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Effectiveness of Ethanol Extract of *Lantana camara* as a Biolarvicide Against the Mortality of *Aedes aegypti* Larvae

[1]Balda Balda, [1*]Sry Sukmawaty, [1]Rusni Bagenda

1. Biology Education Study Program, Faculty of Tarbiyah and Teacher Training, Institut Agama Islam Negeri Kendari, Indonesia

*Correspondence email: srysukmawaty@iainkendari.ac.id

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Abstark: *Aedes aegypti* merupakan vektor utama penyebab demam berdarah dengue (DBD) yang hingga kini masih menjadi persoalan serius dalam kesehatan masyarakat di Indonesia, termasuk di wilayah Sulawesi Tenggara. Pemakaian larvasida kimia secara terus-menerus berpotensi menimbulkan resistensi dan dampak bagi lingkungan, sehingga diperlukan alternatif pengendalian vektor yang lebih ramah lingkungan. Penelitian ini bertujuan untuk menguji efektivitas ekstrak etanol daun dan bunga *Lantana camara* sebagai larvasida alami terhadap mortalitas larva *Aedes aegypti*. Penelitian dilakukan dengan metode maserasi menggunakan etanol 96% dan pengujian bioassay terhadap larva *Aedes aegypti* dengan variasi konsentrasi 10–25 µg/mL. Mortalitas larva diamati dalam interval waktu 24 jam. Hasil menunjukkan bahwa ekstrak daun *L. camara* memiliki efektivitas lebih tinggi dibandingkan bunga, dengan nilai LC_{50} sebesar 3,26 µg/mL dan LC_{90} sebesar 8,31 µg/mL. Mortalitas larva meningkat seiring dengan konsentrasi dan waktu paparan. Kesimpulannya, ekstrak daun *Lantana camara* memiliki potensi sebagai larvasida nabati yang efektif dan ramah lingkungan untuk pengendalian nyamuk DBD.
Kata Kunci : *Aedes aegypti*, *Lantana camara*, Larvasida, LC, Mortalitas

Abstarct : *Aedes aegypti* is the primary vector of dengue hemorrhagic fever (DHF), which remains a major public health concern in Indonesia, including Southeast Sulawesi. Continuous use of chemical larvicides poses risks of resistance development and environmental impacts, thereby necessitating safer alternatives for vector control. This study aimed to evaluate the larvicidal efficacy of ethanol extracts from the leaves and flowers of *Lantana camara* against *A. aegypti* larvae. The extracts were prepared through maceration using 96% ethanol, followed by bioassay tests on third- and fourth-instar larvae at concentrations ranging from 10–25 µg/mL. Larval mortality was observed at 24-hour intervals. The results revealed that the leaf extract of *L. camara* exhibited higher larvicidal activity than the flower extract, with LC_{50} and LC_{90} values of 3.26 µg/mL and 8.31 µg/mL, respectively. Larval mortality increased proportionally with both concentration and exposure time. In conclusion, the leaf extract of *L. camara* demonstrates potential as an effective and environmentally friendly botanical larvicide for dengue vector control.
Keyword: *Aedes aegypti*, *Lantana camara*, Larvicide, LC, Mortality

1. Introduction

Aedes aegypti is an arthropod and the primary vector responsible for transmitting arboviruses such as yellow fever, dengue fever, chikungunya, Zika fever, and others [1][2]. According to data reported by the World Health Organization (WHO), since the beginning of 2024 there have been more than 7.6 million cases, including 3.4 million confirmed cases, over 16,000 severe cases, and more than 3,000 deaths [3]. Previous studies have estimated that 70% of the global burden is found in Asia [4]. In Indonesia, there were 149,866 confirmed cases—approximately three times higher than during the same period in 2023—with 884 reported deaths across 465 districts in 38 provinces [5]. Data from the Southeast Sulawesi Provincial Health Office recorded 918 and 1,083 cases in 2022 and 2023, respectively, with 17 and 9 deaths. Most cases were reported in Kendari City, with 230 cases and six deaths in 2022, and 219 cases with four deaths in 2023.

Efforts to control *Aedes aegypti* larvae have largely been carried out using chemical larvicides. Although effective, continuous use may cause adverse effects such as the death of non-target organisms, environmental pollution, increased resistance, food contamination, and potential health hazards if consumed by humans [6]. This has encouraged the adoption of approaches using natural larvicides. Plant-based natural larvicides are capable of controlling harmful organisms through non-toxic mechanisms, are target-specific, highly effective in small quantities, effectively reduce populations, are biodegradable, and environmentally friendly. One plant species with potential as a natural larvicide is *Lantana camara*, which contains secondary metabolites such as alkaloids, terpenoids, tannins, flavonoids, and saponins [7].

