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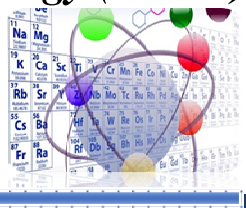

Antioxidant Activity Test of Barangan Banana Peel *(Musa Acuminata Linn)* Etanol Extract With DPPH Method

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ABSTRACT

Barangan banana (Musa acuminata linn) is also called Medan banana which is often found in North Sumatra. Banana peels are known to contain phenolic compounds that have potential as antioxidants. This study aims to determine the antioxidant activity of raw and ripe banana peel extract of Barangan banana (Musa acuminata linn) using the DPPH (1,1-diphenyl-2-pikrihidrazil) method. The data obtained was calculated to determine the antioxidant activity. The results showed that the antioxidant activity of the ethanol extract of Barangan raw banana peel IC50 = 32,52 µg/mL, the ethanol extract of Barangan ripe banana peel IC50 = 83,60 µg/mL, and the vitamin C comparator obtained an IC50 value of 5,00. Raw banana peel extract has higher antioxidant activity than ripe banana peel extract.

Keywords: antioxidant, DPPH, barangan banana peel (musa acuminata linn)

1. INTRODUCTION

Free radicals can be referred to as an atom or molecule which has more than one unpaired electron and is very effective, so when stabilizing it has to take electrons from other molecules which can cause abnormalities in other molecules and start chain reactions that can disrupt tissues. Free radicals are a trigger for degenerative diseases such as cancer, diabetes mellitus and alzheimer[1]. Excessive exposure to free radicals can result from ultraviolet light, cigarette smoke, air pollution, food, insecticides and stress. Excessive free radicals in the body can cause various diseases such as cancer, heart disease, cataracts, premature aging, and other degenerative diseases[2].

To avoiding the accumulation of free radicals which can lead to cancer, antioxidant compounds are needed to reduce, neutralize and limit the formation of free radicals in the body, antioxidants in the body function as donors of electrons to free radicals so that free electrons in the body eventually pair up and are able to inhibit and stop the damage that occurs in the body[3].

Antioxidants have a function as substances that are able to eliminate free radicals and prevent damage caused by free radicals in normal cells, fat, and protein. Antioxidant compounds have a molecular structure that is able to distribute electrons to free radical molecules without disrupting their function and can break chain reactions in free radicals[4]. The use of synthetic antioxidants has been banned and limited by

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the ongoing development of natural antioxidants because the use of natural antioxidants has fewer side effects[5].

Banana plants are one of the most important agricultural products in the world, banana plants are included in the ten plants that have the largest production area. Banana producers in Indonesia have many varieties and types, one of which is Barangan banana. Barangan banana (*Musa acuminata linn*) also called Medan bananas are often found in North Sumatra[6].

Every part of a banana has a positive effect on the health of the body, including the peel which always has the impression of being useless and just a waste. Research on Barangan banana peels has been carried out by Chandra in 2019 who measured the level of antifungal activity of Barangan banana peel extract with the results of phytochemical screening obtained showing results if Barangan banana peel extract has various secondary metabolites, namely flavonoids, saponins, tannins, glycosidants and steroids/tritepenoids which function as antifungalsl[7]. The content of flavonoids is a good source of antioxidants and can prevent the oxidation of body cells by free radicals, so that the body can be free from degenerative diseases and premature aging. Therefore the authors are interested in researching this plant using the DPPH (1,1-diphenyl-2-pikrihidrazyl) method based on IC_{50} (*Inhibition Concentration)*[5].

2. EXPERIMENTAL

2.1 Tools and Materials

The tools used were analytical balance (Fujitsu FSR-A320), volumetric flask, micro pipette, vortex, rotary evaporator, UV-Vis spectrophotometer (Spectroquant prove-300), measuring cup, stir bar, beaker glass, knife, funnel, Whatman No. filter paper. 1, blender, dark bottle, measuring pipette, micropipette, dropper, vortex, vial, 25 mesh sieve.

