



The Determination of Tannin Content and Larvicidal Activity Test Of Ripe Sapodilla Fruit Peel (*Manilkara zapota*)

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ABSTRACT

This study aimed to determine the results of total tannin content determination of sapodilla peel extract (*Manilkara zapota*) and the effectiveness of sapodilla peel extract (*Manilkara zapota*) as a *Aedes aegypti* mosquito larvicide. The method used was a quantitative descriptive with a purposive sampling method. The sample was ripe sapodilla (*Manilkara zapota*) peel. The determination of tannin content in sample extracts was analyzed using UV-Vis spectrophotometry. The extraction of tannins from sapodilla peel (*Manilkara zapota*) was carried out by maceration method using 70 % ethanol. Based on the research results it was found that the total content of sapodilla peel extract was 3,058 mg GAE/g extract. The results of the larvicide test showed that the sapodilla peel extract could be used as an *Aedes aegypti* larvicide with the most effective concentration in killing 50% of the larvae at a concentration of 20,000 ppm.

INTRODUCTION

Phytochemical exploration of natural products aims to find out what contents are present in natural ingredients and observe the bioactivity of natural ingredients that may occur. The results of this exploration are expected to provide benefits for human life, one of which is as an effort to improve the quality of public health. The sapodilla is one of the plants that exist in Indonesia and has been widely explored for its benefits, starting from the roots, stems, bark, leaves, and fruit. Ripe sapodilla fruit has soft flesh, 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 utilization of ripe sapodilla peel is still not optimal, usually it will only be thrown away or used for animal feed. Some research results reported that sapodilla peel contains bioactive compounds such as tannin (Shafii et al, 2017; Pravin & Shashikant, 2019), phenolic and flavonoid compounds (Gomathy et al, 2013; Tulloch et al, 2019), and alkaloids (Sihombing et al, 2015).

Trisnawati & Azizah (2019) reported that raw sapodilla fruit peels can be used as larvicides. The potential of raw sapodilla fruit peel as a larvicide is thought to be due to the presence of tannin compounds. Tannin as a larvicidal agent has the task of inhibiting the ability to digest food in the mosquito's body by reducing the activity of digestive enzymes (protease and amylase) (Wahyudi, et al 2021). In addition, tannin compounds along with other secondary metabolites such as alkaloids, glycosides and saponins function as bioactive components in eradicating bacteria or antibacterial (Bhargavi et al, 2013). Shafii et al (2017) also stated that tannins in plants function as a natural defense mechanism against bacterial infections and can be used as antibacterial agents. Due to the potential of tannin compounds, this study aimed to determine the tannin content in ripe sapodilla peel (*Manilkara zapota*) by UV-Vis spectrophotometry and to determine the activity of sapodilla peel as a larvicide against *Aedes aegypti* mosquitoes. The research results are expected to provide academic and practical knowledge about the benefits of tannins found in ripe sapodilla peel.

LITERATURE REVIEW

Tannins are polyphenolic compounds of plant origin that taste bitter and are chelating. They react with and agglomerate proteins or various other organic compounds, including amino acids and alkaloids. There are two types of tannins: condensed tannins and hydrolyzed tannins. Condensed tannins, namely compounds formed by polymerization of flavonoid units, are abundant in woody plants. Because condensed tannins can often be hydrolyzed to anthocyanidins by strong acid treatment, they are often called pro-anthocyanidins. The second type of tannin is hydrolyzed, which is a heterogeneous polymer containing phenolic acids, especially gallic acid, and simple sugars and having a molecular mass between 600 and 3000. Based on Trisnawati's (2018) research, the ripe sapodilla fruit skin indicates the presence of condensed tannins. The content of tannins in sapodilla fruit is expected to be an alternative in efforts to eradicate Ae.



aegypti in the larval stage. Research conducted by Cahyati et al (2017) showed that hay infusions and papaya leaf juice contain alkaloid, flavonoid, saponin, tanin dan steroid have effect as attractant and bioinsecticide against *Aedes aegypti*. Beside that, research conducted by Cahyati et al (2017) showed that the *Manihot glaziovii* peel extract has larvicidal potency to *Aedes aegypti* larvae with low effective dosage.

