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Larvicidal activity of *Aloe vera* leaf extract against *Aedes aegypti*

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ABSTRACT

Aedes aegypti is the main vector of various arbovirus diseases, so effective and natural-based control alternatives are needed. *Aloe vera* is known to contain various bioactive compounds that have the potential to have insecticidal activity. This study aims to evaluate the effectiveness of Aloe vera leaf extract as a larvicide against fourth-instar *A. aegypti*. The study used fourth-instar *A. aegypti* larvae with *A. vera* extract concentrations of 0%, 0.3%, 0.6%, 0.9%, and 1.1%. Each treatment used 25 larvae with three replications. *Aloe vera* extract demonstrated larvicidal activity against fourth-instar *Aedes aegypti* that consistently increased with concentration. Mortality reached 92% at a concentration of 1.1%, with highly significant differences observed between treatments ($F=166.30$; $p<0.001$). Probit analysis indicated an LC_{50} of approximately 0.58% for the *A. vera* extract, demonstrating that a relatively low concentration was sufficient to cause 50% larval mortality. The dose-response relationship also showed a positive coefficient ($B=0.591$; $SE=0.0655$), reinforcing the pattern of increasing mortality associated with higher concentrations. *A. vera* leaf extract has the potential as a botanical larvicide against fourth instar A. aegypti.

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INTRODUCTION

Dengue is a disease that remains a serious threat to global public health. The World Health Organization reports that the number of dengue cases globally will increase from 505,430 in 2000 to more than 14 million in 2024 (World Health Organization (WHO), 2025). The increase in cases is closely related to the existence and expansion of the distribution of mosquitoes of the genus Aedes, especially *Aedes aegypti* as the main vector of the dengue virus (R. M. da Silva et al., 2026). The ability of *Ae. aegypti* to adapt to urban environments, breed in various water bodies, and live in proximity to humans makes vector population control a key component in preventing dengue transmission. Therefore, the increasing population and spread of *Ae. aegypti* are important concerns, particularly in tropical and subtropical countries with environmental conditions that support the mosquito's life cycle (Pourzangiabadi et al., 2025)

Indonesia is one of the countries with a high dengue burden in Southeast Asia. The dengue situation in Indonesia shows that this disease remains a persistent public health problem and has the potential to cause periodic increases in cases. In 2023, Indonesia reported approximately 114,720 dengue cases with 894 deaths. This number increased significantly in 2024 to 210,644 cases with 1,239 deaths spread across 259 districts/cities and 32 provinces (Nurul 'Ain & Pratiwi, 2025). In fact, in the initial period of the increase in cases in 2024, Indonesia reported tens of thousands of confirmed cases and hundreds of deaths, demonstrating the significant challenge of national dengue control. These high numbers emphasize that strengthening vector control strategies remains a critical need in dengue prevention efforts in Indonesia (Rakhmani & Zuhriyah, 2024)

One important approach to vector control is intervention during the larval stage. Control at the larval stage has the advantage of being implemented before the mosquito develops into an adult, which plays a role in virus transmission. Chemical larvicides have long been used as a method to control Aedes larval populations (Weeratunga et al., 2017). However, continued reliance on synthetic insecticides faces several challenges, including the potential development of resistance in vector populations, impacts on non-target organisms, and potential environmental disruption. Furthermore, the global increase in dengue cases demonstrates that vector control requires a sustainable approach and not a single control strategy (Oxborough et al., 2025). In recent years, the exploration of natural products as sources of candidate insecticides and larvicides has received increasing attention. Plants produce a variety of secondary metabolites as part of their defense mechanisms against herbivores, insects, and other organisms (Engdahl et al., 2022). Compounds such as alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds are known to possess diverse biological activities that could potentially be utilized in insect control. Plant-based larvicides are attractive because they offer alternative active ingredients derived from biological sources, are readily available, and can be developed as part of a more sustainable vector control strategy (Demarque & Espindola, 2021)

One plant with potential for development as a source of natural larvicide is *Aloe vera*. This plant is widely known and has been used in various fields, particularly as an ingredient in traditional medicine, cosmetics, food, and health products. In addition to polysaccharides and various other bioactive components, *A. vera* also contains secondary metabolites with potential biological activity against target organisms. This potential has prompted research into the use of *A. vera* extract as a mosquito larval control agent (Lubis et al., 2018a)

