DETERMINATION OF TANNIN CONTENT OF YOUNG SAPODILLA FRUIT (*Manilkara zapota*) USING UV-VIS SPECTROPHOTOMETRY AND PERMANGANOMETRIC TITRATION

Ade Trisnawati
Universitas PGRI Madiun, Indonesia
adetrisnawati@unipma.ac.id

Abstract

This study aims to determine the tannin content of the young fruit peels and young flesh of sapodilla (Manilkara zapota) using spectrophotometry and permanganometric titration methods. The samples used were the skin and flesh of young sapodilla fruit. The extraction of tannins from the skin and flesh of sapodilla fruit (Manilkara zapota) was carried out by maceration using 95% ethanol. The data analysis used a simple linear regression test. Based on the results of research using the UV-VIS spectrophotometry method, it was found that the total content of young sapodilla rind extract was 2.745 mg GAE/g extract, or 0.2745% extract, whereas in young sapodilla fruit flesh extract, it was 3.353 mg GAE/g extract, or 0.3353% extract. The determination of tannin content using the permanganometric titration method on young sapodilla fruit skin is known to have an average tannin content of 3.756% and on young sapodilla fruit flesh of 6.443%.

Keywords: Manilkara zapota, Tannin Content, UV-Vis Spectrophotometry, Permanganometric Titration, Young Sapodilla

INTRODUCTION

The sapodilla plant is one of the plants in Indonesia, and its benefits have been widely explored, starting from the roots, stems, bark, leaves, and fruit. Ripe sapodilla fruit has soft flesh, a sweet taste, and is rich in fiber. Sapodilla fruit is a good source of nutrition because it contains polyphenolic bioactive compounds (Gomathy et al., 2013). Ripe sapodilla fruit skin is reddish brown to yellowish, with rough scales and the remaining dry pistil stalk at the end. The skin of the sapodilla fruit is part of the sapodilla fruit whose utilization is not optimal; usually it will only be thrown away or used for animal feed. Several research results report that sapodilla fruit skin contains bioactive compounds such as tannin compounds (Shafii et al., 2017; Pravin & Shashikant, 2019), phenolic compounds, and flavonoids (Gomathy et al., 2013; Tulloch et al., 2019), as well as alkaloids (Sihombing et al., 2015).

Tannin is known to be an active secondary metabolite compound that has several properties, namely as an astringent, anti-diarrhea, anti-bacterial, and antioxidant. Tannin is a very complex component of organic substances, consisting of phenolic compounds that are difficult to separate and crystallize, precipitate proteins from the solution, and combine with these proteins (Pratama et al., 2019).

Tannin has several properties, including stopping bleeding and treating burns, stopping internal healing from taking place, and being able to create a protective layer for wounds and kidneys. Tannins have been used for a long time as a quick treatment for diarrhea, dysentery, bleeding, and to reduce tumor size. Various viruses are inactivated by exposure to tannins (Pratama et al., 2019). Tannin can be used as an antibacterial because it has a phenol group, so tannin has properties like alcohol, namely antiseptic properties that can be used as an antimicrobial component (Mihra et al., 2018).

The determination of tannin levels in the skin and flesh of sapodilla fruit (Manilkara zapota) has never been carried out; therefore, it is necessary to carry out analysis to determine tannin levels in these samples. The content of secondary metabolite compounds in a plant can

be determined using an approach that can provide information on the presence of secondary metabolite compounds, in this case tannins. One method that can be used is the phytochemical method. Apart from that, to determine the highest tannin levels in sapodilla plants, use the UV-VIS spectrophotometry method and permanganometric titration. During titration with potassium permanganate solution, the phenol groups in the tannins will be oxidized. The number of phenol groups is directly proportional to the amount of potassium permanganate required for the titration. Tannins are a class of compounds that have phenol groups, so these four phenol groups are assumed to represent the total amount of tannins. Based on the background described above, it is necessary to carry out research regarding the determination of tannin content from the ethanol extract of sapodilla skin and flesh using the UV-VIS spectrophotometry method and permanganometric titration.

