

Larvicidal Activity of Ethanolic Extract of Papaya (*Carica papaya L.*) Fruit Peel Against Dengue Vector Mosquito Larvae *Aedes aegypti*

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ABSTRACT

Larviciding is an insecticide application activity aimed at killing larvae. In this study, the larvicidal activity of ethanol extract of papaya fruit peel (*Carica papaya L.*) against dengue vector mosquito larvae, *Aedes aegypti*, was evaluated. Two stages of testing were conducted, namely a preliminary test and a main test. The preliminary test used 10 larvae for each concentration, with doses ranging from 200 ppm to 2000 ppm at 200-ppm intervals. The main test was conducted at concentrations ranging from 600 ppm to 2000 ppm with 100-ppm intervals, using 25 larvae per concentration. A positive control using abate was included in each test. Since the toxicity threshold of the extract had not been previously determined and could potentially pose risks, LC_{50} and LC_{90} values were assessed. The preliminary test results showed that a concentration of 600 ppm caused 25% larval mortality, which was subsequently used as the initial concentration for the main test. The main test demonstrated 100% larval mortality at a concentration of 2000 ppm. Analysis of variance (ANOVA) indicated no statistically significant differences among the treatments. The LC_{50} and LC_{90} values were determined to be 1121.29 ppm and 1720.81 ppm, respectively.

Keywords: Papaya skin; larvacida; *Aedes aegypti*; ethanolic extract; toxicity

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an infectious disease transmitted by *Aedes aegypti* mosquitoes. DHF remains a significant public health problem in Indonesia. In 2024, Indonesia reported 244,409 DHF cases with 1,430 deaths, making it one of the countries with the highest burden of dengue in Southeast Asia (1,2). The increasing incidence is influenced by various factors, including climate change, which shortens the dengue cycle from every 10 years to only 3 years or less (2). One of the main strategies to control *Aedes aegypti* is the use of larvicides, particularly Temephos (Abate). Temephos is still widely used to eliminate *Aedes aegypti* larvae (3). In Indonesia, Abate was first used in 1976, and by 1980, Temephos (Abate) was officially incorporated into the national mass eradication program against *Aedes aegypti* (4). However, resistance to synthetic larvicides has become an increasing concern, highlighting the need for alternative approaches (4).

Recently, plant-based natural alternatives have gained attention for mosquito control. Plant-derived larvicides contain a mixture of bioactive compounds that affect both behavioral and physiological processes in mosquitoes, thereby limiting the potential for resistance development (5). Papaya (*Carica papaya L.*) is one plant believed to have larvicidal potential. Phytochemical analysis of papaya leaves has revealed the presence of alkaloids, saponins, glycosides, flavonoids, tannins, and other compounds. Among these, flavonoids and alkaloids are considered potential larvicides against *Aedes aegypti* larvae (1). Additionally, papain, an enzyme found in papaya latex, has been shown to inhibit larval growth by disrupting hormonal activity, ultimately causing larval mortality (6).

Several studies have confirmed the larvicidal potential of papaya extracts. I Gusti et al. (2016) demonstrated that ethanol extract of papaya leaves effectively killed *Aedes aegypti* larvae (1). Similarly, Meidy et al. reported the larvicidal activity of ethanolic papaya leaf extracts against *Aedes aegypti* larvae (6). Bhat and Surolia (2003) identified bioactive compounds in various parts of the papaya plant that may contribute to insecticidal properties (7). Malathi (2024) examined larval morphology changes of *Aedes aegypti* exposed to papaya extracts and found physiological disruptions (8). Recent studies have extended investigations to other parts of the papaya plant and different extract types. Adeniran and Fabiyi (2015) reported that aqueous extracts of seeds and fruit peel exhibited larvicidal activity against *Aedes aegypti* (6). Suraj and Sushil (2025) found that methanolic leaf extracts had higher larvicidal potency compared to root and latex extracts (9). Castro et al. (2023) identified proteases from papaya with insecticidal activity (10). Lim et al. (2023) reported similar larvicidal effects from plant extracts including *Carica papaya*, supporting its potential as a botanical larvicide (11).

