



EFFECTIVENESS OF GUAVA LEAF EXTRACT (PSIDIUM GUAJAVA) ON DEATH OF AEDES AEGYPTI MOSQUITO LARVAE IN GORONTALO CITY

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ABSTRACT

Background: Dengue Hemorrhagic Fever (DHF) is a public health problem characterized by an increasing number of cases year after year, including in Gorontalo City. Vector control efforts in the larval stage are an effective strategy for breaking the life cycle of the *Aedes aegypti* mosquito. The use of natural ingredients such as guava leaves (*Psidium guajava*) has the potential to be an environmentally friendly larvicide.

Methods: This type of laboratory experimental research with an equivalent control group research design using guava leaves with concentrations of 10%, 12.5%, and 15% with variations in observation time of 4 hours, 8 hours, 12 hours, and 24 hours. The research sample was 750 instar III larvae divided into treatment and control groups. Data analysis used the Two Way ANOVA test.

Results: The results of the study showed significant differences in larval mortality across observation time variations (4 hours, 8 hours, 12 hours, and 24 hours) across all treatments. Guava leaf extract showed a difference between observation times ($p\text{-value} = 0,000$), with variations in observation time of 4 hours versus 8 hours ($p\text{-value} = 0,000$), 4 hours versus 24 hours ($p\text{-value} = 0,024$), and 8 hours versus 12 hours ($p\text{-value} = 0,002$).

Conclusion: For the community to utilize guava leaves as an environmentally friendly natural larvicide alternative and as an effort to control the *Aedes aegypti* mosquito population.

KEYWORDS: Guava, Larva, *Aedes aegypti*, Dengue Fever

1. Introduction

Dengue Hemorrhagic Fever (DHF) is a public health problem with a rising number of cases. This disease is caused by the dengue virus, which is transmitted through the bite of the *Aedes aegypti* mosquito, the primary vector (Panjaitan, 2021). Globally, over a period of more than 40 years, dengue fever cases in endemic countries reached 4.975.807 cases with 68.977 deaths (1,4%), and Southeast Asia contributed 67.295 deaths (Isna & Sjamsul, 2021).

In Indonesia, in 2024, there were 164.673 cases of dengue fever recorded with 926 deaths, an increase from 114.720 cases in 2023 (World Health Organization, 2024). In Gorontalo City, there was a significant increase from 80 cases (2020) to 455 cases by August 2025 (Gorontalo City Health Office, 2025). Dengue vaccines and antiviral drugs are not yet

available, so control is focused on eradicating the vector, especially the larval phase (Bisyaroh, 2020).

Dengue fever vector control is local in nature by taking into account physical environmental factors (weather/climate), socio-cultural environment (knowledge, attitudes, and behavior) and vector aspects (behavior and vulnerability status of vectors). Dengue fever vector control can be physical, biological, and chemical (Sari et al., 2024).

The people of Gorontalo Province utilize guava leaves as a herbal remedy, primarily to treat digestive disorders such as diarrhea and boost immunity. However, the use of guava leaves among the Gorontalo people is still largely limited to traditional medicine. Public knowledge has yet to reach other scientific benefits, such as the leaves' potential as a natural larvicide to control mosquito populations, particularly *Aedes aegypti*, the primary vector of dengue fever.

Natural, plant-based larvicides are an environmentally friendly, biodegradable, and non-hazardous control alternative. Guava leaves (*Psidium guajava*) contain tannins (9–12%), saponins, flavonoids, alkaloids, and essential oils that have larvicidal properties (Menisasti, 2021).

Pre-laboratory tests conducted by researchers in June 2025 with concentrations of 3% and 5% showed that guava leaves had an effective killing power against the death of *Aedes aegypti* mosquito larvae. At a concentration of 3% guava leaves caused an average death of 15 (75%) *Aedes aegypti* mosquito larvae, and at a concentration of 5% guava leaves caused an average death of 19 (95%) *Aedes aegypti* mosquito larvae. Both have an effective killing power against *Aedes aegypti* larvae, with a mortality rate ranging from 60–95%, meeting WHO standards (2016) which stipulate an effective larvicide if it is able to kill 10–95% of test larvae. This study aims to analyze the differences in the effectiveness of guava leaf extract with concentrations of 10%, 12,5%, and 15% against the death of *Aedes aegypti* larvae.