Experimental evidence regarding the larvicidal activity of *A. vera* is able to produce LC50 and LC90 of 300.059 ppm and 612.96 ppm on *Ae. aegypti* instar IV larvae. This finding is important because instar IV larvae are the final larval stage before entering the pupal phase. At this stage, larvae have undergone more advanced physiological and morphological development compared to previous instars. Thus, specific testing on instar IV can provide relevant information regarding the ability of a larvicide candidate to control larvae at the final developmental stage (Subramaniam et al., 2012). However, the activity of a plant extract is not solely determined by the plant species used. Geographical variation of the plant, environmental conditions of the growing area, plant part, drying process, solvent type, extraction method, extract concentration, and bioassay conditions can influence the composition of bioactive compounds and the resulting biological response (Aryakia, 2026)

Based on previous research, *A. vera* has the potential as a source of larvicide against *Ae. aegypti*. However, research is still needed to strengthen the evidence regarding the effectiveness of *A. vera* leaf extract on the mortality response of *Ae. aegypti* instar IV larvae. Therefore, this study aims to evaluate the effectiveness of *Aloe vera* leaf extract as a larvicide against *Aedes aegypti* instar IV larvae based on the level of larval mortality at various treatment concentrations.

MATERIALS AND METHOD

Research design

This study used a laboratory experimental design to evaluate the effectiveness of *Aloe vera* leaf extract as a larvicide against instar IV *Aedes aegypti* larvae. The treatment consisted of five concentration groups, namely 0%, 0.3%, 0.6%, 0.9%, and 1.1%. The sample criteria in this study included: *Ae. aegypti* mosquito larvae that had reached instar IV (4-5 days after hatching from eggs) and actively moving larvae. Based on the Guidelines for Laboratory and Field Testing of Mosquito Larvicides, the number of larvae used in the study was 25 larvae (World Health Organization (WHO), 2005). Each concentration was tested on 25 larvae with three repetitions. Larval mortality was observed after 24 hours of exposure to the extract. Thus, the number of larvae in each treatment group was 75, while the total sample used was 375 instar IV Aedes aegypti larvae.

Procedure

Egg Rearing. The *Aedes aegypti* used in this study were obtained in dried form from the Parasitology and Pathology Department of the Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Bogor. The *Aedes aegypti* eggs were then placed in plastic trays filled with clean water for larval rearing. The eggs hatch into larvae within 1-2 days. The larvae develop from stages I to IV over 4-5 days. During this development, the larvae are fed fish pellets (Sieumeni et al., 2026a)

Identification of Aedes aegypti larvae. Larvae that are 5-7 days old, measuring 5-6 mm, are then removed using a pipette. Ae. aegypti mosquito larvae hang at an angle to the water surface. The larvae are placed under a glass slide for microscopic morphology examination. *Ae. aegypti* larvae are characterized in the 8th abdominal segment, there are comb teeth with large median spines and side spines (subapical spines). Observe under a microscope at 40x magnification (Anindita et al., 2022)

Aloe vera leaf extraction. The preparation of *A. vera* leaf extract was carried out at the Spice and Medicinal Plants Research Institute (BALITRO), Bogor. *A.vera* leaves were obtained from the Bekasi area, West Java, Indonesia with a height of 30-50 cm, single green leaves and there are thorns on the edges of the leaves. The solvent used was 96% ethanol. The *A.vera* leaves that had been obtained were washed thoroughly with running water, then dried. The *A. vera* leaves that had been cleaned and had their thorns removed were then weighed 1000 grams. The *A. vera* was cut into small pieces then placed in a closed stainless steel container, added with 96% ethanol at a ratio of 1: 5, stirred for 3 hours with a stirrer and precipitated overnight for 24 hours. After being left to stand, the maceration results were filtered 3 times with a Buctner funnel lined with filter paper and collected with an Erlenmeyer flask. The filtrate was concentrated using a rotary evaporator at a temperature of 50-55˚C until a thick extract was obtained (Chore et al., 2014). The extract was then dissolved in 100 ml of distilled water. The *A.vera* leaf extract was then diluted to obtain extract solutions with concentrations of 0%, 0.3%, 0.6%, 0.9%, and 1.1%.