RESEARCH METHODS

Location of Research Implementation

This research was conducted at the Integrated Laboratory of PGRI Madiun University. Young sapodilla fruit used as the main ingredient comes from Bareng Village, Babadan District, Ponorogo Regency, where sapodilla plants are commonly found in people's yards or gardens.

Research Stages

Preparation of Research Tools and Materials

The sapodilla fruit used in this research was taken from Bareng Village, Babadan District, Ponorogo Regency. The young sapodilla fruit used is young sapodilla fruit that falls before it is ripe due to several factors, such as animal disturbance or being carried by the wind. The materials used in this study were 70% ethanol, distilled water, gallic acid, folin ciocalteu reagent, 15% Na2CO3, oxalic acid, sulfuric acid (H2SO4), 0.1 N KMnO4, indigo sulfonate indicator, and FeCl3.

The tools used in this study were beakers, test tubes, volume pipettes, measuring pipettes, stir bars, volumetric flasks, pipettes, analytical balances, rotary evaporators, cuvettes, UV-VIS spectrophotometry, dropper pipettes, water baths, analytical balances, and microscopy. pipette, Erlenmeyer glass, filter paper, beaker, measuring cup, glass stirrer, glass funnel, drip board, burette, 25-ml volume pipette, blender, volumetric flask.

Sample preparation is in the form of dry powder from sapodilla fruit skin and flesh. The skin and flesh of the sapodilla fruit are separated, dried, and ground to obtain powdered skin and pulp. The skin and flesh of the sapodilla fruit were weighed to determine the weight.
Making Ethanol Extract of Sapodilla Fruit Skin and Flesh
100 grams of dry powder of sapodilla fruit skin and flesh were weighed and mixed with 300 mL of 70% ethanol, then the mixture was stirred. The mixture was left for two days. After 2 days, filter until there are dregs and filtrate. Re-maceration of the dregs obtained The filtrate obtained was collected and concentrated using a rotary evaporator.

Determination of Tannin Content Using UV-VIS Spectrophotometry

a. Maximum Wavelength Determination

As much as 10.0 mg of gallic acid was weighed, dissolved with distilled water, put in a 100-mL volumetric flask, and added to a volume of 100 mL to form a standard stock solution of 100 ppm. A certain amount of the gallic acid stock standard solution is pipetted and put into a 10 mL volumetric flask. Add 1 mL of Folin Ciocalteu reagent, shake the solution, and leave for 5 minutes. Add 2 mL of a 15% Na_2CO_3 solution to the solution, shake until homogeneous, and let the solution sit for 5 minutes. Add distilled water to the solution until it is exactly 10 mL, and read at a wavelength in the range of 500–900 nm.

b. Stable timing

A certain amount of gallic acid mother standard solution is taken, put into a 10 mL volumetric flask, and 1 mL of Folin Ciocalteu reagent is added. The solution is shaken and allowed to stand for 5 minutes. 2 mL of a 15% Na2CO3 solution was added to the solution, shaken until homogeneous, and allowed to stand for 5 minutes. Then add distilled water until it is exactly 10 mL. The absorbance of the solution was observed at intervals of up to 110 minutes at the maximum wavelength.

c. Preparation of the Gallic Acid Standard Curve with Folin Ciocalteu Reagent

A certain amount of gallic acid mother standard solution is pipetted, the solution is put into a 10 mL volumetric flask, 1 mL of Folin Ciocalteu reagent is added, and the solution is shaken and allowed to stand for 5 minutes. The solution was added to 2 mL of 75% Na_2CO_3 solution, shaken until homogeneous, and allowed to stand for 5 minutes. Added distilled water to a volume of 10 mL, shaken until homogeneous, and allowed to stand for a stable time. The absorbance was measured on a UV-VIS spectrophotometer and observed at the maximum wavelength. Intake of gallic acid mother stock solution is carried out a certain number of times, five times, so that five concentrations are obtained and a gallic acid standard curve is made.

