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Antioxidant Activity Of Ethanol Extract of Soft Shell, Hard Shell And Seeds On Melinjo (*Gnetum gnemon* L.) With DPPH Method

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ABSTRACT

The human body cannot be avoided from exposure to free radicals in everyday life. Free radicals are very dangerous for the body, one of which causes degenerative diseases. One of the plants that is thought to be able to inhibit free radicals is the melinjo plant. The processing of melinjo plants in the community is only used as a vegetable, so there is a need for research on the benefits of antioxidants in melinjo plants. The purpose of this study was to explore the parts of melinjo which had the best antioxidant activity found in the soft shell, hard shell and seeds of melinjo (Gnetum gnemon L.) with different solvent concentrations. The research method used was to prepare test samples by maceration process using 50% and 70% ethanol, then the extracts were tested for antioxidants using the DPPH method. Data analysis was performed using the ANOVA statistical test with a 98% confidence level. The results showed that the optimal percentage of free radical inhibition was obtained in 50% ethanol solvent, the outer shell of melinjo was 53.29%, and the IC_{50} was 318.18 ppm. Based on the One way Anova test ($p>0.02$) there was no difference between the use of 50% and 70% ethanol solvent on the antioxidant activity of the outer shell, hard skin and melinjo seed samples.

Keywords : antioxidants, melinjo, DPPH.

INTRODUCTION

Free radicals are an unavoidable part of everyday life because of their basic nature in the human body. Free radicals as a result of the process of oxidation and burning of human body cells. When the body is exposed to free radicals, such as motor vehicle pollution, cigarette smoke, an unbalanced diet and low physical activity continuously it will cause tissue damage and cause degenerative diseases. Free radicals are formed in the body which will produce new free radicals through a chain reaction which in the end the number continues to increase and harm the body. One of the compounds that can stop this chain reaction is an antioxidant (Safiera, 2016). Antioxidants can help overcome oxidative damage by inhibiting the activity of free radicals in the body by providing an electron for oxidants (Noviati. et al., 2004) Antioxidants are present in the body, but the formation of antioxidants in the body is not sufficient to combat uncontrolled free radicals, exogenous antioxidants are needed, both natural and synthetic (Permatasari et al., 2020).

Synthetic antioxidants have many drawbacks and can cause problems for the body such as BHA, BHT, sodium thiosulfate . so natural antioxidants are needed which are relatively safer and are thought to help reduce the effects of harm from synthetic antioxidants (Sani & Kunarto, 2018). Melinjo is a plant that is rich in antioxidants. Melinjo seeds, bark, leaves and roots are said to be high in antioxidants because they contain flavonoids and phenols. Melinjo has a phenolic concentration comparable to Butylated Hydroxytolune (BHT) as an antioxidant

Research by Sani and (Kunnaryo & Wikandari, 2021). found the best red melinjo skin anthocyanin extract with a yield of 12.95%, a total anthocyanin content of 43.52 mg/L, and antioxidant activity (DPPH inhibition) of 66.29% using ethanol and water with a ratio of 100: 0. Anthocyanins are antioxidants because they are flavonoids that can neutralize free radicals and peroxyl .

Melinjo seed hard shell extract contains gnetin c derivatives, resveratrol and stilbenoids. Research (Kunarto et al., 2019) found that fractionation with MAR HPD-600 removed excess sugar molecules from the hard shell extract of red melinjo seeds, resulting in high antioxidant activity. Research by (Kunnaryo & Wikandari, 2021), shows that the melinjo seeds are glutinous there is a total phenolic content of 7.033 ± 0.06 mg GAE/g, a total flavonoid content of 335.04 ± 1.32 mg

CE/100 g, resveratrol content of 1.55 ± 0.3%, DPPH radical inhibition of 66.07 ± 0.38%, and reducing power (80.26 ± 0.06%). In the extraction process, this study used the maceration method. The maceration procedure was chosen because it is simple and does not involve high temperatures, which can damage chemical components with antioxidant activity found in the outer simplicia shell, hard skin, and melinjo seeds (Noviati. et al., 2004)

