

Effectiveness of Zodia (*Evodia suaveolens*) leaf ethanol extract spray as a repellent against *Aedes aegypti* Mosquitoes

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Abstract

Aedes aegypti mosquitoes are the primary vector of Dengue Hemorrhagic Fever (DHF), which can cause death if not properly treated. Synthetic insecticides have been used to control this vector; however, they may cause negative effects such as resistance, environmental pollution, and health problems. Therefore, safer alternatives are needed, such as natural insecticides derived from zodia leaves (*Evodia suaveolens*). This study aimed to determine the effectiveness of a spray formulation containing the ethanol extract of zodia leaves as a repellent against *Ae. aegypti* mosquitoes. This research employed an experimental method using a Completely Randomized Design (CRD) with four extract concentrations: 0%, 5%, 10%, and 15%. The observed parameters included phytochemical content, spray formulation stability, and mosquito repellency. The data were analyzed using ANOVA, followed by the Least Significant Difference (LSD) test and probit analysis. The results showed that the ethanol extract of zodia leaves contains alkaloids, flavonoids, saponins, tannins, fenols, terpenoids, and steroids. The spray formulation had a pH of 5, was non-homogeneous, and exhibited increased turbidity with higher extract concentrations. An increase in extract concentration was directly proportional to mosquito repellency. The repellency at concentrations of 5% (13,63%); 10% (36,62%), and 15% (74,64%), with the 15% concentration showing the highest repellency. Based on the probit analysis, the EC50 value of 11.55% showed that the ethanol extract spray formulation of zodia leaves is effective as a repellent against *Ae. aegypti* mosquitoes.

Keywords: *Aedes aegypti*; *Evodia suaveolens*; Repellent; Spray

1. Introduction

Indonesia is a tropical country with warm temperatures and high humidity, conditions that support the presence and proliferation of mosquitoes as vectors of disease transmission, such as Dengue Hemorrhagic Fever (DHF), a disease caused by the dengue virus. DHF can affect all age groups and occurs throughout the year, with the potential to cause serious complications and even death if not properly managed [1]. Globally, approximately 40% of the world's population living in tropical and subtropical regions are at risk of dengue infection, according to the World Health Organization. In 2024, Indonesia recorded 88,593 DHF cases with 621 deaths, indicating that this disease remains a significant public health concern.

Efforts to prevent DHF are generally carried out through vector control, such as mosquito breeding site eradication (PSN) and the use of synthetic insecticides, including fogging. However, the use of synthetic insecticides can cause negative impacts, such as mosquito resistance, environmental pollution, and harmful effects on non-target organisms [2]. Therefore, safer and more environmentally friendly control alternatives are needed.

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One alternative is the use of natural insecticides derived from plants, such as zodia leaves (*Evodia suaveolens*). Zodia is a plant from the Rutaceae family that has a strong aroma in its flowers and leaves. This aroma can disrupt the mosquito's olfactory system, thereby inhibiting its ability to detect host odors and causing it to move away [3]. In addition, zodia leaves are known to contain bioactive compounds such as alkaloids, flavonoids, terpenoids, and tannins, which have repellent and toxic activities against mosquitoes [4]. Repellents in spray form have advantages, including a lower risk of contamination due to tightly sealed and airtight packaging, as well as greater practicality [5]. Therefore, the utilization of zodia leaf extract as a repellent in spray formulation has the potential to be an effective and environmentally friendly alternative for DHF vector control.

2. Methods

2.1. Time and Location

This study was conducted from November to December 2025 at the Botany Laboratory and Zoology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung.

2.2. Tools and Materials

The instruments used included laboratory glassware, an analytical balance, a rotary evaporator, volumetric pipettes, spray bottles, mosquito cages, an aspirator, and a stopwatch. The materials used included *Aedes aegypti* mosquito eggs obtained from Loka Labkesmas Baturaja, zodia leaves, 70% and 96% ethanol, distilled water, larval feed (fish pellets), adult mosquito feed (sucrose solution), and phytochemical reagents (HCl, FeCl₃, Mayer, Dragendorff, and Wagner reagents).