d. Determination of the Total Tannin Content of Sapodilla Fruit Skin

As much as 100 mg of the ethanol extract of young sapodilla rind was dissolved in 50 mL of distilled water and replicated three times. Each of the replicates was taken at a concentration of as much as 9 mL and dissolved in distilled water up to 10 mL (90 ppm). 1 mL of folin ciocalteu reagent was added, allowed to stand for 5 minutes, and then 2 mL of a 75% Na2CO3 solution was added and incubated. Sample absorption measurements were carried out at the maximum wavelength.

e. Determination of the Total Tannin Content of Sapodilla Fruit Flesh

As much as 100 mg of ethanol extract from young sapodilla fruit flesh was dissolved with distilled water up to 50 mL and replicated three times. Each replication was pipetted to as much as 9 mL and dissolved with distilled water up to 10 mL (90 ppm). 1 mL of folin ciocalteu reagent was added, allowed to stand for 5 minutes, and 2 mL of a 75% Na2CO3 solution was added and incubated. Sample absorption measurements were carried out at the maximum wavelength.

Determination of Tannin Content Using Permanganometric Titration

a. Standardization of Oxalic Acid Primer Solution

Weighed 0.693 grams of $2H_2O$ oxalic acid, put it in a beaker, and dissolved it with enough distilled water. The oxalic acid solution was put into a 100-mL volumetric flask, and distilled water was added until the mark on the volumetric flask was reached (Ebry, 2014).

b. Standardization of KMnO4 solution with 0.1 N oxalic acid

10.0 mL of $2H_2O$ 0.1N oxalic acid solution was pipetted and put into a 100-mL Erlenmeyer flask, and 10 mL of 4 N H_2SO_4 solution was added. The solution was heated to a temperature of 70 °C. Then the solution was titrated with a 0.1 N $KMnO_4$ solution. The titration is stopped when the color changes from colorless to pink (the TAT has been reached). Three replications were carried out, and the results were recorded (Ryanata, 2014).

c. Determination of Tannin Content with $KMnO_4$

Take ± 0.1 grams of young sapodilla skin and young fruit flesh extract each and put them into a beaker. The extract was added to 5 mL of distilled water, then heated in a water bath until boiling for 30 minutes while stirring. The solution was left for several minutes until a precipitate appeared. The extract solution is poured on filter paper into a 25-mL volumetric flask to obtain the filtrate and residue. Boiling distilled water is added to the residue and put into the same volumetric flask. The residue was filtered several times until it did not show a color change to blue-black when reacted with $FeCl_3$. The solution was cooled, and distilled

water was added quantitatively to 250 mL in the measuring flask. A total of 2.5 mL of the solution was pipetted and put into a 100-mL Erlenmeyer flask. 75 mL of distilled water and 2.5 mL of LP indigo sulfonic acid indicator were added. The sample solution was titrated with $KMnO_4$ until the color changed from dark blue to golden yellow. The volume of $KMnO_4$ used was recorded and replicated three times (Ryanata, 2014).

d. Blank Titration Preparation and Measurement

A total of 75 mL of distilled water was put into a 100 mL Erlenmeyer flask, and 10 mL of sample solution and 2.5 mL of indigo sulfonic acid indicator were added. The solution was titrated with $KMnO_4$ until the color of the solution changed from dark blue to golden yellow and repeated three times (Ryanata, 2014).

RESULTS AND DISCUSSION

A. Extraction of Skin and Flesh Powder of Sapodilla Fruit (Manilkara zapota)

Results of extracting the skin and flesh of sapodilla fruit (Manilakara zapota) by maceration for 3 days and stirring every 8 hours Weighing 300 grams of powder resulted in 8.1 grams of light sapodilla skin extract and 10.2 grams of light sapodilla pulp extract.

B. Determination of Tannin Content Using UV-VIS Spectrophotometry

This study aims to determine the total tannin content of the skin and flesh of sapodilla fruit. The determination of total tannin content begins with determining the maximum wavelength (λ max) of gallic acid in the range λ 400–800 nm, and the resulting maximum wavelength (λ max) is 788 nm. The determination of the stable time was carried out to create a gallic acid calibration curve. From the experimental results at a wavelength of 788 nm, it shows that the absorbance is stable from the 80th minute to the 84th minute.

