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# Ovicidal and Larvicidal Activity of the *Impatiens rothii* and *Salvia officinalis* extracts against *Anopheles stephensi* (Culicidae: Diptera) in Laboratory conditions

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## Abstract

### Background

Malaria vector control with synthetic insecticides can cause resistance, environmental toxicity, and harm to non-target animal species. To address these issues, it is critical to investigate safe and environmentally friendly botanical extraction methods for mosquito control. This study investigates the effects of crude root solvent extracts from *Impatiens rothii* and *Salvia officinalis* on *Anopheles stephensi* eggs and larvae under controlled environments.

### Methods

Fresh roots of *Salvia officinalis* and *Impatiens rothii* were collected separately, allowed to air dry, ground into a powder, and then sieved. The test plant powders were soaked in ethanol and chloroform solvent, and the extracted product was concentrated, forming a desired concentration solution for testing. Aquatain (AMF) was used as the standard control, while 20 Tween was used as the negative control. Larval mortality was measured after a 24-hour recovery period in each treatment group, and the hatchability of eggs was monitored after 48 hours. Statistical analyses were conducted using R Studio to check normality and identify significant differences between groups, then followed by SPSS, ANOVA to compare extract to standard group and probit regressions for $LC_{50}$ and $LC_{90}$ calculations.

**Results**

There were significant differences ($P < 0.05$) in ovicidal and larvicidal activities between the treatment, negative, and standard control groups. Both the chloroform extract of *S. officinalis* ($LC_{50}$ and $LC_{90}$ values of 83.8 and 305.4 ppm, respectively) and the ethanol extract of *I. rothii* ($LC_{50}$ and $LC_{90}$ values of 64.7 and 214.28 ppm, respectively) demonstrated low $LC_{50}$ and $LC_{90}$ values when tested against eggs. Additionally, the larvae treated with the ethanol extracts of *I. rothii* and *S. officinalis* presented the lowest larval mortality values, with $LC_{50}$ and $LC_{90}$ values of 124.6 ppm and 350.0 ppm, respectively. Aquatain AMF reached 100%, whereas 20 Tween did not result in egg or larval mortality.

**Conclusion**

The study suggests that root extracts of *I. rothii* and *S. officinalis* can be a safe and effective alternative to synthetic mosquitocides for controlling *Anopheles stephensi*, suggesting early-stage mosquito control is more efficient than adult control. Further research is required to understand the essential ingredients, their mechanisms of action, efficacy, and safety in larger-scale applications.

**Keywords**: *Anopheles stephensi*, control, extract larval, Mortality & standard

## Introduction

Malaria is a mosquito-borne disease that poses significant economic and health challenges worldwide, particularly in sub-Saharan Africa, where young children and pregnant women with compromised immune systems are at the highest risk(1). According to a report by the WHO(2), in 2023, malaria cases reached 263 million globally, with an incidence rate of 60.4 cases per 1,000 people at risk, an 11 million case increase from the previous year. The WHO African Region, responsible for 94% of cases, bears the most burden of the diseases(3).

*Anopheles stephensi* is the primary urban malaria vector in South Asia, extending from the Arabian Peninsula to Thailand (4,5). The species was found in western Saudi Arabia (2004), Sri Lanka (2016), and Yemen (2021)(6-8), associated with *Plasmodium vivax* and *Plasmodium falciparum*, two common malaria parasites(9, 10). The global *Anopheles stephensi* invasion is expected to heighten malaria risk for 126 million Africans, necessitating urgent mosquito control measures (11-13).

*An. stephensi*, first detected in Djibouti in 2012(14-16), has since been reported in Ethiopia and Sudan (2016), Somalia (2019), Nigeria (2020), Eritrea, Ghana and Kenya (2022)(4, 17), with further documentation in recent years. *Anopheles stephensi*, initially identified in 2016 in the Somalia region of Ethiopia, has not been reported to spread eastward from its original region (16, 18, 19). The species has been expanded to the Amhara, Oromia, Northeastern, and Southern regions of Ethiopia (5, 18, 20, 21). Awash Sebat Kilo in Ethiopia is home to this prevalent species, which spreads malaria in the area due to the susceptibility to local strains of *P. vivax* and *P. falciparum*(22). The expansion of *An. stephensi* poses a significant threat to ongoing malaria control and eradication efforts in Ethiopia, which focus on both primary and secondary vectors(22).

The World Health Organization suggests that African malaria control programmes should improve their surveillance methods to combat *An. stephensi* invasion(13, 23). Furthermore, malaria may spread more extensively due to the incursion of this invasive vector into Ethiopia and other nations within the Horn of Africa. Laboratory experiments have demonstrated that *An. stephensi* collected in Ethiopia is more effective at transmitting *P. vivax* than *An. arabiensis* (22). The World Health

Organization (WHO) recommends insecticide-treated nets (ITNs) and indoor residual spraying (IRS) for malaria vector control in most malaria risk areas(24, 25). The use of synthetic insecticides to control malaria vectors results in vector resistance, negative environmental effects, and effects on both humans and non-target organisms. The resistance of the malaria vector *An. stephensi* to the four chemical classes approved for both IRS and ITN is a significant issue in the eradication of malaria Synthetic insecticides that are used to control malaria vectors lead to vector resistance and negative effects on the environments, humans and non-target organisms.