Aloe vera leaf larvicide test. The test solution consisted of aloe vera leaf extract, with group 1: 0% concentration (negative control), group 2: 0.3% concentration, group 3: 0.6% concentration, group 4: 0.9% concentration, and group 5: 1.1% concentration. A total of 25 fourth-instar *Ae. aegypti* larvae were placed in each concentration group. Each group was replicated three times. Effectiveness testing was determined by the mortality of 50% of the sample within 24 hours.

Data Analysis. The effect of aloe vera leaf extract on the mortality of fourth-instar Ae. aegypti larvae was tested using one-way ANOVA, while probit analysis was used to determine the LC_{50}.

RESULTS AND DISCUSSION

The effectiveness of *Aloe vera* leaf extract as a larvicide against *Aedes aegypti* instar Fourth was evaluated based on the number and percentage of larval mortality after 24 hours of treatment. Data from three replicates were analyzed and presented as the mean ± standard deviation (SD). Differences in the number of larval mortality between concentration groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to determine differences between treatment groups.

Table 1. Effectiveness of *A. vera* leaf extract on the mortality of instar fourth *Ae. aegypti* larvae after 24 hours of treatment.

Extract concentration (%)	Number larvae per repetition	Number of larvae mortality repetition			Mean mortality ($\bar{X}$ ± SD)
		I	II	III	
0.0	25	0	0	0	0.00 ± 0.00[a]
0.3	25	3	5	4	4.00 ± 1.00[b]
0.6	25	13	15	11	1.00 ± 2.00[c]
0.9	25	17	20	18	18.33 ± 1.53[d]
1.1	25	22	24	23	23.00 ± 1.00[e]

Note : Data are presented as the mean ± standard deviation (SD) of three replicates (n = 3). Different superscript letters in the same column indicate significant differences between treatment groups based on one-way ANOVA followed by Tukey HSD post-hoc test at a significance level of $p < 0.05$. The results of one-way ANOVA showed that the concentration of Aloe vera leaf extract significantly affected the number of deaths of instar IV Aedes aegypti larvae ($F = 166.30$; $p < 0.001$).

Table 1 shows that increasing the concentration of A.vera leaf extract is followed by an increase in the number of deaths of Ae.aegypti fourth-instar larvae. The control group with a concentration of 0% showed no larval deaths. Meanwhile, a concentration of 0.3% resulted in an average mortality of 4.00 ± 1.00 larvae or 16%, then increased to 13.00 ± 2.00 larvae or 52% at a concentration of 0.6%. The highest mortality was found at a concentration of 1.1%, which was 23.00 ± 1.00 larvae with a mortality percentage reaching 92%. The results of one-way ANOVA showed a significant difference in the number of larval mortality between concentration groups ($p < 0.001$). The results of the Tukey HSD post hoc test also showed that each concentration differed significantly from the others ($p < 0.05$). These findings indicate a dose-dependent effect, meaning that the higher the concentration of *A. vera* leaf extract, the higher the mortality of fourth-instar *Ae. aegypti* larvae. Analyze the relationship between extract concentration and larval mortality response and to estimate the effective concentration of extract that can cause larval mortality, a Probit analysis was performed. This analysis was used to illustrate the relationship between increasing extract concentration and the probability of *Ae.aegypti* larval mortality. The results of the Probit regression parameter estimates are presented in **Table 2**.

Table 2. Probit analysis of the relationship between the concentration of aloe vera leaf extract and the mortality of fourth instar Aedes aegypti larvae.

Parameter	Coefficient (B)	Std. Error
Intercept	0.356	0.2749
Concentration	0.591	0.0655
Scale	1[a]	

The results of the Probit analysis showed that the concentration of *A.vera* leaf extract had a positive regression coefficient of 0.591 with a standard error of 0.0655. This positive coefficient indicates a unidirectional relationship between the concentration of the extract and the probability of larval mortality. In other words, increasing the concentration of *A. vera* leaf extract tends to be followed by an increase in the mortality response of fourth-instar *Ae. aegypti* larvae. These results also indicate that a concentration of *A. vera* leaf extract of 0.591% is needed to kill 50% of the total population of *Ae. aegypti* larvae.