Table 1: Determination of Gallic Acid Stable Time

Time (minute)	Absorbance	Time (minute)	Absorbance
68	0,400	90	0,424
70	0,403	92	0,429
72	0,404	94	0,436
74	0,405	96	0,441
76	0,411	98	0,445
78	0,411	100	0,461
80	0,412	102	0,470
82	0,412	104	0,464
84	0,412	106	0,466
86	0,413	110	0,472
88	0,420	112	0,480
89	0,422	114	0,485

The gallic acid standard curve was prepared from a working standard solution with the addition of Folin Ciocalteu reagent, which was observed using UV-VIS spectrophotometry at a wavelength of 748 nm. The results obtained are listed in Table 2 below.

Table 2: Absorbance of Gallic Acid Standard Solution

Concentration Gallic Acid	Absorbance
10	0,125
20	0,148
30	0,326
40	0,512
50	0,771

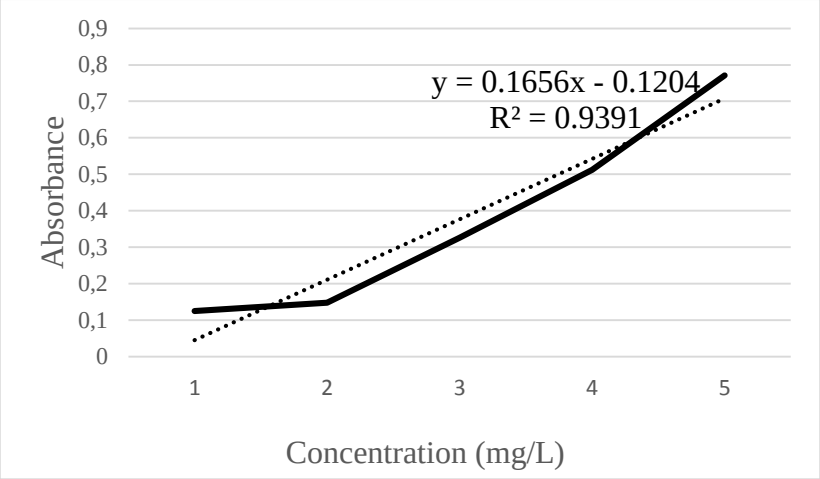

Figure 1: Gallic Acid Standard Curve

The regression results show that the calculated r is 0.9391, which shows that concentration and absorbance have a significant correlation. Followed by measuring the absorption of the sample. Following are the results of determining the absorbance of the two samples with three repetitions.

Table 3: Absorbance Measurements of Light Brown Skin Samples

Repetition	Absorbance	Mean absorbance	Total Tannin Content
I	0,7881	0,7886	2,745 mgGAE/g extract
II	0,7886		
III	0,7892		

Table 4: Absorbance Measurements of Sapodilla Fruit Flesh Samples

Repetition	Absorbance	Mean absorbance	Total Tannin Content
I	0,9871	0,9901	3,353 mg GAE/g extract
II	0,9861		
III	0,9972		

Research has been carried out on the ethanol extract of the skin and flesh of the sapodilla fruit (Manilkara zapota). Tannin is a very complex organic substance component, consisting of phenolic compounds that are difficult to separate and crystallize, precipitate proteins from the solution, and combine with these proteins. Tannin's properties as an astringent can be used as an antidiarrheal to stop bleeding and prevent inflammation, especially in the oral mucosa, as well as being used as an antidote for heavy metal and alkaloid poisoning.

This research aims to determine the levels of tannin compounds contained in the ethanol extract of the skin and flesh of sapodilla fruit using two methods, namely the UV-VIS spectrophotometer method and permanganometric titration.