METHOD

The Preparation of Research Tools and Materials

The plant material used in this study was sapodilla fruit peel taken from Bareng Village, Babadan District, Ponorogo Regency from July to September 2021. The materials used in this study were 70% ethanol, aquades, gallic acid, folin ciocalteu reagent, Na_2CO_3 15% and *Aedes aegypti* mosquito larvae. The tools used in this study were beakers, test tubes, volume pipettes, measuring pipettes, stirring rods, volumetric flasks, pipettes, analytical balances, rotary evaporators, cuvettes, and UV-VIS spectrophotometry.

The Preparation of Ripe Sapodilla Fruit Peel Ethanol Extract

Stir 100 grams of ripe sapodilla peel dry powder with the addition of ethanol 70% as much as 300 mL for 2 days and the dregs and filtrate were obtained. Re-maceration was carried out on the dregs. The filtrate obtained was collected and concentrated using a rotary evaporator.

The Determination of Tannin Levels by UV-VIS Spectrophotometry

a. The Determination of Maximum Wavelength

Weigh gallic acid as much as 10 mg, dissolved and added aquades to a volume of 100 mL so that 100 ppm standard was obtained. A certain amount of gallic acid main standard solution was pipetted and put into a 10 mL volumetric flask, 1 mL of Folin Ciocalteu reagent was added, then shaken and allowed to stand for 5 minutes. Added 2 mL of Na_2CO_3 15% to the solution, shaken homogeneously and allowed to stand for 5 minutes. Then added aquades to exactly 10 mL and read at wavelengths in the range λ 500 – 900 nm.

b. Stable Timing

A certain amount of gallic acid main standard solution was pipetted and put into a 10 mL volumetric flask, added 1 mL of Folin Ciocalteu reagent, then shaken and allowed to stand for 5 minutes. 2 mL of Na_2CO_3 15% was added to the solution, shaken homogeneously and allowed to stand for 5 minutes. Then add aquades until exactly 10 mL. Then the absorbance was observed with an observation time interval of up to 110 minutes at the maximum wavelength.

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

A certain amount of gallic acid main standard solution was pipetted and put into a 10 mL volumetric flask, then 1 mL of Folin Ciocalteu reagent was added, shaken, and allowed to stand for 5 minutes. 2 mL of Na_2CO_3 15% was added to the solution, shaken homogeneously and allowed to stand for 5 minutes. Then add aquades to the exact volume of 10 mL, shaken homogeneously and allowed to stand for a stable time. Then observed the absorbance at the maximum wavelength. A certain amount of gallic acid standard solution was taken seven times, so that seven concentrations were obtained, and a gallic acid standard curve was made.

d. The Determination of Total Tannin Content

1 mg of ethanol extract of ripe sapodilla peel was weighed and dissolved in aquades up to 10 mL (100 ppm) and replicated 3 times. Each of the replications was pipetted as much as 9 mL and dissolved with aquades up to 10 mL (90 ppm). 1 mL of folin denis reagent was added, allowed to stand for 3 minutes, 1 mL of saturated Na_2CO_3 solution was added and incubated for 40 minutes, then the absorbance was read at a wavelength of 788 nm.

The Larvicidal Activity Test of Ripe Sapodilla Fruit Peel

A stock solution of 20,000 ppm was prepared from ripe sapodilla fruit peel extract. The extract was diluted to 2,500 ppm; 5,000 ppm; 7,500 ppm; 10,000 ppm; 12,500 ppm; 15,000 ppm and put in a test tube. 10 *Aedes Aegypti* mosquito larvae were put into test tubes of 0 ppm; 2,500 ppm; 5,000 ppm; 7,500 ppm; 10,000 ppm; 12,500 ppm; 15,000 ppm and 20,000 ppm respectively. Observed and counted dead mosquito larvae for 24 hours. If after 24 hours 50% of the test larvae have not died, then increase the observation time to 48 hours and so on up to a maximum of 96 hours because if it is more than 96 hours the death of the larvae can be caused by other factors. The larvicidal activity test was carried out 3 times and averaged to calculate the percentage of larval mortality.