The resistance of the malaria vector *An. stephensi* to the four chemical classes approved for both IRS and ITN poses a significant challenge in malaria eradication (26). *An. stephensi* is known to have enhanced resistance to various chemical insecticides, including pyrethroids, deltamethrin, alpha-cypermethrin, permethrin, and pirimiphos-methyl(27). Chemical insecticides are used to control mosquito populations, but they have environmental and non-target effects, and resistance poses a global health threat (28). The lack of novel insecticides and botanical extracts contributes to insecticide resistance (26, 29). Therefore, the search for eco-friendly, biodegradable, easily accessible, affordable, and target-specific botanical compounds to repel or kill mosquitoes is crucial for a viable alternative to chemical insecticides(30, 31).Botanical-based ovicidal and larvicidal activities are effective in targeting immature mosquitoes, preventing egg hatching, disrupting larval development, and reducing adult mosquito populations(32).

Traditionally botanical extracts have been used as insecticides since ancient times due to their complex active compounds, which have less resistance and more bioactivity, making it an environmentally friendly method for preventing mosquito-borne diseases (33, 34). However, few botanicals have successfully transitioned from laboratory to field studies, highlighting the significance of botanical bioactive compounds as alternatives(35). The Global Malaria Program aims to eliminate malaria by 2030 by controlling mosquito populations(2). The screening of indigenous medicinal plants for mosquito control techniques could enhance public health, reduce dependency on imports, and generate employment locally(36, 37).

Plant extracts have natural insecticidal properties and can be used as mosquitocides(38). The effectiveness of plant extracts in mosquito control relies on different factors such as plant species, parts, solvents, concentrations, bioassays, exposure times, seasons, extraction techniques, geographical location, and climate(12, 39). *Salvia officinalis*(40), and *Impatient rothii* are medicinal plants in Ethiopia that help prevent vector-borne diseases. Furthermore, these medicinal plants are inexpensive and widely available throughout the country, making them potential long-term solutions for mosquito control.

Salvia officinalis has larvicidal activity against *Ae. aegypti*, *Ae. quadrimaculatus* and *Ae. albopictus* (41-43), as well as oviposition and repellency in *Ae. albopictus*. The medicinal properties of impatiens include larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*(44), secondary metabolites in *I. rothii* roots exhibit insecticidal activity against mosquito species(45). *Salvia officinalis* has antibacterial, antiviral, antioxidant, antimalarial, anti-inflammatory, and anticancer properties (46, 47). However, there is no research on the ovicidal and larvicidal properties of the root extracts of those plants against the invasive *An. stephensi* in Ethiopia. Therefore, the current study focused on assessing the effects of ethanol and chloroform extracts from *S. officinalis* and *I. rothii* roots on invasive *An. stephensi* eggs and larvae under laboratory conditions.

## Methods

### Description of sampling areas

The test plant *Salvia officinalis* was collected from the Dilla University Botanical Garden and Ecotourism Center, with a geographical location of 6° 43′ 79.20″ N and 38° 27′ 62.90″ and located at 1423 meter above. *Impatiens rothii* was collected from a highland area in the southern region of Ethiopia, Haro Beriti village, Gedeb town, with a geographical location of 5° 91' 99.13" N and 38° 27' 77.80" E and located at over 2157 meter above sea level. A susceptible colony of *An. stephensi* were taken from the Insectary Laboratory, Biology Department, Dilla University, and reared at the Insectary Laboratory. But, originally, larvae of this species were collected from Hawassa Town, Southern Ethiopia (5), reared at laboratory using

standard mosquito rearing protocols(48) at 23–27°C Temperature, 60%–80% Relative Humidity (RH), and a Photoperiod of 12 hours light and 12 hours dark.

## Plant identification and processing

The collected test plants were identified by plant taxonomist. The collected root part of the test plant extracted at Dilla University's chemistry laboratory, Department of Chemistry. *S. officinalis* and *I. rothii* roots were first cleaned with clean water, rinsed with distilled water, chopped into pieces and shaded at room temperature to dry, ground separately into powder manually via a mortar and pestle, and then finally passed through a grinder machine and then sieved through a mesh (size 0.5 mm) to obtain a fine powder. The fine powder obtained from the roots of *I. rothii* and *S. officinalis* was then stored in glass bottle containers to prevent moisture absorption and degradation. The containers were labeled with the plant's name.

## Extraction

Two hundred grams of *Salvia officinalis* or *Impatiens rothii* root powder was macerated separately with 80% ethanol or chloroform at a ratio of 1:5 (weight per volume) in Erlenmeyer flasks and then placed on an orbital shaker (Heidolph Unimax 2010, Berlin, Germany) at a speed of 145 rpm at room temperature for 48 hours. *S. officinalis* and *I. rothii* root extracts were filtered through Whatman filter paper No. 1 (size 110 mm) and a Buchner funnel. The solvents from the extracts were removed, and the crude extracts were collected and concentrated in a water bath at 40°C. The collected crude material was subsequently weighed and preserved in a beaker container capped with an aluminum coil and refrigerated at 4°C until it was needed for an experiment (49).

## Test mosquitoes

Originally, larvae of *Anopheles stephensi* were collected from Hawassa Town, southern Ethiopia(5). Larvae of this species were subsequently reared at the Dilla University Insectary Laboratory. These experiments were conducted via the fifth generation of *An. stephensi* eggs and larvae were reared in a laboratory under the following standard insectary laboratory conditions: 23°C_27°C, 60% _80% relative humidity, 12 hr. of darkness, and 12 hr. of photo. The female *An. Stephensi* was fed

rabbit blood three times a week to facilitate oviposition. An egg cup was placed inside the cage to aid oviposition. The eggs were then transferred to a standard larval-rearing tray. The larvae were fed yeast and tetra cichlid flakes (2.82 oz.) two to three times a day. After pupating, the pupae were moved into wire mesh nylon cages 30 × 30 × 30 cm in size. Adult mosquitoes were placed in cages and given 10% sugar in the form of cotton socks (48).

