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Determination of Hydrolyzed Tannin Content in Cocoa Fruit Peel Waste of MCC 01 Clone, Using UV-Vis Spectrophotometry

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Abstract

Cocoa Fruit Peel Waste MCC 01 Clone (Theobroma cacao L.) is a traditional medicinal plant with tannins that can be utilized as an anti-bacterial because it contains phenol groups and has antiseptic properties. This study aims to determine the type of tannin compound and the tannin content in cocoa fruit peel waste extract. The maceration method was used to extract the extract using 70% ethanol as a solvent. Qualitative test of tannin type was conducted using $C_2H_4O_2$ 10%, $C_2H_3O_2$ 10%, HCl, $FeCl_3$, and KBr, while quantitative test was conducted using UV-Vis Spectrophotometry at a wavelength of 765 nm. The qualitative test results to determine the type of tannins obtained from cocoa fruit peel waste indicate that it contains hydrolyzed tannins. In the quantitative test, the average tannin content was 4.73 mg/g extract.

Keywords: Cocoa fruit peel waste, MCC 01 Clone, extraction, tannin, 70% ethanol solvent, UV-Vis spectrophotometry

Introduction

Cocoa, or with the scientific designation Theobroma cacao L., is a tree-shaped plant from tropical forests in Central America and Northern South America (Septiani, 2023). Theobroma cacao L., cocoa, is a plantation commodity with bright prospects because it can be developed towards product diversification with a fairly high selling value. Cocoa fruit consists of cocoa pods and cocoa. In Pada Village, South Lore Subdistrict, the cocoa beans are often utilized in the cocoa processing industry. At the same time, the outer shells, which have become waste, are discarded so that they accumulate. Cocoa fruit peel waste is often not utilized properly and is left as waste among community plantations and the cocoa processing industry (Yumas, 2017). If cocoa fruit peel waste is not managed properly, the resulting impact can cause environmental pollution. Cocoa fruit peel waste contains active compounds that can be developed, such as tannin (Pappa et al., 2019).

Tannin is an active secondary metabolite compound with several properties, such as an antigen, antidiarrheal, antibacterial, and antioxidant (Farhanandi & Indah, 2022). Tannin can also be used as an antibacterial because the tannin content has a phenol group. Tannins have properties such as alcohol, which is antiseptic, and can be used for antimicrobial components. Tannin compounds can also inhibit the workings of enzymes and eliminate substrates (proteins) that bind to lipids and proteins, which can bind protease enzymes that catalyze proteins into amino acids needed for growth (Pappa et al., 2019).

Chemically, there are two main tannin types: hydrolyzed and condensed tannins. Hydrolyzed tannins can occur because they are formed from esterification reactions of phenolic acids and sugars (glucose). In contrast, condensed tannins occur due to polymerization (condensation) reactions between flavonoids (Halimu et al., 2017). Tannins are easily oxidized; depending on the substance exposed to air or hot water, they will easily turn into tannic acid. Meanwhile, tannic acid itself is an example of hydrolyzed tannins. Tannic acid is a polymer of gallic acid and glucose. Tannic acid is a crystalline powder, shimmering, white, yellow, or light brown, and has a distinctive odor. The efficacy of tannic acid is in treating diarrhea. In addition, tannic acid has the function of freezing proteins. Substances that have tannic acid content can harm the gastric mucosa, such as the mucous membrane lining the stomach, which can cause the person to suffer from various problems with the stomach. Tannic acid also has effects as an anti-bacterial, anti-enzymatic, antioxidant, and antimutagen. Hidjrawan (2020) used the extraction method to determine tannin levels in cocoa fruit peel waste.

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Extraction is a method of separating compounds from a matrix or simplisia using a suitable solvent. This is because the analysis process can begin with extraction until the end of the analysis. The purpose of extraction is to withdraw the components of chemical compounds contained in natural materials from plants, animals, and marine biota, using certain solvents. Fakhruzy (2020) states that the extraction process depends on the ability of a solvent to draw dissolved compounds from within, as well as the difference in equilibrium pressure of active substances inside and outside the cell. Extraction, in general, is a process of separating active substances from a solid or liquid using a solvent. Generally, the extraction process can be carried out with solvents based on the solubility of components in relation to other components in the mixture. In the extraction process, solvent selection is needed because the solvent must be able to extract the desired substance without dissolving other unwanted substances (Natsir, 2022). The extraction in this study will be done using the maceration method.

