

Effects of Guava Leaf Extract (*Psidium Guajava*) Against the Killness of *Aedes Larvae Aegypti*

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ABSTRACT

Content the phytochemicals present on guava seeds (*Psidium guajava*) can use to control the mosquito *Aedes aegypti* as vector disease Fever Dengue Hemorrhagic Fever (DHF). Because that need looking for alternative control vector made from ingredients natural. one plants that can be used for vector control is leaf guava seeds. Leaf guava seeds (*Psidium guajava*) aim for know potency extract leaf, and guava seeds (*Psidium guajava*) as larvicide *Aedes aegypti* and know effect extract leaf and guava seeds (*Psidium guajava*) against *Aedes aegypti* design the research is experiment with five treatments and five replicates, respectively consists of 20 larvae. Concentration used namely 5%, 10%, 15%, 20%, and 0% (control). Data analyzed with ANOVA one direction, Real Difference test Smallest and continue with analysis Probit (CI 95% and = 0.05). The study shows that the effective dosage for LD50 is 15% concentration. Analysis Probit got an effective dose which is 11.484%. Dosage of 5% extract leaf guava seeds (*Psidium guajava*) can kill larvae on average 5.4 tails (29%). 10% extract dose leaf and guava seeds (*Psidium guajava*) can kill larvae an average of 8 tails (40%). Dosage of 15% extract leaf guava seeds (*Psidium guajava*) can kill larvae on average 10.2 tails (51%). Dosage of 20% extract leaf guava seeds (*Psidium guajava*) can kill larvae on average 14.6 tails (73%) each dose could kill larvae, and the effective dosage is 11.484%.

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1. Introduction

Dengue is the most common vector-borne disease worldwide and has posed a significant health burden in tropical and subtropical countries [1],[2]. Mosquito-borne diseases cause commercial and labor losses, especially in countries with tropical and subtropical climates [3]. Mosquitoes are a significant threat to millions of people worldwide because they spread various tropical diseases, especially malaria, transmitted by female *A gambiae* mosquitoes [4].

Indonesia, located in Southeast Asia, is a tropical country where the two main DENV mosquito vectors, *Aedes aegypti* species, are endemic in all regions [5]. The DHF prevention and control program has been implemented nationally by the Indonesian Ministry of Health through the Directorate General of Infectious Disease Control since 1968 [6]. The program includes the implementation of adult peri-focal spraying, mass larvicide, and disease control counseling to the community. Despite the efforts of these control programs, dengue has grown in both incidence and geographic range over the years and has become hyperendemic [7].

Vector control is an essential requirement in epidemic situations. New methods for mosquito control must be economical, efficient, and safe for non-target organisms and the environment. The use of impregnated mosquito nets or indoor sprays is a measure to slow disease transmission by killing or preventing infected mosquitoes from biting humans [8].

So far, vector control is generally carried out using synthetic pesticides. The use of synthetic pesticides is considered adequate, practical, efficacious, and economically more profitable. However, the use of synthetic pesticides continuously and repeatedly can cause environmental pollution and the death

of various living things. Pesticides contain ingredients that are easily and quickly degraded in nature and have a negligible impact on the environment, so they are not harmful [9]. Therefore it is necessary to look for vector control alternatives made from natural materials. Several compounds that work as Larvae killers include _ group cyanide, saponins, tannins, flavonoids, alkaloids, steroids, and oil essential [10].

Moment this many Plant parts that can be used whole, powdered, extracted (with water or organic solvent compounds) can be in the form of leaves, flowers, fruit, seeds, bark, and stems. one _ plants that can be used for vector control is leaf guava seeds. Guava leaves (*Psidium guajava*) were also found to have substances toxic to insects, such as tannins, tannins, saponins, triterpenoids, malic acid, and essential oils [11]. Guava leaf extract (*Psidium guajava*) can inhibit the development of the dengue virus and accelerate the increase in blood platelets. Looked at the effect of guava and leaf extract (*Psidium guajava*) as a larvicide because that study; this aim evaluates the Effect of Extract Leaf Guava Seeds (*Psidium Guajava*) Against Power Kill *Aedes Larvae aegypti*. It is hoped that this research can provide information to the management of the dengue eradication and prevention program and the public to carry out dengue vector control, using natural ingredients as a substitute for synthetic pesticides.

