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Investigating the Insecticidal Properties of *Alangium salviifolium* Root Extracts on *Culex quinquefasciatus* Mosquitoe

Alex Yagoo*, M.C. John Milton, Jelin Vilvest
PG & Research Department of Advanced Zoology & Biotechnology, Loyola College (Autonomous), Chennai, India.

Corresponding author*
alexyagoo@loyolacollege.edu

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Abstract

This study evaluated the effectiveness of successive hexane, chloroform, and methanol extracts of *Alangium salviifolium* roots in combating mosquito-borne diseases caused by *Culex quinquefasciatus* mosquitoe in India. The hexane extract showed the highest efficacy with LC_{50} values of 105.0 ppm (larvicidal activity), 108.0 ppm (pupicidal activity), and 65.6 ppm (ovicidal activity). The chloroform extract had LC_{50} values of 156.8 ppm (larvicidal), 376.0 ppm (pupicidal), and 20.0 ppm (ovicidal), while the methanol extract had LC_{50} values of 235.7 ppm (larvicidal), 441.8 ppm (pupicidal), and 30.4 ppm (ovicidal). The hexane extract of *A. salviifolium* roots shows potential as a mosquito control agent, specifically against *Cx. quinquefasciatus* mosquitoes. Further studies are needed to assess the safety and effectiveness of this extract. This research highlights the potential of plant extracts as alternative mosquito control agents for mitigating mosquito-borne diseases in India.

Keywords: *Alangium salviifolium* roots; *Culex quinquefasciatus*; plant extract; toxicity.

Abbreviations: *Cx.quinquefasciatus: Culex quinquefasciatus; A.salviifolium: Alangium salviifolium;* LC: lethal concentration; LL: lower limit; UL: upper limit; PPM: Parts per million; DMSO: Dimethyl sulfoxide

INTRODUCTION

Vector-borne diseases are a group of illnesses that are transmitted to humans and animals through arthropod vectors, such as mosquitoes, ticks, fleas, and sandflies. These diseases are prevalent worldwide and pose a significant threat to public health (Alvarado-Esquivel et al., 2021; Tack et al., 2021). Mosquito-borne diseases are among the most prevalent vector-borne diseases globally and can cause significant illness and death in affected populations. These diseases are transmitted through the bite of infected mosquitoes, which inject the disease-causing pathogens, such as viruses or parasites, into the human bloodstream (WHO, 2021; Bhatt et al., 2013). Globally, mosquito-borne diseases such as malaria, dengue fever, and Zika virus continue to be major public health problems, particularly in low- and middle-income countries. According to the World Health Organization (WHO), there were an estimated 229 million cases of malaria worldwide in 2019, with an estimated 409,000 deaths, most of which occurred in sub-Saharan Africa. Dengue fever is also a significant public health problem, with an estimated 100 million cases worldwide each year. The majority of dengue cases occur in Asia and the Americas. The burden of Zika virus is less well-known, but outbreaks have occurred in several countries in recent years, including Brazil, Colombia, and India (WHO, 2020; WHO, 2020). In India, mosquito-borne diseases are also a significant public health problem. Malaria, dengue fever, chikungunya, filariasis and Japanese encephalitis are the most common mosquito-borne diseases in India. According to the National Vector Borne Disease Control Programme (NVBDCP), there were over 5 million cases of malaria in India in 2019, with 229 deaths. Dengue fever is also a significant problem in India, with over 1.8 lakh cases reported in 2019, including 132 deaths. Chikungunya is less common but still occurs, with over 60,000 cases reported in 2019 (MHFW, 2020; NVBDCP, 2020). Prevention and control of mosquito-borne diseases involve several strategies, including reducing mosquito breeding sites, using insecticides, and wearing protective clothing. Vaccines are available for some diseases, such as yellow fever and Japanese encephalitis, but not for all mosquito-borne diseases. Natural insecticides can be just as effective as synthetic insecticides for controlling insect pests (Tabanca et al., 2017). Mosquitoes have been known to develop resistance to synthetic insecticides, which has led to the exploration of alternative control methods, including the use of natural products or plant-derived products. These products may have insecticidal properties that can effectively control mosquito populations, and they may also be less harmful to the environment and non-target organisms than synthetic insecticides (Das and Dhiman, 2015; Govindarajan et al., 2015). Furthermore, the use of synthetic insecticides has been associated with negative effects on the environment and non-target organisms, such as pollinators and aquatic organisms. In contrast, natural products and plant-derived products are often biodegradable and less toxic, which can reduce their impact on the environment (Isman, 2006).