The maceration method was used in this study because the process is relatively simple and the equipment is easy to obtain. Maceration is the process of soaking samples using organic solvents at room temperature. In the process of soaking, plant samples will experience the breakdown of cell walls and membranes due to pressure differences between inside and outside the cell, so that secondary metabolism in the cytoplasm will be dissolved in organic solvents (Handayani & Nurcahyanti, 2014). The organic solvent used in the maceration method is 70% ethanol. 70% ethanol solvent was used in this study because 70% ethanol is a solvent that can extract or separate various kinds of polar compounds from polar to non-polar. 70% ethanol solvent has a level of polarity closest to the polarity of bioactive compounds. In addition, 70% ethanol is the only type of solvent that is safe or non-toxic when used because of the low level of toxicity content compared to other solvents, and 70% ethanol has a low boiling point of 79 ℃. for the tannin content determination test using the UV-Vis spectrophotometric method (Wahyuni, 2021).

UV-Vis spectrophotometry measures the wavelength and intensity of ultraviolet light and visible light absorbed by the sample used. Ultraviolet and visible light have enough energy to promote electrons on their outer shells to higher energy levels (Ryanata et al., 2015). Ultraviolet light is at a wavelength of 200-400 nm, while visible light is at a wavelength of 400-800 nm. The UV-Vis spectrum has a broad shape, and there is little information about the structure obtained from this spectrum, but this spectrum is also very useful for quantitative measurements. The concentration of the analyte in a solution can be determined by measuring the absorbance at a certain wavelength using the Lambert-Beer law (Natsir, 2022). To determine the hydrolyzed tannin content of cocoa pod waste of the MCC 01 clone type, the following objectives were carried out

This study aims to determine the tannin content and type of hydrolyzed tannin in the cocoa pod waste of the MCC 01 clone.

Methods

The research used was experimental quantitative research. The experiment conducted was to determine the total tannin content of cocoa fruit peel waste using the UV-Vis spectrophotometric method.

Research Tools and Materials

Tools

The tools used in this study include stirring rod, glass jar (*canister*), blender (*miyako*), spray bottle, funnel (*Iwaki*), analytical balance (*kern*), vortex (*maxi mix II*), water bath (*robusta*), dropper pipette, measuring pipette (*Iwaki*), test tube rack, test tube (*Iwaki*), vacuum rotary evaporator (*eyela*), maceration container (*Iwaki*), and UV-Vis spectrophotometry (*nanbei*).

Units

The materials used in this study include cocoa pod waste of MCC 01 clone (Theobroma cacao L.) originating from Pada Village, South Lore Subdistrict. The cocoa selected is fresh, not easy, and not old. Chemicals used included 70% ethanol, distilled water (H_2O), iron (III) chloride ($FeCl_3$) 1%, gallic acid 10 mg, Folin-Ciocalteu 3 mL, sodium carbonate (Na_2CO_3) 15%, acetic acid solution 10%, Pb acetate solution 10%, HCl 3 mL, and bromine reagent (KBr) 3 mL.

Research Procedure

Sample Preparation

The cocoa fruit skins were washed and dried by aerating in the room's open air. After the sample is dry, it is ground with a milling machine to obtain crude simplisia. The crude simplisia obtained is then dried in the sun and covered with a black cloth for 30 minutes to reduce the moisture content of the cocoa fruit skin, which is still a lot. Furthermore, the crude simplisia that has been dried in the sun is then blended to obtain finer simplisia.

Extraction Process

The cocoa fruit peel waste extraction begins with weighing 50 grams of cocoa fruit peel waste powder. After that, the sample was put into an Erlenmeyer flask and extracted using 70% ethanol for as long as 250 mL. Then, the Erlenmeyer containing the mixture was closed using aluminum foil and soaked for 3 days. After 3 days, the extract was filtered using filter paper to get the filtrate. Then the filtrate is put into a round-bottom flask and evaporated using a vacuum rotary evaporator at a temperature of 40-50 °C for 1 hour to obtain a concentrated and thick extract (Wahyuni & Marpaung, 2020).