Lantana camara belongs to the Verbenaceae family and is considered by the IUCN as one of the world's ten worst invasive weeds that can cause significant ecological impacts [8]. *Lantana camara* is an ornamental plant that is green, clustered, upright, and shrubby, with hairy leaves that have a distinctive aroma and can grow up to three meters tall. Numerous studies have reported on the medicinal properties of *Lantana camara*. Its leaf extract has demonstrated

strong larvicidal activity, while its flowers have been reported to repel mosquitoes due to the aroma they produce [9].

However, significant research gaps remain that warrant further investigation. Most previous studies have primarily focused on the use of crude extracts of *Lantana camara* without specifically identifying the contribution of individual secondary metabolites to the mechanisms of larval mortality. In addition, studies that quantitatively evaluate the larvicidal efficacy of *Lantana camara* under standardized experimental conditions are still limited, particularly those addressing dose–response relationships and its potential application as a sustainable bioinsecticide. Therefore, this study aims to evaluate the effectiveness of secondary metabolites of *Lantana camara* on the mortality of *Aedes aegypti* larvae as an environmentally friendly alternative larvicide.

2. Research Method

a. Research Instruments

The materials used in this study included *Lantana camara* leaves, *Lantana camara* flowers, third and fourth instar larvae, ethanol, distilled water, fish food, and Tween 80. The equipment utilized in the study consisted of a blender, flour sieve, analytical balance, Petri dishes, rotary evaporator, measuring cylinder, dropper pipette, Erlenmeyer flask, and stirrer.

b. Preparation of plant extract

Healthy leaves and flowers of *Lantana camara* were collected from the South Konawe area, Southeast Sulawesi, Indonesia. The study was conducted at the Biology Education Laboratory, Faculty of Tarbiyah and Teacher Training, State Islamic Institute (IAIN) Kendari. For the extraction process, one kilogram of *Lantana camara* was washed thoroughly to remove residual impurities. The plant material was then air-dried at room temperature, cut into small pieces, and ground into fine powder. Subsequently, 250 g of the powdered simplicia was macerated with 96% ethanol for 72 hours at room temperature. After the

maceration step, the solution was filtered using Whatman filter paper, and the filtrate was evaporated using a rotary evaporator (Stuart RE 400 with RE400DB) under reduced pressure at a temperature of 40–45 °C. This process was repeated until a concentrated ethanolic extract of *L. camara* leaves and flowers was obtained [10].

c. Larvacidal biossay

Aedes aegypti larvae were collected and maintained at the Biology Education Laboratory, Faculty of Teacher Training and Education, IAIN Kendari. The larvae were reared in containers filled with water, fed with fish food, and maintained at a temperature of 28 ± 2 °C. The experimental samples consisted of 10 larvae per treatment group, with six treatments and three replications, resulting in a total of 360 larvae used in this study [11]. A total of 10 third- or fourth-instar *Aedes aegypti* larvae were transferred from the stock container into Petri dishes containing 100 ml of distilled water. The leaf and flower extracts of *Lantana camara* were prepared at concentrations of 10, 15, 20, and 25 µg/ml. The positive control was treated with abate, while the negative control consisted only of distilled water with the addition of Tween 80 in each dish [12]. Larval mortality was observed for 24 hours and recorded at 30, 60, 120, 420, 840, and 1440 minutes of exposure.

d. Statistical analysis

The mortality percentage in this study was calculated based on different concentrations, exposure periods (m), and vector species, which were used as variables to determine the significance between parameters and mortality rates. Data analysis was carried out using analysis of variance (ANOVA) to assess the efficacy and effectiveness of the biolarvicide at each concentration, while probit regression analysis was applied to determine the LC_{50} and LC_{90} values for each treatment concentration at a 95% confidence interval (SPSS Version 16.0) [13].

3. Results and Discussion

Administration of *Lantana camara* leaf and flower extracts was performed on 360 larvae, as presented in Table 1 and Table 2.

