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Research Article

The Effect of a Combination of Ethanol Extract from Leaves and Flowers of *Plumeria acuminate L.* Against *Aedes aegypti* Larvae

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Abstract
Aedes aegypti is the primary vector that causes dengue virus (DENV) infection. *Ae. aegypti* resistance to abate (Temephos) has occurred in Indonesia and several other countries. Innovations are needed to develop alternative ingredients that can be used as natural larvacides. The *Plumeria acuminate L.* is an Indonesian plant with metabolites that have the potential to act as larvicides. This study aims to determine the potential combination of ethanol extract of *P. acuminate L.* leaves and flowers as a larvicide for *A. aegypti*. A laboratory experimental study with a post-test-only control group design was carried out on *Ae. aegypti* larvae instar III. The larvicide test was carried out according to WHO standards in 2005 with concentrations of 10000, 7500, 5000, and 2500 ppm, as well as control (water and 1% DMSO), with 20 larvae for each concentration. The observation was carried out at the 24th and 48th hours with 3 repetitions. Larval mortality data was analyzed using one-way ANOVA statistical tests to determine significant differences and lethal concentrations (LC_{50} and LC_{90}) were calculated using probit analysis using SPSS software. The mortality of *Ae. aegypti* larvae due to the administration of a combined ethanolic extract of *P. acuminate L.* leaves and flowers was the highest at doses of 10000 and 7500 ppm, which was 100% with lethal concentrations of LC_{50} and LC_{90} of 3364.715 and 6293.759 ppm at the 24th hour observation, whereas at the 48th-hour observation, lethal concentrations were detected at 1767.998 and 2941.138 ppm, respectively. One-way ANOVA analysis test showed a significant difference in *Ae. aegypti* larval mortality due to the administration of a combined ethanol extract of *P. acuminate L.* leaves and flowers at 24th-hour observation ($p = 0.000$) and ($p = 0.013$) at 48th hour observation. The combination of ethanol extract of *P. acuminate L.* leaves and flowers showed larvicidal activity against *Ae. aegypti* larvae as evidenced by larvae mortality which were influenced by concentration and observation time.

Keywords: larvacide, *P. acuminate L., Ae. aegypti*

1. INTRODUCTION

Aedes aegypti is a primary vector that causes dengue virus infection (DVI), yellow fever, and chikungunya [1]-[4]. The Centers for Disease Control and Prevention (CDC) states that 4000 million inhabitants, or approximately half of the world's population, are at risk of contracting DVI [5]. According to the Ministry of Health of the Republic of Indonesia in 2023, DVI cases in Indonesia during the last five years from 2019–2023 have fluctuated. DVI cases that occurred in Indonesia in 2019 were 138,270 cases with a total of 919 deaths, while from 2020 to 2021 the number of DVI cases decreased by 20 to 50%, but in 2022 the number of DVI cases increased by 143,000 cases with the number of deaths 1,236 cases and in 2023 DVI cases decreased by 114,720 cases with a total of 894 deaths [6][7]. The increase in the spread of DVI is influenced by several factors, temperature, mobility, population density, and community behavior, which are the foundation of efforts to prevent and control DVI [8]-[10]. The spread of DVI is often found in the rainy season when there is lots of standing water and pots or bathtubs are used as breeding places for *Ae. aegypti* mosquitoes [11]. The *Ae. aegypti* female mosquitos have a behavior of repeatedly sucking blood until their stomachs are full to meet their protein intake when female mosquitoes produce eggs, thereby increasing the efficiency of spreading DVI epidemics [11].

One way to control DVI in Indonesia is using vaccine, which is a preventive key to reduce the risk of transmission. The dengue vaccine in Indonesia is the tetravalent dengue vaccine (Dengvaxia) [2][12]. This vaccine is designed to protect against four types of dengue viruses, namely DENV1, DENV2, DENV3, and DENV4 [12][13]. Even though the dengue vaccine has been proven safe and effective, the role of the community is very important in the

Table 1. Phytochemical screening of the ethanol extract of *P. acuminate* leaves and flowers.