## Ovicidal and larvicidal Bioassay

One gram of each crude ethanol and chloroform root extract was dissolved in 100 ml of 3% Tween 20. Then, 900 ml of distilled water was added to make a 1000 ppm stock solution. The stock solution was subsequently serially diluted to prepare 200 ml of 50, 100, 200, 300, and 400 ppm solutions for ovicidal and larvicidal activity. Two hundred milliliters of 3% tween 20 was used as the negative control, (48)and 0.5ml/0.5$m^2$ of Aquatain AMF was used as the positive control, as recommended, 1ml/$m^2$ (78% polydimethyl siloxane, Aquatain Products Pty, Ltd., Australia)(50).

### Ovicidal bioassay

The ovicidal bioassay protocol, which was modified from (51), involves exposing insect eggs to a compound solution and calculating the percentage of hatching inhibition. Twenty-five *An. stephensi* eggs were collected, counted with a magnifying glass, and then placed in a 250 ml glass beaker with 200 ml of plant extracts at concentrations of 50, 100, 200, 300, and 400 ppm. As a negative control, eggs were treated with a 20-Tween solution mixed with distilled water. Each bioassay procedure was repeated three times, with all five concentrations tested. After 48 hours, ovicidal activity was assessed via a magnifying glass to determine whether the eggs in the treated, positive, and negative controls had hatched. The mortality percentage is then determined via the following formula(48).

$$\% \text{ Mortality} = \frac{\textbf{Number of unhatched larvae}}{\textbf{Total no.of exposed eggs}} \times 100$$

### Larvicidal bioassay

The larvicidal effect of each root crude extract was tested for 24 hours, following WHO guidelines, with three replicates. Batches of 25 *An. stephensi* larvae were pipetted into 250 ml glass beakers that held 200 ml of extracted plants at 50, 100, 200, 300, and 400 ppm. Following WHO guidelines, the water depth in the beaker was maintained between 5 and 10 cm to avoid excessive mortality from deeper levels (48). The test was carried out via a 12-hour photo session that alternated between light and dark, with temperatures between 23°C and 27°C and relative humidity levels between 60% and 80%. Larval deaths were observed following a 24-hour exposure period to each test solution. Moribund larvae, which would not surface within an acceptable time if disturbed, were counted and added to dead larvae. The mortality of the control larvae was corrected via the Abbott formula(52).

$$\% \text{ mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

The mortality rate of the control group was 5–20%. Nevertheless, following 24 hours of exposure, no dead larvae were observed in the controls.

## Statistical analysis

Quantitative statistical methods were used to analyses and interpret the numerical data. RStudio software versions 4.1.1 were used to check the normality of the mortality data and dose-dependent mortality. The percentage mortality of larvae and adult were checked for normality by the 1-Sample Kolmogorov-Smirnov Z test (K-S). When the percent mortality did not conform to the normal distribution, the non-parametric equivalent tests of Kruskal-Wallis were followed. P-values were adjusted with the Bonferroni correction. ANOVA were used to calculate the means, and standard error comparisons of the means were also performed. A probit regression model was used to determine the $LC_{50}$ and $LC_{90}$ values to evaluate and compare the ovicidal and larvicidal potencies of each plant against *An. stephensi.* The concentration at which 50% of the test larvae died was the $LC_{50}$, and the concentration at which 90% of the test larvae died was the $LC_{90}$. With SPSS version 20 software (SPSS Inc., Chicago, IL, USA), probit regression is used to calculate chi-square values, upper and lower confidence limits (UCL and LCL), and other statistics at 95% confidence limits.

# Results

## Ovicidal activity of the extracts on *An. stephensi*

Table 1 shows the effects of chloroform and ethanol crude root extracts on egg hatchability, which were compared with a control group. The standard positive control (Aquatain) achieved 100% egg mortality, while both ethanol and chloroform extracts significantly reduced egg hatchability. At 50 ppm, the lowest concentrations of the ovicidal activities of the crude root ethanol and chloroform extracts of *I. rothii* were 38.66±1.2% and 16±1.52%, respectively. At 400 ppm, the effective ovicidal activity of the chloroform and ethanol extracts resulted in 100% mortality.

The mean mortality rate of the crude ethanol extract of *I. rothii* was significantly different from that of the negative control at all concentrations tested (50, 100, 200, 300, and 400 ppm) ($p < 0.05$), but at 400 ppm, there was no significant difference from the positive control. Compared with the negative control, the chloroform extract significantly differed at concentrations of 100, 200, 300, and 400 ppm but did not significantly differ at 50 ppm ($p < 0.05$).

Table 1.Mean eggs mortality rate of *I. rothii* crude root solvents extracts at vary concentrations rates on eggs of *An. stephensi* after the exposure of 48 hours.

| | **Mean % mortality ± SE** | |
|---|---|---|
| | **Extracts** | |
| **Concentrations (ppm)** | **Ethanol** | **Chloroform** |
| Negative control | $0.00\pm0.00^{Aa}$ | $0.00\pm0.00^{Aa}$ |
| 50 | $38.66\pm1.2^{Bb}$ | $16\pm1.52^{Aa}$ |
| 100 | $73.33\pm1.85^{Cc}$ | $42.66\pm1.76^{Bb}$ |
| 200 | $80\pm1.15^{Cd}$ | $74.66\pm0.88^{Cc}$ |
| 300 | $96\pm0.57^{De}$ | $97.33\pm0.66^{Dd}$ |
| 400 | $100\pm0.00^{Ee}$ | $100\pm0.00^{Dd}$ |
| Positive control | $100\pm0.00^{Ee}$ | $100\pm0.00^{Dd}$ |

*Mean mortality ± SE represents the value of three replicates.
*Means values followed by the same letters across column (uppercase) and row (lowercase) are not statistically significantly different ($p < 0.05$).

