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Antioxidant Activity Test of Bride's Tears (*Antigonon leptopus*) Leaves Using the DPPH Method

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Abstract

Bridal tear leaves can be used to treat various health and beauty problems because they contain various active compounds, such as flavonoids, saponins, and tannins. These compounds have antioxidant, anti-inflammatory, and antibacterial properties. This flower contains antioxidants that can help protect the skin from damage caused by free radicals. This research aims to see the effectiveness of antioxidants from bridal tear leaves. The research method involves leaf extraction using 96% ethanol and testing antioxidant activity using the DPPH method. The research results showed that the extract sample from bride's tear leaves was (IC50 – 69.7146 ug/mL). This shows that the bride's tear leaf extract is quite strong in inhibiting free radical reactions.

Keywords: Antioxidant; Etanol Extract; DPPH.

INTRODUCTION

Indonesia has a relatively high number of diabetes cases, based on data obtained in 2018 Basic Health Research (RISKESDAS) results, showing the prevalence of Diabetes Mellitus in Indonesia based on doctor's diagnosis in the population aged ≥15 years by province in 2013 by 1.5%, increasing in 2018 by 2.0%. The diversity of plant species in Indonesia has various benefits. The benefits of plants are obtained from the compounds in plants. Active plant compounds are obtained by extraction. One of the benefits of plant extracts is that they can withstand or block the occurrence of free radicals. Compounds with the ability to ward off free radicals are called antioxidants. Antioxidants are compounds that can inhibit and prevent oxidation (Simanjuntak, 2012). The way antioxidants work is by stopping free radical reactions from metabolism in the body or from the environment (Meigaria et al., 2016). Free radicals are molecules with one or more unpaired electrons.

Free radicals in the body cause cancer, stroke, heart disease, and premature aging (Rahman et al., 2016). One of the plants that can act as an antioxidant and prevent free radicals is the leaves of bridal tears (Antigonon leptopus). Bride's tears is a type of creeping herb that is also often found wrapped around tree trunks. This plant has a twisting tool that functions as a flower support. Bride's tears flowers can grow well in conditions exposed to full and direct sunlight or also in a slightly shaded place. A suitable place for planting this flower is between 1 - 1800 m above sea level (Meidita, 2023). The content of the bride's tears leaves includes Alkaloids, saponins, tannins, sterols and / or unsaturated triterpenes, flavonoids, glycosides, coumarins, phytosterols, triterpenoidal sapogenins, phenols, amino acids, steroids, xanthoproteins, carboxylic acids, quinines, resins, n-hentriacontane, ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside, kaempherol-3-glucoside along with ß-sitosterol, ß-sitosterol-glucoside, and d-mannitol (Suryowinito, 1997).

In research conducted by Lestari (2021), the content in bride's tears leaves has properties antioxidant and can inhibit diabetes. Testing the antioxidant content is needed to find out how strong the bride's tears leaves are as antioxidants using the DPPH test. The DPPH method is a method that can be used to determine the antioxidant activity in the sample to be tested by looking at its ability to counteract DPPH free radicals, the use of the DPPH test is used because it has several advantages including a simple, easy, fast, sensitive method, and requires a small amount of sample (Cavalcante, 2018). Based on the description that has been presented, it is necessary to test

antioxidants in bride's tears leaves using the DPPH method (Camero et al, 2018).

MATERIALS AND METHODS

Tools and Materials

The tools used in this study include analytical scales, blender, piper, micropipette, measuring cup, Durham tube, oven, test tube and rack, DPPH kit, sieve, aluminum foil, spectrophotometer, shaker, filter paper, erlenmayer, glass funnel, evaporator flask and rotary evaporator. The materials used include bride's tears leaves, methanol, DPPH solution and quercetin.

Procedures

A. Sample Preparation

Sample preparation begins with collecting or picking the bride's tears leaves, which is carried out in Kebonagung village, Pakisaji District, Malang Regency.