Identification of Tannin Types

The 70% ethanol extract of cocoa fruit peel waste obtained can be tested as follows:

a. Hydrolyzed Tannins
   1) A total of 5 mL of extract filtrate was put into a test tube, and then 2 mL of a 10% acetic acid solution and 1 mL of a 10% Pb acetate solution were added, shaken, and allowed to stand for 5 minutes. A positive test for hydrolyzed tannins will produce a precipitate after standing for 5 minutes.
   2) A total of 3 mL of filtrate from the extraction was put into a test tube, and then 1 mL of HCl was added and heated to boiling. A positive test will not form an insoluble phenolphthalein red color.
   3) 3 mL of the extracted filtrate was put into a test tube, FeCl3 was added, and the mixture was shaken. A positive test will form a black color.
   4) 3 mL of extracted filtrate was put into a test tube, then 1 mL of bromine reagent (KBr) was added and shaken. A positive test will not have a precipitate.

b. Condensed Tannins
   1) A total of 5 mL of extract filtrate was put into a test tube, and then 2 mL of 10% acetic acid solution and 1 mL of 10% Pb acetate solution were added, shaken, and then allowed to stand for 5 minutes. A positive test for the presence of condensed tannins does not produce a precipitate after standing for 5 minutes, or remains in the form of a solution
   2) A total of 3 mL of the extracted filtrate was put into a test tube, and then 1 mL of HCl was added and then heated to boiling. A positive test will form an insoluble phenolphthalein red color
   3) A total of 3 mL of the extracted filtrate was put into a test tube and then added FeCl3 then shaken. A positive test will form a greenish black color
   4) 3 mL of extracted filtrate was put into a test tube, then 1 mL of bromine reagent (KBr) was added and shaken. A positive test will have a precipitate.

(Suwena, 2019).

Preparation of Gallic Acid Standard Solution 1000 ppm

A total of 100 mg of gallic acid is dissolved in distilled water until the volume is 100 mL. This standard solution must always be new every time you do the test (Hamzah et al., 2024).

Preparation of 250 ppm Gallic Acid Standard Solution

Piped 25 mL of 1000 ppm gallic acid standard solution made previously into a 100 mL volumetric flask, then dissolved with distilled water to the limit mark and homogenized (Hamzah et al., 2024).

Preparation of Standard Curve

250 ppm gallic acid standard solution was pipetted 0.2, 0.4, 0.6, 0.8, and 1 mL, put into a 25 mL volumetric flask, and distilled water was added to the limit mark. The resulting standard solution concentrations were 2, 4, 6, 8, and 10 ppm, after which 3 mL was pipetted from each concentration and 1 mL of 15% Na_2CO_3 solution was added, then vortexed and allowed to stand for 2 hours at room temperature. Then measure the absorbance using UV-Vis spectrophotometry at a wavelength of 765 nm (Hamzah et al., 2024).

Determination of Tannin Content by UV-Vis Spectrophotometry

1 mL was taken and put into a test tube for the thick extract obtained. 4 mL of distilled water, 1 mL of Folin-Ciocalteu reagent, and 15% Na2CO3 were added. The solution was then vortexed and allowed to stand for 2 hours at room temperature. Then, the absorbance was measured using UV-Vis spectrophotometry at a wavelength of 765 nm (Hamzah et al., 2024).

Data Analysis Technique

Data obtained in the study of tannin determination in cocoa fruit peel waste extract is from the UV-Vis spectrophotometric method. Spectrophometric method data in the form of absorbance data calculated by a linear regression equation:

$$y = ax + b$$

Where:
y = absorbance
x = concentration
a, b = constant

The determination of tannin levels by the UV-Vis spectrophotometric method can be calculated using the formula:

$$Total\ tannin = \frac{c \times x}{g}$$

Where:
c = tannin concentration (x value)
v = volume of extract used (mL)
g = weight of sample used

(Ulfasari, 2021).

Results and Discussion

Data on Results of Cocoa Fruit Peel Waste Extract

50 grams of cocoa fruit peel waste powder were extracted using 70% ethanol solvent for as long as 250 mL. The filtrate obtained was then concentrated using a vacuum rotary evaporator. A dark brown cocoa fruit peel waste extract was obtained from the extraction process.

Qualitative Test of Tannin Type Determination

Qualitative research on hydrolyzed and condensed tannin types was conducted on a 70% ethanol extract of cocoa fruit peel waste. Data from the identification test results for the presence of tannins are shown in **Table 1**.