Table I. Mortality Rate and Percentage of *Aedes aegypti* Larvae After Exposure to *Lantana camara* Leaf Extract for 24 Hours

Concentration µg/ml	Total Tested Larvae	Cumulative Larval Mortality			Total	Percentage Mortality (%)
		I	II	III		
0 (-)	30	1	0	0	1	3.33
10	30	1	4	4	9	30.00
15	30	8	6	6	20	66.67
20	30	5	6	9	20	66.67
25	30	10	8	4	22	73.33
Abate (+)	30	2	8	3	13	43.33

Table 2 Mortality Rate and Percentage of *Aedes aegypti* Larvae After Exposure to *Lantana camara* Flower Extract for 24 Hours

Concentration µg/ml	Total Tested Larvae	Cumulative Larval Mortality			Total	Percentage Mortality (%)
		I	II	III		
0 (-)	30	0	0	0	0	0.00
10	30	1	2	0	3	10.00
15	30	1	1	2	4	13.33
20	30	2	2	1	5	16.67
25	30	3	2	2	7	23.33
Abate (+)	30	9	10	10	29	96.67

Table 1 shows that the mortality of *Aedes aegypti* larvae increased with increasing concentrations of *Lantana camara* leaf extract. The highest mortality was observed at 25 µg/mL (73.33%), while the lowest occurred in the negative control (3.33%). Moderate mortality rates were recorded at 15 and 20 µg/mL (both 66.67%), and lower mortality at 10 µg/mL (30.00%). The positive control (Abate) resulted in 43.33% mortality.

Similarly, Table 2 indicates a concentration-dependent increase in larval mortality following exposure to *Lantana camara* flower extract, although the overall effectiveness was lower than that of the leaf extract. The highest mortality was observed at 25 µg/mL (23.33%), while no mortality occurred in the negative control. Mortality gradually increased from 10 µg/mL (10.00%) to

20 µg/mL (16.67%). In contrast, the positive control (Abate) showed a high mortality rate of 96.67%.

The observation results at exposure intervals of 30, 60, 120, 420, 840, and 1440 minutes with *Lantana camara* leaf and flower extracts on *Aedes aegypti* larvae are presented in Table 3.

Table 3. Mortality intervals of *Aedes aegypti* larvae after exposure to *Lantana camara* leaf and flower extracts.

Extract	Concentration µg/ml	Percentage of *Aedes aegypti* Mosquito Larvae Mortality During Observation Peroid (%)					
		Exposure Duration (Minutes)					
		30	**60**	**120**	**420**	**840**	**1440**
leaf *Lantana camara*	0 (-)	0	0	0	0	0	3.33
	10	0	0	6.67	20	26.67	30
	15	0	0	3.33	16.67	43.33	66.67
	20	0	0	13.33	23.33	46.67	66.67
	25	0	0	3.33	13.33	73.33	73.33
	Abate (+)	0	0	6.67	6.67	6.67	50
flower *Lantana camara*	0 (-)	0	0	0	0	0	0
	10	0	0	0	0	0	10
	15	0	0	0	3.33	6.67	13.33
	20	0	0	0	0	6.67	13.33
	25	0	0	0	0	10	23.33
	Abate (+)	0	0	10	36.67	86.67	96.67

Based on Table 3, shows that larval mortality of *Aedes aegypti* increased with longer exposure time and higher concentrations of *Lantana camara* extracts. For the leaf extract, mortality began to appear after 120 minutes and increased substantially over time, reaching the highest value at 25 µg/mL (73.33%) after 840–1440 minutes.

In contrast, the flower extract showed delayed and lower larvicidal activity, with mortality observed only after prolonged exposure and reaching a maximum of 23.33% at 25 µg/mL after 1440 minutes.

Table 4. LC_{50} and LC_{90} Values of *Aedes aegypti* Larval Mortality after 24 Hours of Treatment

Extract Type	Exposure Time	Lethal Concentration	
		LC_{50}	LC_{90}
Ethanolic Leaf Extract of *Lantana camara*	24 Jam	3.26	8.31
Ethanolic Flower Extract of *Lantana camara*	24 Jam	5.02	9.42

Table 4 shows that the leaf extract of *Lantana camara* had lower LC_{50} and LC_{90} values (3.26 and 8.31 µg/mL) compared to the flower extract (5.02 and

9.42 µg/mL). This indicates that the leaf extract required a lower concentration to achieve 50% and 90% larval mortality, reflecting higher toxicity and effectiveness than the flower extract.

This study evaluated the larvicidal activity of ethanol extracts from the leaves and flowers of *Lantana camara* against *Aedes aegypti* larvae under controlled laboratory conditions. The results indicate that both extracts possess larvicidal potential, with a clear dose- and time-dependent increase in larval mortality. However, their efficacy varies significantly between different plant parts, with the leaf extract exhibiting significantly higher larvicidal activity than the flower extract.