Table 2 shows the ovicidal activity of *S. officinalis* ethanol and chloroform extracts against *An. stephensi* mosquitoes across varying concentrations. At 400 ppm, the ethanol extract exhibited a mortality rate of 97.33 ± 0.66%, while the chloroform extract achieved 100 ± 0.00% mortality. At the lower concentration of 50 ppm, the ethanol and chloroform extracts recorded mortality rates of 34.66 ± 1.20% and 25.33 ± 1.76%, respectively. The results demonstrated that the mean mortality rates of the crude root ethanol and chloroform extracts at 50, 100, 200, 300, and 400 ppm were significantly higher than those of the negative control ($P < 0.05$). However, for the ethanol extract at concentrations of 200, 300, and 400 ppm, no statistically significant difference was observed compared to the positive control. Likewise, the chloroform extract at 300 and 400 ppm showed no significant difference from the positive control ($P < 0.05$).

| | **Mean % Mortality ± SE** | |
|---|---|---|
| | **Extracts** | |
| **Concentrations (ppm)** | **Ethanol** | **Chloroform** |
| Negative control | $0.00 \pm 0.00^{Aa}$ | $0.00 \pm 0.00^{Aa}$ |
| 50 | $25.33 \pm 1.76^{Bb}$ | $34.66 \pm 1.20^{Bb}$ |
| 100 | $54.33 \pm 1.76^{Cc}$ | $53.33 \pm 0.88^{Cc}$ |
| 200 | $84 \pm 0.57^{Dd}$ | $74.66 \pm 0.88^{Dd}$ |
| 300 | $90.66 \pm 0.66^{De}$ | $90.66 \pm 0.88^{Ee}$ |
| 400 | $100 \pm 0.00^{Df}$ | $97.33 \pm 0.66^{Ef}$ |
| Positive control | $100 \pm 0.00^{Df}$ | $100 \pm 0.00^{Ef}$ |

*The value of three replicates is represented by the mean mortality ± SE represents.
*Means values followed by the same letters across column (uppercase) and row (lowercase) are not statistically significantly different ($p < 0.05$).

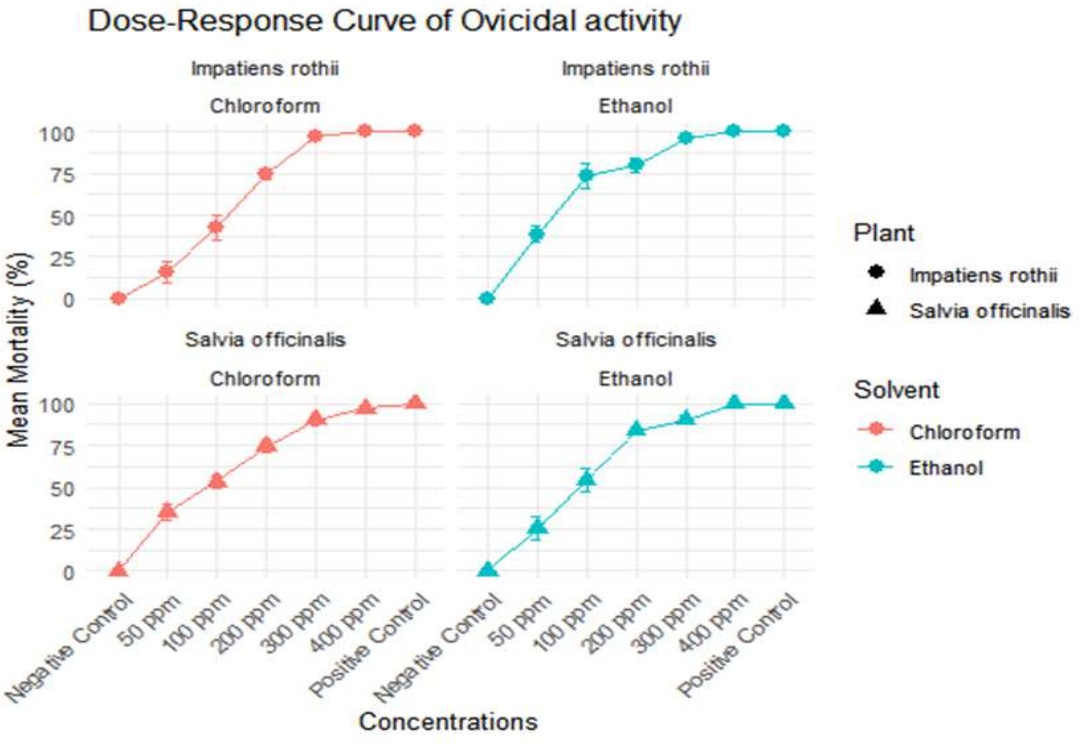
Table 3 displays the values of the $LC_{50}$ and $LC_{90}$ of the *I. rothii* and *S. officinalis* crude root extracts against *An. stephensi* eggs. The $LC_{50}$ values of the crude root ethanol and chloroform extracts of *I. rothii* against *An. stephensi* eggs were 64.7 and 108.06, respectively, and the $LC_{90}$ values were 214.28 and 254.00 ppm, respectively. Similarly, the $LC_{50}$ and $LC_{90}$ values for the *S. officinalis* ethanol and chloroform crude root extracts were 88.50 and 83.86, respectively, and 246.44 and 305.44, respectively.