Reviews and recent experimental studies further support the promise of papaya as a vector control agent. Maideen et al. (2025) highlighted the potential of papaya leaves for dengue vector control (12). Sudarwati et al. (2020) demonstrated that methanol fractions from papaya leaves significantly reduced larval survival (13). Ahmed et al. (2025) studied the Red Lady variety of papaya leaves and confirmed its efficacy as a larvicide (14). Despite these findings, the fruit peel of papaya remains underexplored for larvicidal activity. Papaya fruit peel contains alkaloids, steroids, saponins, quinones, monoterpenes, and sesquiterpenes, of which alkaloids and saponins are believed to induce physiological disruptions in larvae, interfering with growth and development (5). Therefore, investigating the ethanolic extract of papaya fruit peel as a potential larvicide against *Aedes aegypti* larvae is scientifically warranted.

METHODS

Tool

The tools used in this study included containers for larva rotary evaporator, sterile bottles, and other standard laboratory glassware used for extraction and testing procedures. Test (Tukey Test). Acute toxicity, defined as harmful effects occurring within 24 hours, was assessed to determine the lethal concentrations (LC₅₀ and LC₉₀). The determination of LC₅₀ was carried out using the probit analysis method, which compares the mortality rates across different concentrations or doses.

Materials

The materials used in this study included papaya fruit peel, *Aedes aegypti* mosquito larvae, distilled water, well water, 95% ethanol, 2 N hydrochloric acid (HCl), Mayer's reagent, Dragendorff's reagent, Liebermann-Burchard reagent, ferric chloride (FeCl₃), gelatin, vanillin, toluene, 5% potassium hydroxide (KOH), chloroform, amyl alcohol, and sulfuric acid (H₂SO₄).

Detailed Procedure

At the initial stage, phytochemical screening was performed on papaya leaf simplicia to detect the presence of alkaloid compounds. The sample was alkalized with 1 mL of ammonia, followed by the addition of chloroform, and then ground vigorously. The chloroform layer was collected and subsequently treated with 2 N hydrochloric acid (HCl). After vigorous shaking, two layers were formed, and the acidic layer was separated and divided into three portions. The first portion was treated with Mayer's reagent; the formation of turbidity or a white precipitate indicated the presence of alkaloids. The second portion was treated with Dragendorff's reagent; the formation of turbidity or an orange-yellow precipitate confirmed the presence of alkaloids. The third portion was used as a blank control.

Subsequently, tannin compounds were identified using two qualitative tests. In the ferric chloride (FeCl_3) test, a few drops of 5% FeCl_3 solution were added to 1 mL of the sample solution. The formation of a blue coloration indicated the presence of tannins. In the gelatin test, the sample was mixed with water in a test tube, heated, and filtered. The resulting filtrate was then treated with 1% gelatin solution, and the formation of a white precipitate confirmed the presence of tannins.

Subsequently, the presence of steroid and triterpenoid compounds was evaluated. The sample was ground with ether, and the filtrate was collected and placed in an evaporating dish, then allowed to dry. Liebermann-Burchard reagent was added to the dried residue. The formation of a purple coloration indicated the presence of triterpenoids, whereas a blue-green coloration indicated the presence of steroids.

Detection of Saponin Compounds, the sample was mixed with water, heated, and then filtered. The filtrate was transferred into a test tube and shaken vigorously. The formation of stable foam that persisted for at least 10 minutes with a minimum height of 1 cm indicated the presence of saponins. Detection of Flavonoid Compounds, the sample was placed in a test tube and treated with magnesium powder and 2 N hydrochloric acid (HCl). The mixture was heated and filtered. Amyl alcohol was then added to the filtrate and shaken vigorously. The presence of flavonoids was indicated by the formation of a yellow to red coloration that was extractable into the amyl alcohol layer.

Detection of Monoterpene and Sesquiterpene Compounds, the sample was ground with ether, and the filtrate was collected and placed in an evaporating dish, then allowed to dry. A 10% vanillin solution in concentrated sulfuric acid (H_2SO_4) was added to the dried residue. The development of characteristic colors indicated the presence of monoterpene and sesquiterpene compounds.

Papaya peel simplicia powder was extracted using a cold extraction method (maceration) in 96% ethanol at a ratio of 1:10 (w/v). The mixture was then allowed to stand for 3×24 hours at room temperature. After three days, the mixture of papaya peel powder and ethanol was filtered to separate the extract solution from the residue. The resulting filtrate was subsequently concentrated using a rotary evaporator to obtain a viscous extract. The concentrated extract was then transferred into sterile bottles for further use.