Table 1. Determination of hydrolyzed tannin type

Reagent	Results	Information
$C_2H_4O_2$ 10% + $C_2H_3O_2$ 10%	A precipitate formed	Hydrolzed
HCl + heated	No red pholiphen color is formed	Hydrolzed
$FeCl_3$	A bluish black color is formed	Hydrolzed
KBr	No precipitate is formed	Hydrolzed

The experimental data show that cocoa pod shell waste is positive for hydrolyzed tannins.

Sample preparation in this study is cocoa fruit skin waste, cleaned and cut into smaller pieces to produce rough simplisia, then dried by aerating in the open air indoors, after which the rough simplisia obtained is then dried in the sun and covered with a black cloth. The purpose of drying is to reduce the water content of the cocoa fruit skin, which is still a lot, and to inhibit bacterial growth. Drying is covered with a black cloth so that the secondary metabolites sensitive to heat are not damaged. Furthermore, the rough simplisia that has been dried in the sun is then blended to get simplisia that is finer so that the surface of the material with the solvent dissolves quickly. The compounds are expected to be well absorbed (Handoyo & Pranoto, 2020).

Extraction is the separation of materials from their mixture using a suitable solvent. The extraction method used in this study is the maceration method, which separates compounds by immersion using certain organic solvents. The extraction process is influenced by temperature, time, and the type of maceration solvent used. The right temperature will produce high tannin yields; otherwise, using high temperatures and too long a time will reduce the yield of tannins produced and solvents. Appropriate solvents will increase tannin levels (Fakhruzy, 2020). Samples that have been mashed are taken as much as 50 grams and then extracted using 70% ethanol solvent, and 70% ethanol solvent is soaked for 3 days, soaking the sample aims to break the cell wall and cell membrane caused by the pressure difference between inside and outside the cell. This can cause secondary metabolites in the cell cytoplasm to be dissolved in organic solvents (Handoyo & Pranoto, 2020). Then the samples that have been macerated are filtered using a funnel and filter paper, and the filtrate obtained is concentrated using a rotary evaporator at a temperature of 40-500C °C. The rotary evaporator aims to change part or all of a solvent from liquid form to a more concentrated form, or as needed. In evaporation, the concentrated solution is the expected product (Artini et al., 2022).

In the tannin type identification test with 70% ethanol extract of cocoa fruit peel waste, hydrolyzed tannin results were obtained (Table 1). Where the first test is the addition of 10% acetic acid and 10% Pb acetate, the function of adding the solution is to form a precipitate. This precipitate is formed because the results of tannin hydrolysis produce polyhydroxy alcohol and gallic acid (Desinta, 2015). The second test of adding HCl and then heating the results obtained did not form a brick red color. The third test of adding $FeCl_3$ hydrolyzes the tannin group and produces a greenish-black precipitate (Halimu et al., 2017). The fourth test of adding bromine (KBr) reaction did not produce a precipitate.

Hydrolyzed tannins are ester compounds derived from a simple sugar and have one or even more carboxylic acid polyphenol compounds. In the center of the hydrolyzed tannin molecule, there is a carboxylate (D-glucose). The hydroxyl group of the carbohydrate will be partially or almost completely esterified by phenolic groups such as gallic acid in (galotanin) or can also be known as ellagic acid in (ellagitanin). Condensed tannin is a flavonoid polymer and is included in the type of phenol compounds. This type of tannin will produce hydrochloric acid. Condensed tannins are also known as proanthocyanidins, which are polymers of 2-50 flavonoid units bound by carbon-carbon bonds and are less susceptible to being broken down by hydrolysis. Condensed tannins can dissolve in water, but some large condensed tannins are insoluble, while hydrolyzed tannins are soluble in water (Kodir & Moektiwardoyo, 2022).