$$\text{Percentage of Larvae Mortality (\%)} = \frac{\text{average dead larvae}}{\text{number of initial larvae}} \times 100 \%$$

Data Analysis

Data analysis in this study used SPSS 16.0 for Windows software. It was preceded by a normality test with the Shapiro Wilk test because the number of samples was less than 50. The regression test was used to determine how much



influence each concentration of sapodilla peel extract had on the number of deaths of *Aedes aegypti* mosquito larvae. The R value could indicate the degree of association between the independent variable (extract concentration) and the dependent variable (the number of deaths of *Aedes aegypti* mosquito larvae), the R^2 value indicated the effect of the treatment. Furthermore, to determine the differences between treatments (different extract concentrations) with the average cumulative number of larvae deaths, if the data is normally distributed, a one-way ANOVA test is carried out and then the Turkey test. However, if the normal conditions are not met, then the analysis uses the Kruskal Wallis test followed by the Mann Whitney test. The Mann Whitney follow-up test is used to determine the difference between the two treatments.

RESULT

This study aimed to determine the total tannin content and larvicidal activity of ripe sapodilla peel on *Aedes aegypti* mosquito larvae. Determination of total tannin content began 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. Stable timing was determined to create a gallic acid calibration curve. From the experimental results at a wavelength of 788 nm, the absorbance was stable from 80 to 84 minutes.

Table 1
Stable Time Determination of Gallic Acid

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 788 nm. The results obtained have been listed in Table 2 below.

Table 2
Absorbance of Gallic Acid Standard Solution

Concentration of Gallic Acid (mg/L)	Absorbance
1	0,125
2	0,148
3	0,326
4	0,512
5	0,771

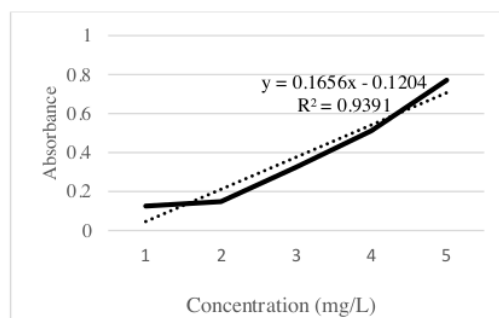


Fig. 1 Gallic Acid Standard Curve



The regression results showed that r count was 0.9391 which indicated that there was a significant correlation between concentration and absorbance. Followed by measuring the absorption of the sample. In measuring the tannin content in the sapodilla fruit peel sample, the Folin Ciocalteu reagent was used as a reducing agent. Based on the research results on determining the total tannin content of sapodilla peel extract using UV-VIS spectrophotometry, the total tannin content was obtained 3.058 mg GAE/g.

Table 3
Determination of Total Tannin Content from Sapodilla Fruit Peel Extract

Mass Extract (gram)	Absorbance	Total Tannin (mg GAE/g)
0,0012	0,4881	3,058

The larvicidal test of sapodilla peel extract activity using instar III *Aedes aegypti* mosquito larvae. The death percentage of instar III *Aedes aegypti* larvae using sapodilla fruit peel extract can be seen in Table 4.

Table 4
Average Percentage of *Aedes aegypti* Larvae Mortality at Various Concentrations of Ripe Sapodilla Fruit Peel Extract