The raw and ripe banana skins used were obtained from Koga Galuh Village, Perbaungan District, Serdang Bedagai District, North Sumatra Province. The materials used are DPPH powder, 96% ethanol (Merck), pro-analytical ethanol, Vitamin C, aquadest.

2.2 Research Procedure

Simplicia Powder Preparation

As much as 2 kg of banana peels cleaned of dirt adhering to the running water until completely clean. Then the banana peel was cut into small pieces and dried and then mashed and sieved using a 26 mesh sieve.

Preparation of Banana Peel Ethanol Extract

Extraction was carried out using the maceration method. As much as 300 grams of simplicia powder was macerated with 96% ethanol for 3 × 24 hours. It is stirred every 2 hours and remaceration is carried out after that it is filtered with Whatman No. paper. 1 until the filtrate is obtained. The filtrate is then evaporated with a Rotary Evaporator until a thick extract is obtained.

Preparation of 0.1 mM DPPH Blank Solution

DPPH powder was weighed as much as 3.9 mg and dissolved in ethanol p.a to 100.0 mL.

Preparation of Vitamin C Sample and Comparison solutions

Weigh raw and cooked banana peel extract as much as 5 mg each, then dissolve with ethanol p.a in a 50 ml volumetric flask up to the mark, to obtain a concentration of 10%. From the 10% concentration, concentration series of 10, 20, 40, and 80 µg/ml were made.

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Vitamin C as much as 1 mg was dissolved with water up to 100 ml and a concentration of 1% was obtained. From this concentration, a series of concentrations of 1, 2, 4, and 8 µg/ml was obtained.

Determination of the Maximum Absorption Wavelength of DPPH

Measurement of wavelength (λ) by measuring 4.0 mL of 0.1 mM DPPH solution on a spectrophotometer with a wavelength of 400-600 nm to obtain an absorbance of ± 0.2 - 0.8.

Determination of operating time of 0.1 mM DPPH solution

Determination of operating time was determined by reacting 50 µl of reference standard of vitamin C plus 4.0 mL of 0.1 mM DPPH solution, homogenized with a vortex for 1 minute and the absorbance was measured at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes at the maximum λ that had been obtained.

Antioxidant Activity Test with DPPH Method

A total of 4.0 mL of 0.1 mM DPPH was put into the vial, added 50 µL of ethanolic extract of unripe Barangan banana peels with various concentrations then vortexed for 1 minute until homogeneous and incubated for 30 minutes at 37^0C, read the absorbance at the maximum λ obtained. Test the activity of the standard standard of vitamin C with the same treatment.

Data analysis

The antioxidant activity of the samples was determined from the amount of DPPH radical uptake inhibition by calculating the percentage of DPPH uptake inhibition. Calculated by the formula**:**

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100\%$$

Information :

Control absorbance : Absorption of DPPH radical solution

Sample absorbance : Absorption of sample solution in DPPH solution

The calculation of the IC_{50} value uses a linear regression equation. IC_{50} is a value or number that indicates the concentration in the sample that is able to inhibit the activity of a radical by 50%. In determining the IC_{50}, the standard curve equation of percent inhibition is needed as the y axis and the concentration of antioxidant extract as the x axis. Where the smaller the IC_{50} value, the higher the antioxidant activity.

3. RESULT AND DISCUSSION

In this experiment, a condensed extract of unripe Barangan banana peels was obtained with a yield of 31.33% and a thick extract of Barangan ripe banana peels with a yield of 29%.

Maximum Absorption Wavelength

From the results of the scanning performed, the maximum absorption wavelength of DPPH corresponds to the theoretical maximum wavelength of DPPH, which is 517 nm with an absorbance value of 0.643. Sample and comparison testing was carried out at a wavelength of 517 nm.

