ANALYSIS OF TANNIN IN SAPODILLA FRUIT (*Manilkara zapota* (L) VAN ROYEN)

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ABSTRACT

Sapodilla fruit (*Manilkara zapota* (L) Van Royen) contains chemical compounds including flavonoids, saponins and tannins. This study aims to determine the types and levels of tannins in sapodilla fruit. Sapodilla fruit was extracted using the maceration method. Sapodilla fruit extract (*Manilkara zapota* (L) Van Royen) was then tested qualitatively using the following tests: 1) FeCl₃ 2) potassium fericyanide and 3) gelatin. The test results showed that sapodilla fruit was positive for tannin. The type of tannin contained in sapodilla fruit is a type of condensed tannin. The quantitative test was performed using the permanganometric method and obtained an average tannin content of 0.84% with a standard deviation and a coefficient of variation of 0.0710 and 8.45%, respectively.

Keywords: Sapodilla Fruit (*Manilkara zapota* (L) Van Royen), Maceration, Analysis, Tannin.

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INTRODUCTION

Sapodilla fruit (*Manilkara zapota* (L) van royen) is a tropical fruit originating from America, precisely in Mexico to Guatemala, Salvador, and North Honduras. It is also called neesbery or sapodilla. Sapodilla fruit has soft fruit flesh, but there is a taste texture like sand because sapodilla fruit contains stone cells (sclereida). Sapodilla fruit has properties as a medicine for diarrhea and dysentery. In addition, sapodilla can also be used as an ingredient in processed foods, such as jam, syrup, or fermented into wine or vinegar.¹

Sapodilla plants contain chemical compounds including flavonoids, saponins and tannins. The three compounds have antibacterial properties. In young sapodilla fruit, the tannin content is higher than the old sapodilla fruit. Antibacterial power that is high in sapodilla due to the high content of tannins so that the sapodilla taste becomes bitter and bitter.²

Tannin is defined as phenol polyhydric which is complex with molecular size and shape that allows it to dissolve in water, glycerol, alcohol, and hydroxy alcohol. Tannin, also called tanic acid, C₁₄H₁₀O₉, is a group of vegetable compounds that are acidic, aromatic, and give a feeling of roughness. Tannin is an active compound of secondary metabolites that is known to have several properties including anti-diarrhea, anti-bacterial and antioxidant.³

Tanin generally consists of two types, namely condensed tannins and hydrolyzed tannins. These two types of tannins are found in plants, but the most dominant ones in plants are condensed tannins. Identification of tannin can be done by using a solution of FeCl₃, gelatin, potassium ferricyanide and ammonia. For quantitative testing of tannins using the permanganometry method.⁴

The desired compound in a plant can be obtained by the extraction process. Extraction is the process of withdrawing metabolites from plants using appropriate solvents. Extracting tannin from *Phyllanthus niruri* Linn, using various organic solvents (petroleum ether, dikhoromethane, chloroform, methanol, ethanol

and acetone).⁵ Extracting tannins from avocado (*Persea americana* Mill) seeds with 95% ethanol.⁶ Extracting tannins from betel nut (*Areca catechu*. L) with water and ethanol.⁷ Extracting tannins in tea bags with 70% ethanol using maceration method.⁸ The use of solvents for extraction is of course adapted to the nature of the content of these plant compounds, generally organic solvents are used for plants containing lipophilic compounds.⁹

The levels of tannins in each plant have different contents in a region, this is caused by the condition of climate, and environmental factors where it grows. This article is intended to analyze tannin levels in sapodilla fruit (*Manilkara zapota* (L) van royen) which grows in the area of Central Sulawesi.

EXPERIMENTAL

Sample Preparation

Choosing sapodilla fruit whose size is not too large, if the outer part of the sapodilla fruit is rubbed it will look green on the inner skin which indicates the sapodilla fruit is still young and there is still much sap. Then wash with water until clean, then dry (aerated at room temperature). Next, cut into small sapodilla fruit with a knife and dioven at a temperature of 45° C to dry and mashed with a blender.

Extraction of samples in this study using maceration method. Young sapodilla fruit powder weighed 50 grams, put into maceration container, then added 70% ethanol 150 mL until all samples were submerged by solvent, then shaker for 2 hours then covered with aluminum foil, then left for 12 hours. The maceration obtained is filtered with Whatman no.1 paper obtained by filtration and residue. On the dregs, maceration is repeated (repeated 1 time). The obtained filtrate is collected. Then concentrated using a vacuum rotary evaporator at 45° C.¹⁰

Identification of The Type of Tannin

Sapodilla fruit extract (*Manilkara zapota* (L) van royen), then performed a qualitative test using: (1) The extracted filtrate is added with 1 mL of FeCl₃. A positive test for the presence of tannins will give a blue-black precipitate to the hydrolyzed tannin and give a greenish-black precipitate to the condensed tannin. (2) The extract was added with 1 ml of potassium fericyanide and 1 ml of ammonia. A positive test for the presence of tannins will give a dark red color. (3) The extract was added with 1 mL of 1% gelatin solution containing NaCl. A positive test for the presence of tannins will result in a sediment¹¹. (4) The extract is added with 1 mL of ammonia solution then exposed to open air. Test positive for the presence of tannins with the appearance of green color.