Table 3. $LC_{50}$ and $LC_{90}$ (ppm) of crude root ethanol and chloroform extracts of tests plants against *An. stephensi* egg.

| **95 % CI** | | | | |
|---|---|---|---|---|
| Plants | Solvents | **$LC_{50}$ (LCI_ UCI)** | **$LC_{90}$ (LCI_UCI)** | **$\chi 2$ df=3** |
| ***Impatiens rothii*** | Ethanol | 64.7 (22 _99.2) | 214.28 (138_662) | 4.71 |
| | Chloroform | 108.06(95_120) | 254.0 (221_304.6) | 4.55 |
| ***Salvia officinalis*** | Ethanol | 88.5 (75.7_101) | 246.44 (209_303) | 3.6 |
| | Chloroform | 83.86 (68_98.6) | 305.44 (249_402) | 4.3 |

Figure 1 below indicates that all crude extracts of *Impatiens rothii* and *Salvia officinalis* showed dose-dependent ovicidal effects against *Anopheles stephensi* eggs, with significant differences based on solvent type. Chloroform extracts produced steep response curves and induced rapid egg mortality, achieving 100% lethality at concentrations above 300 ppm. The chloroform extract of *I. rothii* was particularly potent, causing high mortality at lower doses compared to its ethanol counterpart. Ethanol-based extracts also demonstrated ovicidal activity, though with a more gradual increase in mortality, peaking at 80 to 90% at 400 ppm.

Figure1. Dose-response curve of ovicidal activities against *Anopheles stephensi*.



## Larvicidal effect of plant extracts against *An. stephensi*

Table 4 presents the larvicidal efficacy of *I. rothii* crude root ethanol and chloroform extracts against third instar *An. stephensi* larvae following a 24-hour exposure. Both extracts demonstrated larvicidal activity across the tested concentrations. At 400 ppm, the ethanol extract exhibited a mean mortality of $92 \pm 1.15\%$, while the chloroform extract yielded $85.33 \pm 0.88\%$. The lowest mortality rates were observed at 50 ppm, with the ethanol and chloroform extracts recording $9.33 \pm 2.33\%$ and $5.33 \pm 0.88\%$, respectively. No larval mortality occurred in the negative control groups. At 50 ppm, the ethanol extract's mean mortality was not significantly different from that of the negative control ($P < 0.05$). In contrast, the chloroform extract demonstrated statistically significant differences from both positive and negative controls at all tested concentrations (50, 100, 200, 300, and 400 ppm) ($P < 0.05$).

Table 4.Mean larvae mortality rate of *I. rothii* root crude solvents extracts at vary concentration rates on third instar larvae of *An. stephensi* after exposure of 24hr.

| | **Mean % mortality±SE** | |
|---|---|---|
| | **Extracts** | |
| **Concentrations (ppm)** | **Ethanol** | **Chloroform** |
| Negative control | $0.00 \pm 0.00^{Aa}$ | $0.00 \pm 0.00^{Aa}$ |
| 50 | $9.33 \pm 2.33^{Aa}$ | $5.33 \pm 0.88^{Aa}$ |
| 100 | $45.33 \pm 0.88^{Bb}$ | $20 \pm 1.15^{Ba}$ |
| 200 | $73.33 \pm 1.20^{Cc}$ | $45.33 \pm 1.20^{Cb}$ |
| 300 | $84 \pm 1.52^{Cc}$ | $72 \pm 1.15^{Dc}$ |
| 400 | $92 \pm 1.15^{Cd}$ | $85.33 \pm 0.88^{Dd}$ |
| Positive control | $100 \pm 0.00^{Dd}$ | $100 \pm 0.00^{Ee}$ |

*The value of three replicates is represented by the mean mortality ± SE represents.
*Means values followed by the same letters across column (uppercase) and row (lowercase) are not statistically significantly different ($p < 0.05$).

Table 5 presents the larvicidal activity of *S. officinalis* crude root ethanol and chloroform extracts against third instar *An. stephensi* larvae across a range of concentrations. At 50 ppm, the ethanol and chloroform extracts induced mean mortalities of $5.33 \pm 1.33\%$ and $4 \pm 0.57\%$, respectively. Mortality rates increased markedly at 400 ppm, reaching $85.33 \pm 0.88\%$ and $80 \pm 0.57\%$ for ethanol and

chloroform extracts, respectively. The results indicated that *S. officinalis* extracts exerted significant larvicidal effects compared to the negative control, although no statistically significant difference was observed at the lowest concentration of 50 ppm. Notably, the chloroform extract exhibited statistically significant differences from the positive control at all tested concentrations ($P < 0.05$).

Table5. Mean of larval mortality rate of *S. officinalis* root crude solvents extracts at varies concentration rates on third instar larvae of *An. stephensi* after exposure of 24 hr.

| | **Mean % mortality±SE** | |
|---|---|---|
| | **Extracts** | |
| **Concentrations (ppm)** | **Ethanol** | **Chloroform** |
| Negative control | $0.00\pm0.00^{Aa}$ | $0.00\pm0.00^{Aa}$ |
| 50 | $5.33\pm1.33^{Aa}$ | $4\pm0.57^{Aa}$ |
| 100 | $32\pm0.57^{Bb}$ | $20\pm1.15^{Ba}$ |
| 200 | $46.66\pm1.45^{Bb}$ | $45.33\pm0.88^{Cb}$ |
| 300 | $68\pm0.57^{Cc}$ | $61.33\pm0.88^{Dc}$ |
| 400 | $85.33\pm0.88^{Dd}$ | $80\pm0.57^{Ed}$ |
| Positive control | $100\pm0.00^{De}$ | $100\pm0.00^{Fe}$ |

*The value of three replicates is represented by the mean mortality ± SE represents.
*Means values followed by the same letters across column (uppercase) and row (lowercase) are not statistically significantly different ($p < 0.05$).