Group	Treatment	Positive result	*P. acuminate*	
			Leaves	Flowers
Alkaloids	Mayer's Reagent	White precipitate	Positive	Positive
	Wagner's reagent	Brown precipitate	Positive	Positive
Flavanoids	Mg and HCl reagents	Appear red	Positive	Positive
Saponins	Shaking and HCl	Stable foam	Positive	Positive
Tannin	$FeCl_3$ reagents	Blackish-green color	Positive	Positive
Steroids	Liebermann-Burchard test	Blue–green ring	Negative	Negative
Terpenoids	Liebermann-Burchard test	A brown or red ring	Positive	Negative

success of the vaccination program. However, prevention efforts such as controlling mosquito populations and environmental cleanliness must be implemented consistently [13]. Efforts made to overcome DVI include fogging and eradicating mosquito nests by draining, closing, burying, monitoring, and using synthetic or natural larvacides [14]. *Ae. aegypti* larval resistance against abate (Temephos) has occurred in Indonesia and several other countries, including Brazil, Bolivia, Argentina, Cuba, the Caribbean, and Thailand [5][9][15]. According to Kandi's report in 2023, the larvae of *Ae. aegypti* experienced resistance to Temephos in Surabaya, Bandung, and Palembang [16]. So there is a need for natural, environmentally friendly larvicides as a substitute for chemical larvicides such as abate.

The Cambodian cendana plant (*Plumeria acuminate L.*) is one of the plants in Indonesia that has the potential to act as a larvicide which is often found on Java and Bali islands [17]. Metabolic compounds that have the potential to act as larvicides are found in *P. acuminate*, including sap (resin), which contains plumierid (plumeria acid $C_{10}H_{10}O_5$ (oxymethyl dioxykaneelzuur)), and is a bitter substance found in the stem of the skin, roots, and leaves of *P. acuminate L.* that contains saponins, flavonoids, alkaloids, and polyphenols [18][19]. This statement has been proven by previous researchers that the metabolite compound from the ethanol extract of *P. acuminate L.* flowers has the potential as a larvicide against *Ae. aegypti* larvae [17][19].

Ae. aegypti is a vector of DVI in Indonesia. Vector control efforts to reduce the population of dengue fever cases [9][20][21]. Breaking the life cycle of the *Ae. aegypti* mosquito by administering larvicide so that the larvae do not grow into adult mosquitoes. Excessive use of chemical larvicides can cause environmental pollution, so there is a need for innovation in using natural larvicides that are safe for the environment [22]. *P. acuminate* is one of the plants chosen as a natural larvicide because of its metabolite compounds which are taken from its leaves and flowers. So it is necessary to research administering a combination of ethanol extracts of *P. acuminate L.* leaves and flowers to *Ae. aegypti* to analyze its effectiveness as a natural larvicide.

2. MATERIALS AND METHODS

2.1. Materials

This study used experimental laboratory research with a post-test-only control group design. This study was conducted in February 2024. The samples used for this study were randomly obtained from *Ae. aegypti* instar III larvae obtained from the Laboratory Entomology Health Office in Surabaya. The materials used as samples were parts of leaves and flowers of *P. acuminate L.* taken in the Kebaraon sub-district area, Karang Pilang sub-district, Surabaya city and identified in the biology laboratory of the Faculty of Science and Technology, Airlangga University.

2.2. Methods

2.2.1. Extraction of Plumeria acuminate L. Flowers and Leaves

The leaves and flowers that have been picked are then cut into small pieces to speed up drying so that

dried leaves and flowers (simplicia) are obtained. In the next process, the simplicia can be ground until it becomes simplicia powder (Figure 1). The research ingredients used were ethanol extracts of *P. acuminate L.* leaves and flowers obtained using the maceration method. The extract was prepared from December 2023 to January 2024 at the Laboratory of the Faculty of Health Sciences at Ma'arif Hasyim Latif University. The extraction process for simplicia powder of *P. acuminate L.* leaves and flowers uses the maceration method. As much as 500 g of simplicia powder soaked in 1 L of solvent for 3 × 24 h. The solvent used is 96% ethanol. The results of the maceration filtration are continued with the evaporation stage until a thick extract is obtained which is ready to be used as a test material.

2.2.2. Phytochemical Test

Phytochemical tests include those for alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. This test was performed to identify the secondary metabolite compounds contained in the ethanol extract of *P. acuminate L.* leaves and flowers.

2.2.2.1. Alkaloid Test

The sample (0.5 g) was weighed, and then 1 mL of 2 M hydrochloric acid (HCl) and 9 mL of distilled water were added, heated in a water bath for 2 min, cooled, and filtered. Filtrate was used for alkaloid testing. In the 2-tube reaction, the filtrate (0.5 mL) was added to each test tube. Two drops of Wagner's reagent and two drops of Meyer's reagent Alkaloids were added into the tube. The positive is considered if they occur as white precipitates (Mayer's reagent) or brown precipitates (Wagner's reagent) [23].