B. Sample Preparation

Sample preparation begins with collecting or picking bride's tears leaves carried out in Kebonagung village, Pakisaji District, Malang Regency.

C. Making simplisia

Fresh bride's tears leaves are first cleaned and sorted then dried using an oven at 50 degrees Celsius. After drying the sample is crushed using a blender until smooth, the bride's eye powder is then sifted to get the finest powder and check the water content. Water content in simplisia must be below 10%, which aims to avoid the rapid growth of fungi in the extract.

D. Extraction

Simplisia was weighed as much as 30 grams and then added 96% ethanol solvent in a mass-to-mass ratio with a volume ratio of 1:10. The sample was heated using a hot plate at a temperature of 40 ° C - 50 ° C for 2 hours with 130 rpm. simplisia powder was put into a maceration container and ethanol was added and then allowed to stand for 24 hours. After 24 hours continued with evaporation using a Rotary Evaporator with an rpm of 50 at 70 ° C for 1 hour to get a paste-shaped extract. The paste obtained was weighed and the result was 3.515 grams.

E. Calculation of yield

The yield is obtained by comparing the weight of the red pomegranate leaf extract and the weight of the simplisia before extraction. According to Kurniawan (2016) the calculation of yield can be calculated with the following equation:

$$Yield\ (\%) = \frac{Weight\ of\ Extract\ (g)}{Weight\ of\ Simplisia\ (g)}\ x\ 100$$

F. Preparation of DPPH

DPPH powder was weighed as much as 0.007 grams and then dissolved with 50 mL of ethanol, vortexed until dissolved. Furthermore, the DPPH solution was taken 1 mL then ethanol was added to 5 mL and allowed to stand for 30 minutes. Then a master solution was made with 50 mg of extract and 50 ml of methanol, made a solution with a concentration of 200, 100, 50, 12 and 5 ppm. Each solution was taken 1 mil and added DPPH as much as 3 ml and incubated for 30 minutes.

G. Maximum wavelength measurement

The maximum wavelength was calculated using a spectrophotometer using a comparison solution, namely quercetin. Whether it is lower or higher than the comparison solution, or even has the same antioxidant activity.

H. Antioxidant activity test of bride's tears leaf extract

The results of the maximum wavelength calculation from the spectrophotometer were analyzed using Excell to determine the sample's IC50.

RESULTS AND DISCUSSION

Extraction and Yield Calculation

Extraction of bride's tears leaf extract simplisia using maceration method obtained the yield value in Table I.

Table 1. Extraction Results and Calculation of the Yield of Bride's Tear leaves (Antigonon leptopus).

Simplisia Weight Bride's tears leaf	Extract Caracteristic		
	Extract Weight	Yield Weight	Shape, colour, and smell
30 gram	3,515 gram	11,71 gram	thick, green leafy odor

The yield value shows the bioactive value contained in plants, the higher the yield value, the higher the bioactive value of plants (Budiyanto, 2015). According to the Indonesian Ministry of Health, the yield requirement can be used if the yield value is more than 10%. Table 1 shows that the yield can be used with a value of 11, 71%.

Qualitative Test

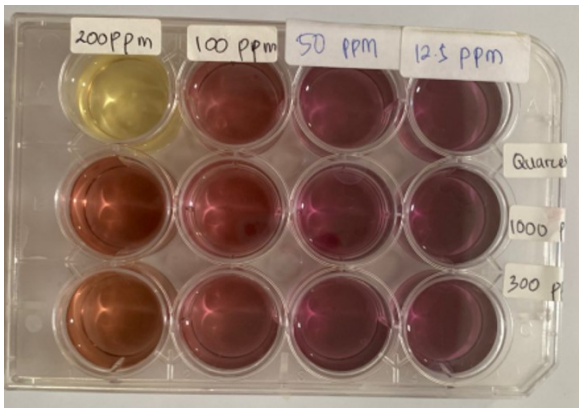

Figure 1. The result of DPPH addition to Bride's Tear Leaf Extract (Antigonon leptopus).