2. Methods

The type of research used in this study was pure experimental with 5 treatments, namely the concentration of guava leaf extract (*Psidium guajava*) namely 5%, 10%, 15%, 20% and control (0%) with 5 repetitions (Federer's formula (Federer's formula). $(n-1)(t-1) > 15$)³¹

$$4t - 4 > 15$$

$$4t > 15 + 4 \rightarrow 4t > 19$$

$$t > 19/4 = 4.75 \rightarrow 5$$

This research was conducted at the Biology Laboratory of Syiahkuala and the Chemical Laboratory of the Department of Environmental Health from March 5 to September 2021. The population in this study was *Aedes aegypti* larvae derived from *Aedes aegypti* mosquito eggs, so homogeneity was maintained. *Aedes aegypti* larvae samples were 500 tails. **Research materials include** 1000 grams of guava leaves (*Psidium guajava*) crushed, 2 L 96% ethanol as solvent, *Aquades* is a breeding ground for mosquitoes, and for dilution of extracts **and** 500 *Aedes aegypti* eggs. Research tools include Tools for the preparation of test materials, namely larval containers, small containers filled with clean water, *Hand counter* to count the number of larvae. Tools for making guava leaf extract, namely:

- a. Scales to weigh guava leaves needed
 - b. *Blender* to smooth the dried guava leaves
 - c. Jars and gauze for the guava leaf maceration process
 - d. *Rotary evaporator* for making guava leaf extract
 - e. Dropper to take guava leaf extract
 - f. 6) Measuring cup and closed bottle as a place for guava leaf extract
 - g. 25 ml measuring cup for measuring guava leaf extract
- The research is divided into several stages, namely:
- 1) Preparation Stage Mosquito eggs are transferred to a tray containing water media for 1-2 days until the eggs hatch and become larvae. The larvae will develop from stage I to IV, which lasts about five days.
 - 2) Making Guava Leaf Extract (*Psidium guajava*), The extract is made according to the *Harbone method*, the extract used is 1000 grams of guava leaves, then cleaned with water, then finely chopped or finely sliced. After finely sliced guava, leaf pieces dried by drying in the sun for 3 x 24 hours. After drying, the pieces of guava leaves were weighed and mashed; then, the guava leaf *Simplicia* was macerated for 3 x 24 hours using 2 L of 96% ethanol solution, then filtered and concentrated at room temperature. 400C-500C in a *rotary evaporator* to produce a concentrated extract of guava leaves with a concentration of 100%
 - 3) Determination of Dose of Guava Leaf Extract (*Psidium guajava*), The dosage of guava leaf extract was determined based on the objectives, namely 5%, 10%, 15%, and 20%. 5% is 5gram extract plus liquid up to 100ml, 10% is 10gram extract plus liquid up to 100ml ,15% is 15gram extract plus liquid up to 100ml, 20% is 20gram extract plus liquid up to 100ml

- 4) The stage of assessing the effective dose of guava leaf extract (*Psidium guajava*) as the killing power of *Aedes aegypti* larvae was carried out by assessing the number of dead larvae with concentrations of 5%, 10%, 15%, 20%, and 0%. Each was repeated five times.

The data obtained from the observations are processed using statistical software. Data from the research results were analyzed descriptively and statistically with the normality test (*homogeneity*). The homogeneity test got the value of $sign = 0.597 > 0.05$, which means there is no difference between the average distribution data), followed by using the one-way ANOVA test, the LSD (*Least Significant Difference*) test, which is also called BNT (Least Significant Difference) and then continued with Probit analysis with CI 95% and alpha 0.05. Data is presented in the form of tables, graphs, and narratives.

