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ANTIOXIDANT ACTIVITY TEST OF GUAVA LEAVES ETHANOL EXTRACT (*Syzygium aqueum Burm F*) WITH DPPH METHOD (1,1-Diphenyl-2-picrylhydrazil)

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ABSTRACT

Free radicals are compounds that can cause damage to human body cells, which can be prevented with antioxidants. Antioxidants can be found in several natural ingredients, such as water guava leaves. This study aims to determine the antioxidant activity of an ethanol extract from water guava leaves (*Syzygium aqueum Burm F*). The water guava leaves used came from the Kempek Palimanan Cirebon area. Extraction was carried out using the maceration method using a 96% ethanol solvent until a concentrated extract was obtained. The extract was identified for secondary metabolite content using a reagent test. An antioxidant activity test was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Analysis of the strength of antioxidant activity is carried out by calculating the IC_{50} value, which is based on the percent inhibition of free radicals by the test sample by comparing Vitamin C. Identification of active compounds in the concentrated ethanol extract of water guava leaves indicated the presence of flavonoids, alkaloids, saponins, and tannins. The antioxidant activity of an ethanolic 96% extract of water guava leaves (Syzygium aquarium) in this study had an IC_{50} value of 6.89 ppm, which shows very strong antioxidant activity in warding off free radicals.

Keywords: antioxidants, water guava leaves, DPPH

INTRODUCTION

Cell and tissue damage can trigger the emergence of degenerative diseases (Susantiningsih, 2015). Antioxidants are compounds that can prevent or inhibit oxidation reactions that damage human body cells. This compound can be found in various types of food, such as fruit, vegetables, grains, and fish oil (Dewi, 2012). The development of science regarding bioactive-based medicine from plants has increased rapidly. More and more researchers are exploring medicinal plants to find out the various types of bioactive compounds contained in them and their benefits for improving the quality of human life. To date, many medicinal plants have been proven to treat disease (Indrawati, 2013). One of them is the water guava plant (*Syzygium aqueum*).

Research on water guava leaves extract conducted by Soetikno *et al.*, (2019) has shown that water guava leaves extract has antioxidant activity. Water guava leaves (*Syzygium aqueum*) have also long been used in traditional medicine because they contain many bioactive compounds that have various health benefits. Research by Albab *et al.*, (2018) has also shown that the antioxidant activity of water guava leaves extract has an IC_{50} value of 41.01 ppm. In addition, Pandey *et al.*, (2020) have shown that water guava leaves extract contains several compounds, such as flavonoids and phenolic acids, which have strong antioxidant activity. Another study by Sim *et al.*, (2019) has shown that water guava leaves extract has strong antioxidant activity, with the ability to inhibit lipid peroxidation and protect cells from oxidative damage. Apart from being a medicine, water guava leaves can also be used as a source of natural antioxidants.

RESEARCH METHODS

Tools and Materials

The tools used in this research were UV-spectrophotometers. Vis (Perkin Elmer Lambda 25), shaker, measuring flask, Erlenmeyer flask, *analytical balance* (Ohaus), volume pipette, maceration bottle, *rotary evaporator* (Heidolph Laborota 4000), glassware (Pyrex), oven. Meanwhile, the materials used include freshwater guava leaves obtained from Kempek Village, Cirebon, West Java, ethanol 96% (Bratachem), ascorbic acid, acetic acid, distilled water, HCl, $FeCl_3$ 0.1%, dragendorff, and DPPH (*1.1-diphenyl-1-picrylhydrazyl*).

Research Procedure

A. Plant Determination

The determination of water guava leaves plants was carried out at the IAIN Syekh Nur Jati Cirebon Laboratory. The results of determining the plant used in this research were water guava leaves (*Syzygium aqueum*) from the *Myrtaceae family*.

B. Sample Preparation

Fresh young leaves are selected, then the water guava leaves are washed with running water, drained, and placed in an oven at 50°C (Budiarti *et al.*, 2014). The dried leaves were blended until smooth and sieved using a 40-mesh sieve (Albab *et al.*, 2018).

C. Extract Preparation

Extract preparation was carried out using the maceration method using 96% ethanol solvent. Water guava leaves powder is soaked in a maceration bottle with 96% ethanol for 5 days and stored in a closed vessel with stirring at least once a day. In the filtering process using a paper filter, the filtrate, and residue are separated and then concentrated using a *rotary evaporator* at a temperature of less than 50 ^{0}C until a thick extract is obtained (Budiarti *et al.*, 2014).