The clearer the color resulting from the addition of DPPH to the extract, the higher the antioxidant content in the extract. In Figure 1, it is known that the clearest color is at a concentration of 12, 5 ppm and the darkest at a concentration of 200 ppm. This color change indicates the antioxidant activity that is happening. If DPPH is reduced, it is indicated by a decrease in purplish color to yellow color due to the antioxidant activity of the extract (Miksusanti, 2012).

Antioxidant activity test

Measurement of the ethanol extract of bride's tears leaves (Antigonon leptopus) was carried out at various concentrations, namely 12.5, 50, 100, and 200 µg/mL. The results of the antioxidant activity test by looking at the results of the calculation of absorbance or absorbance carried out on a spectrophotometer were analyzed to determine the percentage of inbibition and IC50 on the leaves of the bride's tears, can be seen in Table 2.

Table 2. Analysis of activity test and IC50 on bride's tear leaves (Antigonon leptopus).

Concentration	Review	Absorbance	Range	Inbibition (%)	IC50
12,5 ppm	1	0,737	0,620	61,89	69,7146
	2	0,568			
	3	0,555			
50 ppm	1	0,557	0,521	67,97	
	2	0,523			
	3	0,483			
100 ppm	1	0,351	0,266	83,65	
	2	0,221			
	3	0,483			
200 ppm	1	0,087	0,128	92,11	
	2	0,176			
	3	0,122			

The IC50 test results on the sample have a value of 69.7146 ug/mL as presented in table 2. Table 2 shows that bride's tears leaves with the maceration method have antioxidant activity. The results of the linear regression equation can be seen in Figure 2. shows that the equation between %inbibition with extract concentration and obtained the standard equation curve y = ax + b, with a value of y = 0.0025x + 0.5752, y with a value of 50 obtained from IC50. The high and low antioxidant activity in the sample is seen from the Efficient Concentration (EC50) or Inhibition Concentration (IC50) value, which is the value of 50% DPPH losing its free radical properties. The smaller the IC50 value, the higher the antioxidant activity of the sample. According to (Sepriyani et al., 2020) a compound is said to be a very strong antioxidant if the IC50 value is less than 50 µg/mL, strong if the IC50 value is between 50-100 µg/mL, moderate if the IC50 value is 100-150 µg/mL, weak if the IC50 value ranges from 150-200 µg/mL. Based on this statement, bride's tears leaves (Antikodon leptopus) are classified in the strong category.

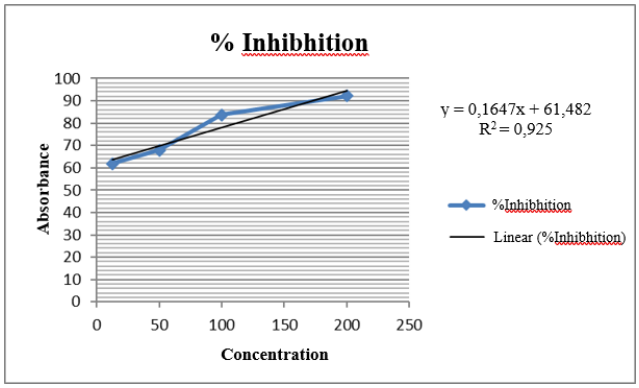

Figure 2. Linear regression of bride's tears (Antikodon leptopus) leaf antioxidant assay

The test results of the comparison solution in the form of quercetin have an IC50 value of 35.189 can be seen in table 3. The IC50 value of the test solution is smaller than the IC50 value of the bride's tears leaf sample. Although the IC50 value of the comparison solution is smaller, the antioxidant activity of the comparison solution is included in the strong category. The smaller the IC50 price, the stronger the antioxidant in counteracting free radicals or can be said to have stronger antioxidant activity (Bujang, 2014).

Table 3. Analysis of activity test and IC50 on bride's tear leaves (*Antigonon leptopus*).