3. Results and Analysis

This study was conducted to examine the effect of guava leaf extract (*Psidium guajava*) on *Aedes aegypti* mosquito larvae mortality. Five types of guava leaf extract were tested in 25 containers, each containing 20 larvae of the *Aedes aegypti* mosquito. The number of dead larvae was counted at 2 hours of observation. The results of the study can be seen in the following table:

Table 1

Percentage of larval mortality per treatment (number of larvae per treatment 20 individuals)

Repetition	Treatment									
	control		5%		10%		15%		20%	
	f	%	f	%	f	%	f	%	f	%
1	0	0	5	25	8	40	11	55	15	75
2	0	0	6	30	8	40	11	55	13	65
3	0	0	6	30	7	35	9	45	15	75
4	0	0	5	25	9	45	10	50	14	70
5	0	0	7	35	8	40	10	50	16	80
Average	0	0	5.8	29	8.0	40	10.2	51	14.6	73

The highest 5% treatment was in the fifth repetition, the highest 10% treatment was in the fourth repetition, the highest 15% treatment was in the 1&2 repetitions, and the highest 20% treatment was in the fifth repetition. The average value of larval mortality in a row was the 5% dose was 5, 8 animals (29%), 10% dose was eight animals (40%), 15% dose was 10.2 animals (51%), and 20% dose was 14.6 animals (73%)

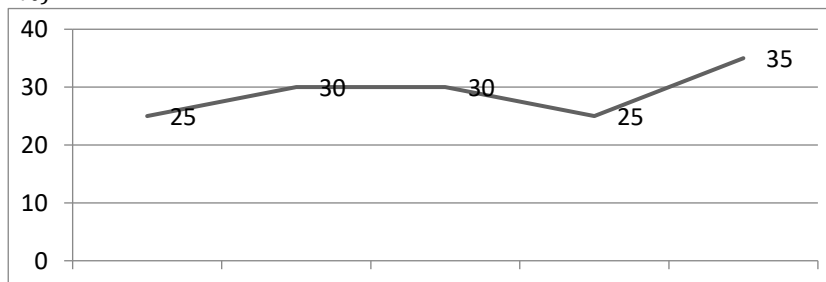


Figure 1 . Graph of Percentage of Dead Larvae With 5% Dose, 5 Repetitions.

In the first and second repetitions, it increases, the second and third stays, the fourth repetition decreases, and in the last repetition, it increases up to 35%. The lowest number of dead larvae was 5(25%), and the highest was 7(35%). The highest number of scores was also found in the third repetition with a dose of 10%. The average number of dead larvae was 5.8 tails.

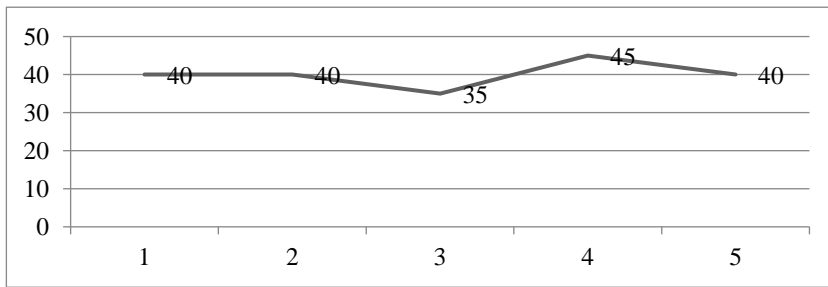


Figure 2 . Graph of the Percentage of Dead Larvae With a Dose of 10%, 5 Repetitions

In the first and second repetitions, the third repetition goes down, the fourth repetition goes up, and on the last repetition, it drops to 40%, the same as the initial condition. The lowest number of dead larvae was 7 (35%) in the third repetition, and the highest was 9 (45%) in the fourth repetition. The lowest number of dead larvae (7=35%) was also found in the last repetition with a dose of 5%. The average number of dead larvae was eight tails.