2.3. Research Design

This study employed an experimental method using a Completely Randomized Design (CRD), consisting of four treatment concentrations of zodia leaf extract: 0% (control), 5%, 10%, and 15%. Each treatment used 25 mosquitoes with four replications. Observations were carried out for 5 minutes at 1-hour intervals.

2.4. Preparation of Zodia Leaf Extract

The collected zodia leaves were first identified (determined) to confirm the plant species. The leaves were then cleaned and washed under running water, followed by cutting them into small pieces. The leaves were air-dried for five to seven days and then ground into a fine powder (simplicia). A total of 500 grams of simplicia was extracted using the maceration method with 70% ethanol as the solvent at a ratio of 1:10 (500 g material : 5000 mL solvent). The maceration process was carried out for three days (3 × 24 hours) in a closed container and stirred daily [6].

2.5. Preparation of Test Animals

The test animals were *Ae. aegypti* mosquitoes. Rearing was carried out by placing mosquito eggs into a container filled with water. Within approximately 1–2 days, the eggs hatched into larvae. The larvae were fed with finely ground fish pellets until they reached the fourth instar stage, which took about 4–7 days. They then developed into pupae and were transferred into a new container filled with water up to half its volume, and placed in a mosquito cage for 1–2 days until they emerged as adult mosquitoes. Adult mosquitoes were fed with a sucrose solution. The mosquitoes used in this study were 3–5-day-old female mosquitoes that had been starved for 12 hours [7].

2.6. Phytochemical Screening

Phytochemical screening of the ethanol extract of zodia leaves was conducted at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. This test aimed to determine the presence of bioactive compounds in zodia leaves. The procedures for phytochemical screening of the ethanol extract are as follows [8].

Flavonoid Test. One milliliter of zodia leaf extract was placed into a test tube, followed by the addition of magnesium powder and 5 mL of HCl. A positive result was indicated by the appearance of reddish-black, yellow, or orange coloration.

Alkaloid Test. Five drops of HCl were added to 1 mL of zodia leaf extract in a test tube. The solution was then divided into three separate test tubes. Each tube was treated with Mayer, Dragendorff, and Bouchardat reagents, respectively.

A positive result was indicated by the formation of a white precipitate (Mayer), an orange precipitate (Dragendorff), and a brown precipitate (Wagner), along with changes in solution color.

The saponin test was carried out by adding 1 mL of zodia leaf extract into a test tube, followed by 10 mL of distilled water, and then shaken vigorously for 30 seconds. A positive result was indicated by the formation of stable foam.

The tannin test was performed by adding 1 mL of zodia leaf extract into a test tube, followed by 5 mL of distilled water. The mixture was heated to boiling, and then 3–5 drops of FeCl_3 solution were added. The appearance of a green or blackish color indicated a positive result. The phenol test was conducted in a similar manner to the tannin test; however, the mixture was not heated. The terpenoid and steroid tests were conducted by adding 1 mL of zodia leaf extract into a test tube, followed by the addition of 5 mL of chloroform, 2 mL of sulfuric acid, and 2 mL of acetic anhydride. A positive result was indicated by a color change of the mixture to red or brown for terpenoids, while the presence of steroids was indicated by blue, purple, or green coloration.

2.7. Preparation of Spray Formulation

The spray formulation was adapted from [9]. The concentrations of zodia leaf ethanol extract used were 0%, 5%, 10%, and 15%. The formulation consisted of zodia leaf ethanol extract as the active ingredient, 96% ethanol as the primary solvent, propylene glycol and glycerin as co-solvents to dissolve the active compounds, maintain the physical and chemical stability of the formulation, and adjust viscosity, and distilled water as the solvent. The formulation is presented in Table 1.