Alangium salviifolium, commonly known as Sage-leaf alangium, is a plant species belonging to the family Alangiaceae. It is distributed throughout the Indian subcontinent, Southeast Asia, and parts of China. The plant is known for its traditional medicinal uses, with its bark, roots, and leaves being utilized to treat various ailments such as fever, inflammation, wounds, and snake bites. Phytochemical studies have revealed that *A. salviifolium* contains a diverse array of chemical constituents, including flavonoids, alkaloids, steroids, terpenoids, and phenolic compounds. These compounds are believed to be responsible for the plant's pharmacological activities. It has been found to possess pharmacological activities such as anti-inflammatory, antioxidant, antitumor, antimicrobial, antidiarrheal, and hepatoprotective properties. Its extracts have shown promising results in various studies, indicating its potential use in treating various diseases. The plant is also of ecological significance, as it serves as a food source for various animals, including birds and insects. The wood of *A. salviifolium* is hard and durable, and it is used for various purposes, such as construction, furniture, and tool handles (Sharma et al., 2015; Dhanasekaran et al., 2018; Saxena et al., 2013). In this communication, we present the effect of successive extracts of hexane, chloroform, and methanol of root of the *A. salvvfolium* on the larvicidal, pupicidal, and ovicidal activities of *Cx. quinquefasciatus.*

MATERIALS AND METHODS

Collection of plant materials

In February 2022, the roots of *Alangium salviifolium* were collected from Palayamkottai, Tirunelveli district of Tamil Nadu, India. The plant material was then authenticated by Dr. K. N. Sunilkumar, a Research Officer from the Department of Pharmacognosy at the Siddha Central Research Institute in Chennai. A voucher specimen with an authentication code (No: A24012302S.) was deposited in the institute's herbarium.

Extraction of the plant materials

The plant material, consisting of 1 kg of dried roots, was first ground into a coarse powder and then extracted using a Soxhlet apparatus with hexane, chloroform, and methanol in successive order. The resulting extracts were filtered and concentrated with a vacuum rotary evaporator, and the dry extracts were stored in airtight containers at a temperature of 4º C until further use. The yield obtained from the three extractions were 4.15 g, .7.22 g, and 10.48 g for hexane, chloroform, and methanol, respectively.

Insect rearing

Culex quinquefasciatus third instar larvae were procured from the Entomology Research Institute, Chennai for insect rearing. The larvae were bred in a controlled environment of 27±2°C temperature, 75-85% relative humidity, and a 13:11 L/D photoperiod using chlorine-free tap water. The larvae were provided with a diet consisting of a mixture of dog biscuits and Brewer's yeast in a ratio of 3:2 (Reegan et al., 2021).

Larvicidal and pupicidal assays

To evaluate the larvicidal and pupicidal activities of the extracts, the guidelines provided by the World Health Organization (WHO, 2005) were followed. Various concentrations of the extracts, including 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm, were tested with five replicates for each concentration. An emulsion in 1.0% aqueous DMSO was prepared for all three activities. Plastic containers with a volume of 150 ml were used for the tests, and 20 larvae or pupae were added to 100 ml of the extract solution. A negative control consisting of a 1% aqueous DMSO solution was used, while Temephos was used as a positive control. Dead larvae or pupae were identified when they showed no movement upon being touched with a glass rod. The percentage mortality and corrected percentage mortality were calculated using standard formulas (Abbot, 1925).