2.2.2.2. Flavonoid Test

Flavonoid compounds were identified using magnesium powder (Mg) and concentrated HCl. The purpose of the Mg ribbon is to form a bond with carbonyl groups in flavonoids. The purpose of adding HCl is to form flavylium salts that are characterized by changes in color to orange and red. The test procedure was as follows: 0.5 g of sample and 2 mL of 50% methanol were added. The samples were heated to 50 °C and then cooled. Then, Mg metal and five drops of concentrated HCl were added to the solution. A red/orange color indicates a positive flavonoid response [24].

2.2.2.3. Saponin Test

The saponin test was carried out by dissolving a 0.5 g sample in distilled water heated for 15 min and then shaking for 10 s. If stable foam formed for approximately 10 min and a few drops of 2 M HCl were added, then the sample was positive for saponin [25].

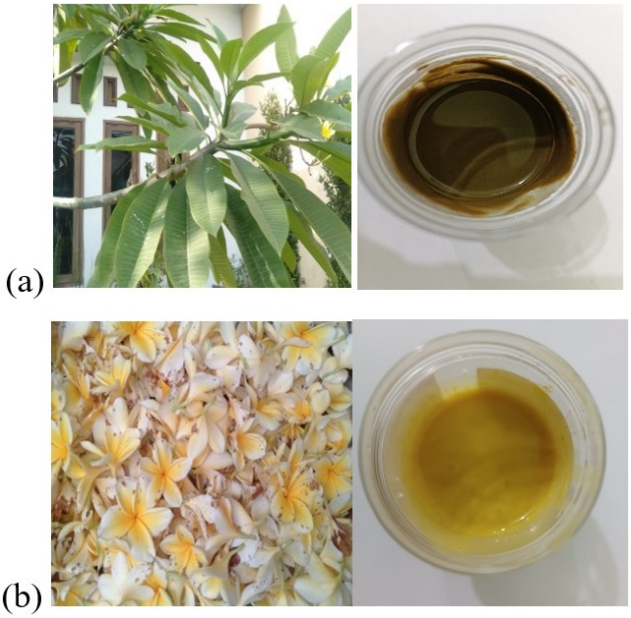

Figure 1. The ethanol extract of *P. acuminate L.* (a) leaves and (b) flowers.

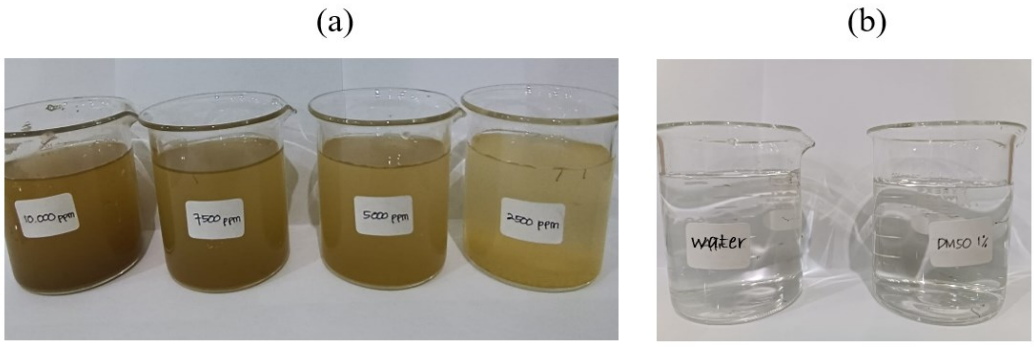

Figure 2. Larvicide test solution, (a) combination of ethanol extract of *P. acuminate L.* leaves and flowers, (b) control (PDAM water and 1% DMSO).

2.2.2.4. Tannin Test

The sample (0.5 g) was filtered through 10 ml of distilled water. The sample was filtered and diluted with distilled water until it was colorless. Then, 2 mL of the solution was added to 1–2 drops of iron (III) chloride reagent. The blackish-blue or green color indicates the presence of tannins [26][27].