The larvicidal assay was carried out in two stages: a preliminary test and a main test. In the preliminary test, the ethanolic extract of papaya fruit peel was prepared in a series of concentrations: 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, 1200 ppm, 1400 ppm, 1600 ppm, 1800 ppm, and 2000 ppm in 100 mL of water. A stock solution of 2000 ppm in 1 liter of water was first prepared to facilitate serial dilution. The test solution was placed into a container, and 10 *Aedes aegypti* larvae at instar III and IV stages were introduced. Larval mortality was recorded after 24 hours of exposure. Each concentration was tested in duplicate, and the number of dead larvae was documented. Commercial Abate was used as the positive control.

The main test was conducted using the same technique as the preliminary assay. A stock solution of 2000 ppm in 1 liter of water was prepared, from which a series of test concentrations were obtained: 600 ppm, 700 ppm, 800 ppm, 900 ppm, 1000 ppm, 1100 ppm, 1200 ppm, 1300 ppm, 1400 ppm, 1500 ppm, 1600 ppm, 1700 ppm, 1800 ppm, 1900 ppm, and 2000 ppm in 100 mL of water. Each solution was placed into a container, and 25 *Aedes aegypti* larvae at instar III and IV stages were introduced. Larval mortality was recorded after 24 hours of exposure. Each concentration was tested in triplicate, and the number of dead larvae was documented. Commercial Abate was used as the positive control.

In this study, statistical analysis was performed using two-way ANOVA, followed by a Post Hoc Test (Tukey Test). Acute toxicity, defined as harmful effects occurring within 24 hours, was assessed to determine the lethal concentrations (LC₅₀ and LC₉₀). The determination of LC₅₀ was carried out using the probit analysis method, which compares the mortality rates across different concentrations or doses.

RESULT AND DISCUSSION

Results Subsection

Phytochemical screening was conducted to identify the secondary metabolite compounds present in the simplicia and extract of papaya fruit peel. The results indicated that both the simplicia and the extract contained alkaloids, steroids, saponins, quinones, monoterpenes, and sesquiterpenes. Results of phytochemical screening of papaya fruit peel simplicia and extract are presented in Table 1.

Table 1. Results of phytochemical screening of papaya fruit peel simplicia and extract

Compounds	Result	
	Simplicia	Extract
Alkaloids	+	+
Steroids	+	+
Saponin	+	+
Monoterpene and Sesquiterpene	+	+
Quinone	+	+
Tannin	-	-
Triterpenoid	-	-
Flavonoid	-	-

Notes: (+) = Compound detected

(-) = Compound not detected

The preliminary test data on the larvicidal activity of the ethanolic extract of papaya (*Carica papaya* L.) fruit peel against *Aedes aegypti* larvae are presented in Table 2.

Table 2. Preliminary test results and percentage of *Aedes aegypti* larvae mortality at various concentrations of papaya (*Carica papaya* L.) peel extract

No	Concentration	Number of larvae tested	Results		Average mortality	Larva mortality (%)
			I	II		
1.	Control Positive (+) (Abate)	10	10	10	10	100%
2.	Control Negative (-)	10	-	-	0	0%
3.	200 ppm	10	-	-	0	0%
4.	400 ppm	10	-	-	0	0%
5.	600 ppm	10	3	2	2.5	25%
6.	800 ppm	10	3	3	3	30%
7.	1000 ppm	10	4	4	4	40%

8.	1200 ppm	10	5	6	5.5	55%
9.	1400 ppm	10	7	7	7	70%
10.	1600 ppm	10	7	8	7.5	75%
11.	1800 ppm	10	9	10	9.5	95%
12.	2000 ppm	10	10	10	10	100%

The percentage of larval mortality was calculated by multiplying the average number of mortality larvae by 100%. The results of the preliminary test showed that concentrations of 200 ppm and 400 ppm exhibited no larvicidal activity. Larvicidal activity was first observed at 600 ppm, which resulted in 25% larval mortality. At the highest concentration of 2000 ppm, 100% mortality of *Aedes aegypti* larvae was achieved. Probit analysis was then applied to determine the toxic concentrations, yielding an LC_{50} value of 1121.29 ppm, indicating that the ethanolic extract of papaya fruit peel was able to kill 50% of the larval population. The LC_{90} value was calculated at 1720.81 ppm, meaning that at this concentration, 90% mortality of the larvae occurred. These findings were used as the basis for selecting concentrations in the main test, namely 600 ppm, 700 ppm, 800 ppm, 900 ppm, 1000 ppm, 1100 ppm, 1200 ppm, 1300 ppm, 1400 ppm, 1500 ppm, 1600 ppm, 1700 ppm, 1800 ppm, 1900 ppm, and 2000 ppm.

