Antioxidant Activity Test Of Barangan Banana Hump's Ethanol Extract (Musa Paradisiaca (L.)) With Dpph (1,1 Diphenyl-2-Picrylhydrazyl) Methods

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Abstract.
This study aims to determine the secondary metabolites contained in Barangan banana weevil (Musa paradisiaca (L.)) and antioxidant activity based on the IC_{50} value. The stages of this research include processing plant materials, making ethanol extracts, characterization examinations, phytochemical screening, and testing the antioxidant activity of Barangan banana weevil. The ethanol extract of Barangan banana weevil was processed by the maceration method using 96% ethanol, and the antioxidant activity was tested using the DPPH method. The results showed that the ethanol extract of Barangan banana weevil contained chemical compounds such as alkaloids, glycosides, flavonoids, saponins, tannins, and triterpenoids. The determination of the antioxidant activity of Barangan banana weevil obtained an IC_{50} value of 180.8 µg/mL. This means that the ethanol extract of Barangan banana weevil contains secondary metabolites and has the potential as an antioxidant with a weak category.

Keywords: *Ethanol extract, barangan banana hump, antioxidants*

I. INTRODUCTION

Free radicals are molecules, atoms or groups that have one or more unpaired electrons in their outer shell so they are highly reactive. Free radicals are very dangerous for the human body because they can damage body cell components such as lipids, proteins, and DNA. This happens because of an imbalance in the number of free radicals with the activity of antioxidant enzymes produced by the body. So we need antioxidants that can help protect the body from the effects of free radicals and reduce their negative impacts (Parwata, 2016).Antioxidants are compounds that can absorb or neutralize free radicals so as to prevent degenerative diseases such as cardiovascular, carcinogenesis, and other diseases. Because antioxidants can donate electrons to stop free radical chain reactions that can damage the body (Sauhoka et al., 2019).According to Khaira (2017), free radicals will attack other molecules in the absence of antioxidants. Unlike the case when there are antioxidants, free radicals will immediately react with antioxidants to form molecules that are stable and harmless. The reaction stops here. There are 2 kinds of antioxidants, namely endogenous antioxidants, which are produced by the body itself, and exogenous antioxidants, which are antioxidants ingested from outside the body. The human body does not have excess antioxidant reserves, so if there is excessive exposure to radicals, the body needs exogenous antioxidants. Adequate antioxidant consumption can help to reduce the occurrence of degenerative diseases.

Indonesia has a variety of plants that are used as ingredients for traditional medicines, such as agarwood bark, Barangan banana weevil, cocoa leaves, and so on. Agarwood bark contains chemical compounds such as flavonoids. The group of compounds contained in cocoa leaves (Theobroma cacao L.) with phytochemical screening methods are alkaloids, flavonoids, tannins, saponins, steroids, and glycosides (Rani et al., 2022). Gaharu bark contains chemical compounds such as flavonoids, saponins, and tannins. The determination of antioxidant activity was carried out using the UV-Vis spectro with the DDPH method. The results were obtained from the methanol extract of the bark of agarwood (Aquilaria malaccensis Lam.), which has antioxidant activity in the strong category with an IC_{50} value of 94.59 µg/mL and vitamin C in the very strong category with an IC_{50} value of 22.11 µg/mL (Ridwanto et al., 2022). While the Barangan banana is one of the fruits consumed by the people of Aceh as a food additive in typical Acehnese dishes that contain antioxidant compounds, the banana plant also has secondary metabolite compounds such as flavonoid compounds and saponins (Hilma, 2016). The content and activity of secondary metabolites produced depends on one of them being the process of extracting the plant. Seeing the state of the plant, it is

very necessary to do research to find out what secondary metabolite content is in the plant to be studied (Ainil et al., 2022).