Ethanol is polar , so it can penetrate the walls of active chemical substances from plants and extract active molecules. The use of ethanol as a solvent has been found to be very successful in the production of metabolites in several studies. Ethanol is used as a universal solvent because it dissolves polar, semi-polar and non-polar active substances (Riwanti et al., 2020). The DPPH method (1 ,1 Diphenyl 2 Picrilhidrazil) was used to determine antioxidant activity in this study. The DPPH method was chosen as the antioxidant activity test method because it is a simple, easy, fast, and sensitive method that only requires a small sample to evaluate the antioxidant activity of natural compounds. So it is widely used to test the ability of compounds to act as electron donors, and has been proven to be accurate, reliable, and practical (Kertawati et al., 2022). Melinjo has the ability as a natural antioxidant, preventing damage caused by free radicals. As a result, deliberate steps must be taken to determine which part of the melinjo has the highest antioxidant activity (IC_{50}) so that it can be used in future applications.

Therefore, comprehensive research is needed on the potential of the outer shell, hard shell, and seeds of melinjo (Gnetum gnemon L) as a source of natural antioxidants, which will compare the antioxidant capacity of the outer shell, hard shell, and seed extracts using the DPPH method.

TOOLS AND MATERIALS

The tools used in this study are: Analytical scales, knives, Tray, Blender, Beaker glass, Erlenmeyer, sieve, aluminum foil, paper filters, funnels, dropping pipettes, volume pipettes, maceration containers, stir bars, rotary evaporator, tube rack, test tube, volumetric flask, thermometer, water bath, oven, cuvette, and UV-Vis Spectrophotometry.

The materials used in this study were red melinjo shell 200 g, hard shell melinjo 150 g, melinjo seeds 300 g, DPPH 10 mg Ethanol 70% 7 L, Ethanol 50% 7 L, Methanol 1 L, Magnesium

Metal 0.5 mg, concentrated HCl , Pure Vitamin C 20 mg, Aquadest 1 L, Disodium Hydrogen Phosphate 5 g, Sodium Hydrogen Phosphate

METHODS

Research procedure

1. Determination of Plants

   The first step in this research is to identify the melinjo plant (Gnetum gnemon L.). The purpose of this determination is to see if the plant is correct based on the morphological characteristics of the melinjo plant (Gnetum gnemon L.) as described in the literature. 2. Making Simplisia Melinjo (Gnetum gnemon L.) The outer skin, hard skin and melinjo seeds obtained are washed with water flowing then wet sorting to remove impurity components. Melinjo fruit skin is red, hard skin, and then the seeds are dried in the oven at 500 Celsius, for 30 minutes. After that The sample was mashed using a blender and sieved using a 40 mesh sieve.

2. Extraction of Melinjo powder (Gnetum gnemon L.)

   Extraction is done by soaking the melinjo simplicia (outer skin, skin hard, and melinjo seeds) with ethanol solvent concentration of 50% and 70% with a ratio of 1:10. Maceration was carried out for 3x24 hours. Yield results are filtered using filter paper. The extract is then concentrated using a rotary evaporator at 40°C and 50 rpm to produce a thick extract.

3. Phytochemical screening

   Examination of flavanoids with concentrated HCl and MgSO4 powder Weigh 0.5 gram of sample ethanol extract (outer skin, skin, and seeds melinjo) put into a cup, add 2 ml of 70% ethanol then stir and add 0.5 g of magnesium powder and 3 drops of concentrated HCl . Mixture shaken vigorously. The presence of flavonoids was indicated by the appearance of a yellow color. red , or orange.

4. Preparation of 200 ppm DPPH solution

   A total of 10 mg of DPPH was put into a 50 ml volumetric flask, continued with the addition of methanol to the boundary mark, so that a solution was obtained DPPH with a concentration of 200 ppm. The solution is used immediately and placed in a dark container.

5. Preparation of test solutions

A 0.1% mother liquor is prepared by weighing 0.1 g of the extract (extract, skin hard, melinjo seeds) then put into a volumetric flask 100 ml dissolved with ethanol up to the mark.

6. Preparation of standard solution of Vit C A 20,000 ppm standard solution is prepared by weighing 2 g of Vit . C then put into a 100 ml volumetric flask dissolved with distilled water up to the mark limit. From the mother liquor a series of 20 ppm, 40 ppm, 80 ppm, 120 ppm was made. and 360 ppm. Each of these concentrations 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml and 1.6 ml of mother liquor, put into a volumetric flask 10 ml is sufficient volume up to the mark with aquadest .