D. Identification of the Secondary Metabolite Contents

1. Alkaloids. Identification of alkaloids was carried out by placing 1 gram of water guava leaves extract in a test tube and adding 5 ml of 2N HCl, heating it, then cooling it, and adding 1 ml of dragendroff reagent. If it produces a precipitate, the sample is declared positive for containing alkaloids (Ergina, 2014).
2. Flavonoids. Identification of flavonoids was carried out by placing 1 gram of water guava leaves extract in a test tube then adding 4 ml of HCl and 2 drops of H_2SO_4 and shaking vigorously. A positive sample contains flavonoids if there are very striking colors, namely yellow, red, brown, and green (Huliselan *et al.*, 2015).
3. Saponin. Saponin identification was carried out by placing 1 gram of extract in a test tube, adding 10 ml of hot water, cooling it, then shaking it vigorously for 10 seconds. It is positive for containing saponin if foam forms 1-10 cm in no less than 10 minutes and when 1 drop of 2N HCl is added, the foam does not disappear (Muthmainah, 2017).
4. Tanin. Tannin identification was carried out by dissolving 1 gram of extract in 4 ml of water, then the filtrate was taken from the dissolved extract, then 1 ml of 0.1% $FeCl_3$ was added. A positive reaction is formed, indicated by the formation of a dark blue or greenish-black color (Simaremare, 2014).

E. Antioxidant Activity Test

1. Preparation of DPPH Solution
   A 40 ppm DPPH solution is made by dissolving 4 mg DPPH powder in 100 ml of ethanol p.a. to the limit mark. The DPPH solution is kept at a low temperature and protected from light (Sinala and Dewi, 2019).
2. Determination of DPPH Maximum Wavelength
   Pipette 5 ml of 40 ppm DPPH solution then put into a test tube and add 1 ml of 96% ethanol then vortex until homogeneous, incubate for 30 minutes, then measure the wavelength in the 400-800 nm range. The results obtained will be the

maximum length and absorbance value of the DPPH solution of 40 ppm (Sinala and Dewi; Nurisyah, *et al*., 2020).

3. Determination of Antioxidant Activity of Vitamin C
   Vitamin C was used as a comparison with samples of water guava leaves extract (*Syzygium aqueum*) because it is a source of water-soluble antioxidants, is easy to obtain, and is widely consumed by the public. Make a stock comparison solution of 1000 ppm vitamin C by weighing 100 mg and dissolving it with 96% ethanol to 100 mL. Then dilution of the 1000 ppm vitamin C stock solution was carried out to produce a series of vitamin C solutions with concentrations of 2, 4, 6, 8, and 10 ppm. 1 ml of each solution was pipetted into a test tube that had been covered with aluminum foil and 5 ml of 40 ppm DPPH solution was added. The solution was incubated for 30 minutes, and then the absorbance at the maximum wavelength was measured using UV-Vis spectrophotometry (Salim, 2018; Sinala & Dewi, 2019).
4. Determination of Antioxidant Activity of Water Guava Leaves Extract
   An antioxidant activity test was carried out by making a 500 ppm stock solution by weighing 0.025 grams of water guava (*Syzygium aqueum*) leaves extract and dissolving it in a 50 ml volumetric flask with 96% ethanol. Then the stock extract solution was diluted at 500 ppm to produce a series of extract solutions with concentrations of 50, 75, 100, 125, and 150 ppm. 2 ml of 40 ppm DPPH solution was pipetted into each extract solution and then put into a test tube and covered with aluminum foil, the solution was incubated for 30 minutes, then the absorbance at the maximum wavelength was measured using UV-Vis spectrophotometry (Sinala and Dewi, 2019)

Data Analysis

Absorbance data obtained from each extract can be used to calculate % inhibition using equation 1.

$$\% \text{ Inhibition} = \frac{(\text{Blank Absorbance} - \text{Sample Absorbance})}{\text{Blank Absorbance}} \text{ X } 100$$

The determination of antioxidant activity was carried out by calculating inhibitory concentration (IC_{50}). The IC_{50} value is the concentration value of the extract and vitamin C needed to provide antioxidant activity against 50% of DPPH radicals. The IC_{50} value is obtained from the intersection of the line between % inhibition and the concentration axis, then the calculation results are entered into the regression equation y = a+ bx where the y value = 50 and the x value shows IC_{50} (Rahmawati & Fauzi 2019).

RESULTS AND DISCUSSION

Plant Determination

The identification results stated that the plant used was correct, *Syzigium aqueum* with the following identification keys: 1b, 2b, 3b, 4b, 6b, 7b, 9b, 10b, 11b, 12b, 13b, 14b, 16a.....Van steenis, (2003) De Guzman. C.C., and Sieomansma J, S (1999).