The findings of this study complement those of a study testing *A. vera* extract on third-instar *A. aegypti* larvae. In that study, after 60 minutes of exposure, larval mortality reached 69.2% at a 10% extract concentration, increasing to 76.0% at a 20% concentration, and reaching 86.6% at a 30% concentration (Arianto & Aditama, 2024). Research on *A. vera* leaf extract at concentrations of 20, 40, 60, 80, and 100 ppm on third and fourth instar *A. aegypti* larvae after 24 hours resulted in a mortality percentage of 29% at 20 ppm, 32% at 40 ppm, 39% at 60 ppm, 50% at 80 ppm, and 66% at 100 ppm with an LC_{50} value of 80.5 ppm at 1,440 minutes (Lubis et al., 2018b). Tests of extract concentrations of 80, 160, 240, 320, and 400 ppm resulted in mortality of 19%, 28%, 41%, 50%, and 68%, with LC_{50} values for instar IV larvae of 300.05 ppm and LC_{90} reaching 612.96 ppm. The LC_{50} value for instar IV larvae was higher than instars I, II, and III (Sieumeni et al., 2026b). Larvicidal activity in the genus Aloe is also influenced by plant species and solvent type, which shows that the hexane extract of *Aloe fibrosa* has the highest activity with an LC_{50} of 0.05 mg/mL and an LC_{90} of 0.09 mg/mL; the hexane extract of *Aloe ngongensis* shows an LC_{50} of 0.11 mg/mL. In comparison, the ethyl acetate

extract of *Aloe turkanensis* has an LC_{50} of 0.11 mg/mL; the methanol extract of *A. fibrosa* shows much lower activity with an LC_{50} of 3.89 mg/mL (Chore et al. 2014). The form and composition of the test material also determine the larvicidal activity of *Myrciaria floribunda* leaves against fourth instar *A. aegypti* after 48 hours in the form of essential oils showing LC_{50} values of 201.73 ppm and LC_{90} of 604.78 ppm, water extracts producing LC_{50} of 15.85% and LC_{90} of 19.92%, rutin compounds showing LC_{50} of 22.46 ppm and LC_{90} of 43.55 ppm, while hydrolates did not show larvicidal activity (Santos et al. 2025).

Phytochemically, *A. vera* is a plant rich in secondary metabolites, particularly anthraquinones/anthrones, chromones, flavonoids, phenolic compounds, phytosterols, and various other components. The most studied compounds in *A. vera* include aloin, aloe-emodin, emodin, aloesin, and acemannan. A comprehensive study of *A. vera* indicates that aloe-emodin and aloin are important components. In contrast, the overall chemical composition of *A. vera* is very complex and can vary depending on the plant part, environmental conditions, and extraction process (Sánchez et al., 2020). The anthraquinone group, particularly aloin and aloe-emodin, is an important candidate for explaining the biological activity of *A. vera* against mosquito larvae. Studies of various quinone compounds have demonstrated their toxicity against *A. aegypti* larvae (R. L. Silva et al., 2020) Quantitative analysis of *A. vera* from 12 agro-climatic regions of India found aloin content between 0.15–0.45 mg/g and aloe-emodin 0.09–0.29 mg/g, and showed that environmental factors and the origin of the plant material can influence the metabolite composition and biological activity (Kumar et al., 2017). Thus, the larvicidal activity obtained in this study is likely not only determined by the overall extract concentration, but also by the concentration and proportion of bioactive metabolites successfully extracted from *A. vera* leaves.