2.2.2.5. Terpenoid and Steroid Tests

The solution (0.5 g) was evaporated in an evaporating cup. The residue was dissolved in chloroform (0.5 mL), and anhydrous acetic acid (0.5 mL) was added. Then, 2 mL of concentrated sulfuric acid was added to the tube walls. The formation of a brownish or red ring on the solution boundary indicates the presence of triterpenoids, whereas a blue ring that appears greenish in color indicates the presence of steroids [27].

2.2.3. Larvacide Bioassay

Mortality tests were performed according to the based on the guidelines for laboratory and field testing of mosquito larvacides in 2005. Standard bioassay tests were used for third- or fourth-instar mosquito larvae, and concentration tests were used to determine their activity. The concentration test used is between 4 and 5 concentrations and produces a mortality rate of between 10 and 95% within 24 or 48 h. The results of this data are used to determine the lethal concentration values of 50 and 90 (LC_{50} and LC_{90}) [28]. A main ethanol extract of *P. acuminate L.* leaves and flowers at a concentration of 10000 ppm (0.25 g in 25 mL of 10% DMSO) was prepared, and the concentrations of the ethanol extract of *P. acuminate L.* leaves and flowers at 10000, 7500, 5000, and 2500 ppm were used for the *Ae. aegypti* larval mortality test solution. A combination solution of the ethanol extract of *P. acuminate L.* flowers and leaves was prepared by mixing one of the various concentrations (10000, 7500, 5000, or 2500 ppm). Variations in the combination of the ethanol extract of *P. acuminata L.* leaves and flowers at each concentration were tested in triplicates. A total of 20 *Ae. aegypti* larvae (Instar III) were added to each concentration variation container, and larval mortality was observed for 24 and 48 h, after which the mortality of the *Ae. aegypti* larvae (Instar III) was calculated.

Ethics approval was obtained from the Ethic Committee of Health Research of Universitas Nahdlatul Ulama Surabaya with registration number 0052/EC/KEPK/UNUSA/2024.

2.2.4. Data Analysis

The data obtained in the form of larvae mortality in each test group was analyzed using the statistical product and service solution (SPSS) program for Windows Release 26.0 calculating LC_{50} and LC_{90} using the probit log regression analysis, brine shrimp lethality test (BSLT) method, and one-way ANOVA test.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Screening of the Ethanol Extract of P. acuminate L. Leaves and Flowers

The results of the phytochemical screening tests on extracts of *P. acuminate L.* leaves and flowers ethanol are presented in Table 1. Leaf and flower extracts of *P. acumnate L.* were extracted using the maceration method with 96% ethanol solvent. The

process of separating metabolite compounds is based on the level of polarity, where higher polarity is bound or retained in a polar solvent, and metabolite compounds with a relatively low polarity are eluted first [29][30]. However, the process of withdrawing these compounds can damage the cell walls and plasma membranes in plants, making it easier for the eluent to enter the cell walls and vacuoles to dissolve metabolite compounds. Alkaloids, flavonoids, tannins, and saponins are metabolites found in cell walls and vacuoles that prevent tissue decay in plants [29] [31].

3.2. pH of the Larvicide Test Solution

The combination solution of the ethanol extract of *P. acuminate L.* leaves and flowers had a gradient color, namely, brownish yellow at the highest concentration (10,000 ppm) and cloudy yellow at the lowest concentration (2500 ppm), while in the control (PDAM water and 1% DMSO), the solution was colorless and clear (Figure 2). The combination solution of the ethanol extract of *P. acuminate L.* leaves and flowers and 1% DMSO had the same pH (6.5), while the pH of the PDAM water was 6.8. The larvae of *Ae. aegypti* grew well in the pH range of 6.0 to 8.0.

The survival of *Ae. aegypti* larvae are influenced by pH. In the larvicidal activity test, the combination of the ethanol extract of *P. acuminate L.* leaves and flowers and the 1% DMSO control had a pH of 6.5, while the water control had a pH of 6.8. *Ae. aegypti* larvae grew well at pH 6–8. pH conditions that are either too acidic (≤ 3) or too alkaline (≥12) can inhibit the growth of *Ae. aegypti* larvae [11][32]. According to Iswara (2021), larval growth is hampered at pH 5, which causes the death of *Ae. aegypti* larvae [33]. The combination solution of the ethanol extract of *P. acuminate L.* leaves and flowers had a pH of 6.5, so it was categorized as safe for use in larvicidal activity tests (Table 2).