Tannin Content

The study results of tannin content analysis from cocoa fruit peel waste extract from Pada Village using UV-Vis spectrophotometry through a standard curve of gallic acid. The standard curve of gallic acid can be seen in **Figure 1**. The initial step taken to analyze the tannin content of cocoa fruit peel waste extract using the UV-Vis spectrophotometric method is by making a standard solution of gallic acid by weighing as much as 100 mg of gallic acid and dissolving it in distilled water to a volume of 100 mL, so that a standard solution of 1000 ppm can be obtained, then pipetted 25 mL of 1000 ppm gallic acid standard solution that has been made before into a 100 mL volumetric flask, dissolved with distilled water until the limit mark and homogenized, so that a standard solution of 250 ppm can be obtained. Furthermore, the standard solution of gallic acid 250 ppm was pipetted 0.2, 0.4, 0.6, 0.8, and 1 mL, entered into a 25 mL volumetric flask, and distilled water was added until the limit mark, until a concentration series of 2, 4, 6, 8, and 10 ppm. The function of making several series of gallic acid solution concentrations is to determine

the levels of tannin compounds in the sample using the linear regression equation of the gallic acid standard curve. The results of UV-Vis spectrophotometry measurements can be made into a linear regression equation, y = 0.0085x + 0.1071. Where y: absorption strength, x: concentration and coefficient, r2 = 0.9915, proving the regression is linear (Dalming & Karim, 2023).

Analysis of tannin content, thick extract of cocoa fruit peel waste, using UV-Vis spectrophotometry, was carried out at a wavelength of 765 nm. The thick extract obtained was taken as much as 5 mL and put into a test tube, then 1 mL of Folin-Ciocalteu reagent was added, after which 1mL of 15% Na_2CO_3 solution was added, vortexed, and allowed to stand for 2 hours at room temperature. The absorbance was measured at a wavelength of 765 nm. The function of the folin ciocalteu reagent and 15% Na_2CO_3 is used to form a colored solution that can occur based on colorimetric oxidation and reduction reactions to measure all tannin compounds present in the test sample. It can be measured for absorbance (Andriani et al., 2019).

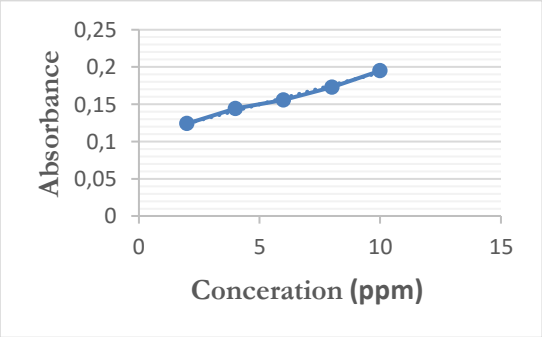

Figure 1. Gallic acid standard curve

The following presents the absorbance of cocoa pod shell waste samples in **Table 2**. In Pappa et al. (2019) research on tannin content in cocoa fruit skin (Theobroma cacao L.), the results of phytochemical tests showed that cocoa fruit skin contains terpenoids, saponins, and tannins. The total tannin content of the cocoa fruit skin of the North Toraja regency was higher at 12.679% compared to 4.981% from the Poliwalimandar regency. What distinguishes this study from the research by Pappa et al. (2019) is the location of the sample, the type of sample, and the solvent used, because the chemical content of plants can also be influenced by the type of fruit and soil content where the plant grows. The soil content can affect the composition of cocoa bioactive compounds, one of which is found in the fruit skin. In addition, what distinguishes this study is that the previous study only examined the total tannin content, while this study examined the hydrolyzed tannin type test.

Table 2. Absorbance data of cocoa pod weste extract samples

Sample	Absorbance	Concentration Sample (ppm)	Tannin Levels (mg/g extract)
Cocoa fruit skin waste 1	0.139	3.752941	3.752941
Cocoa fruit skin waste 2	0.161	6.341176	6.341176
Cocoa fruit skin waste 3	0.142	4.105882	4.105882
Average		4.73 mg/g Extract	

In the research on tannin content analysis using UV-Vis spectrophotometry conducted with three repetitions, the tannin content of cocoa fruit peel waste extract can be obtained; the first sample is 3.752941 ppm, the second sample is 6.341176 ppm, and the third sample is 4.105882 ppm. The cocoa fruit peel waste extract calculation results amounted to 4.73 mg/g extract. The purpose of repetition is to get more accurate results and minimize the possibility of data errors.

Conclusions

Based on the research results, it can be concluded that cocoa fruit peel waste MCC 01 Clone (Theobroma cacao L.) is classified as hydrolyzed tannins. In determining the tannin content of cocoa fruit peel waste (Theobroma cacao L.) using UV-Vis spectrophotometry, an average tannin content of 4.73 mg/g extract was obtained.

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