Percentage mortality:

$$\frac{No.of\ dead\ larvae\ or\ pupae}{No.of\ larvae\ or\ pupae\ exposed} \times 100$$

Corrected percentage mortality:

$$[1 - nT/nC] \times 100$$

The corrected percentage mortality formula is used when the mortality rate in the control group is less than 5%. In this case, the formula adjusts for the natural mortality rate in the control group. It is calculated by subtracting the mortality rate in the control group from the mortality rate in the treated group, and then dividing by the difference between 100 and the mortality rate in the control group.

Ovicidal activity

The ovicidal activity of the extracts was determined using a modified version of the method described by

Yagoo et al., 2023. Freshly laid eggs of *Cx. quinquefasciatus* were exposed to different concentrations of the extracts in five replicates. The same concentrations used for larvicidal and pupicidal activities were tested. After 120 hours post-treatment, the hatchability of the eggs was observed under a compound microscope. The percentage of ovicidal activity was calculated using the following formula:

Percentage of Ovicidal activity:

$$\frac{No. of\ unhatched\ eggs}{Total\ number\ of\ eggs\ exposed} \times 100$$

The results were compared with those of the standard control, Temephos.

Statistical analysis

In order to determine the concentration at which larvicidal, pupicidal, and ovicidal agents resulted in a certain percentage of mortality, statistical analysis was performed using probit analysis software (version 1.5) from the US EPA. This analysis allowed us to estimate the LC_{50} and LC_{90} values for each concentration. A p-value of ≤ 0.05 was considered significant, indicating that the observed differences were unlikely to have occurred by chance (Finney, 1971).

RESULTS AND DISCUSSION

In this study, we evaluated the effectiveness of hexane, chloroform, and methanol extracts of *A. salviifolium* roots against third instar larvae of *Cx. quinquefasciatus*. We conducted larvicidal and pupicidal assays and the outcomes are presented in Table 1 respectively.

Table 1. Lethal concentration (in ppm) of crude extracts of roots of *A. salviifolium* against the larvae and pupae of *Cx. Quinquefasciatus.*

Species	Extract	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ^2
			LL	UL		LL	UL			
Cx .quinquefasciatus larvae	Hexane	105.0	28.2	205.4	227.0	138.4	1289.1	3.8 ± 0.7	-2.7 ± 1.5	9.6^*
	Chloroform	156.8	36.0	499.4	391.2	209.1	7244.2	3.2 ± 0.6	-2.0 ± 1.4	1.5^*
	Methanol	235.7	88.3	2335.9	711.2	331.8	1332.7	2.6 ± 0.5	-1.3 ± 1.3	5.9^*
Cx .quinquefasciatus Pupae	Hexane	108.0	45.8	184.7	238.7	150.4	2322.1	3.7 ± 0.6	-2.5 ± 1.3	7.7^*
	Chloroform	376.0	319.5	463.6	1389.5	984.7	2335.5	2.2 ± 0.2	-0.8 ± 0.5	1.5^*
	Methanol	441.8	376.1	547.4	1407.4	1011.8	2330.4	2.5 ± 0.2	-1.7 ± 0.7	4.4^*

LC_{50}-lethal concentration that kills 50% of the exposed larvae; LC_{90}-lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). $^*p \leq 0.05$, level of significance of chi-square values.

The results of the study revealed that the hexane extract exhibited the highest larvicidal activity against both *Cx. quinquefasciatus*, with LC_{50} values of 105.0 ppm, respectively. The chloroform extract showed moderate activity, with chloroform being more active than the methanol extract, as shown in Table 1. In terms of pupicidal activity, the hexane extract was also more active compared to the chloroform and methanol extracts, with LC_{50} values of 108.0 ppm, respectively, for *Cx.quinquefasciatus* mosquito species as shown in Table 1. Notably, the hexane extract also exhibited significant ovicidal activity, with LC_{50} values of 65.6 ppm for *Cx. quinquefasciatus*, respectively. On the other hand, the ovicidal activity of the chloroform and methanol extracts was low for mosquito species, even at the highest concentration of 500 ppm. The range of ovicidal activity for the other two extracts was 16.8% to 30.4%.