3.3. Larvicidal Activity of Ethanol Extracts of P. acuminate L. Leaves and Flowers toward Ae. aegypti

The results show that there are 2 concentrations, namely 7500 and 10,000 ppm, which have adequate potential for the death of *Ae. aegypti* larvae as much as 100% (Table 3, Figure 3).

The mortality of *Ae. aegypti* larvae occur due to the inability of the larvae to detoxify chemical compounds contained in the ethanol extract solution of *P. acuminate L.* leaves and flowers. A reaction that arises from mosquito larvae begins with a stimulation process. The flowers and leaves of *P. acuminate L.* contain several compounds that can act as toxic larvicides. The compounds contained in the plant that are thought to function as insecticides include saponins, tannins, flavonoids, alkaloids, and terpenoids [23][34][35]. Larvicide can kill larvae as a stomach poison through the mouth of *Ae. aegypti* larvae and through food [36].

Obtained from the results of microscopic observations showed that the living *Ae. aegypti* larvae had intact body morphology and looked healthy, while the dead *Ae. aegypti* larvae showed damage to the chitin layer on the body of the larvae, this condition was caused by exposure to toxic substances from metabolite compounds of *P.*

Table 2. pH of the combination solution of the ethanol extract of *P. acuminate L.* leaves and flowers and the control solution (water and 1% DMSO).

Extract Concentration (ppm)*	Average pH	Standard deviation
10000	6.5	0
7500	6.5	0
5000	6.5	0
2500	6.5	0
Control		
DMSO 1%	6.5	0
Water	6.8	0

* Combination of ethanol extract *P. acuminate L.* leaves and flowers

Table 3. Mortality of *Ae. aegypti* larvae due to the administration of a combination of an ethanol extract of *P. acuminate L.* to leaves and flowers and a control (water and 1% DMSO).

Variabel	n	Mortality *Ae. Aegypti* larvae			
		24th-h		48 th-h	
		Amount	Percent	Amount	Percent
10000 ppm*	60	60	100	-	-
7500 ppm*	60	60	100	-	-
5000 ppm*	60	40	67	60	100
2500 ppm*	60	25	40	48	80
Control					
Water	60	-	-	2	3
DMSO 1%	60	-	-	2	3

* Combination of ethanol extract *P. acuminate L.* leaves and flowers

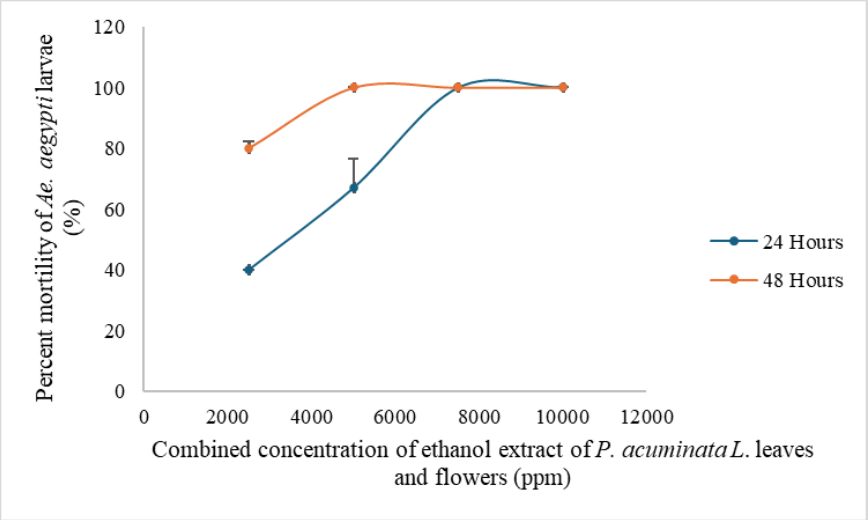

Figure 3. Graph of the percentage of mortality of *Ae. aegypti* larvae due to the administration of a combination of ethanol extracts of *P. acuminate L.* to leaves and flowers.

acuminate L. leaves and flowers resulting in the death of *Ae. aegypti* larvae [5][37]. The dead *Ae. aegypti* larvae showed damage to the siphon which functions as a respiratory tract, due to exposure to toxic substances from metabolite compounds of *P. acuminate L.* leaves and flowers causing the larvae to fail to breathe and eventually die (Figure 4) [37].