The study found that all crude extracts exhibited concentration-dependent larvicidal effects against *An. stephensi* larvae, as shown in Figure 2. Mortality rates continually increased with dose, reaching near-complete mortality at 400 ppm across all treatments. Chloroform extracts of both *I. rothii* and *S. officinalis* showed steep dose-response curves, indicating rapid action and high potency. Ethanol extracts also achieved 100% mortality at the highest concentration, though their curves were more gradual, suggesting a slower but effective response.

Figure 2. Dose-response curve of ovicidal activities against *Anopheles stephensi.*

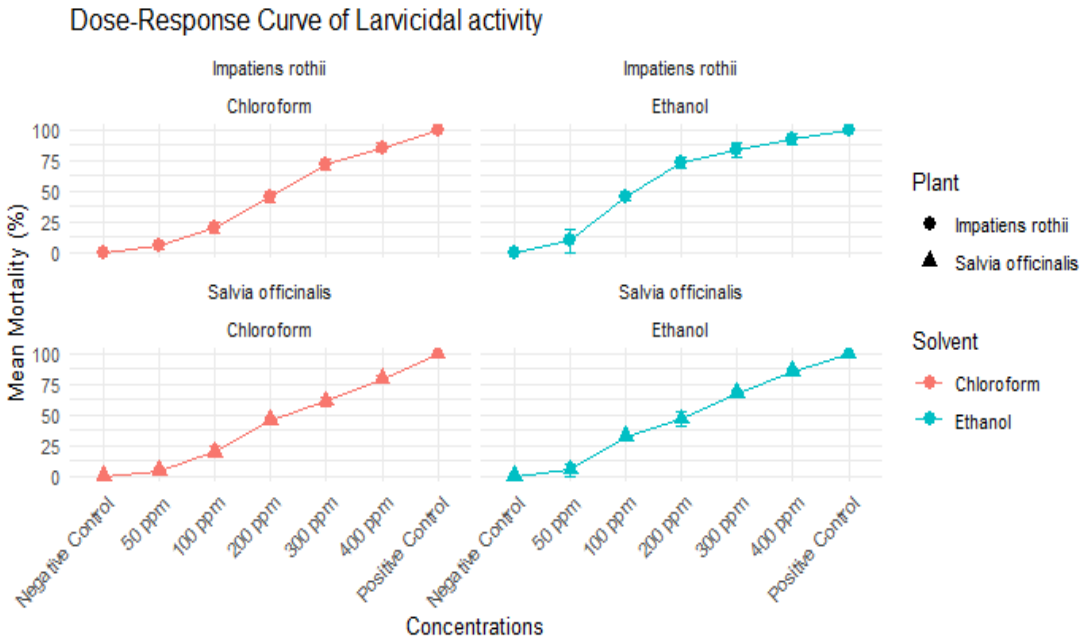



Table 6 reports the $LC_{50}$ and $LC_{90}$ values of *I. rothii* and *S. officinalis* crude root ethanol and chloroform extracts against third instar *An. stephensi* larvae. For *S. officinalis*, the ethanol extract exhibited $LC_{50}$ and $LC_{90}$ values of 64.27 ppm and 88.83 ppm, respectively, while the chloroform extract presented values of 215.46 ppm and 638.85 ppm. For *I. rothii*, the ethanol extract showed $LC_{50}$ and $LC_{90}$ values of 124.63 ppm and 350.68 ppm, respectively, whereas the chloroform extract recorded values of 194.05 ppm and 526.41 ppm. These findings suggest that *S. officinalis* ethanol extract demonstrates the highest larvicidal potency. However, the overall toxicity profiles indicate that *I. rothii* may exhibit relatively higher toxic effects across both solvent treatments when considering broader concentration ranges.

Table 6. $LC_{50}$ and $LC_{90}$ of ethanol and chloroform crude root extracts of tests plants against the third instar larvae of *An. stephensi*.

| **95 % CI** | | | | |
|---|---|---|---|---|
| **Plants** | **Solvents** | **$LC_{50}$ (LCI_ UCI)** | **$LC_{90}$ (LCI_UCI)** | **$\chi 2$ df=3** |
| ***Impatiens*** | Ethanol | 124.63 (108 _140) | 350.67 (296_436.48) | 2.40 |
| ***rothii*** | Chloroform | 194.05 (172 _218) | 526.41 (436_680.4) | 1.94 |
| ***Salvia*** | Ethanol | 64.27 (56 _74.17) | 88.83 (76.4 _117.4) | 2.40 |
| ***officinalis*** | Chloroform | 215.46 (190_245) | 638.85 (511.7 _875 | 1.14 |

## Discussion

Botanical extracts are an alternative to synthetic insecticides; they are cost-effective, environmentally friendly, and readily available. These extracts address issues related to vector ecology, synthetic chemical resistance and minimal harm to non-targeted animals and environment(53), while decreasing vector populations, has been gaining popularity(12). *An. stephensi* mosquito are highly susceptible to disease transmission in urban areas due to their adaptability and thriving in human-made environments like stagnant water pools and waste areas (54).

The study examined the ovicidal and larvicidal effects of *S. officinalis* and *I. rothii* root ethanol and chloroform extracts on *An. stephensi*. However, the impact of *Salvia officinalis* and *Impatiens rothii* on *An. stephensi* eggs and larvae has not been previously studied. The study found that *I. rothii* had higher $LC_{50}$ and $LC_{90}$ values with chloroform extract than ethanol extract, while *S. officinalis* had higher $LC_{50}$ and $LC_{90}$ values with ethanol extract against *An. stephensi* eggs. The study indicates that these plant extracts have the potential to act as effective ovicidal and larvicidal agents against the malaria vector *An. stephensi*.