Determination of Tannin Level by Permanganometry

Making of Primary Standard Solution of 0.1N Oxalic Acid

0.67 grams of solid oxalic acid is weighed and then dissolved with enough aquadest in the beaker. Enter into a 100 mL volumetric flask, then add aquadest to the limit mark on the measuring flask. Then calculate N oxalic acid.¹² The formula for calculating N oxalic acid:

$$N \text{ Oxalic acid} = \frac{\text{gram}}{\text{Mr}} \times \frac{1000}{p} \times \text{equivalent}$$

Standardization of KMnO₄ with 0.1N Oxalic Acid

10 mL of 0.1N oxalic acid solution was put into 100 mL Erlenmeyer, 10 mL of 4N H₂SO₄ solution was added, heated to 70°C then titrated with KMnO₄ 0.1N. The titration is stopped if there has been a change in color or has reached the endpoint of titration¹². Repeated 5 times. The results of determining the normality of KMnO₄ are determined by the formula¹²:

$$N \text{ KMnO}_4 = \frac{\text{Oxalic acid volume} \times N \text{ Oxalic acid}}{\text{KMnO}_4 \text{ volume}}$$

Determination of Total Tannin Level

2 grams of dried sapodilla powder which has been mashed is put into a beaker. Then added 50 mL of aquadest, heated over the water bath until boiling for 30 minutes while stirring. Let stand for a few minutes, deposited, then filtered using filter paper and obtained the filtrate. The pulp is filtered again with hot aquadest. Screening is done several times until the residue does not show a change in color to black blue when reacted with FeCl₃.

The filtrate obtained was taken 25 ml and then entered into a 250 mL volumetric flask and added aquadest to the measuring flask limit. 2.5 mL of solution was put into 250 mL erlenmeyer, 75 mL of aquadest was added and 2.5 mL of the indigo carmine indicator. Then, titrated with KMnO_4 until the color changes from dark blue to golden yellow. Recorded the volume of KMnO_4 used. Repeat 5 times.^{13,14} The results of determining the total tannin can be determined by the formula:^{13, 14}

$$\% \text{ Tannin} = \frac{10(A-B) \times N \times 0,0415}{\text{Sample (g)}} \times 100\%$$

Where A is the volume of tannin titration (mL); B is the volume of blank titration (mL); N is the normality of standard KMnO_4 (N); and 10 is a dilution factor. 1ml of KMnO_4 0.1N equals 0.0415 grams of tannin.

RESULTS AND DISCUSSION

Sapodilla Extract

Tannin is an organic substance that is very complex and consists of phenolic compounds that are found in various plants. Generally, tannins are distributed in almost all parts of the plant, such as the bark, stems, leaves and fruit.¹⁵ Tannin is an active secondary metabolite compound that is known to have several properties including anti-diarrhea, anti-bacterial, and antioxidant properties. The sapodilla fruit extraction was carried out by using the kinetic maceration method. This method is a cold maceration that does not go through a heating process to obtain the extract to avoid damage to the compounds contained in sapodilla fruit at high temperatures.¹⁶ The extract of sapodilla fruit (*Manilkara zapota* (L) Van Royen) obtained 29.3 mL of dark brown sapodilla fruit extract.

Qualitative Analysis of Tannin

The test to determine the presence of tannins in sapodilla fruit, used the addition of FeCl_3 to determine whether the sample contains phenol groups. The presence of phenol groups is indicated by a blackish-green or dark blue color after adding FeCl_3 , so that if the FeCl_3 test gives positive results, the sample may contain phenolic compounds and one of them is tannins because tannins are polyphenolic compounds.¹⁷

The results showed that the addition of FeCl_3 solution to sapodilla fruit extract resulted in a black-green color change. This shows the presence of a phenol group in the tannin that binds to FeCl_3 to form a black-green complex because tannins form complex compounds with Fe^{3+} ions (Fig.-1). These results indicated that sapodilla fruit was positive for tannin.