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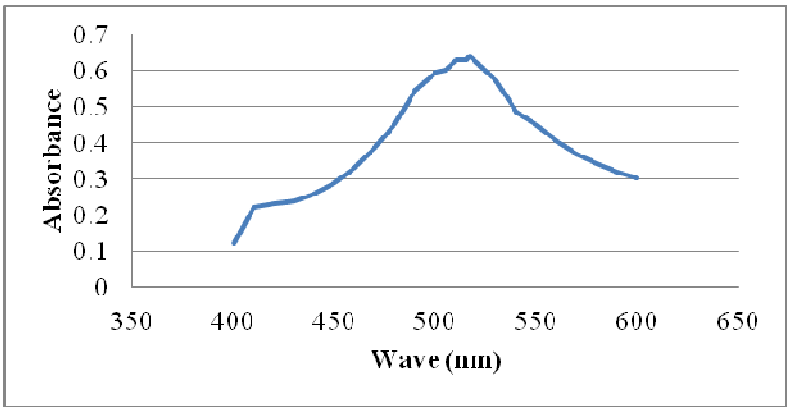

Figure 1. Maximum wavelength curve

From the results of the scanning performed, the maximum absorption wavelength of DPPH corresponds to the theoretical maximum wavelength of DPPH, which is 517 nm with an absorbance value of 0.643. Sample and comparison testing was carried out at a wavelength of 517 nm.

Determination of Operating Time

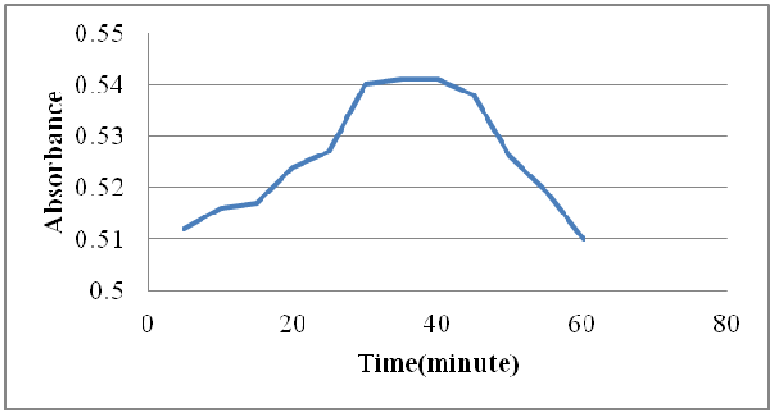

Figure 2. Operating time wavelength curve

The results of determining the operating time of vitamin C with 0.1 mM DPPH were obtained at 30 to 40 minutes with an absorbance of 0.541. Then the antioxidant activity test was carried out at 30 to 40 minutes.

Antioxidant Activity with DPPH Method

The basic principle in the antioxidant test using the DPPH method is that there is a chemical reaction between the antioxidant compound and the DPPH free radical which results in a change in the color of the solution from purple to yellow or from deep purple to faded purple.

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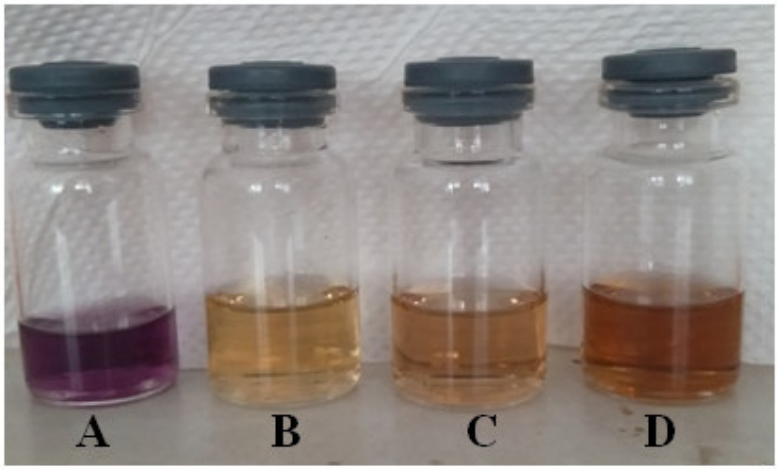

Figure 3. Color change (a) DPPH solution, (b) Vitamin C + DPPH solution, (c) raw banana peel extract + DPPH, and (d) ripe banana peel extract + DPPH

The antioxidant activity of the vitamin C solution showed positive results which could be seen by the color change from purple to yellow, and the solution of raw and ripe banana peel extracts also showed positive results indicated by a decrease in the intensity of the purple color.