2. Methods

The experimental research method to determine the effectiveness of guava leaf extract (*Psidium guajava*) in this study used a quasi-experimental research design. Quasi-experiments are research that approaches real experiments. This study aims to directly test the effect of one variable on another variable and test the hypothesis of a cause-and-effect relationship (Pitaloka et al., 2023). This research design uses an Equivalent Control Group Design, which is a study used to test the effects of a particular treatment by comparing the results between the group receiving the treatment and the group not receiving the treatment (control group), where the two groups are selected randomly so that they are considered equal at the beginning of the study (Pratiwi et al., 2024). The research sample consisted of 250 third-instar *Aedes aegypti* larvae divided into treatment and control groups. Each treatment group consisted of 25 larvae, representing the median number or middle value of

the larvicide test guideline with three repetitions. Observations of larval mortality were conducted at 4, 8, 12, and 24 hours after treatment and at concentrations of 10%, 12,5%, and 15%.

This research was conducted at the Public Health Laboratory, Gorontalo State University. The sampling location for *Aedes aegypti* larvae was in Padebuolo Village, East Kota District, Gorontalo City. The independent variable was the type of guava leaf extract, while the dependent variable was the number of *Aedes aegypti* larvae deaths.

The extraction process used a maceration method with 96% ethanol as a solvent. Data were analyzed using the Shapiro-Wilk normality test, Levene's homogeneity test, and Two-Way ANOVA to determine differences in effectiveness between treatments and variations in observation time. If significant differences were found, a Post Hoc test (Tukey HSD) was performed with a confidence level of $\alpha = 0,05$.

3. Result

Larval Mortality in Guava Leaf Extract (*Psidium guajava*)

Table 1. Mortality of *Aedes aegypti* Larvae Using Guava Leaf Extract (*Psidium guajava*) in Gorontalo City

Extract	Concentration (%)	Larva used (Tail)	Observation Time	Larva Death			Average	
				P I	P II	P III	Mean	Presentation
Leaf Guava Seed	0	25	4 Hours	0	0	0	0	0%
		25	8 Hours	0	0	0	0	0%
		25	12 Hours	0	0	0	0	0%
		25	24 hours	0	0	0	0	0%
	10	25	4 Hours	1	1	1	1	4%
		25	8 Hours	2	4	3	3	12%
		25	12 Hours	6	4	3	4	16%
		25	24 hours	12	1	5	6	24%
	12,5	25	4 Hours	4	1	1	2	8%
		25	8 Hours	8	5	4	6	24%
		25	12 Hours	3	4	3	3	12%
		25	24 hours	6	4	7	6	24%
	15	25	4 Hours	4	3	3	3	12%
		25	8 Hours	12	13	11	12	48%
		25	12 Hours	0	0	4	1	4%
		25	24 hours	6	3	1	3	12%

Description: PI = First Repetition; PII = Second Repetition; PIII = Third Repetition

