cannot be said to be weak because it has a strong ability as an antioxidant if we look at the ability of its servants. Therefore, methanol extract of young rattan bark can be used as a natural antioxidant alternative. In addition, it is hoped that further research will be able to isolate and further research on the skin of *Semambu rattan* rods.

Conclusion

Based on the results of research testing the antioxidant activity of the leaves and stems of the balsam plant (Polygala paniculata Linn) using the DPPH method which has been carried out, it can be concluded that the IC_{50} value obtained from the leaf and stem extract of the balsam plant is 44.987 µg/mL and is categorized as a powerful antioxidant.

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