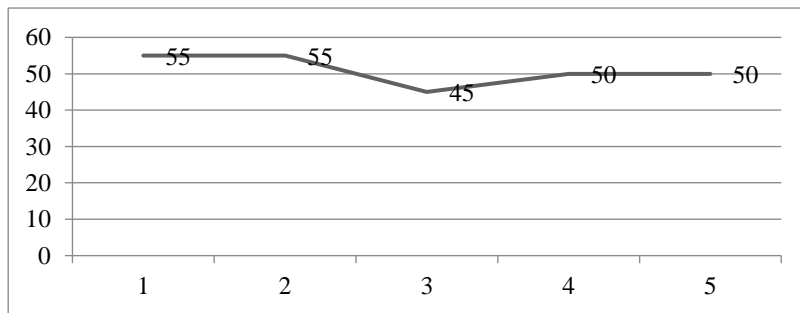


Figure 3 . Graph of the Percentage of Dead Larvae With a Dosage of 15%, 5 Repetitions

In the first and second repetitions, the third repetition goes down, the fourth repetition goes up, and on the third, it drops down to 45% lower than the initial condition. The lowest number of dead larvae was 9 (45%) in the third repetition, and the highest was 11 (55%) in the first and second repetitions. The lowest number of dead larvae (9 = 45%) was also found in the fourth iteration with a dose of 10%. The average number of dead larvae was 10.2.

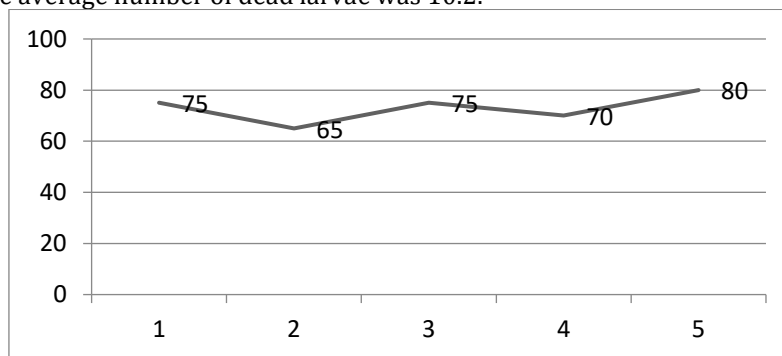


Figure 4 . Graph of Percentage of Dead Larvae With 20% Dose, 5 Repetitions

The first and second repetitions go down, the third goes up, the fourth goes down, and the fifth repetition goes up again to 80%. The lowest number of larvae that died was 13 (65%) in the second repetition, and the highest was 16 (80%) in the last repetition. The exact number of dead larvae was not found with the lower treatment dose. The average number of dead larvae was 14.6 tails.

Table 2

Combined 5 repetitions per dose

Change	5%	10%	15%	20%
1 to 2	Up (25-30)	Fixed (40-40)	Fixed (55-55)	Down (75-65)
2 to 3	Fixed (30-30)	Down (40-35)	Down (55-45)	Up (65-75)
3 to 4	Down (30-25)	Up (35-45)	Up (45-50)	Down (75-60)
4 to 5	Up (25 -35)	Down (45-40)	Fixed (50-50)	Up (60-75)

The number 35% is in the fifth repetition of the 5% dose and the third repetition with a 10% dose. The figure of 45% was in the fourth repetition of the 10% dose and the third repetition at the 15% dose. At the same time, the 20% dose ranged between (60 – and 75)%, where there was no exact percentage figure at lower doses. If a lethal dose above 50% is considered adequate, then a 15% guava leaf extract is an effective dose to kill *Aedes aegypti* larvae. This is also following Dikki Triyadi's research which states that the dose of guava leaf extract, which is effective as a killer of *Aedes aegypti* larvae, is above 10%.