Table 1 shows the results of antioxidant measurements from raw and ripe banana peel extracts, as well as vitamin C as a standard based on the resistance given to DPPH radicals.

Table 1. Percent free radical inhibition of banana peel extract and vitamin c samples

Sample	Concentration (µg/ml)	Absorbance			Average Absorbansi	Absorbance Blank (µg/ml)	% Inhibition
		1	2	3			
Raw Banana Peels	10	0.357	0.359	0.359	0.358	0.576	37.85
	20	0.311	0.309	0.313	0.311		46.01
	40	0.268	0.266	0.265	0.266		53.82
	80	0.164	0.167	0.163	0.164		71.53
Ripe Banana Peels	10	0.486	0.490	0.490	0.488	0.576	15.28
	20	0.436	0.435	0.440	0.437		24.13
	40	0.384	0.385	0.384	0.384		33.33
	80	0.304	0.304	0.304	0.304		47.22
Vitamin C	1	0.387	0.384	0.385	0.385	0.576	33.16
	2	0.363	0.362	0.364	0.363		36.98
	4	0.292	0.295	0.292	0.293		49.13
	8	0.226	0.226	0.227	0.226		60.76

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From table 1. it can be seen that the higher the concentration of the sample used, the greater the antioxidant activity, marked by the reduced color intensity which reflects the decrease in the absorbance of the DPPH given the sample or the comparison. The IC50 value was determined using the linear regression equation from the relationship curve of sample concentration to percent inhibition with the equation Y = ax + b, where the sample concentration (µg/ml) is the axis (X) and the inhibition percentage value is the axis (Y). The curve of the relationship between the concentrations of raw and ripe banana peel extract and the percentage of inhibitors against DPPH radicals is shown in Figure 4, while the curve of the relationship between the concentrations of Vitamin C and the percentage of inhibition of DPPH radicals is shown in Figure 5.