The mean mortality rates of *I. rothii* against *An. stephensi* eggs were significantly different at concentrations of 50, 100, 200, 300, and 400 ppm from the negative control, but no significant difference were observed at 400 ppm from the positive control. Moreover, the mortality rates of *I. rothii* chloroform extract significantly differed from the negative control against *An. stephensi* eggs at concentrations of 100, 200, 300, and 400 ppm. Furthermore, the mortality rates of *I. rothii* chloroform extract against *An. stephensi* eggs significantly varied among the 50, 100, and 200 ppm groups compared to the positive control. The mean mortality rate of *S. officinalis* ethanol and methanol extract against *An. stephensi* eggs were significantly different ($P < 0.05$) at concentrations of 50, 100, 200, 300, and 400 ppm compared to the negative control. However, at concentrations of 300 and 400 ppm, no significant difference was observed from the positive control. *I. rothii* showed $LC_{50}$ values of 64.7 ppm (ethanol) and 108.06 ppm (chloroform), with corresponding $LC_{90}$ values of 214.28 ppm (ethanol) and 254.0 ppm (chloroform), while, for *S. officinalis,* the $LC_{50}$ values were 88.5 ppm (ethanol) and 83.86 ppm (chloroform), with $LC_{90}$ values of 246.44 ppm (ethanol) and 305 ppm (chloroform).

The study found that the ethanol and chloroform extracts of *I. rothii* root exhibited high ovicidal activity, with $LC_{50}$ values of 64.7 and 108.06 ppm, respectively. Similarly, the ethanol and chloroform crude root extracts of *S. officinalis* from *An. stephensi* eggs showed $LC_{50}$ values of 88.5 and 83.81 ppm, respectively. The results indicated that the root extracts of both plants have potential for use as natural ovicidal agents against *An. stephensi* mosquito eggs,(55) suggesting that the ethanol extract of the *G. sepium* (Jacq.) had 100% ovicidal and 96.0±2.4% larvicidal effects on *An. stephensi*, with $LC_{50}$ and $LC_{90}$ values of 121.79 and 231.98 ppm, respectively. (56) reported the ovicidal, larvicidal and adulticidal activities *of E. indica* root extracts against *An. stephensi*. The methanol extract was the most effective against larvae, with $LC_{50}$ and $LC_{90}$ values of 90.97 and 168.82 ppm, respectively.

In a study conducted by (57), showed *A. indica* leaf extracts (benzene, chloroform, ethyl acetate, and methanol) exhibited ovicidal and larvicidal activity against *An. stephensi,* with $LC_{50}$ values of 19.25, 27.76, 23.26, and 15.03 ppm, respectively. Similarly, (58) investigated the larvicidal and ovicidal properties of the benzene extract of *C. vulgaris* against *An. stephensi,* with an $LC_{50}$ value of 18.56 ppm. The ovicidal and larvicidal properties of *P. dulce* against *An. stephensi* were examined by (59), where the methanol extract was found to have the highest larval mortality, with $LC_{50}$ and $LC_{90}$ values of 145.43 and 251.23 ppm, respectively. The crude extract of the test plants at a low concentration (50 ppm) inhibited *An. stephensi* eggs. According to this study, *I. rothii* ethanol and chloroform extracts have a more effective insecticidal effect on eggs than *S. officinalis*.

The present study, similar to(60), demonstrated that *S. verticillata* extracts exhibited the highest efficacy in terms of oviposition and repellency against *Ae. albopictus,* with success rates of -0.9942 and 99.7%, respectively. Similarly, the study is in accordance with those of (43), who studied plant species and solvent extracts and reported that the essential oil of *S. officinalis* decreased the percentage of hatched eggs with increasing EO concentration from 74.7% to 1 mg. L-1 to 0% for 50 mg L-1 against *Ae. aegypti* and the highest larvicidal activity at 63 and 76 mg L-1, with 27 ± 13.4 and 37 ± 18.6%, respectively. (39) suggested that the polarity of the extraction solvent may impact mosquito vector mortality and disease transmission. Additionally, the ovicidal

activity of the study may have been affected by the solvent extraction and extraction technique.

The mean mortality rate of crude root ethanol from *I. rothii* on *An. stephensi* larvae were 9.33% at a concentration of 50 ppm, which was not significantly different from the negative control ($p < 0.05$). However, the mean mortality rate of the ethanol crude root extract from *I. rothii* at concentrations of 50, 100, and 200 ppm was significantly different from that of the positive control ($p < 0.05$). The mean mortality rates of crude root chloroform from *I. rothii* on *An. stephensi* larvae significantly differed from the positive and negative controls at concentrations of 50, 100, 200, 300, and 400 ppm. The mean mortality rates of *S. officinalis* crude root ethanol extract significantly increased mortality in *An. stephensi* larvae at concentrations of 100, 200, 300, and 400 ppm compared to the control ($p < 0.05$).

At the values of 50, 100, 200 and 300 ppm, concentrations were significantly different from the positive control. Similarly, the mean mortality rates of the crude root chloroform and ethanol extract of *S. officinalis* at 100, 200, 300 and 400 ppm were significantly different from the negative and positive controls ($p < 0.05$). The study revealed that the larvicidal activity of *I. rothii* displays higher $LC_{50}$ values with chloroform (194.05 ppm) than ethanol (124.63 ppm), whereas *S. officinalis* exhibits lower $LC_{50}$ values with ethanol (64.27 ppm) than chloroform (215.46 ppm). The study found that the crude root extracts of *I. rothii* and *S. officinalis*, containing ethanol and chloroform, show larvicidal properties.