The preliminary test data on the larvicidal activity of the ethanolic extract of papaya (*Carica papaya* L.) fruit peel against *Aedes aegypti* larvae are presented in Table 2.

Table 2. Main test results and percentage of *Aedes aegypti* larvae mortality at various concentrations of papaya (*Carica papaya* L.) peel extract

No	Concentration	Number of larvae tested	Results			Average mortality	Larva mortality (%)
			I	II	III		
1.	Control Positive (+) (Abate)	25	25	25	25	25	100%
2.	Control Negative (-)	25	-	-	-	-	0%
3.	600 ppm	25	6	7	5	6	24%
4.	700 ppm	25	6	6	7	6.3	25.2%
5.	800 ppm	25	8	6	7	7	28%
6.	900 ppm	25	8	8	7	7.6	30.4%
7.	1000 ppm	25	8	9	8	8.3	33.2%
8.	1100 ppm	25	9	9	9	9	36%
9.	1200 ppm	25	11	9	9	9.6	38.4%
10.	1300 ppm	25	9	13	12	11.3	45.2%
11.	1400 ppm	25	13	13	15	13.6	54.4%
12.	1500 ppm	25	17	16	14	15.6	62.4%
13.	1600 ppm	25	16	19	18	17.6	70.4%
14.	1700 ppm	25	19	23	23	21.6	86.4%
15.	1800 ppm	25	23	23	25	23.6	94.4%
16.	1900 ppm	25	24	25	25	24.6	98.4%
17.	200 ppm	25	25	25	25	25	100%

Based on Table 2, the ethanolic extract of papaya (*Carica papaya* L.) fruit peel at a concentration of 2000 ppm produced the highest mortality rate of *Aedes aegypti* larvae, reaching 100%. Probit analysis

of larval mortality revealed that the LC_{50} value was 1195.86 ppm, while the LC_{90} value was 1885.87 ppm.

In this study, *Aedes aegypti* larvae at instar III and IV stages were used, as larvae at these stages are larger in size and possess higher tolerance to toxins compared to instar I and II larvae, which are more vulnerable. The *Aedes aegypti* larvae were obtained from the Entomology Laboratory, School of Life Sciences and Technology, Bandung Institute of Technology (ITB), Indonesia.

The findings of this study indicate that the ethanolic extract of papaya (*Carica papaya* L.) fruit peel exhibits larvicidal effects, thereby demonstrating the ability to kill *Aedes aegypti* larvae. No larval mortality was observed in the negative control, as it did not contain the ethanolic extract of papaya fruit peel, whereas the positive control using commercially available Abate showed effective larvicidal activity.

The Probit analysis of the main test on the ethanol extract of papaya peel against *Aedes aegypti* larvae showed that the LC_{50} value was obtained at a concentration of 1195.86 ppm, which means that at this concentration the extract was able to kill 50% of the test larvae population. Meanwhile, the LC_{90} value was obtained at a concentration of 1885.87 ppm, indicating that at this concentration the extract was capable of killing 90% of the larvae (Malathi, 2024). To determine the differences in effectiveness among treatments, a Two-Way ANOVA test was conducted. The statistical analysis results showed that there were no significant differences among treatments, with a p-value < 0.05 at a 95% confidence level. The analysis was then continued with a Post Hoc Tukey test, which also revealed that there were no significant differences among the treatment groups. This finding indicates that although the ethanol extract of papaya peel demonstrated larvicidal activity against *Aedes aegypti* larvae, the differences in concentrations applied did not result in statistically significant variations in larvicidal effectiveness.

CONCLUSION

Based on the research conducted, it can be concluded that the ethanol extract of papaya peel (*Carica papaya* L.) possesses larvicidal effects against *Aedes aegypti* larvae, attributed to its secondary metabolites, namely alkaloids and saponins. The concentration of ethanol extract of papaya peel required to kill 50% of the larval population (LC_{50}) was 1121.29 ppm in the preliminary test and 1195.86 ppm in the final test, whereas the concentration required to kill 90% of the larvae (LC_{90}) was 1720.81 ppm in the preliminary test and 1885.87 ppm in the final test within 24 hours.

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