The addition of potassium ferric chloride and ammonia produces a dark red color which indicates the presence of tannins in sapodilla fruit. The reaction of tannins with potassium ferric chloride and ammonia can be seen in the following reaction:

Ammonia test ethanol extract of 70% sapodilla fruit produces a green color after exposure to open air, with these results, the sapodilla fruit extract contains positive tannins. The detailed results of the tannin identification test can be seen in Table-1.

Table-1: Identification of Tannin

Reagent	Result	Concl.
FeCl_3	Greenish Black	+
Gelatin Salt Solution	Precipitation	+
$\text{K}_3\text{Fe}(\text{CN})_6$ + Ammonia	Dark Red	+
Ammonia Test	Green	+

Tannins are divided into two groups, namely easily hydrolyzed tannins and condensed tannins.¹⁸ Based on the data in Table-1, the formation of a greenish black color and the presence of sediment (positive test for condensed tannins) indicates the type of tannin contained in sapodilla fruit. The results obtained indicate that the type of tannin contained in sapodilla fruit (*Manilkara zapota* (L) van royen) is a type of condensed tannin. Condensed tannins are polymers of flavonoid compounds with carbon-carbon bonds in the form of catechin and gallo catechin.¹⁹

The next test used a gelatin solution (Table-1). This test is a preliminary test to strengthen the suspicion of tannin compounds in sapodilla fruit extract. All tannins create a little or a lot of deposits when added with gelatin. The addition of a salt solution to which gelatin is added forms a yellowish white precipitate. The

presence of a yellowish-white precipitate indicates that tannins coagulate proteins from gelatin, because tannins can react with proteins to form copolymers that are insoluble in water.¹⁷ The reaction between tannins and gelatin can be seen in Fig.-2.

Fig.-1: Reaction between Tannin and FeCl_3 ¹⁷

Fig.-2: Reaction between Tannin and Gelatin²⁰

Quantitative analysis to determine the total tannin content of sapodilla fruit peel (*Manilkara zapota* (L) van royen) was carried out by permanganometric titration method. This method uses the principle of the redox reaction (reduction-oxidation), potassium permanganate as the oxidizing agent and the reducing

agent is oxalic acid (primary standard solution). The indicator used in this titration is indigo carmine (TAT) which is shown by the color of the solution changing to golden yellow, in determining the levels of tannins.²¹

Blank titration was also carried out which aims to determine the volume of KMnO_4 which reacts with indigo carmine. The volume of the blank titration is used as a reduction factor in the titration volume of the sample. From the results of the titration which was carried out five times, the percent of the total tannin content was determined. The results of determining the total tannin content in sapodilla fruit can be seen in Table-2.

Table-2: Determination of Tannin in Sapodilla Fruit

Powder Weight (g)	Titran Vol. (ml)		Tannin Level (%)
	Sample (A)	Blank (B)	
4.02	2.1	1.4	0.74
4.03	2.1	1.3	0.84
4.01	2.2	1.4	0.85
4.02	2.2	1.3	0.95
4.01	2.2	1.4	0.85
Average Tannin Level (%)			0.84
SD			0.071
KV(%)			8.45

The results of the quantitative determination of the tannin content in sapodilla fruit (*Manilkara zapota* (L) van royen) by permanganometry showed that the average tannin content was 0.84%. The sample weight is obtained from reducing the sample weight before extraction by the weight of the sample after extraction.

The results of the determination of the tannin content in sapodilla fruit, when compared with several previous studies on different types of fruit, obtained different results (smaller). Determination of total tannin content from old coconut fibers obtained a tannin content of 4.28% and young coconut fibers obtained a tannin content of 5.62%¹², determination of tannin content from young flower fruit obtained a tannin content of 7.98%.²² Determination of tannin content from ripe banana fruit peels obtained a tannin content of 0.8%¹³, lower than the tannin content in sapodilla fruit.

The standard deviation (standard deviation) shows the degree or degree of variation in the data set from the mean. This standard deviation is used to show how different the existing data is compared to the average data. The coefficient of variance (variability) is a comparison between the standard deviation and the average value and is expressed as a percentage. The coefficient of variance shows the proportion of the standard deviation to the mean. The results of the calculation of Standard Deviation (SD) and the Coefficient of Variance (KV) on sapodilla fruit (Table-2) are 0.0710 and 8.45%. The accuracy in this analysis can be said to be good, because the coefficient of variance obtained is small. The small coefficient of variance also shows good homogeneity, because the results obtained are not too far away.²³ This value indicates that this method is suitable for use in the analysis of tannin determination.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the sapodilla fruit (*Manilkara zapota* (L) van royen) contains tannin which is classified as a type of condensed tannin. In the determination of tannin content using the permanganometry method, the average tannin content obtained in sapodilla fruit (*Manilkara zapota* (L) van royen) was 0.84%.