Table 1 Formulation of Spray Containing Ethanol Extract of *E. suaveolens* Leaves

Concentration	<i>E. suaveolens</i> extract (mL)	Propylene glycol (mL)	Gliserin (mL)	Ethanol 96% (mL)	Aquadest (mL)
0%	-	10	5	1	Ad 100
5%	5	10	5	1	Ad 100
10%	10	10	5	1	Ad 100
15%	15	10	5	1	Ad 100

2.8. Evaluation of Spray Formulation

The evaluation of the spray formulation was conducted to determine its physical characteristics, stability, homogeneity, and safety, including homogeneity test, clarity test, pH test, and irritation test.

The homogeneity test was performed by spraying the formulation onto a glass slide and observing it visually. The spray formulation was considered homogeneous if no solid particles or clumps were observed on the surface of the glass slide [10].

The clarity test was conducted through direct visual observation to assess the level of transparency. The formulation was considered clear if it had a transparency comparable to water and showed no turbidity [10].

The pH test was carried out using universal pH indicator paper. The indicator paper was immersed in the formulation for approximately 3 seconds, and the resulting color change was compared with the standard color scale provided on the indicator packaging to determine the pH value of the formulation [10].

The irritation test was conducted by spraying the formulation onto the skin area from the wrist to the elbow. Observations were made for 15 minutes after application [11].

2.9. Effectiveness Test of Spray Formulation

The effectiveness test of the spray formulation containing ethanol extract of zodia leaves was conducted with the assistance of four volunteers who met the following criteria: aged 18–35 years, physically healthy, having no history of allergies to the tested materials, not using fragrances, and not smoking during the testing period [12].

Before and after the test, the volunteers' arms were cleaned by washing, rinsing with clean water, and then drying. The left arm was used as the control; it was treated with 1 mL of 70% alcohol and allowed to dry, followed by spraying with the formulation without extract (0% concentration). The arm was then inserted into a mosquito cage labeled as control for 5 minutes.

The right arm was first cleaned with 70% alcohol, then sprayed with 1 mL of ethanol extract spray of *E. suaveolens* leaves at a concentration of 5%. The same testing procedure was repeated for spray formulations with concentrations of 10% and 15%. Observations were conducted over 4 hours, with data collected four times for each concentration. Each data collection was performed at 1-hour intervals with an observation duration of 5 minutes.

The percentage of mosquito repellency was calculated using the following formula

$$\% \text{Daya Tolak Nyamuk} = \frac{K - P}{K} \times 100\%$$

Description

K: Number of mosquitoes landing on the untreated arm (without zodia leaf extract).

P: Number of mosquitoes landing on the treated arm (with zodia leaf extract).

2.10. Data Analysis

Data obtained from phytochemical screening, homogeneity test, clarity test, pH test, and irritation test were analyzed descriptively. To determine differences in mosquito repellency at various concentrations, statistical analysis was performed using one-way analysis of variance (ANOVA). This was followed by the Least Significant Difference (LSD) post hoc test and probit analysis to evaluate the effectiveness of the spray formulation containing ethanol extract of *E. suaveolens* leaves against the repellency of *Ae. aegypti* mosquitoes.

3. Results and Discussion

3.1. Phytochemical Screening of Ethanol Extract of *E. suaveolens* Leaves

The results of phytochemical screening of the ethanol extract of *E. suaveolens* leaves showed that the extract contains secondary metabolites, including flavonoids, saponins, tannins, alkaloids, phenols, terpenoids, and steroids, as presented in Figure 1.