In addition to anthraquinones, flavonoids can also contribute to the larvicidal activity of *A. vera*. Research evidence suggests that several flavonoids can inhibit glutathione S-transferase Noppera-bo (Nobo) in A. aegypti. The Nobo enzyme plays a role in the biosynthesis of the insect steroid hormone ecdysone, so its inhibition can disrupt the larval development process. Flavonoids will interact with the AeNobo protein. Among the identified flavonoid-type inhibitors, desmethylglycitein (4,6,7-trihydroxyisoflavone) is one of the flavonoids that shows the highest inhibitory activity and is able to suppress the development of *A. aegypti* larvae more effectively than daidzein. These findings at the molecular level provide a strong basis for the belief that plant flavonoids can cause death or disrupt the development of *A. aegypti* through disruption of the hormonal system and insect development (Inaba et al. 2022).

The saponin group also has the potential to contribute to larvicidal effects. Biologically, saponins have amphipathic properties, allowing them to interact with cell membranes and disrupt membrane integrity. Experimental evidence on mosquito larvae indicates that commercial saponin from *Quillaja saponaria* is toxic to instar III–IV larvae of *A. aegypti*, and at a concentration of 800 mg/L results in 100% mortality after 1 day (Pelah et al., 2002). Although the study did not use *A. vera*, the results provide evidence that saponins are a group of plant metabolites that do possess larvicidal activity against *A. aegypti*. Therefore, if saponins were present in the *A. vera* extract used, they could theoretically have contributed to the observed mortality.

The mechanism of activity of plant extracts against *A. aegypti* larvae does not always originate from a single compound. A review of secondary metabolites of larvicides indicates that plant compounds can act through multiple targets, including the nervous system, acetylcholinesterase, the GABA system, mitochondrial activity, the digestive system, and larval development. Therefore, crude extracts containing multiple metabolite groups have the potential to produce combined or synergistic effects (De Souza Wuillda et al., 2019). In the context of this study, the increase in mortality from 16% at 0.3% to 52%, 72%, and 92% at concentrations of 0.6%, 0.9%, and 1.1%, respectively, after 24 hours may indicate that increasing the extract concentration increases the amount of bioactive compounds available to interact with the physiological targets of the larvae.

Interestingly, more specific histopathological evidence for *A. vera* has also been reported. Studies of the methanol-soluble fraction of *A. vera* latex/skin found that it contained aloin and caused damage to the midgut of *A. aegypti* larvae, particularly to the peritrophic membrane and lumen. The LC_{50} value of the methanol-soluble fraction was reported to be 120.56 ppm, while the chloroform extract showed an LC_{50} of 192.87 ppm. Midgut damage also increased with increasing fraction concentration. These findings suggest that *A. vera* components are not only associated with mortality but may also cause structural changes in key larval tissues, particularly the digestive system (Rahmayani et al. 2019).

Thus, the high mortality observed in this study is likely a consequence of the multifactorial activity of *A. vera* secondary metabolites, particularly the anthraquinone group such as aloin and aloe-emodin, flavonoids, and possibly saponins and other phenolic compounds. Anthraquinones have been shown to have direct larvicidal activity against *A. aegypti*, flavonoids are known to interfere with hormonal regulators of

development through the Nobo protein, and saponins have been shown to be toxic to *A. aegypti* larvae. However, because this study has not yet identified and quantified the active compounds, the relationship between each chemical component and mortality cannot be stated as a causal relationship. Therefore, the results of this study are more appropriately interpreted as evidence of larvicidal activity of *A. vera* extracts. In contrast, future studies should use LC-MS/GC-MS, HPLC, or bioassay-guided fractionation approaches to determine the compounds most contributing to this activity.

CONCLUSION

This study shows that *Aloe vera* leaf extract has potential as a botanical larvicide against fourth-instar *Aedes aegypti*, with increasing effectiveness with increasing concentration. This activity strengthens the evidence that *A. vera* contains bioactive components that have the potential to disrupt the survival of *A. aegypti* larvae. Overall, *A. vera* leaf extract has the potential to be developed as an alternative plant-based larvicide in controlling *A. aegypti*. Further research is needed to identify the active compounds involved, elucidate their toxicity mechanisms, and evaluate their safety against non-target organisms and their effectiveness under semi-field and field conditions.

AUTHORS CONTRIBUTION

I. Izharoh designed and conducted the study, analyzed and interpreted the data, R. Anindita wrote a draft of the manuscript, analyzed and interpreted the data.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest, and will take full responsibility for the content of the article, including implications of AI-generated art.

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