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REFERENCES

1. J.H. Crane, C.F. Balerdi, and I. Maguire, The Institute of Food and Agricultural Sciences (IFAS), Florida, p.1,7 (2019)
2. P. Saranraj, and S. Sivasakthi, *Global Journal of Pharmacology*, **8(3)**, 316(2014), DOI:

[10.5829/idosi.gjp.2014.8.3.83194](https://doi.org/10.5829/idosi.gjp.2014.8.3.83194)

3. Y. Desmiaty, H. Ratih, M.A. Dewi and R. Agustin, *Ortocarpus*, **8**, 106(2008)
4. T. Desinta, *Calypatra: Jurnal Ilmiah Mahasiswa Universitas Surabaya*, **4(1)**, 1(2014)
5. M. Markom, M. Hasan, W.R.W. Daud, H. Singh and J.M. Jahim, *Separation and Purification Technology*, **52(3)**, 487(2007), [DOI:10.1016/j.seppur.2006.06.003](https://doi.org/10.1016/j.seppur.2006.06.003)
6. L.P. Malangngi, M.S. Sangi and J.E.P. Jessy, *Jurnal MIPA*, **1(1)**, 5(2012), [DOI:10.35799/jm.1.1.2012.423](https://doi.org/10.35799/jm.1.1.2012.423)
7. T. Sulastrri, *Jurnal Chemica*, **10(1)**, 59(2009), [DOI:10.35580/chemica.v10i1.401](https://doi.org/10.35580/chemica.v10i1.401)
8. A. Fajrina, J. Jubahar and S. Sabirin, *Jurnal Farmasi Higea*, **8(2)**, 133(2016)
9. F. Khachik and A.N. Chang, *The Journal of Organic Chemistry*, **74(10)**, 3875(2009), [DOI:10.1021/jo900432r](https://doi.org/10.1021/jo900432r)
10. D. H. S. Palupi, D. S. Retnoningrum, M. I. Iwo, and A. A. Soemardji, *Rasayan Journal of Chemistry*, **13(1)**, 636(2020), [DOI:10.31788/RJC.2020.1315519](https://doi.org/10.31788/RJC.2020.1315519)
11. Cut Nuraskin, Marlina, R. Idroes, C. Soraya and Djufri, *Rasayan Journal of Chemistry*, **13(1)**, 18(2020), [DOI:10.31788/RJC.2020.1315434](https://doi.org/10.31788/RJC.2020.1315434)
12. F.R. Lisan and S. Palupi, *Calypatra: Jurnal Ilmiah Mahasiswa Universitas Surabaya*, **4(1)**, 1(2015)
13. E. Ryanata, S. Palupi, and A. Azminah, *Calyptra : Jurnal Ilmiah Mahasiswa Universitas Surabaya*, **4(1)**, 1(2015)
14. A. R. Prihadi, A. Maimulyanti, B. Mellisani and Nurhasanah, *Rasayan Journal of Chemistry*, **13(2)**, 955(2020), [DOI:10.31788/RJC.2020.1325613](https://doi.org/10.31788/RJC.2020.1325613)
15. S. Dur, *Jurnal Al-Irsyad*, **3**, 106(2013)
16. A. Dewi, I.U. Pri and A.D. Binar, *Pharmacy*, **7(2)**, 1(2010), [DOI:10.30595/pji.v7i1.552](https://doi.org/10.30595/pji.v7i1.552)
17. J. B. Harborne, *Metode fitokimia: Penuntun cara Modern menganalisis tumbuhan*, Bandung, Institut Teknologi Bandung(1987)
18. A.E. Hagerman, C.T. Robbins, Y. Weerasuriya, T.C. Wilson and C.M. Arthur, *Journal of Range Management*, **45(1)**, 57(1992)
19. A.K. Patra and J. Saxena, *Phytochemistry*, **71(11-12)**, 1198(2010), [DOI:10.1016/j.phytochem.2010.05.010](https://doi.org/10.1016/j.phytochem.2010.05.010)
20. R.H.M.J. Leemans and S.N. Wulijarni, *Journal of the Society of Dyers and Colourists*, **107**, 414 (1991), [DOI:10.1111/j.1478-4408.1991.tb01289.x](https://doi.org/10.1111/j.1478-4408.1991.tb01289.x)
21. A.L. Underwood and R.A. Day, *Analisa Kimia Kuantitatif*. (L. Spyan, Ed.), Erlangga, Jakarta(2006).
22. F.R. Amelia, *Calyptra : Jurnal Ilmiah Mahasiswa Universitas Surabaya*, **44(22)**, 1(2015)
23. I.G. Gandjar and A. Rohman, *Kimia Farmasi Analisis*, Pustaka Pelajar, Yogyakarta(2007).

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