The highest larval mortality rate for the leaf extract was observed at a concentration of 25 µg/mL, reaching 73.33% after 24 hours of exposure, whereas the flower extract at the same concentration resulted in a significantly lower mortality rate of 23.33%. This difference indicates that the bioactive compounds responsible for larvicidal activity are more abundant or more potent in the leaves than in the flowers.

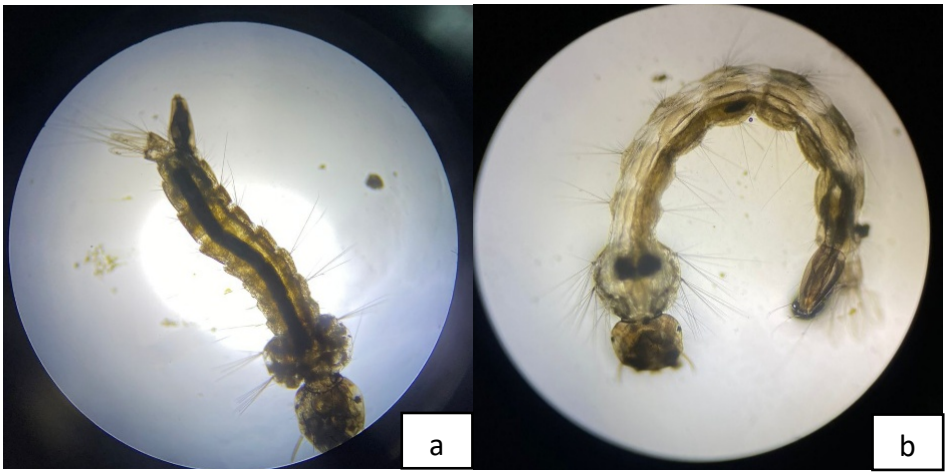

Figure 1. Microscopic Observation of Larvae: (a) *Aedes aegypti* Larva Alive and (b) *Aedes aegypti* Larva Dead.

The increase in larval mortality observed as concentration and exposure time increased confirms the presence of a positive dose-response relationship, which is consistent with general toxicological principles stating that biological effects are influenced by dose and duration of exposure [14][15]. It is worth

noting that larval mortality began to increase significantly after 120 minutes of exposure, particularly in the leaf extract treatment, indicating the onset of relatively rapid toxic effects.

However, some inconsistencies in the control groups warrant further consideration. The negative control showed a low level of mortality (3.33%) in the leaf extract experiment but no mortality (0%) in the flower extract experiment. This variation may be attributed to minor environmental fluctuations, handling stress, and natural biological variability among larvae, as bioassay outcomes are known to be influenced by mosquito age, handling before and after exposure, acclimatization, and environmental conditions such as temperature and humidity[16]. Recent methodological studies have also highlighted that small organism numbers and changing environmental conditions can contribute to variability in mortality data [17]. According to the updated WHO guidance, control mortality below 5% is considered acceptable and does not require correction, indicating that the present bioassay remained valid despite the slight discrepancy observed between control groups[18]**.** Nevertheless, this variation should be acknowledged as a limitation of the study.