The mortality of *Ae. aegypti* larvae due to the administration of a combination of the ethanol extract of *P. acuminate L.* leaves and flowers was the highest at concentrations of 10000 and 7500 ppm, which was 100% of the lethal concentrations of LC_{50} and LC_{90} of 3364.715 and 6293.759 ppm and squared correlation coefficient (R^2) 0.8554 in the 24th-h observation (Table 4, Figure 5), whereas in the 48th-h observation, the mortality of *Ae. aegypti* larvae due to the administration of a combination of the ethanol extract of *P. acuminate L.* leaves and flowers was the highest at concentrations of 10000, 7500, and 5000 ppm were100% with the lethal concentrations of LC_{50} and LC_{90} of 1767.998 and 2941.138 ppm and squared correlation coefficient (R^2) 0.7763 (Table 5, Figure 5). The squared value of the correlation coefficient (R^2) is close to 1 indicating a relationship between the two variables, so the squared correlation coefficient (R^2) values obtained indicate a very strong relationship between extract concentration and *Ae. aegypti* larvae mortality [38].

Comparative analysis of groups of ethanol extract concentrations of *P. acuminate L.* leaves and flowers on the mortality of *Ae. aegypti* larvae using a posthoc test that there were significant differences in the 7500, 5000, and 2500 ppm concentration groups in 24-h observations with a *p-value: 0.000; p: 0.001; p: 0.003 (p<0.05),* while in the 48-h

observation, there was a significant difference in the 5000 and 2500 ppm concentration groups with a *p-value: 0.036 (p<0.05)* (Table 6).

Analysis of the mortality of *Ae. aegypti* larvae after the administration of a combination of the ethanol extract of *P. acuminate L.* leaves and flowers, it was found that there was a significant difference in one-way ANOVA with *p-value: 0.000; p: 0.036 (p<0.05).* So it can be concluded that the mortality of *Ae. aegypti* was significantly influenced by the concentration of the extract solution and the observation time (Table 7). Based on the results of observations of larval death due to the administration of a combination of ethanol extracts of *P. acuminate L.* leaves and flowers for 24 and 48 h, there is a relationship between the concentration of the test solution and the duration of observation. The higher the concentration of the test solution, the higher the level of larval death. *Ae. Aegypti* larvae that receive higher toxins have a higher chance of dying faster [5].

The activity of crude plant extracts is often attributed to the complex mixture of active compounds. Compounds obtained from the leaves and flowers of *P. aciminate L.* such as Alkaloids, flavonoids, tannins, and saponins have an unpleasant taste in plants so they are used as plant defense to repel insects [39][40]. Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920 [41]. These compounds exhibit antioxidant properties and antibacterial effects. Saponins have a bitter taste and irritate the stomach, which can inhibit the activity of digestive enzymes, resulting in damage to the cells in the digestive tract of *Ae. aegypti* larvae. The damage begins with the swelling of the midgut to reach the body walls, resulting in an acellular peritrophic membrane apart from the midgut cells and death in *Ae. aegypti* larvae [9][23][26]. Rotenone compounds (flavonoids), such as larvicides and natural insecticides, can inhibit respiratory enzymes between coenzyme Q (the respiratory coenzyme is responsible for carrying electrons in the electron transport chain) and NAD+ (coenzymes involved in oxidation and reduction in metabolic processes), which results in breathing failure in *Ae. aegypti* larvae [15]. Alkaloids play an important role in nerve poisoning. The compound enters through the cell wall on the hydrophobic side, causing the micelle; so that permeability is disrupted and toxins can enter the body of the *Ae. aegypti* larvae [15][34][42]. Toxicity to enter the larval nervous system