Concentration (ppm)	Average of <i>Aedes aegypti</i> Larvae Mortality at minute												Average Percentage (%)
	5	10	20	40	60	120	240	480	1440	2880	4320	5760	
0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0
2500	0	0	0	0	0	0	0	0	0	0	0	0	1
	0	0	0	0	0	0	0	0	0	0	0	0	3,3
	0	0	0	0	0	0	0	0	0	0	0	0	0
5000	0	0	0	0	0	0	0	0	0	0	0	0	2
	0	0	0	0	0	0	0	0	0	0	0	1	13,3
	0	0	0	0	0	0	0	0	0	0	0	1	0
7500	0	0	0	0	0	0	0	0	0	0	1	2	16,7
	0	0	0	0	0	0	0	0	0	0	1	2	0
	0	0	0	0	0	0	0	0	0	0	1	1	0
10000	0	0	0	0	0	0	0	0	0	1	1	3	26,7
	0	0	0	0	0	0	0	0	0	0	0	2	0
	0	0	0	0	0	0	0	0	0	0	1	3	0
12500	0	0	0	0	0	0	0	0	0	1	1	3	23,3
	0	0	0	0	0	0	0	0	0	0	1	2	0
	0	0	0	0	0	0	0	0	0	0	1	2	0
15000	0	0	0	0	0	0	0	0	1	1	2	5	46,7
	0	0	0	0	0	0	0	0	0	1	1	5	0
	0	0	0	0	0	0	0	0	1	1	1	4	0
20000	0	0	0	0	0	0	0	0	1	2	3	6	53,3
	0	0	0	0	0	0	0	0	1	2	3	5	0
	0	0	0	0	0	0	0	0	1	2	2	5	0
Positive control (Abate)	0	4	6	8	10	10	10	10	10	10	10	10	100
	0	4	6	8	10	10	10	10	10	10	10	10	0
	0	4	6	8	10	10	10	10	10	10	10	10	0

The results of the larvicide test were then analyzed statistically with the help of SPSS 16.0 for Windows software. This analysis began with a normality test and the results can be seen in Table 5 below.

Table 5
Normality Test Results of Test Larvae Mortality that were given Ripe Sapodilla Fruit Peel Extract with the Shapiro-Wilk Test

Treatment	Concentration	Mean \pm SD	P-value
Ripe Sapodilla Fruit Peel Extract	2500	0,33 \pm 0,58	0,000
	5000	1,33 \pm 0,57	
	7500	1,67 \pm 0,58	
	10000	2,67 \pm 0,58	
	12500	2,33 \pm 0,58	
	15000	4,67 \pm 0,58	



20000 5,33±0,58

Based on the Shapiro Wilk test results, a significant value of $0,000 < 0,05$ was obtained, so it can be said that the results of the data analyzed were not normally distributed. Then the next test, namely the regression test was used to find out how much influence each concentration of sapodilla fruit peel extract had on the number of deaths of *Aedes aegypti* mosquito larvae. The results of the regression analysis can be seen in Table 6 below.

Table 6
Regression Test Results Average Mortality of Test Larvae Given Ripe Sapodilla Fruit Peel Extract

Treatment	r	R ²	P-Value
Ripe Sapodilla Fruit Peel Extract	0,957	0,917	0,001

Based on the results in Table 6, an r value of 0,957 was obtained, which mean that the correlation between the administration of sapodilla peel extract with various concentrations and the death of *Aedes aegypti* larvae had a very close correlation because it was close to number 1. The R² value of 917 means that the effect of giving sapodilla peel extract with various concentrations in killing *Aedes aegypti* larvae was 91,7%, the rest was influenced by other variables.

The next test was to find out the difference between treatments (different extract concentrations) with the average cumulative number of larvae deaths. The tests carried out were the Kruskal Wallis test followed by the Mann Whitney test because the data obtained was not normally distributed. The results of the Kruskal Wallis and the Mann Whitney test can be seen in Table 7 and Table 8.

Table 7
The results of Kruskal Wallis Test to Know the Average Value of Each Treatment

Model	Treatment	n	Average
Mortality	2500 ppm	3	2,50
	5000 ppm	3	6,17
	7500 ppm	3	7,83
	10000 ppm	3	12,50
	12500 ppm	3	11,00
	15000 ppm	3	17,67
	20000 ppm	3	19,33
	Positive Control	3	23,00
		24	
P-value		0,003	

Table 8
Mann Whitney Test

Ripe Sapodilla Fruit Peel Extract								
	2500 ppm	5000 ppm	7500 ppm	10000 ppm	12500 ppm	15000 ppm	20000 ppm	K+
2500 ppm		0,100	0,068	0,0215	0,0215	0,0215	0,0215	0,017
5000 ppm			0,228	0,034	0,0495	0,0215	0,0215	0,017
7500 ppm				0,0495	0,0985	0,0215	0,0215	0,017
10000 ppm					0,228	0,0215	0,0215	0,017
12500 ppm						0,0215	0,0215	0,017
15000 ppm							0,017	0,017
20000 ppm								0,017
K+								

The results of the Kruskal Wallis test showed a p-value of 0,003, which means there was a difference in the average number of *Aedes aegypti* larvae deaths using sapodilla peel extract with various concentrations. The results of the Mann Whitney test stated that sapodilla peel extract with various concentrations had a significant difference with the positive control (abate).