The samples used in this research were the skin and flesh of sapodilla fruit obtained from Bareng Babadan village, Ponorogo, East Java. Next, the samples were dried in a place not exposed to direct sunlight. The dried skin and flesh of the sapodilla fruit are then powdered to expand the surface so that during extraction, contact between the solvent and the sample is more effective and the compounds can be extracted optimally. Drying is done so that enzymatic reactions do not take place and prevent microbial growth on the simplicia. After obtaining the simplicia powder, extraction was carried out using the maceration method.

Before carrying out quantitative analysis, a standard curve is first constructed. The aim of making a standard curve is to determine the relationship between the concentration of the gallic acid solution and the absorbance value so that the concentration of sapodilla fruit skin

and flesh samples can be known. Standard solutions were made into concentration series of 10, 20, 30, 40, and 50 ppm, and a linear regression was made as in Figure 4.1. The standard curve equation obtained from the gallic acid concentration, namely y = 0.1656 x 0.1204 with a value of r2 = 0.9391, was used to determine the tannin content in the skin and flesh of sapodilla fruit (Manilkara zapota).

Tannins that are read using UV-Vis spectrophotometry must be reacted with color-forming reagents, namely folin ciocalteu and sodium carbonate. The formation reaction that occurs between tannin and folin ciocalteu reagent is an oxidation-reduction reaction, where tannin acts as a reducing agent while folin ciocalteu reagent acts as an oxidizer. The result of oxidation will be a blue color that can be read at the maximum wavelength. Folin ciocalteu is used as a reagent because phenolic compounds can react with folin to form a colored solution whose absorbance can be measured. The principle of the Folin-Ciocalteu method is that a blue complex compound is formed and can be measured at a maximum wavelength in the range 400–800 nm. This reagent oxidizes phenolics (alkaline salts), or the phenolic-hydroxy group reduces the heteropoly acid (phosphomolybdic-phosphotungstic) contained in the folin ciocalteu reagent into a molybdenum-tungsten complex compound (Ryanata, 2015). This can be seen in Figure 4.2 below.

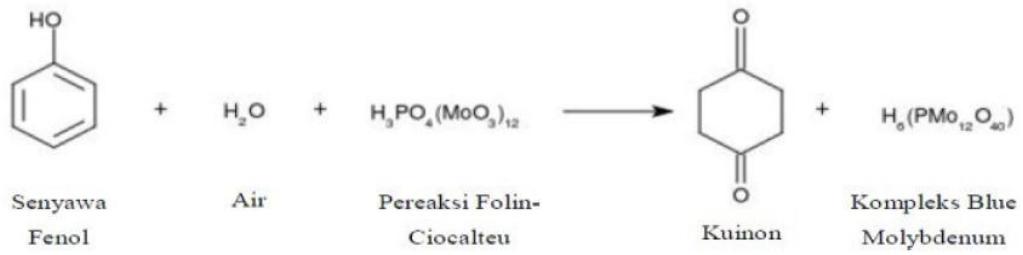

Figure 2: Reaction of Tannins with Folin Ciocalteu (Fatonah et al., 2021).

The samples were weighed three times, with each weighing 0.1 gram. Then measured using UV-Vis spectrophotometry to determine the absorbance of the sample. To obtain the absorbance of the actual sample, it is done by reducing the absorbance value of the sample by the absorbance value of the extract control. After obtaining the absorbance of the actual sample, the value of the total tannin content of each sample was calculated. The average total tannin content value of extracts from the skin and flesh of sapodilla fruit (Manilkara zapota) is obtained by adding up the total value of the total tannin content and then dividing it by 3. The results obtained are that each gram of sapodilla peel extract contains 0.2745% tannin, and that of the flesh extract of young sapodilla fruit contains tannins of 0.3353%, which is equivalent to gallic acid.