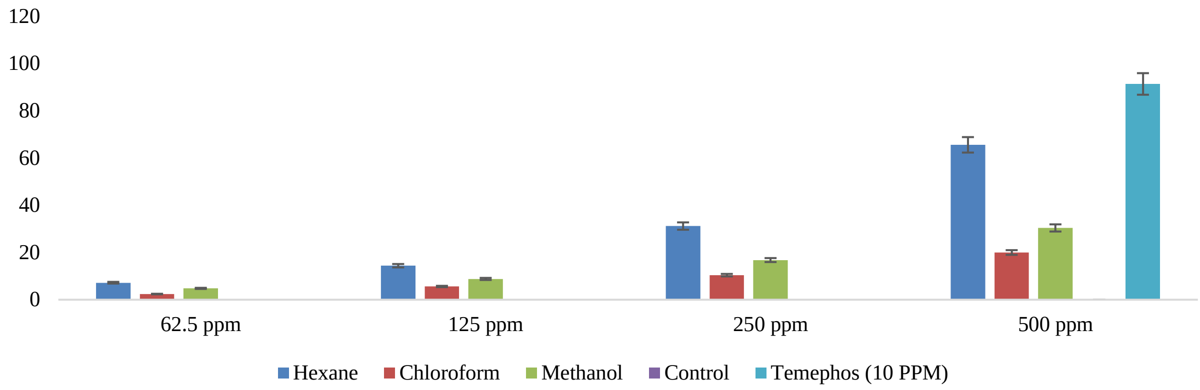

Figure 1. Percent ovicidal activity of crude extracts of roots of *A. salviifolium* against *Cx. quinquefasciatus* eggs.

These findings were further supported by Figure 1, which clearly demonstrated the superior efficacy of the hexane extract against mosquito species, while the chloroform and methanol extracts showed limited activity. In summary, the results indicate that the hexane extract is highly effective against *Cx. quinquefasciatus*, exhibiting potent larvicidal, pupicidal, and ovicidal activities. On the other hand, the chloroform and methanol extracts showed comparatively lower activity, with the hexane extract being the most promising for further investigation as a potential mosquito control agent.

The insecticidal activity of *A. salvifolium* has been reported in several studies, highlighting its potential as a natural source of insect control agents. Several studies have reported on the insecticidal activity of different parts of *A. salvifolium* against various insect species. For instance, a study investigated the insecticidal activity of the methanol extract of *A. salvifolium* bark against, *Helicoverpa armigera*, *Spodoptera litura*, and *Dysdercus cingulatus*. The study reported significant insecticidal activity of the extract against all species, with the highest activity observed against *Dysdercus cingulatus* (Baskar et al., 2011). Moreover, a study examined the insecticidal activity of the leaf extract of *A. salvifolium* against the stored grain pest *Sitophilus oryzae*, and reported significant insecticidal activity of the extract. The study found that the extract caused mortality in the insect at concentrations as low as 0.5%, and also exhibited repellent activity against the pest (Kavitha et al., 2018). Another study evaluated the insecticidal activity of different solvent extracts of *A. salvifolium* leaves against the red flour beetle, *Tribolium castaneum*. The study found that the chloroform and ethyl acetate extracts of *A. salvifolium* leaves exhibited significant insecticidal activity against *T. castaneum*, with mortality rates of up to 90% at higher concentrations (Ansari et al., 2017).