Source: Primary Data, 2025

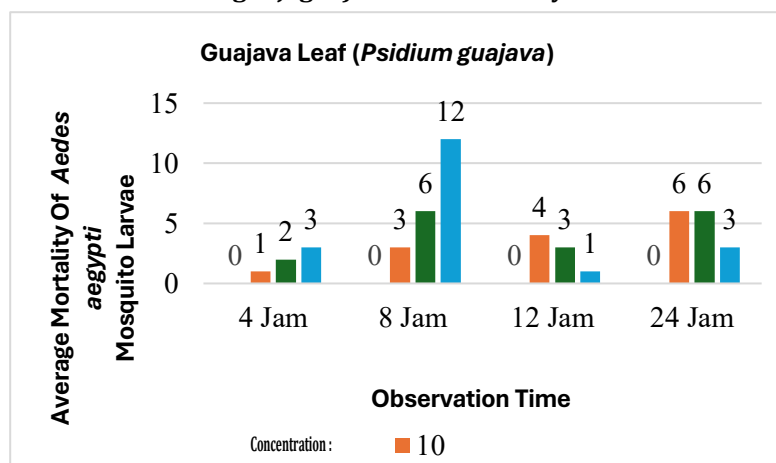
Guava leaf extract (*Psidium guajava*) with a concentration of 10%, 12,5%, and 15% in the observation time of 4 hours, 8 hours, 12 hours, and 24 hours which is most effective against the death of *Aedes aegypti* mosquito larvae is a concentration of 15% with an observation time of 8 hours with the number of deaths seen from the average (mean) of 12 larvae (48%) and the least effective level with an average number of larval deaths at a

concentration of 10% at an observation time of 4 hours and a concentration of 15% at an observation time of 12 hours of 1 larva (4%).

The mortality of *Aedes aegypti* larvae on guava leaves showed a fluctuating pattern at several concentrations and observation times. At concentrations of 10% (observation time 4 hours) and 15% (observation time 12 hours) showed the same value, namely an average percentage of 4%. In addition, at concentrations of 12,5% (observation time 8 hours and 24 hours) showed the same value, namely an average percentage of 24%. Fluctuations were also seen at concentrations of 12.5% (observation time 12 hours) and 15% (observation time 24 hours) with an average percentage of 12%. This is caused by differences in the response and resistance of each larva to the active compounds contained in guava leaves, such as flavonoids, saponins, tannins, alkaloids and essential oils (Farich et al, 2021). These compounds are known to have larvicidal activity that works through various mechanisms, including disrupting the nervous system, inhibiting metabolic processes, disrupting the digestive system of larvae, and affecting the toxicity level of the active compounds, resulting in differences in the level of larval mortality in each treatment (Hari & Mathew, 2021).

The results of the two-way ANOVA test of guava leaf extract for the concentration factor obtained 0,234, which indicates that there is no significant difference in the mortality of *Aedes aegypti* mosquito larvae between the three concentrations ($p\text{-value} > 0,05$). Meanwhile, for the observation time factor, a $p\text{-value}$ of 0,000 is obtained, which indicates that there is a significant difference in the mortality of *Aedes aegypti* mosquito larvae between the four observation times ($p\text{-value} < 0,05$). As for the interaction of the two factors, a $p\text{-value}$ of 0,000 is obtained, which indicates that there is a significant interaction between the two factors ($p\text{-value} < 0,05$).

Figure 1. Graph of mortality of *Aedes aegypti* mosquito larvae on guava leaf extract (*Psidium guajava*) in Gorontalo City



Source: Primary Data, 2025

The results of the study showed that there were differences in the average number of deaths of *Aedes aegypti* mosquito larvae at each concentration and observation time of 4

hours, 8 hours, 12 hours, and 24 hours. During the 4-hour observation period, the number of larvae was still low at all concentrations, ranging from 1-3 larvae. Furthermore, during the 8-hour observation period, there was an increase in larval mortality, especially at a concentration of 15% which showed the highest number of deaths, namely 12 larvae, followed by a concentration of 12,5% with 6 larvae and a concentration of 10% with 3 larvae. During the 12-hour observation period, the number of larval deaths tended to decrease at a concentration of 15% to 1 larva, while at a concentration of 10% and 12,5% respectively, there were 4 larvae and 3 larvae. Meanwhile, during the 24-hour observation period, the number of larval deaths increased again, at a concentration of 10% and 12,5% each reaching 6 larvae, and at a concentration of 15% with 3 larvae.