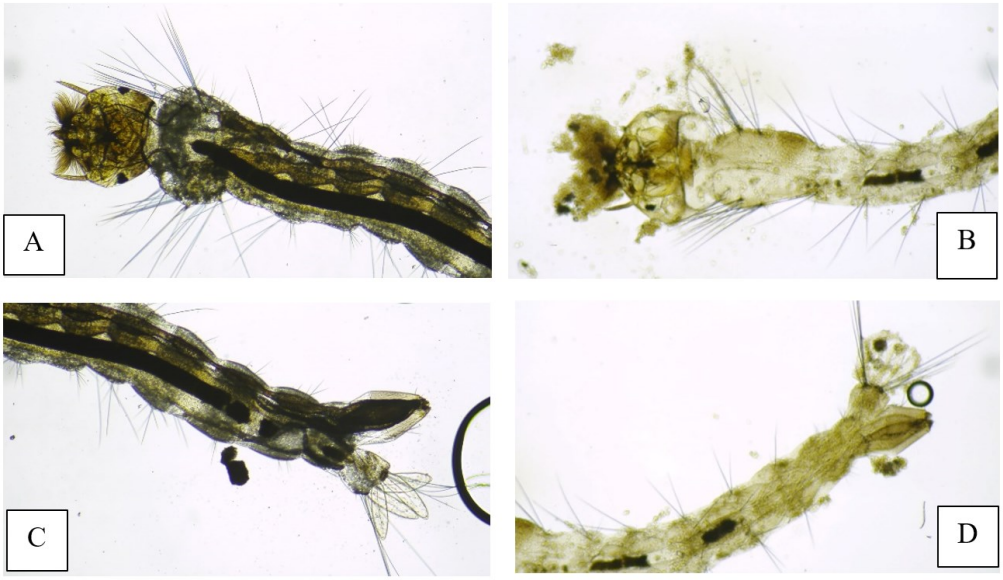

Figure 4. Morphology of (a) live *Ae. aegypti* larvae and (b) morphology of dead *Ae. aegypti* larvae due to the administration of a combination of ethanol extract of *P. acuminate L.* leaves and flowers (B). Morphology of the siphon of (c) live *Ae. aegypti* larvae and (d) morphology of the siphon of dead *Ae. aegypti* larvae due to administration of a combination of ethanol extract of *P. acuminate L.* leaves and flowers.

Table 4. Mortality of *Ae. aegypti* larvae and lethal concentration due to the administration of a combination of ethanol extracts to *P. acuminate L.* leaves and flowers during the 24th-hour of observation.

Dose (ppm)	Number of larvae		Mortality (%)	LC_{50} (95%CI)	LC_{90} (95%CI)
	n	Dead			
2500	60	24	40	3364.715	6293.759
5000	60	40	67		
7500	60	60	100		
10000	60	60	100		

Table 5. Mortality of *Ae. aegypti* larvae and lethal concentration due to the administration of a combination of ethanol extracts of *P. acuminate L.* leaves and flowers during the 48th-hour of observation.

Dose (ppm)	Number of larvae		Mortality (%)	LC_{50} (95%CI)	LC_{90} (95%CI)
	n	Dead			
2500	60	48	80	1767.998	2941.138
5000	60	60	100		
7500	60	60	100		
10000	60	60	100		

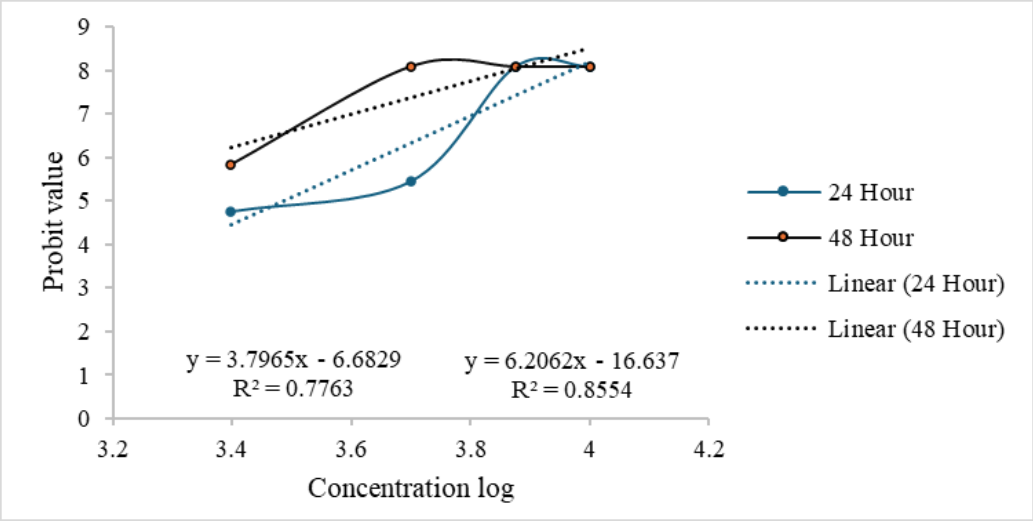

Figure 5. Graph of lethal concentration due to the administration of a combination of ethanol extracts to *P. acuminate L.* leaves and flowers during the 24th and 48th-hour of observation.

inhibits the activity of acetylcholinesterase enzyme (AChE), which results in the buildup of acetylcholine, causing a decrease in the implied delivery system to muscle cells; therefore, the next events cannot continue, and the larvae experience a continuous span and eventually die [36][42]. In the research of Yanuar et al (2023), the results of qualitative and quantitative phytochemical screening found alkaloids, flavonoids, terpenoids and phenolic compounds in Plumeria sp. plants [43].

