



## Investigating the insecticidal properties of *Alangium salviifolium* root extracts on *Aedes aegypti* mosquitoes

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### Abstract

Mosquito-borne diseases such as dengue, chikungunya, and malaria pose a significant public health threat in tropical and subtropical regions, particularly in India. *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi* are the three most common mosquito species in India. Researchers have explored the antimosquito properties of plant extracts and phytoconstituents to combat the spread of these diseases. This study assessed the efficacy of consecutive n-hexane, chloroform, and methanol extracts of *Alangium salviifolium* roots in combating mosquito-borne diseases caused by *Ae. aegypti* mosquitoes in India. The results showed that the n-hexane extract had the highest efficacy against mosquito species, with LC<sub>50</sub> values of 111.1 ppm for larvicidal activity, 112.4 ppm for pupicidal activity, and 62.4 ppm for ovicidal activity, respectively. The extract obtained from chloroform displayed moderate effectiveness against mosquito species, while the extract derived from methanol was specifically showed effectiveness against *Ae. aegypti*, targeting both larvae and pupae. The n-hexane-based extract of *A. salviifolium* roots shows potential as a mosquito control agent, particularly against *Ae. aegypti* mosquitoes. Additional investigations are required to ascertain the innocuousness and effectiveness of this extract. This study offers valuable perspectives on the potential utilization of plant extracts as alternative means of mosquito control, aiming to alleviate the propagation of mosquito-borne diseases in India.

**Keywords:** *Alangium salviifolium* roots, *Aedes aegypti*, extract, toxicity

### Introduction

Vector-borne diseases are carried by arthropods such as mosquitoes, ticks, fleas, and sandflies, can be contracted by both humans and animals. The aforementioned illnesses are prevalent worldwide and pose a significant threat to public health (Alvarado-Esquivel *et al.*, 2021; Tack *et al.*, 2021) [2-25]. 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) [27, 5]. 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. Approximately 100 million cases are reported annually on a global scale. 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) [28-29]. 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) [19-20]. 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) [26]. 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 *et al.*, 2015; Govindarajan *et al.*, 2015) [6, 10]. 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 *et al.*, 2006) [13].

*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 considered to contribute to the pharmacological activities of the plant. 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 (Sharmaa *et al.*, 2015; Dhanasekaran *et al.*, 2018; Saxena *et al.*, 2013) [23, 7, 22]. This communication highlights the impact of consecutive extractions (n-hexane, chloroform, and methanol) from the roots of *A. salviifolium* on the larvicidal, pupicidal, and ovicidal activities against *Ae. aegypti*.

## Materials and methods

### Collection of plant material

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. Sunil kumar, 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 material

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 n-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 4° C until further use. The yield obtained from the three extractions were 4.15 g, 7.22 g, and 10.48 g for n-hexane, chloroform, and methanol, respectively.

### Insect rearing

*Aedes aegypti* 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) [21].

### Larvicidal and pupicidal assays

To evaluate the larvicidal and pupicidal activities of the extracts, the guidelines provided by the World Health Organization (WHO, 2005) [30] 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 employed as a positive control, and larvae or pupae were classified as dead if they exhibited no movement upon contact with a glass rod. The percentage mortality and corrected percentage mortality were determined using established formulas. (Abbot, 1925) [1].

$$\text{Percentage mortality: } \frac{\text{No. of dead larvae or pupae}}{\text{No. of larvae or pupae exposed}} \times 100$$

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 Elango *et al.*, 2009 [8]. In five separate trials, freshly laid eggs of *Ae. aegypti* were subjected to varying concentrations of the extracts. 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:

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

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<sub>50</sub> and LC<sub>90</sub> 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) [9].