The current study, similar to those of (61) reported that Salvia essential oils can cause 100% *An. albopictus* larval mortality. The study agrees with (62), finding that all the Salvia essential oils have larvicidal activities except for *S. apiana,* which showed no action against *An. quadrimaculatus* and *Ae. aegypti* at high concentrations of 125 ppm. Additionally, the study supports the findings of(44) the *I. balsamina l*eaf methanol extract has the highest larvicidal activity against *An. stephensi, Ae. aegypti,* and *Cx. quinquefasciatus,* with $LC_{50}$ and $LC_{90}$ values of 98.04, 119.68, 125.06, 172.93, 210.14, and 220.60 mg/L, respectively. The studies were also similar to who reported that *S. sclarea* essential oil showed high larvicidal and repellent activity against *Ae. aegypti*, *An. stephensi, and Cx. quinquefasciatus* larvae, with $LC_{50}$ and LC90 values of 66.13, 71.47, 76.06, 130.19, 142.63, and 144.28 ppm, respectively.

Similarly, the study aligns with **(**64**),** found that Salvia spps. Water and ethanol extracts have the lowest mortality rates at 26.1% and 31.2%, respectively, with LC50 and $LC_{90}$ values of 18620.6, 89198.9, 10800.00, and 32622.7 ppm, respectively, against *Cx. quinquefasciatus*. Additionally, (65) and (66) reported that the secondary metabolites in *I. rothii* root extracts have strong insecticidal effects on mosquito species. The effectiveness of the studies were better than the study by (67), who reported that ethanol *C. philippinum* extract demonstrated the highest larvicidal activity (100 ± 1.9%) and pupicidal activity (58 ± 0.8%) at 600 ppm for controlling *An. stephensi.*

However, the effectiveness of the studies were lower than (68), suggesting that the extracts of ethanol and chloroform from *L. aspera* on *An. stephensi* larvae, with $LC_{50}$ and $LC_{90}$ values of 37.076 and 65.137 ppm for the ethanol extract respectively, and 39.609 and 27.281 ppm for the chloroform extract respectively. The effectiveness of the current studies were also better than (69), which revealed that the ethanol and aqueous extracts of *P. oleracea* L. leaves has larvicidal effects on *An. stephensi,* with an $LC_{50}$ value of 386.23 ppm.

The ovicidal and larvicidal effects of *S. officinalis* and *I. rothii* ethanol and chloroform extracts against *An. stephensi* were lower than (70), who reported that the ethanol extract of *D. stramonium* had $LD_{50}$ and $LD_{90}$ values of 16.0783 ppm and 41.959 ppm, respectively, against *An. stephensi* larvae, and (71) reported that the methanol extract of *S. costus* root had a strong effect on *An. stephensi* larvae, with $LC_{50}$ and $LC_{90}$ values of 7.97 and 34.4 ppm, respectively. However, the current study is more effective than the study by (72), which revealed that the chloroform leaf extract of *P. longifolia* has a mortality rate of 99.58% at 2900 ppm, with $LC_{50}$ and $LC_{90}$ values of 1054.34 and 2849.87 ppm, respectively. However, the study of *S. officinalis* and *I. rothii* extracts exhibit larvicidal and ovicidal activities compared to some potent botanicals; their effectiveness exceeds other plant-based alternatives, highlighting their potential as eco-friendly options for integrated vector control.

### Limitations

This study was conducted under controlled laboratory conditions using crude ethanol and chloroform root extracts of *Impatiens rothii* and *Salvia officinalis*. While the results demonstrate significant ovicidal and larvicidal activity against *Anopheles stephensi*, several limitations must be acknowledged. First, the active compounds responsible for these effects were not isolated or characterized, and instrumental analyses such as GC–MS or IR were not performed. Second, the study did not evaluate the effects of these extracts on non-target organisms, which restricts conclusions about environmental safety. Finally, the findings are limited to laboratory bioassays and may not fully reflect efficacy under field conditions. These limitations highlight the need for future research to identify bioactive constituents, investigate their mechanisms of action, and assess safety and effectiveness in real-world applications.

## Conclusion

This study demonstrates that crude ethanol and chloroform root extracts of *Impatiens rothii* and *Salvia officinalis* exhibit measurable ovicidal and larvicidal activity against *Anopheles stephensi* under controlled laboratory conditions. The ethanol extract of *I. rothii* roots showed relatively higher potency compared to its chloroform counterpart, while both ethanol extracts of *I. rothii* and *S. officinalis* displayed comparable activity. These results provide preliminary evidence that locally available botanicals may serve as potential candidates for mosquito control at the egg and larval stages. However, the findings are limited to laboratory bioassays using crude extracts, without isolation or characterization of active compounds, and without assessment of non-target effects. Further research is required to identify bioactive constituents, clarify mechanisms of action, and evaluate safety and efficacy under field conditions. Within these constraints, the present study contributes baseline data that may inform future efforts to develop eco-friendly alternatives to synthetic insecticides in regions where *An. stephensi* poses a growing public health threat.

## Abbreviations

Hr.: Hour

IRS: Indoor residual spraying.

$LC_{50}$: Lethal concentration that is expected to kill 50%

$LC_{90}$: Lethal Concentration that is expected to kill 90%

LLINs: Long-lasting insecticidal nets

PPM: Parts Per Million

SPSS: Statistical Package for the Social Sciences

WHO: World Health Organization

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### Availability of data and materials

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

### Declarations

### Ethical approval and consent for participate

Rabbits used for laboratory mosquito feeding were cared for in full compliance with the International Guiding Principles for Biomedical Research Involving Animals and adhered to the Standards for the Humane Care and Use of Laboratory Animals.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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