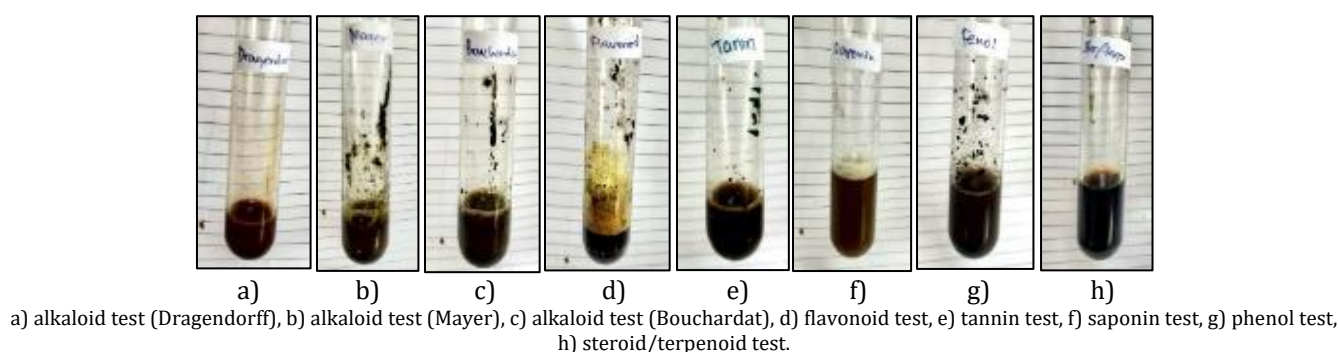


Figure 1 Phytochemical Screening Results of Ethanol Extract of *E. suaveolens* Leaves

Alkaloids exhibit neurotoxic effects on insects, leading to mortality. Zodia leaves contain an alkaloid compound, evodiamine, which can disrupt the insect nervous system and act as a toxin [4]. Terpenoids can interfere with the mosquito's olfactory system, thereby inhibiting its ability to detect and approach hosts [3]. Terpenoids also act as antifeedants and exert toxic effects on the insect nervous system, while steroids can disrupt insect growth [13]. Saponins function as stomach poisons and are capable of lysing the waxy layer (cuticle) on the outer surface of the insect body. Tannins cause constriction of the throat and shrinkage of the mosquito's mouthparts, thereby inhibiting blood-feeding [14]. Flavonoids act as respiratory toxins; these compounds enter the body through inhaled air and disrupt the respiratory process, ultimately causing death [15].

3.2. Evaluation of Spray Formulation

The results of the homogeneity test showed that the spray formulation at 0% concentration was homogeneous, as indicated by the absence of visible particles. In contrast, the spray formulations at concentrations of 5%, 10%, and 15% were not homogeneous due to the presence of visible particles, as shown in Figure 2.

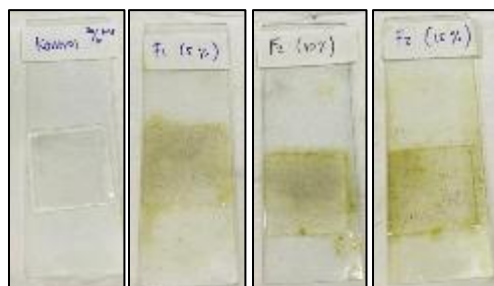


Figure 2 Homogeneity Test Results of Spray Formulation Containing Ethanol Extract of Zodia Leaves

The differences in the level of homogeneity of the formulations were caused by the improper sequence of mixing the ingredients. In addition, differences in the polarity of the components can also affect the homogeneity of the formulation. As a result, the components were not evenly distributed. In the spray formulation, distilled water (aquades), which is polar, was used as the solvent. Meanwhile, the extract of *E. suaveolens* leaves contains various phytochemical compounds with different levels of polarity. Based on the study [16], phytochemical compounds such as flavonoids and phenols are polar, tannins and alkaloids are semi-polar, while terpenoids and saponins are non-polar. These differences in polarity can influence the mixing process of the formulation.

Therefore, 96% ethanol, which is semi-polar, was added as a carrier solvent to help dissolve the phytochemical compounds in the extract so that they could be evenly distributed in the formulation. However, in this study, the amount of 96% ethanol used was relatively small, only 1 mL, which was insufficient to optimally dissolve all extract components, resulting in a non-homogeneous formulation.

The lack of homogeneity in the formulation caused the active compounds from the *E. suaveolens* leaf extract to be unevenly distributed. This condition may result in each spray containing different amounts of active compounds, leading to inconsistent effectiveness of the formulation as a repellent against *Ae. aegypti* mosquitoes. In addition, poor homogeneity can reduce the physical stability of the formulation, as inadequately mixed components may settle over time.