According to Rahmawati et al. (2015), to research antioxidants, several methods can be used, namely the frap method, the cupra method, and the radical scavenger method (DPPH). The reason researchers use the radical scavenger method is because the method is simple, easy, fast, sensitive, and requires small samples. It is easy to apply because the DPPH radical compound used is relatively stable compared to other methods. The principle of this method is the donation of a hydrogen atom (H+) from the tested substance to the DPPH radical into a non-radical compound, diphenyl picryl hydrazine, which will be indicated by a color change. The color change that occurs is a color change from purple to yellow, where the intensity of the color change of DPPH is directly proportional to the antioxidant activity to reduce these free radicals. Until now, there has been no research done on the banana weevil of Barangan, so the researcher is interested in conducting research on (Test of Antioxidant Activity of Ethanol Extract of Barangan Banana Weevil (Musa paradisiaca (L.)) with the DPPH Method).

II. METHODS

2.1 Sampling

The sample of Barangan banana weevil (Musa paradisiaca (L.)) used in this study was obtained in the Panton Labu area, Aceh, as much as 7 kg. The sampling method is done by purpose; the sample is taken in one place or area without comparing it with other areas.

2.2 Materials

The samples used in this study were banana weevil (Musa paradisiaca (L.)), ethanol, aquadest, chloroform, concentrated hydrochloric acid (HCl), magnesium metal (Mg), ferric reagent (III) chloride ($FeCl_3$), acetic acid anhydrous $(CH_3CO)_2O$, concentrated sulfuric acid (H_2SO_4), chloroform-ammonia 0.05 N, Mayer's reagent and DPPH (1,1-diphenyl-2-picrylhydrazyl).

2.3 Preparation of Ethanol Extract of Barangan Banana Weevil (Musa paradisiaca (L.))

Simplicia powder 10 parts (500 g) were put into a vessel and then poured into 75 parts (3750 mL) of ethanol filter liquid. The vessel was then closed and left for 5 days protected from sunlight while stirring occasionally. After five days, the mixture with the pulp is squeezed out. Wash the dregs with sufficient ethanolic solvent to obtain 100 parts (5 liters) of maserate. Then it was transferred to a closed vessel, left in a cool place protected from light for 2 days, and filtered. The maserate is then concentrated using a rotary evaporator and then weighed (Depkes RI, 1989).

2.4 Phytochemical Screening Test

2.4.1 Alkaloid Examination

The simplicia powder and extract were weighed as much as 0.5 grams each, then added 1 mL of hydrochloric acid and 9 mL of distilled water, heated water for 2 minutes, cooled and then filtered. The filtrate was used for the following experiments:

1. Take 3 drops of filtrate, then add 2 drops of Mayer's reagent.
2. Take 3 drops of filtrate, then add 2 drops of Bourchardat reagent.
3. Take 3 drops of the filtrate, then add 2 drops of Dragendroff's reagent.

Alkaloids are considered positive if there is a precipitate or at least two or three of the above experiments (Depkes RI, 1989).

2.4.2 Flavonoid Examination

The weighed Simplicia powder and extract of 10 grams each were weighed, then added to 100 mL of hot water, boiled for 5 minutes and filtered in hot conditions. The filtrate obtained was then taken to 5 mL and added to 0.1 gram of Mg powder, 1 mL of concentrated HCl, and 2 mL of Amyl alcohol, shaken, and allowed to separate. Positive flavonoids if there is a red or yellow-orange color on the amyl alcohol layer (Depkes RI, 1995).

2.4.3 Tanin Examination

The simplicia powder and extract were each weighed at 0.5 grams. The sample was extracted with 10 mL of distilled water, then the filtrate was diluted with distilled water until it was colorless. Take 2 mL of

the solution and add 1 to 2 drops of iron (III) chloride reagent. The occurrence of a blue or green-black color indicates the presence of tannins (Depkes RI, 1995).

2.4.4 Saponin Examination

The Simplicia powder and extract each weighed up to 0.5 grams, and the sample was placed in a test tube with 10 mL of hot distilled water, cooled, and vigorously shaken for 10 seconds, yielding a steady foam as high as 1-10 cm in less than 10 minutes. If you add 1 drop of 2N hydrochloric acid solution, if the foam does not disappear, it indicates the presence of saponins (Depkes RI, 1989).