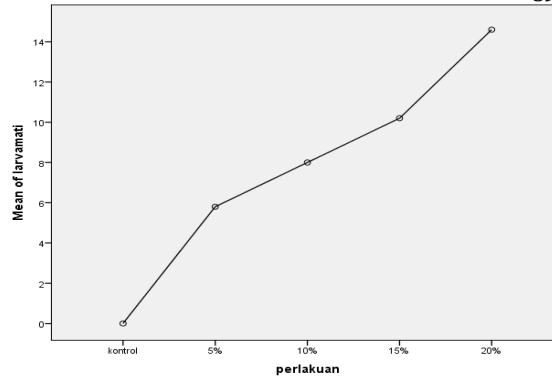


Figure 5. Graph of the average dead larvae per dose

In the treatment with a dose of 5%, the line is slightly upright, forming an angle of about 45 ° (control – 5%); at a dose of 10% and 15%, the line forms an angle of about 30 ° while at a dose of 20% the line returns to the same as at the beginning, which is forming an angle of about 45 °. The conclusion is that the increase in the number of doses with an increase in the number of dead larvae is not a straight line, although there is also an increase in larval mortality with every increase in the dose. The above analysis is based on table descriptions and pictures without statistical tests, but the results are not much different from the analysis using statistics with the help of computer software as follows:

Table 3
Distribution of the results of repeated treatments

R	Average	Std. Deviation	Std. Error	95% CI	
				Lowest value	The highest score
control	,00	,000	,000	,00	,00
5%	5.80	,837	,374	4.76	6.84
10%	8.00	,707	,316	7.12	8.88
15%	10,20	,837	,374	9.16	11.24
20%	14.60	1,140	,510	13.18	16.02
Total	7.72	4,988	,998	5.66	9.78

The average value and the lowest and highest values for each treatment are the same as those listed in the average, minimum and maximum values. Table 3 shows that the Std Deviation and Std Error at doses of guava leaf extract of 5% and 15% are the same and higher than those at 10% doses. The slightest error occurred at a dose of 10%, but at a dose of 10%, the dead larvae were still below 50%, while at a dose of 15%, the average number of dead larvae was average 10.2 (51%). It can be concluded that the dosage of guava leaf extract (*Psidium guajava*), which is effective, is 15%. The most significant error is at a dose of 20%, which can kill an average of 14.6 larvae (73%).

Table 4.
ANOVA

	sum of squares	df	Square mean	F	Sig.
Between groups	584,240	4	146.060		
In Group	12,800	20	,640	228,219	,000
Total	597,040	24			

Based on the one-way ANOVA test, the significance value (p) is 0.000001 < 0.05, which means that there is an effect of guava leaf extract (*Psidium guajava*) against the killing power of *Aedes aegypti* larvae.

Table 5
Multiple Comparison (Least Significant Difference)

(I) Treatment	(J) Treat	Different Mean (II)	Std. Error	Sig.	95% Confidence Interval	
					Lower limit	Upper limit
control	5%	-5,800 *	,506	,000	-7.40	-4.20
	10%	-8,000 *	,506	,000	-9.60	-6.40
	15%	-10,200 *	,506	,000	-11.80	-8.60
	20%	-14,600 *	,506	,000	-16,20	-13.00
5%	control	5,800 *	,506	,000	4.20	7.40
	10%	-2,200 *	,506	,003	-3.80	-,60
	15%	-4,400 *	,506	,000	-6.00	-2.80
	20%	-8,800 *	,506	,000	-10.40	-7.20
10%	control	8,000 *	,506	,000	6.40	9.60
	5%	2,200 *	,506	,003	,60	3.80
	15%	-2,200 *	,506	,003	-3.80	-,60
	20%	-6,600 *	,506	,000	-8,20	-5.00
15%	control	10,200 *	,506	,000	8.60	11.80
	5%	4,400 *	,506	,000	2.80	6.00
	10%	2,200 *	,506	,003	,60	3.80
	20%	-4,400 *	,506	,000	-6.00	-2.80
20%	control	14,600 *	,506	,000	13.00	16,20
	5%	8,800 *	,506	,000	7.20	10,40
	10%	6,600 *	,506	,000	5.00	8.20
	15%	4,400 *	,506	,000	2.80	6.00

*. The mean difference is significant at the 0.05 level.