The clarity test results of the spray formulation containing *E. suaveolens* extract showed that the 0% concentration (control) appeared clear or transparent, whereas the formulations with concentrations of 5%, 10%, and 15% appeared turbid with a dark green color. The turbidity occurred because zodia leaf extract contains phytochemical compounds with varying solubility in the solvent used. Increasing the extract concentration resulted in a higher number of components in the solution. Consequently, some components did not dissolve completely and formed fine particles, causing the solution to appear turbid. These results are presented in Figure 3.



Figure 3 Clarity Test Results of Spray Formulation Containing Ethanol Extract of Zodia Leaves

These results are consistent with the study on a repellent spray formulation of ethanol extract of mint leaves (*Mentha piperita*), which reported that increasing extract concentration in the formulation led to higher turbidity levels [17]. Similar findings were also reported that a repellent spray formulation of jackfruit leaf extract (*Artocarpus heterophyllus*), where only the control formulation appeared clear, while formulations containing the extract showed color changes ranging from clear yellow to dark green [9].

The pH test results showed that the spray formulations of ethanol extract of *E. suaveolens* leaves at concentrations of 0%, 5%, 10%, and 15% all had the same pH value, namely pH 5. This pH value is still within the acceptable range for topical formulations applied to the skin. According to [18], topical preparations should have a pH close to the physiological pH of the skin, which is approximately 4.5–6.5. The pH value also plays an important role in maintaining the physical and chemical stability of the formulation. An appropriate pH can help preserve the solubility and stability of secondary metabolite compounds in the extract [19].

Based on the irritation test conducted on four volunteers using formulations with concentrations of 0%, 5%, 10%, and 15%, none of the formulations caused irritation symptoms such as itching, redness, swelling, or a burning sensation on the skin. These results indicate that the spray formulation containing ethanol extract of *E. suaveolens* leaves is safe for topical application without causing irritation. This is because each component in the formulation was used within safe concentration limits, thereby minimizing the risk of adverse skin reactions. One of the components used in the formulation is propylene glycol, which functions as a co-solvent to help dissolve extract components and maintain formulation stability. According to [20], propylene glycol has been widely used in various topical formulations due to its ability to enhance the solubility of active ingredients. Although at high concentrations it may cause mild irritation in sensitive skin, it is generally considered to have low toxicity and is safe for use.

3.3. Effect of Different Concentrations of Spray Formulation Containing Ethanol Extract of *E. suaveolens* Leaves on the Repellency of *Ae. aegypti* Mosquitoes

The results of the repellency test of the spray formulation containing *E. suaveolens* leaf extract against *Ae. aegypti* mosquitoes showed that the number of mosquitoes landing on the arm treated with the control formulation was higher compared to those treated with concentrations of 5%, 10%, and 15%. These data are presented in Figure 4.

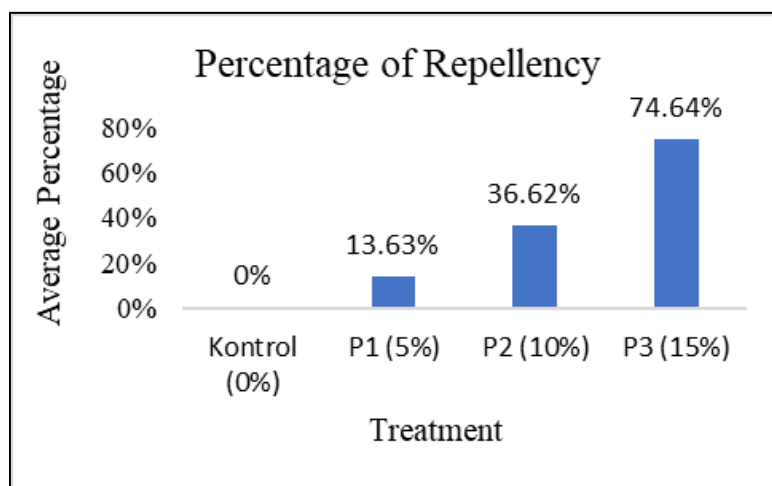


Figure 4 Percentage of Repellency Against *Ae. aegypti* Mosquitoes

The results showed that increasing the concentration of the spray formulation containing ethanol extract of *E. suaveolens* leaves led to an increase in the percentage of repellency against *Ae. aegypti* mosquitoes. The repellency percentage of the control formulation (0% concentration) was 0%, which increased to 13.63% at 5% concentration, 36.62% at 10% concentration, and reached the highest value of 74.64% at 15% concentration.