2.4.5 Steroid/Triterpenoid Examination

The simplicia powder and extract were weighed as much as 1 gram of the sample, macerated with 20 mL of n-hexane for 2 hours, and then filtered. The filtrate is evaporated in a vaporizer cup. Add 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid to the remainder. A purple-red color appears, indicating the presence of triterpenoids, or a green color indicates the presence of steroids (Ditjen POM, 1995).

2.5 Antioxidant Activity Test Using DPPH Method

2.5.1 DPPH Solution Preparation

The DPPH solution was prepared by weighing 20 mg of DPPH and dissolved in methanol, then put into a 100 mL volumetric flask, filled with methanol to the mark line (DPPH solution 0.5 mM, concentration 200 µg/mL (Molyneux, 2004).

2.5.2 Preparation of Sample Solution

The extract was weighed as much as 25 mg, then put into a 25 mL volumetric flask dissolved with methanol and then the volume was filled with methanol to the mark line (concentration 1000 µg/mL)

2.5.3 Determination of Maximum Wavelength DPPH

The DPPH solution with a concentration of 200 µg/mL, was pipetted and put into a 10 mL measuring flask filled with methanol to the mark line to obtain a DPPH solution of 40 µµµg/mL. The absorbance was measured at a wavelength of 400-800 nm, so that the absorbance was obtained. maximum as the maximum wavelength of the DPPH.

2.5.4 Measurement of Antioxidant Activity

Pipette the ethanol extract solution (from a concentration of 1000 µg/mL). Each 2; 3; 4; and 5 mL volumetric flask was filled with 2 mL of DPPH solution (from a solution of 200 µg/mL), then the volume was filled with methanol to the mark line, yielding a solution of concentration 100, 200, 300, 400 and 500 µg/mL are the different concentrations. Then it was allowed to stand for a few minutes according to the stable time. The absorbance was measured at the maximum wavelength obtained. The treatment was repeated up to three times in order to obtain absorbance data from a mixture of DPPH and Barangan banana weevil extract with various concentrations.

2.5.5 IC_{50} Value Determination

The IC_{50} value is a number that indicates the concentration of the test sample (µg/mL) that provides 50% DPPH reduction (capable of inhibiting or reducing the oxidation process by 50%). A value of 0% means that it does not have antioxidant activity, while a value of 100% means that the total attenuation by testing needs to be continued with dilution of the test solution to see the limit of its activity concentration. The calculation results are entered into the regression equation with the extract concentration (µg/mL) as the abscissa (X axis) and the value of % absorption (antioxidant) as the ordinate (Y). Then, from this equation, the IC_{50} value is calculated to get the antioxidant value.

III. RESULT

3.1 Phytochemical Screening Test

Phytochemical screening in this study was carried out on the Barangan banana weevil extract with the aim of knowing the class of secondary metabolites contained in the Barangan banana weevil extract. The results of phytochemical screening of Barangan banana weevil extract can be seen in the table 1 below:

Table 1. Phytochemical Screening of Ethanol Extract (Musa paradisiaca (L.)

No.	Group of Chemical Compounds	Identification
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4	Saponins	+
5	Steroid/Triterpenoid	+

Description: (+) = Contains a group of compounds
(-) = Does not contain a group of compounds

Phytochemical screening was carried out to determine the chemical compounds of secondary metabolites in the ethanol extract of the Barangan banana weevil, including flavonoids, alkaloids, tannins, saponins, and steroids/terpenoids. Positive results were obtained in the flavonoid test, which were indicated by a yellow-orange color change in the alcohol layer. From the results of the examination of flavonoids, the addition of concentrated hydrochloric acid to Mg powder and amyl alcohol formed a yellow-orange colored layer on the amyl alcohol layer. This shows that the ethanol extract of the Barangan banana hump contains flavonoid compounds (Syahputra et al., 2021). These secondary metabolites are thought to have anti-free radical activity.