Sample Preparation

Table I. Percentage of dry weight to wet weight of water guava leaves

Wet weight (grams)	Dry weight (grams)	Yield (% w/w)
1300	300	23

The percentage of dry weight to wet weight of water guava leaves (*Syzigium aqueum*) was 1300 grams of wet weight, then dried and a dry weight of 300 grams was obtained. The yield of dry weight to wet weight was 23% (w/w).

Extract Preparation

Table II. Percentage weight of water guava leaves extract

Powder (grams)	Condensed extract (grams)	Yield (%)
100	5,02	5,02

The extract yield obtained was 5.02%, meaning that the higher the yield value, the greater the value of the macerated extract produced.

Identification of Secondary Metabolite Contents

Table III. Phytochemical Screening Test Results

Compound	References	Results
Alkaloids	There is a precipitate or the solution turns cloudy (Ergina *et al*., 2014).	Positive
Flavonoids	There are striking colors, namely yellow, red, brown, or green (Huliselan *et al*., 2015).	Positive
Saponin	Formation of stable white foam (Kasitowati *et al*., 2017)	Positive
Tanin	Formation of dark blue or greenish-black color (Simarema, 2014)	Positive

Phytochemical screening test results of the extract was carried out to determine the type of secondary metabolites contained in each sample and also to estimate what compounds have antioxidant activity. Based on the data produced, water guava leaves contain flavonoid, alkaloid, tannin, and saponin compounds.

Antioxidant Activity Test

Determination of DPPH Maximum Wavelength

Measurement of the wavelength of the DPPH solution with UV-Vis spectrophotometry yielded a maximum absorbance result of 0.196 at a wavelength of 516 nm.

Determination of Antioxidant Activity of Vitamin C

Table IV. Results of antioxidant activity test of vitamin C

Concentration	Absorbance	% Inhibition	Linear regression	IC_{50} (µg/mL)
2 ppm	0,282	62,99	y = 6,47x + 59,51	0,731
4 ppm	0,19	75,06		
6 ppm	0,157	79,39		
8 ppm	0,087	88,58		
10 ppm	0,087	88,58		

The Vitamin C absorbance values in **Table IV** were used to calculate the antioxidant activity of vitamin C as a comparison solution. The % inhibition of vitamin C was obtained from the calculation results, where the % inhibition value obtained was 2 ppm antioxidant activity. These results show that the greater the concentration of vitamin C, the smaller the percentage of extract inhibition of DPPH radicals to form a stable DPPH-H compound. The IC_{50} value of Vitamin C in this study was 0,731 which is classified as very strong.

Determination of Antioxidant Activity of Extract

Table V. Result of antioxidant activity test of water guava leaves extract

Concentration	Absorbance	% Inhibition	Linear regression	IC_{50} (µg/mL)
50 ppm	0,665	15,71	y = 6,835x + 8,635	6,89
75 ppm	0,589	23,34		
100 ppm	0,574	27,25		
125 ppm	0,554	29,78		
150 ppm	0,413	47,65		

The absorbance value obtained from the 96% ethanol extract of water guava leaves in **Table V** can be used to determine the strength of the antioxidant activity of each extract obtained from the calculation results, where the result of a concentration of 50 ppm is that the antioxidant activity is 15,71%. a concentration of 75 ppm had an antioxidant activity of 23,34%, a concentration of 100 ppm had an antioxidant activity of 27,25%, 125 ppm had an antioxidant activity of 29,78%, and finally, 150 ppm had an antioxidant activity of 47,65%.

The results obtained show that the greater the extract concentration value used in measuring the antioxidant activity of water guava leaves extract, the smaller the absorbance obtained due to the large number of DPPH radicals being reduced to DPPH-H by the extract. Salim (2018) and Nurisyah *et al.*, (2020) stated that DPPH as a free radical is stabilized by an antioxidant by releasing hydrogen atoms to form reduced and stable DPPH-H which is characterized by a change in color from purple to yellow followed by a decrease in absorbance at a predetermined wavelength.

CONCLUSION

The content of active compounds found in water guava leaves (*Syzygium aqueum*) are flavonoids, saponins, tannins, and alkaloids. The antioxidant activity value of 96% ethanol extract of water guava leaves (*Syzygium aqueum*) using the DPPH method showed activity antioxidants are very strong in warding off free radicals with an average IC of 50 average 6,89 ppm

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