Based on the *multi comparisons test* (BNT), the standard error of all treatment levels is the same, namely 0.506; the value of the difference between the lower limit and the upper limit of 95% confidence interval is also the same, namely = 3.2. All p-values between groups <0.05, which means that all dose differences (starting from a dose of 5% to 20% can kill *Aedes aegypti* larvae. Larval death is caused by the presence of *quercetin* in guava leaf extract (*Psidium*). *guajava*). The results of probit analysis is estimated that the LD₅₀ value is 11.384%

3.1 Discussion

Guava Leaf Extract also contains various efficacious components in overcoming dengue fever. Based on the results study is known that there is an influence extract leaf and guava seeds (*Psidium guajava*) against power kill the larvae with a value (p) 0.000001 < 0.05. On dose extract leaf guava, 5% and 15% are the same and taller than the 10% dose. A minor mistake occurred on the 10% dose; however, on 10% dose of dead larvae was still below 50%, while on the dose of 15%, the average larvae that died were 10.2 (51%). Dose extract leaf and guava seeds (*Psidium guajava*) effective is 15%. The biggest mistake is on the dose of 20 %, which could kill larvae on average 14.6 tails (73%). This thing showed that guava leaf extract could inhibit the development of the dengue virus and accelerate the increase in the number of blood platelets. Guava leaf extract contains flavonoid and tannin compounds [11]. Flavonoids are a group of plant pigments that give color to fruits [12]. Flavonoids are components of phenols, which are bioactive that will change the body's reaction to other compounds. Thus, flavonoids have activity as antiviral and antioxidant. The group of tannin and flavonoid compounds expressed as quercetin is commonly found in plants of the class Angiospermae. Quercetin in guava leaf extract can inhibit the reverse transcriptase enzyme activity, which is an enzyme needed by the virus to replicate itself. Based on the multi comparisons test (BNT), the standard error of all treatment levels is the same, namely 0.506; the value of the difference between the lower limit and the upper limit of 95% confidence interval is also the same, namely = 3.2. All p-values between groups were <0.05, meaning that all dose differences (ranging from 5% to 20%) can kill *Aedes aegypti* larvae. Larval death was caused by the presence of quercetin in guava leaf extract (*Psidium guajava*). The results of probit analysis were estimated. The LD₅₀ value is 11.384%. Guava leaf extract has been clinically proven to increase the platelet count up to 100 thousand per mm without causing side effects [13]. This increase can be achieved within 8-48 hours after consuming guava leaf extract. Amino acids in guava leaves can form thrombopoietin from serine and threonine, which functions in the maturation process of megakaryocytes into platelets. Research has also been carried out to make dengue antiviral drugs from

guava leaf extract. The drugs are in supplements and marketed to the public, including capsule form for adults and syrup for children.

4. Conclusion

Dosage of 5% extract leaf guava seeds (*Psidium guajava*) can kill larvae on average 5.4 tails (29%). 10% extract dose leaf and guava seeds (*Psidium guajava*) can kill larvae on average eight tails (40%). Dosage of 15% extract leaf guava seeds (*Psidium guajava*) can kill larvae on average 10.2 tails (51%). Dosage of 20% extract leaf guava seeds (*Psidium guajava*) can kill larvae on average 14.6 tails (73%). Dose extract leaf and guava seeds (*Psidium guajava*) which is 15% effective, and according to results analysis, Probit value (LD50) is 11.814%

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