This statement was proven by previous researchers by Suari et al. that metabolite compounds from the ethanol extract of *P. acuminate L.* flowers have the potential to cause the death of *Ae. aegypti* larvae was proven by damage to larval morphology [19]. According to previous research, ethanol extract of *P. acuminate L.* flowers has the potential to kill *Ae. aegypti* larvae as much as 6.2 to 100% within 24 h [17]. The potential for death of *Ae. aegypti* larvae was 100% in line with several other studies which also used natural plants

to kill mosquito larvae using different plants such as *M. atropurpureum* leaf extract [44], *Annona reticulata* extract [45], *Bougainvillea glabra leaf extract, Delonix regia, Lantana camara*, and *Platycladus orientalis* [46]. These results support the idea that natural plant extracts can be used to control larval life forms.

Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils, and phenolics from different plants have been reported previously for their insecticidal activities [47]. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varities and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, *etc*., of larger plants or trees [48][49]. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control. The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species.

Previous research also shows that the ethanol extract of *P. acuminate L.* leaves contains various secondary metabolites such as terpenoids, flavonoids, tannins, alkaloids, steroids, saponins, glycosides and carbohydrates. It also contains bioactive steroids, flavonoids and alkaloids, while fresh leaves and stems contain pluieride, rosinic acid, fulvoplumerin, and a mixture of terpenoids,

Table 6. Comparative analysis between combined concentrations of ethanol extracts of *P. acuminat*e L. leaves and flowers with the length of observation on the mortality of *Ae. aegypti* larvae using posthoc tests.

Concentrations of ethanol extracts of *P. acuminate* (ppm)		***P value (p:< 0.05)***	
Leaves	**Flowers**	**24-Hour**	**48-Hour**
10000	7500	1.000	1.000
	5000	0.001	1.000
	2500	0.000	0.036
7500	5000	0.001	1.000
	2500	0.000	0.036
5000	2500	0.003	0.036

Table 7. Analysis of the effect of the combined concentration of ethanol extract of *P. acuminate L.* leaves and flowers with the length of observation on the mortality of *Ae. aegypti* larvae using One-way ANOVA test.

Concentration (ppm)*	**The mortality value of mean ±SD**		***P value (p: <0,05)***	
	24-Hour	**48-Hour**	**24-Hour**	**48-Hour**
10000	20.000±0.000	20.000±0.000		
7500	20.000±0.000	20.000±0.000	0.000	0.013
5000	13.330±1.333	20.000±0.000	(*p:<0.05*)	(*p:<0.05*)
2500	8.000±0.000	16.000±1.528		

* Combination of ethanol extract *P. acuminate L.* leaves and flowers

sterols, and plumieride [50]. It has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world.

The metabolite compounds produced by the combination of ethanol extracts of *P. acuminate L.* leaves and flowers have the activity of inhibiting the growth of *Ae. aegypti* larvae, resulting in larval death. Furthermore, further research is needed to identify the metabolite compounds of the combination of ethanol extracts of *P. acuminate L.* leaves and flowers that have special activity in inhibiting the growth of *Ae. aegypti* larvae and molecular observations of dead larvae are carried out. So the combination of ethanol extract of *P. acuminate L.* leaves and flowers can be recommended as a natural larvicide that is safe for the environment. Dengue fever vector control strategies that can be applied in the surrounding environment include social mobilization campaigns (education, community relations) [51][52]. Environmental management, and legislation (law enforcement and incentives) which shall be also considered effective components of a sustainable mitigation program for controlling dengue fever vectors. Application of larvicide from botanical origin was extensively studied as an essential part of Integrated Mosquito Management (IMM), and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin were developed.

4. CONCLUSIONS

The combination of ethanol extracts of *P. acuminate L.* leaves and flowers showed larvicidal activity against *Ae. aegypti* larvae is proven by the number of deaths of *Ae. aegypti* larvae are influenced by concentration and observation time. Thus, the combination of ethanol extract of *P. acuminate L.* leaves and flowers can be recommended as a natural larvicide that is safe for the environment.

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Conflicts of Interest

The authors declare no conflict of interest.

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