Concentration	Review	Absorbance	Range	Inbibition (%)	IC50
20	1	1,243	1,207	25,7939	35,189
	2	1,167			
	3	1,207			
22,5	1	1,023	1,08	33,6202	
	2	1,147			
	3	1,07			
25	1	0,908	0,884	45,6771	
	2	0,902			
	3	0,842			
27,5	1	0,768	0,676	58,4306	
	2	0,638			
	3	0.623			
30	1	0,503	0,473	70,9096	
	2	0,513			
	3	0,404			

The results of the linear regression equation can be seen in Figure 3. shows that the equation between %inbibition and the concentration of the comparator solution obtained a standard equation curve y = ax + b, with a value of y = 4.6017x - 68.156.

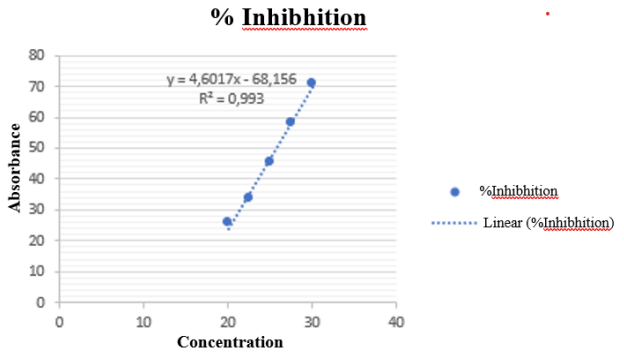

Figure 3. Linear regression of the antioxidant assay of the comparison solution (quercetin).

An antioxidant activity test in a plant is very important to know whether the plant has been proven to have binding activity against free radicals. Bride's tear leaves can be used to treat various health and beauty problems because they contain various active compounds, such as flavonoids, saponins, and tannins. These compounds have antioxidant, anti-inflammatory, and antibacterial properties. In this study, the samples used were extracts of bride's tears leaves that had been macerated using 96% ethanol and quercetin as a comparator, Quercetin is the main flavonoid compound included in the flavonol group (Materska, 2008). The selection of 96% ethanol as a solvent is preferred because it presents a better amount of poly phenols compared to water extracts, is more efficient in penetrating cell walls and pulling polyphenols out of cells, and is preservative against microorganisms (Lapornik et al., 2005).

DPPH compound is a molecule containing unstable nitrogen free radical compounds that can bind hydrogen ions so it is used for testing antioxidant activity (Permana, 2003). The presence of antioxidant activity in the test solution of bride's tears leaf extract in various concentrations is proven qualitatively by the gradual color change to a pale color at various concentrations of the test solution. It can be seen that the bride's tear leaf extract test solution with ethanol solvent at a concentration of 125 ppm still has a purplish color while at the largest concentration of 100 ppm the color of the solution is more purple, this change shows an indication of the activity of antioxidant content and shows that almost all free electrons in DPPH have bonded with antioxidant hydrogen atoms in the sample so as to convert DPPH to DPPH-H which has lost its free radical properties (Juniarti, 2018), The greater the color change to a pale color in the solution indicates that the greater the concentration of antioxidants in the solution.

CONCLUSIONS

From the results, it can be concluded that the antioxidant activity of the bride's tears leaf extract sample (Antigonon leptopus) is in the strong category with an IC59 value (69.71463 ug/mL).

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Authors' Contributions: Majidah Ramadan1 & Faisal designed the study. Terra Januarista & Ramizard Rafsanjan carried out the laboratory work. Nailil Wafiq Azizah, Devana Rahma Aldina & Ramadina Novia Putri analyzed the data. Nur Laila Anggraini, Sabilatul Mufarrohah, Satria Gemilang, Firadika Fitriansyah, Hosiyah, Nuvia Harini Wulan Suci, Nihlatus Sofiya wrote the manuscript. All authors read and approved the final version of the manuscript

Competing Interests: The authors declare that there are no competing interests.

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