Furthermore, a Least Significant Difference (LSD) post hoc test was conducted to determine specific differences between treatments. Significant differences indicate that the spray formulations at these concentrations had a measurable effect on the repellency of *Ae. aegypti* mosquitoes.

Table 2 Results of LSD Test on the Percentage of Repellency of Spray Containing Ethanol Extract of *E. suaveolens* Leaves against *Ae. aegypti* Mosquitoes

Concentration	Average $\pm$ Std. Dev
0%	00.00 $\pm$ 00.00a
5%	13.63 $\pm$ 5.55b
10%	36.62 $\pm$ 9.99c
15%	74.64 $\pm$ 9.28d

Description: Numbers followed by different letters indicate significant differences at the 5% level ($\alpha = 0.05$).

Based on the LSD test results; each concentration showed a significant difference from one another. Higher concentrations of zodia leaf extract in the spray formulation resulted in increased repellency against mosquitoes. This is related to the presence of phytochemical compounds in the extract, such as alkaloids, flavonoids, terpenoids, tannins, and phenols, which are known to have natural repellent activity. Increasing the extract concentration leads to a higher number of phytochemical compounds distributed on the skin surface. This condition produces a stronger aroma, which can interfere with mosquito receptors and make it more difficult for them to detect the presence of a host.

3.4. Effectiveness of Spray Formulation Containing Ethanol Extract of *E. suaveolens* Leaves

The effectiveness test of the spray formulation was conducted to determine its ability to provide a repellent effect against *Ae. aegypti* mosquitoes. Therefore, probit analysis was performed to obtain the Effective Concentration 50% (EC_{50}), as presented in Table 3.

Table 3 Results of Probit Analysis of EC_{50} for the Repellency of Spray Containing Ethanol Extract of *E. suaveolens* Leaves against *Ae. aegypti* Mosquitoes

Parameter	Value (%)
EC_{50}	11,55
Interval cepacian 95%	10,75 – 12,46

Based on the probit analysis results, the spray formulation containing ethanol extract of *E. suaveolens* leaves had an EC_{50} value of 11.55%. This EC_{50} value is lower than the highest tested concentration (15%), indicating that a formulation with a concentration of 11.55% is capable of repelling 50% of the test mosquitoes before reaching the maximum concentration. This finding suggests that the ethanol extract of *E. suaveolens* leaves has strong potential as an active repellent agent.

The effectiveness of the spray formulation is associated with the activity of its bioactive compounds, which work by inhibiting the ability of mosquitoes to approach the host. In addition, the increase in spray concentration is directly proportional to the increase in mosquito repellency. Therefore, the EC_{50} value of 11.55% indicates that the ethanol extract of *E. suaveolens* leaves is effective as a natural repellent against *Ae. aegypti* mosquitoes. The highest tested concentration (15%) produced the greatest repellency effect, suggesting that this extract has strong potential to be developed as an active ingredient in natural repellent formulations.

4. Conclusion

The conclusions drawn from this study are as follows:

- The ethanol extract of zodia leaves contains secondary metabolites, including alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, and steroids, which have potential as mosquito-repellent agents.
- The spray formulation of ethanol extract of zodia leaves was non-homogeneous, turbid in appearance, had a pH of 5, and did not cause irritation.
- Differences in spray formulation concentrations significantly affected the repellency against *Ae. aegypti* mosquitoes. The repellency percentages were 5% (13,63%); 10% (36,62%); and 15% (74,64%).
- The spray formulation of ethanol extract of zodia leaves was effective as a mosquito repellent against *Ae. aegypti*, with the highest repellency observed at 15% concentration (74.64%).

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared no conflict of interests.

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