3.2 Antioxidant Activity Test

Determination of antioxidant activity using the DPPH method or 1,1-diphenyl-2-picrylhydrazyl as a free radical. Antioxidants are electron-donating compounds that can inactivate oxidation reactions by complementing the electron deficiency of free radicals so that the chain reaction will be inhibited and free radicals will become stable (Ainil et al., 2022).The DPPH radical, which has unpaired electrons, has a purple-violet complementary color with a maximum absorbance at a wavelength of 515-520 nm with methanol as a solvent (Rohmaniyah, 2016). The wavelength of a DPPH solution with a concentration of 40 µg/mL that had been incubated in the dark for 10 minutes at 37^{o}C was measured and the maximum wavelength was 516 nm. may be more than 1 (Molyneux, 2004). The measurement results can be seen in Figure 1:

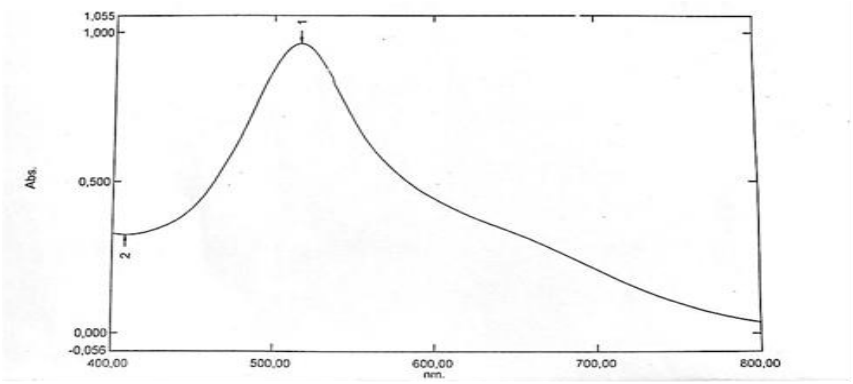

Fig 1. Maximum wavelength of DPPH 40 µg/mL

Measurement of antioxidant potential was carried out on a sample of Barangan banana weevil. Samples were made in various concentrations, namely 100, 200, 300, 400, and 500 µg/mL, then their antioxidant potential was measured using the DPPH method using UV-Vis at an absorption wavelength of 516 nm with a stability time obtained in the previous stages. The principle of this method is that DPPH compounds that do not react with antioxidants (remaining) will be read as absorbance values at a wavelength of 516 nm in methanol solvent and can be seen organoleptically through a color change from purple to light purple, or light yellow. The reduction in color intensity that occurs is related to the number of DPPH electrons that capture hydrogen atoms. DPPH radical is an organic compound containing unstable nitrogen and is dark purple in color. After reacting with antiradical compounds, the DPPH will be reduced and the color will turn yellow. The color change was caused by the reduction of the conjugated double bond in DPPH. The capture of one electron by an antiradical substance causes no opportunity for the electron to

resonate where this change can be measured and recorded with a spectrophotometer (Asih & Setiawan, 2008).

Table 2. Antioxidant strength based on IC_{50} value (Blois, 2018).

No	Category	Consentrasi (µg/mL)
1	very strong	< 50
2	Strong	50 – 100
3	Currently	101 – 150
4	Weak	151 – 200
5	Very weak	>200

Tabel 3. Calculation result of IC_{50} value

No	Sample	IC_{50}	Category Antioxidant Strength
1	Ethanol Extract	180,8 µg/mL	Weak

The table above shows that the ethanol extract of Barangan banana weevil has antioxidant activity with a weak category of "weak" antioxidant activity. This is reasonable considering that the sample used is a weevil from the banana plant, so it is suspected that the content of compounds contained in it is not as much as that contained in the banana itself.Compounds belonging to natural antioxidants that can be extracted from the test compounds are phenols or polyphenols, which can be in the form of flavonoids, cinnamic acid derivatives, and tocopherols. The flavonoid group that has antioxidant activity includes flavonols, isoflavones, flavones, catechins, flavonones, and chalcones (Kumalaningsih, 2006). These flavonoid compounds act as free radical scavengers because the hydroxyl groups they contain donate hydrogen to radicals. These compounds are able to neutralize free radicals by donating electrons to free radicals so that atoms with unpaired electrons get electron pairs and are no longer radicals (Silalahi, 2006).

IV. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the ethanolic ethanol extract of Barangan banana hump contains secondary metabolites of alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids. The ethanol extract of Barangan banana weevil has antioxidant activity with an IC_{50} value of 180.8 µg/mL and is in the weak category.

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