7. UV-Vis Spectophotometry

a. Determination of the maximum wavelength of DPPH A total of 2 ml of 200 ppm DPPH solution was put in a cuvette. this solution The absorption spectrum was determined using UV-Vis spectrophotometry on wavelength 400-800 nm.

b. Absorption measurement with UV-Vis spectrophotometry

Each concentration of the test solution and 2.5 ml of vitamin C was added into a test tube. Added 1 ml of DPPH solution, covered with aluminum foil. Then incubated at 30 0C for 30 minutes in a dark bottle, then the absorbance was measured.

8. Determination of antioxidant activity

Percent inhibition is the ability of a compound to inhibit DPPH oxidation as free radicals. Percentage inhibition data required can be obtained using the following formula:

$$\% \text{ Inhibition} = \frac{DPPH\ absobance - Sample\ absorbance}{DPPH\ absorbance} \text{x } 100\ \%$$

RESULTS

1. Extraction of moringa leaf.

Table 1. Mount of melinjo extract

Melinjo extract	Solvent	sample weight (grams)	sample weight (grams)	% extract (grams)
Soft shell extract	Ethanol 50%	200	48,72	24,36
	Ethanol 70%		32.05	16.02
Hard shell extract	Ethanol 50%	150	13.50	9.00
	Ethanol 70%		12.71	8,47
Seed extract	Ethanol 50%	300	27,81	9,27
	Ethanol 70%		26,33	8.77

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2. Phytochemical screening

Table 2. Flavonoid test results

sample	Flavonoids	result
Softshell extract	+	Orange
Hard shell extract	+	red
Seed extract	+	Orange

3. Maximum wavelength of DPPH

Table 3. Maximum wavelength of DPPH

Maximum wavelength (nm)	Absorbance
546,0	0,017
545,0	0,019
544,0	0,011
543,0	0,065
543,0	0,166
541,0	0,516

4. Antioxidant activity of melinjo

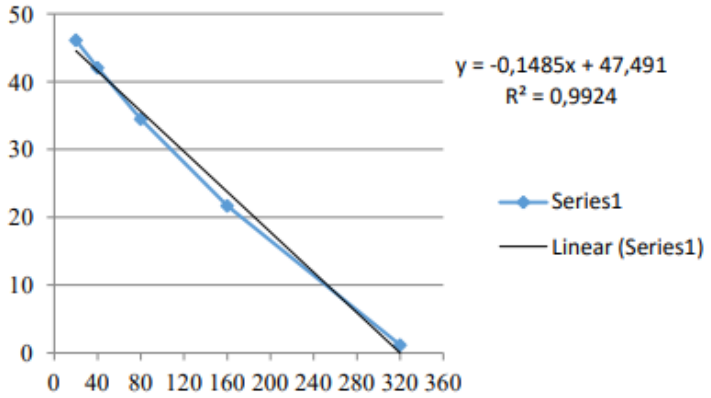


Figure 1. Concentration (x) with % inhibition (y)

Table 4. Result analysis of antioxidant activity

Melinjo extract	Absorbance (nm)	% inhibition	IC_{50}
soft shell 50%	0,241	53,29	318,18
soft shell 50%	0,333	35,46	317,56
Hard shell 50%	0,256	50,36	318,08
Hard shell 70%	0,370	28,29	317,31
Seed extract 50%	0,361	30,03	317,37
Seed extract 70%	0,420	18,60	316,97