C. Results of the Determination of Tannin Levels in Sapodilla Fruit Peel Using the Permanganometric Titration Method

1. Standardization of the KMnO4 Solution with 0.1 N Oxalic Acid

Table 5 Results of Standardizing Solutions with Oxalic Acid

Titrasi	Larutan $H_2C_2O_4$	Larutan H_2SO_4	Larutan $KMnO_4$	Normalitas $KMnO_4$
1	10 mL	10 mL	28,5 mL	0,03509 N
2	10 mL	10 mL	29,3 mL	0,03413 N
3	10 mL	10 mL	27,5 mL	0,03637 N
Rata-rata				0,03520 N

2. Results of sample volume titration with $KMnO_4$

Table 6: Titration Results of Sapodilla Skin Samples

Titrasi	Larutan Sampel + Indikator	Larutan $KMnO_4$
1	10 mL	10,8 mL
2	10 mL	11,2 mL
3	10 mL	10,1 mL

Table 7 Results of blank titration volumes

Titrasi	Larutan Sampel + Indikator	Larutan $KMnO_4$
1	10 mL	1,0 mL
2	10 mL	0,9 mL
3	10 mL	1,1 mL

Table 8: Calculation results of % Tannin

Titrasi	Kadar Tanin
1	7,2484 %
2	7,54125 %
3	6,58944 %

D. Results of the Determination of Tannin Content of Sapodilla Fruit Flesh Using the Permanganometric Titration Method

1. Standardization of the KMnO4 Solution with 0.1 N Oxalic Acid

Table 9: Results of Standardizing Solutions with Oxalic Acid

Titrasi	Larutan $H_2C_2O_4$	Larutan H_2SO_4	Larutan $KMnO_4$	Normalitas $KMnO_4$
1	10 mL	10 mL	28,5 mL	0,03509 N
2	10 mL	10 mL	29,3 mL	0,03413 N
3	10 mL	10 mL	27,5 mL	0,03637 N
Rata-rata				0,03520 N

2. Results of sample volume titration with $KMnO_4$

Table 10: Titration Results of Sapodilla Meat Samples

Titrasi	Larutan Sampel + Indikator	Larutan $KMnO_4$
1	10 mL	10,8 mL
2	10 mL	11,2 mL
3	10 mL	10,1 mL

3. Results of the blank titration volume

Table 11: Blank Titration Results

Titrasi	Larutan Sampel + Indikator	Larutan $KMnO_4$
1	10 mL	1,0 mL
2	10 mL	0,9 mL
3	10 mL	1,1 mL

4. Calculation results of % Tannin

Table 12: Calculation results for tannin content

Titrasi	Kadar Tanin
1	7,2484 %
2	7,54125 %
3	6,58944 %

The determination of tannin content by the permanganometric titration method involves oxidation-reduction reactions. $KMnO_4$ solution is used as a standard solution because it is a strong oxidizing agent, commonly used, easy to obtain, and inexpensive. The principle of this

method is to measure the volume of $KMnO_4$ required in the sample titration process until a golden yellow color changes in the solution. The $KMnO_4$ solution to be used as a titrant in sample titrations must be standardized. Determination of the tannin content of the ethanol extract of the skin and flesh of young sapodilla fruit using a standardized $KMnO_4$ titrant solution. In determining this level, the Indigocarmin indicator is used, which functions to help detect the occurrence of the end point of the titration with a color change from dark blue to golden yellow. $KMnO_4$ acts as an oxidizing agent, which will oxidize phenolics in the sample. For 1 ml of 0.1N $KMnO_4$, it will oxidize 0.00146 grams of tannins. After all the assay processes have been carried out, from the existing data it is possible to calculate the % tannin content, namely the results obtained in the young sapodilla fruit flesh extract of 6.443% and in the young sapodilla fruit skin extract of 3.756%.

CONCLUSION

Based on the results of the analysis and discussion related to the problem formulation, several conclusions can be drawn from the results of the research, namely that the total tannin content using UV-Vis spectrophotometry in the sapodilla fruit peel extract was 2.745 mg GAE/g extract, or 0.2745% extract, and in the sapodilla fruit flesh extract, it was 3.353 mg GAE/g extract, or 0.3353% extract. The determination of total tannin content using permanganometric titration in sapodilla fruit skin extract was 3.756%, and in young sapodilla pulp extract it was 6.443%.

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