Table 2. Analysis of the Effectiveness of Guava Leaf Extract (*Psidium guajava*) on the Death of *Aedes aegypti* Mosquito Larvae

Variables	<i>p-value</i>
Concentrations of 10%, 12,5%, and 15% guava leaf extract (<i>Psidium guajava</i>)	0,234
Observation time 4 hours, 8 hours, 12 hours, and 24 hours	0,000
Interaction of Two Factors	0,000

Source: Primary Data, 2026

Based on table 2, the results of the two-way ANOVA test of guava leaf extract for the concentration factor were obtained at 0,234, indicating that there was no significant difference in the mortality of *Aedes aegypti* mosquito larvae between the three concentrations ($p\text{-value} > 0,05$). Meanwhile, for the observation time factor, a $p\text{-value}$ of 0,000 was obtained, indicating that there was a significant difference in the mortality of *Aedes aegypti* mosquito larvae between the four observation times ($p\text{-value} < 0,05$). As for the interaction of the two factors, a $p\text{-value}$ of 0,000 was obtained, indicating that there was a significant interaction between the two factors ($p\text{-value} < 0,05$).

Table 3. Post Hoc Test

Observation Time Variation	<i>p-value</i>
4 hours against 8 hours	0,000
4 hours against 24 hours	0,024
8 hours against 12 hours	0,002

Source: Primary data, 2026

Based on table 3, it was found that only between observation times of 4 hours versus 8 hours, 4 hours versus 24 hours, and 8 hours versus 12 hours there was a significant difference in the mortality of *Aedes aegypti* mosquito larvae ($p\text{-value} < 0,05$).

4. Discussion

Effectiveness of Guava Leaf Extract (*Psidium guajava*)

The death of *Aedes aegypti* mosquito larvae in each treatment showed that guava leaf extract (*Psidium guajava*) was effective. According to Syazana & Porusia (2022), that a larvicide is said to be effective if the number of larval deaths ranges from 10-95% of the total larvae tested. The results of the study showed that the effectiveness of guava leaf extract with concentrations of 10%, 12,5%, and 15%, the most effective was the 15% concentration (observation time 8 hours) resulting in an average percentage of deaths of 48%. This is due to the content of active compounds found in guava leaves such as flavonoids, alkaloids, tannins, and saponins which act as natural larvicides (Maksum et al., 2024). These compounds work by disrupting the larvae's digestive and nervous systems, as well as damaging the larvae's protective layers, causing death. Furthermore, the lowest mortality rate for *Aedes aegypti* larvae was at a concentration of 10% (4 hours of observation) and 15% (12 hours of observation), with an average percentage of 4% (Kumara et al., 2021). This is because at a concentration of 10%, the active compound contained in guava leaf extract is still insufficient to provide a significant larvicidal effect. Furthermore, the relatively short observation time (4 hours) meant the active compound did not interact with the larvae's bodies for long enough, resulting in a less than optimal toxic effect (Wahyuni et al., 2023).

Based on the results of statistical tests using the Two-Way ANOVA test, the observation time factor showed a significant difference in mosquito larval mortality among the four observation periods. This indicates that the length of exposure to the extract significantly influences larval mortality. The longer the exposure time, the greater the accumulation of active compounds in the larvae's bodies, which causes physiological disorders and even death. In theory, the effectiveness of a toxic substance is influenced by the dose-response relationship and the length of observation time (Grant & Kergoat, 2023). However, within a given dose range, increasing concentrations does not always result in significantly increased effects. Instead, the duration of observation plays a significant role in increasing the accumulation of the substance in the organism, thus increasing the level of toxicity.

The results of this study are in line with research Sari et al. (2024), which states that guava leaf extract has larvicidal activity, but does not always show significant differences at certain concentrations. Furthermore, research by Putri et al. (2022) also showed that observation time significantly influences the increase in *Aedes aegypti* larval mortality.

5. Conclusion

The conclusion is that guava leaf extract (*Psidium guajava*) with concentrations of 10%, 12,5%, and 15% Based on the results of the study, it can be concluded that there is a significant difference in the effectiveness of guava leaf extract (*Psidium guajava*) on the death of *Aedes aegypti* larvae at various observation times of 4, 8, 12, and 24 hours ($p = 0,000$).

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