DISCUSSION

1. Extraction of melinjo

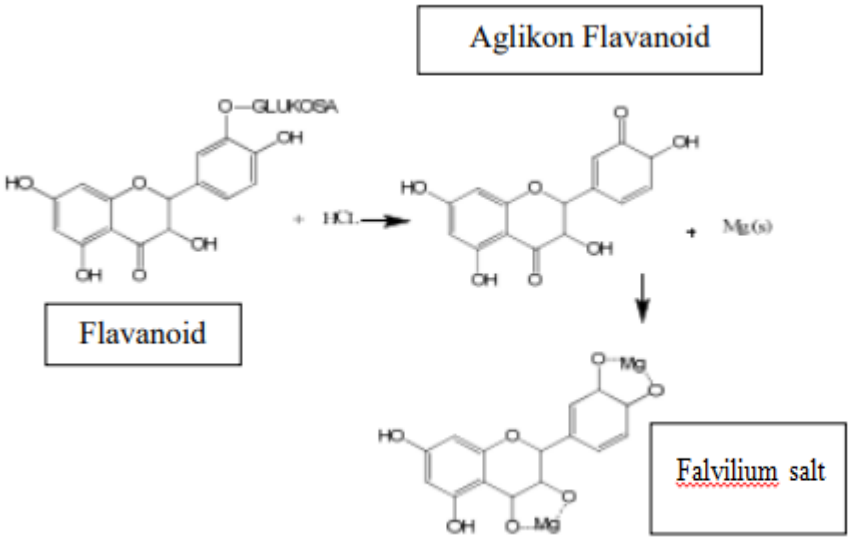
Melinjo extract obtained using 50% and 70% ethanol solvent yield yield in table.1 it can be seen that the percentage yield of 50% ethanol solvent is higher than the yield using 70% solvent. The highest yield was obtained in the outer shell ethanol extract 50% by 24.36% while the lowest yield was in the outer shell ethanol extract 70% of 8.47%. The mass of the extract obtained is relatively large because the sample extracted is a sample that has been in the oven so that the water content contained therein has been reduced. The reduced water content in the sample causes the extraction results to increase because in the extraction process all the compounds contained in the sample are extracted perfectly (Dewi et al., 2012)

2. Phytochemical screening

Phytochemical screening aims to determine which secondary metabolites contained in the sample. One of the compounds contained in melinjo suspected of containing flavanoids. Flavonoid test is done to make sure it is there whether or not flavanoids in the outer shell, hard shell and ethanol extract samples melinjo seeds. The presence of flavanoids in the sample is indicated by the formation of solution is yellow, red or orange after the addition of HCl and powder magnesium.

In the screening test for flavonoid compounds, the color changes to red orange due to the addition of concentrated HCl and magnesium powder which will change into red or orange flavilium salts (Andasari et al., 2020). The addition of HCl aims to hydrolyze flavanoids to its

aglycone, namely by hydrolyzing the O-glycosyl. Glycosyl will be replaced by H+ of acids due to its electrophilic nature Mg powder produces red, yellow or orange colored complexes.



Mg and HCl are used in the oxidation reaction, so that when In the same way, a reduction reaction occurs at the flavanoid glycoside bond (redox reaction). The results of this study indicate that the sample forms an orange color on melinjo outer skin extract, red on melinjo hard skin extract and orange in melinjo seed extract.

3. Maximum wavelength

The maximum wavelength of DPPH is done to find out the length maximum wave of highest absorption by measuring the absorbance of DPPH concentration of 200 ppm using UV-Vis spectrophotometry. Absorbance the maximum wavelength can be seen in Table 3. The results of measuring the wavelength in this study obtained the length maximum wave of 541 nm with an absorbance value of 0.516. The wavelength obtained by DPPH in this study are not in accordance with the theory that DPPH solution showed maximum absorbance at 512-520 nm.

4. Antioxidant activity

Testing the free anti-radical activity of the soft shell, hard shell and seed extracts melinjo is done by measuring the inhibition of DPPH with using a UV-Vis spectrophotometer at maximum wavelength DPPH solution. The ability of antioxidant compounds in samples to inhibiting DPPH oxidation as a free radical can be expressed in percent inhibition.

In determining the percentage of absorbance inhibition can be seen in table.4 that the percentage of DPPH inhibition of antioxidants found in the outer skin, hard skin and melinjo seeds indicates that the use of ethanol solvent 50% produces optimal antioxidant activity compared to 70% ethanol. The greatest percentage of inhibition is found in the outer skin with using 50% ethanol solvent of 53.29%. This shows more high percent inhibition indicates the number of hydrogen atoms that given by antioxidant compounds to DPPH radicals so that DPPH reduced to DPPH-H.

CONCLUSION

In this study it can be concluded that the antioxidant activity of using 50% ethanol is better than 70% ethanol in the outer shell, hard shell and melinjo seeds. The optimal percentage of free radical inhibition is obtained in 50% ethanol solvent, the outer shell of melinjo is 53.29%, and IC50 of 318.18 ppm. Based on the One way Anova test ($p>0.02$) there was no difference between the use of 50% and 70% ethanol solvent on the antioxidant activity of the outer shell, hard skin and melinjo seed samples.

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