The natural larvicide used in this study was sapodilla peel extract. It was prepared in several concentrations, namely 2000 ppm, 5000 ppm, 7500 ppm, 10000 ppm, 12500 ppm, 15000 ppm and 20000 ppm. The larvae of the *Aedes aegypti* mosquito used were in accordance with WHO standards and easily observable, namely instar III larvae. The larvicidal test time was up to 96 hours until there was a concentration that could kill 50% of the test larvae, in this case a



concentration of 20,000 ppm. This research stopped only up to 96 hours because if it was more than 96 hours, the death of the larvae might be caused by other factors.

DISCUSSION

The research results on the larvicidal test using sapodilla peel extract showed that the higher the concentration and the longer the exposure time of the extract, the higher the mortality of the larvae. Increasing the concentration of the extract causes a decrease in larval activity such as movement up and down the surface which has slowed down, and the response to touch has decreased. Sapodilla peel extract is known to contain alkaloids, flavonoids, and tannins. This means that the higher the concentration of the extract, the more chemical compounds it contains and the longer the exposure time, the more active the chemical compounds are.

The content of alkaloids, flavonoids, and tannins in the sapodilla fruit peel is a chemical compound that can be used as a basis for utilizing the extract as a natural larvicide. The research results by Hayatie et al (2015) stated that papaya seed extract showed larvicidal activity against *Aedes aegypti* mosquitoes due to the effect of the phytochemical components, namely flavonoids, alkaloids, and tannins. The regression test results which stated that the effect of giving sapodilla peel extract with various concentrations in killing *Aedes aegypti* larvae was 91.7%. These results indicated that the number of active compounds in the extract greatly influences the ability to kill larvae.

Alkaloids are in the form of salts so they can degrade cell membranes to enter and damage cells and can also interfere with the larval nervous system by inhibiting the action of the acetylcholinesterase enzyme. The color change of the larvae's body becomes more transparent, and its movement slows down when stimulated by touch and always bends its body caused by alkaloid compounds (Cania & Setyaningrum, 2012).

Flavonoid compounds work to wither the nerves of the insect's respiratory system (Nurhaifah & Sukes, 2015), resulting in weakness in the nerves and damage to the spiracles so that the larvae cannot breathe and eventually die (Yuliawati, 2017). Tannin compounds can reduce the ability to digest food by reducing the activity of enzymes in digesting food in mosquitoes (proteases and amylase) (Ahdiyah, 2015). Tannin compounds can affect molting failure in larvae so that they die before developing into pupae (Nurhaifah & Sukes, 2015).

Natural ingredients such as ripe sapodilla fruit peel extract have an effective ability to kill *Aedes aegypti* mosquito larvae. The basis can be said to be effective if it is able to kill $\geq 50\%$ of experimental animals (Yuliawati et al, 2017). This research results are known only on the ripe sapodilla fruit peel extract with a concentration of 20,000 ppm which can kill $\geq 50\%$. In this study the concentration used was only up to a concentration of 20,000 ppm due to the limitations of the samples obtained.

Currently, natural larvicides, especially in Indonesia have been developed with other natural ingredients. Natural larvicides are popular because the basic ingredients are biodegradable in nature, so they do not pollute the environment and are relatively safe for humans (Banerjee et al, 2011).

CONCLUSION

Based on the research results, it can be concluded that the total content of sapodilla peel extract is 3.058 mg GAE/g extract. The results of the larvicide test showed that sapodilla fruit peel extract could be used as an *Aedes aegypti* larvicide with the most effective concentration in killing 50% of the larvae at a concentration of 20,000 ppm.

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