While the present study investigated the larvicidal, pupicidal, and ovicidal activities of leaf extracts of *A. salvifolium* against *Cx. quinquefasciatus*, other studies have also explored the efficacy of different parts of the plant against mosquitoes. For instance, a study evaluated the larvicidal activity of root leaf extracts of *Saussurea costus* (Falc.) Lipsch. against three mosquito vectors:*Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus.* The methanol extract of the roots recorded the highest larvicidal activity against *An. stephensi*, with LC_{50} and LC_{90}values of 7.96 and 34.39 ppm, respectively (Ali et al., 2020). Another study investigated the effectiveness of using crude methanol extracts from the roots of *A. salvifolium* to kill mosquito larvae (*Ae. aegypti*). They found that the plant extract was able to kill the larvae, with an LC_{50} value of 128.01 ppm. Additionally, they investigated how the plant extract worked and found that it was able to inhibit certain enzymes that are important for the development of mosquito larvae, specifically acid and alkaline phosphatases (Shukla et al., 2019).

Interestingly, a study by Marimuthu et al. (2019) compared the larvicidal activity of *A. salvifolium* extracts from different parts of the plant, including the roots, stems, and leaves, against *Ae. aegypti* and *Cx. quinquefasciatus*. The study found that the root extract exhibited the highest larvicidal activity against both mosquito species, with LC_{50} values of 98.59 ppm and 101.36 ppm for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Another study investigated the biocontrol potential of ethyl acetate extract from *A. salviifolium* fruit pericarp against *Cx. quinquefasciatus* larvae, demonstrating significant larval mortality with 100% at 72-hour exposure, revealing the most susceptibility in 3rd instar larvae (LC_{50} = 3.60 ppm), and identifying bioactive compounds including Benzoyl bromide and 3-Amino-5 (2-Furyl) Pyrazole with minimal impact on non-target organisms (Mondal et al., 2022). Overall, the findings of this study suggest that the hexane extract of *A. salvifolium* roots may be a promising natural mosquito control agent. Interestingly, the results of these studies suggest that different parts of the *A. salvifolium* plant may possess varying degrees of activity against mosquitoes, with the roots showing promise for further investigation. Nevertheless, more studies are needed to fully explore the potential of *A. salvifolium* as a mosquito control agent, including investigations into the toxicity and safety of the plant extracts.

CONCLUSIONS

The investigation conducted on the larvicidal, pupicidal and ovicidal activities of the root extracts of *A.salviifolium* on *Cx.quinguefasicatus* has shown that the hexane extract demonstrated significant activities against these mosquito species. The larvicidal and pupicidal activities indicate that the hexane extract can effectively kill the larvae and pupae of these mosquitoes, which are the stages in which they are most vulnerable and responsible for the transmission of mosquito-borne diseases such as dengue fever, Zika virus, and malaria. Additionally, the ovicidal activity suggests that the hexane extract can also inhibit the hatching of mosquito eggs, which can further reduce the mosquito population. The significant results obtained from the investigation indicate that the hexane extract of *A.salviifolium* can be considered for commercial application as an antimosquito agent. The use of natural products as mosquito control agents has gained considerable interest due to their potential for being eco-friendly and cost-effective. The hexane extract of *A.salviifolium* being a natural product, has the potential to be used as an alternative to chemical insecticides, which can be harmful to the environment and non-target organisms.

RECOMMEDATION FOR FUTURE STUDIES

It is essential to conduct further studies before commercializing the hexane extract of *A.salviifolium* as a mosquitocidal agent. These studies should include toxicity evaluations, which will help to determine the safety of the extract on non-target organisms, including humans, pets, and wildlife. Toxicity studies will also help to establish the safe concentration and application rates of the extract. Field trials are also necessary to evaluate the efficacy of the extract in real-world situations. These trials will involve testing the extract in various mosquito breeding sites and assessing its ability to reduce mosquito populations. The field trials will also help to identify any limitations or challenges associated with the use of the extract. Furthermore, the practicality of the extract in terms of production, storage, and application should also be evaluated. The extraction process, storage conditions, and application methods should be optimized to ensure the extract's effectiveness and stability. The results obtained from these studies will also provide valuable information that can be used to optimize the extract's production, storage, and application methods.

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