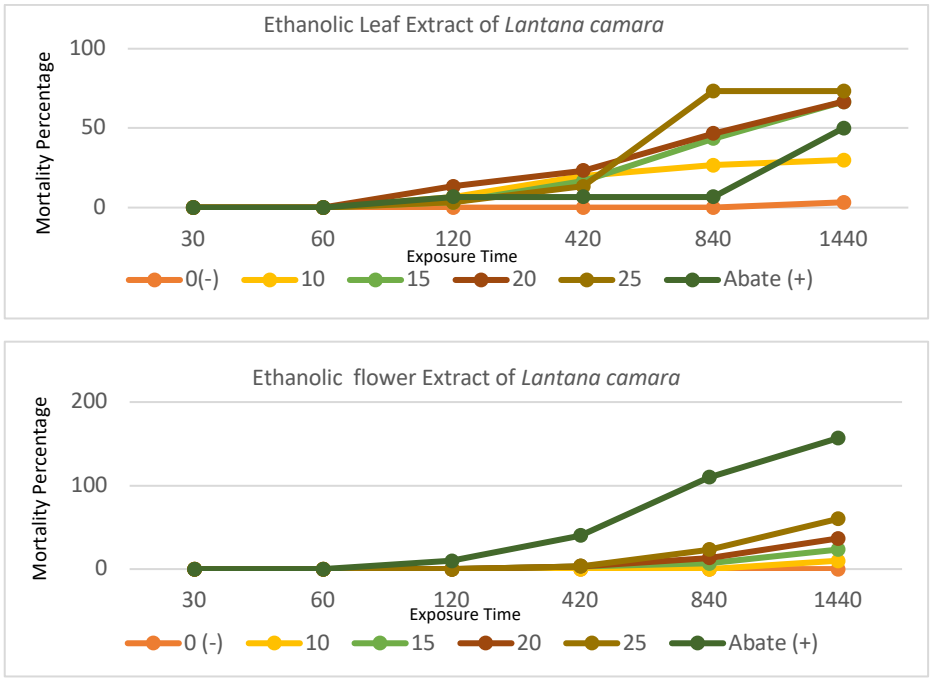

Figure 2. Graph of leaf and flower extracts of *Lantana camara* on the mortality of *Aedes aegypti* larvae.

A more striking inconsistency was observed in the positive control (abate). In the leaf extract test, Abate caused a larval mortality rate of 43.33%, whereas in the flower extract test, Abate caused a mortality rate of 96.67%. Given that Abate is a standard organophosphate larvicide and that the WHO emphasizes the use of standardized bioassay procedures for reliable susceptibility assessment, this striking discrepancy suggests that factors other than the treatment effect may have influenced the results. These factors may include variations in larval susceptibility. Recent evidence suggests that *Aedes aegypti* populations may exhibit significant variation in susceptibility to abate, while methodological differences in mosquito bioassay tests can also significantly influence mortality results. Therefore, comparative interpretation of these findings should be conducted with caution, and future studies should adopt stricter standardization regarding larval sources and testing conditions.

From a mechanistic perspective, the larvicidal activity of *Lantana camara* can be attributed to its various secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and terpenoids [19][20][21]. Compounds such as alkaloids and flavonoids in *Lantana camara* are known to possess neurotoxic potential by inhibiting acetylcholinesterase (AChE) activity, an enzyme responsible for terminating nerve impulses by hydrolyzing the neurotransmitter acetylcholine at the synaptic cleft. This inhibition results in overstimulation, convulsions, and muscle paralysis, ultimately leading to mortality [22][23].

Furthermore, flavonoids and tannins derived from *Lantana camara* leaves have been reported to disrupt larval digestion by damaging the midgut epithelium, thereby reducing enzymatic activity. Tannins act by binding to proteins in the gut wall, causing impaired nutrient absorption and structural damage to the tissue. Saponins, functioning as stomach poisons, are believed to increase intestinal membrane permeability, leading to electrolyte and essential fluid loss, which results in cellular dehydration [24]. Terpenoids denature the larval digestive system by destabilizing intestinal cell membranes, inhibiting

feeding, and consequently inducing mortality [25]. The mechanism of action is multifaceted and varies from compound to compound, resulting in synergistic toxicity.

The findings of this study are consistent with previous reports indicating that Lantana camara possesses significant larvicidal properties. However, the LC_{50} values obtained in this study are relatively lower than those reported in some previous studies, indicating a higher level of toxicity, which may be influenced by several factors, including environmental conditions that affect physiological and biochemical differences among larvae, thereby influencing sensitivity to larvicides. Additionally, the effectiveness of larvicides depends on their penetration mechanism and mode of action within the larval body. These significant differences in mortality highlight the importance of considering larval-specific responses in the development of plant-based larvicides [26].

Although these results are promising, several limitations must be acknowledged. First, this study was conducted under laboratory conditions, which may not fully reflect field conditions where environmental factors can influence the effectiveness of larvicides [27]. Second, this study did not isolate or measure individual bioactive compounds, thereby limiting the ability to determine the specific contribution of each metabolite. Third, the variability observed in the control group highlights the need for better experimental standardization.

Overall, this study indicates that Lantana camara, particularly its leaf extract, has great potential as a natural larvicide for controlling Aedes aegypti. These findings are consistent with previous research, which further confirms the potential of *Lantana camara* as an effective larvicide and supports its possible use as an environmentally friendly alternative [28][29]. However, further research is needed to isolate the active compounds, evaluate their toxicity to non-target organisms, and validate their effectiveness under field conditions.

4. Conclusion

This study demonstrates that secondary metabolites of *Lantana camara*, particularly from leaf extracts, are effective in increasing the mortality of *Aedes*

aegypti larvae. Larvicidal activity showed a clear dose- and time-dependent pattern, with the highest effectiveness observed at 25 µg/mL. These findings indicate that *Lantana camara* has strong potential as an environmentally friendly larvicide. However, further studies are required to assess its safety for non-target organisms and its application under field conditions.

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