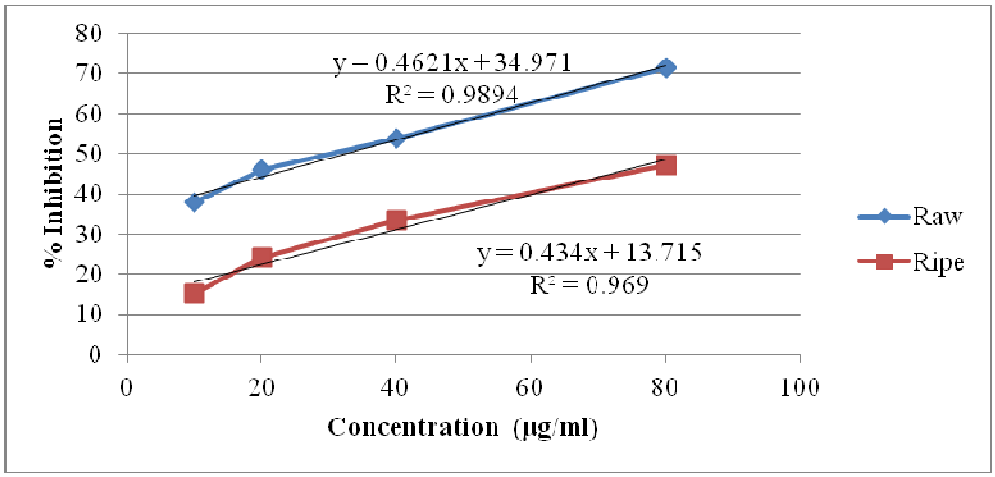

Figure 4. Relationship Curve of Sample Extract Concentration to % Inhibition

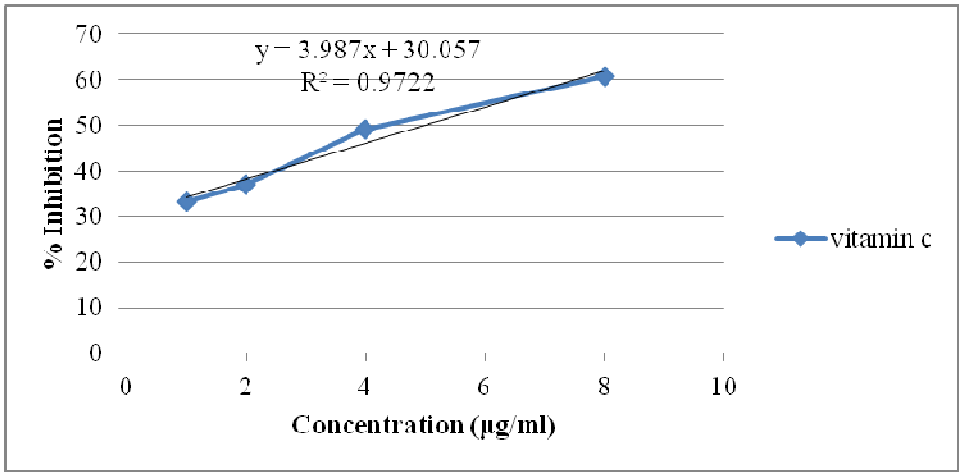

Figure 5. Relationship Curve of Vitamin C Concentration to % Inhibition

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The IC_{50} value is calculated based on the linear equation formula. Based on the curve of the relationship between the concentration of the test solution and the reference solution with the percentage of inhibition of DPPH radicals. Determining the value of IC_{50} is done by entering the number 50 into the variable Y so that the value of X will be known. The X value is the IC_{50} value. The IC_{50} value of raw and ripe banana peel extract and vitamin C comparison is shown in table 2.

Table 2. Free IC50 values of banana peel extract and vitamin C

Sample	Linear Regression Equation	IC50 (µg/ml)
Raw Banana Peels	y = 0.4621x + 34.971 R^2 = 0.9894	32,52
Ripe Banana Peels	y = 0.434x + 13.715 R^2 = 0.969	83,60
Vitamin C	y = 3.987x + 30.057 R^2 = 0.9722	5,00

Specifically, a compound can be said to be a very strong antioxidant if the IC_{50} value is <50 µg/ml, strong if the IC_{50} value is 50-100 µg/ml, moderate if the IC_{50} value is 100-150 µg/ml, and weak if the IC_{50} value is 151-200 µg/ml8. The results showed that the antioxidant activity of the ethanol extract of Barangan ripe banana peel IC_{50} = 32.52 µg/mL (very strong), and the ethanol extract of Barangan ripe banana peel IC_{50} = 83.60 µg/mL (strong). Meanwhile, for the vitamin C standard, the IC_{50} value was 5.00 µg/mL (very strong).

The IC_{50} value of vitamin C has very strong antioxidant activity because vitamin C is a pure compound that has very strong antioxidant activity, while the extract is still in the form of a mixture of compounds that may have various properties[9]. Raw Barangan banana peel extract has higher antioxidant activity than ripe Barangan banana peel extract, this is in accordance with research conducted by Haikal, et al in 2021 with the results obtained showing that more significant antioxidant activity was found in banana peel extract which was still green in color with a fairly high content of flavonoids and in yellow banana peel samples showing lower antioxidant activity[10].

4. CONCLUSION

The ethanolic extract of raw and cooked banana peels has antioxidant activity using the DPPH method. The IC_{50} value of the ethanolic extract of unripe banana peels was 32.52 µg/mL, and that of the cooked barangan banana peel extract was 83.60 µg/mL. The results of this analysis indicate that the ethanol extract of Barangan Ripe banana peels has stronger antioxidant activity than the peel extract of Barangan Ripe Bananas. However, the antioxidant activity value of the ethanol extract of Barangan banana peel was not better than the standard Vitamin C which had an antioxidant activity value of 5.00 µg/mL which was classified as very good.

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