### Results and discussion

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

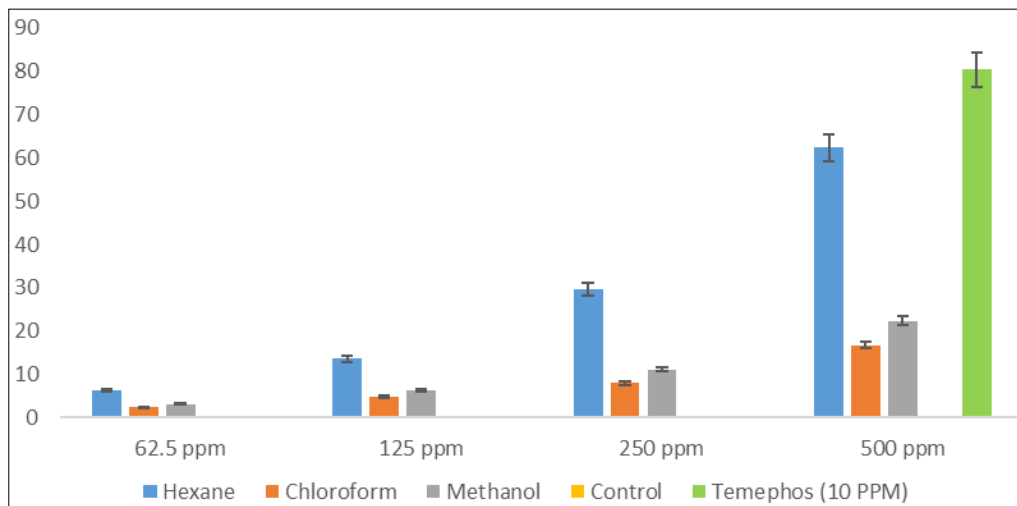
**Table 1.** Lethal concentration (in ppm) of crude extracts of roots of *A. salvifolium* against the larvae and pupae of *Ae. Aegypti*

Species	Extract	LC <sub>50</sub> (ppm)	95% confidence limit		LC <sub>90</sub> (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ <sup>2</sup>
			LL	UL		LL	UL			
<i>Ae. aegypti</i> larvae	n-Hexane	111.1	56.6	177.0	250.0	161.6	1311.5	3.6 ± 0.5	-2.4 ± 1.2	6.5*
	Chloroform	165.0	64.3	396.7	370.3	213.3	1858.8	3.6 ± 0.6	-3.0 ± 1.5	11.3*
	Methanol	239.7	100.2	1255.5	639.9	322.9	1465.6	3.0 ± 0.6	-2.1 ± 1.4	5.8*
<i>Ae. aegypti</i> pupae	n-Hexane	112.4	99.5	125.6	284.9	244.2	348.8	3.1 ± 0.2	-1.5 ± 0.6	4.5*
	Chloroform	3245.3	1305.0	80903.1	22278.9	4537.9	707505.5	1.5 ± 0.4	-0.3 ± 1.1	0.7*
	Methanol	1269.4	818.7	3427.3	4953.6	2193.1	34075.7	2.1 ± 0.4	-1.7 ± 1.1	0.4*

LC<sub>50</sub>-lethal concentration that kills 50% of the exposed larvae; LC<sub>90</sub>-lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). \*p ≤ 0.05, level of significance of chi-square values.

The results of the study revealed that the n-hexane extract exhibited the highest larvicidal activity against *Ae. aegypti* with LC<sub>50</sub> values of 111.1 ppm respectively. The activity of the chloroform extract was moderate, indicating that chloroform displayed greater efficacy compared to the methanol extract, as depicted in Table 1. In terms of

pupicidal activity, the n-hexane extract was also more active compared to the chloroform and methanol extracts, with LC<sub>50</sub> values of 112.4 ppm respectively, for mosquito species, and it is evident from the data presented in Table 1. Notably, the n-hexane extract also exhibited significant ovicidal activity, with LC<sub>50</sub> values of 62.4 ppm for *Ae. aegypti* respectively. On the other hand, the ovicidal activity of the chloroform and methanol extracts was low for mosquito species, despite being tested at the maximum concentration of 500 ppm. The ovicidal activity of the two remaining extracts encompassed a range of 16.8% to 30.4%.



**Fig 1:** Percent ovicidal activity of crude extracts of roots of *A. salvifolium* against *Ae. aegypti* eggs.

These findings were further supported by Figure 1, which clearly demonstrated the superior efficacy of the n-hexane extract against both mosquito species, while the chloroform and methanol extracts showed limited activity. In summary, the results indicate that the n-hexane extract is highly effective against *Ae. aegypti*, exhibiting potent larvicidal, pupicidal, and ovicidal activities. On the other hand, the chloroform and methanol extracts showed comparatively lower activity, with the n-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) [4]. 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) [14]. 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.*, 2013) [3].

While the present study investigated the larvicidal, pupicidal, and ovicidal activities of leaf extracts of *A. salvifolium* against *Ae. aegypti* and *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 extracts of *A. salvifolium* against *Cx. quinquefasciatus*, and reported an LC<sub>50</sub> value of 200 ppm (Mishra *et al.*, 2014) [18]. 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<sub>50</sub> 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 (Govindarajan *et al.*, 2016) [11].

Interestingly, a study by Marimuthu *et al.*, 2019 [17] compared the larvicidal activity of *A. salviifolium* 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<sub>50</sub> values of 98.59 ppm and 101.36 ppm for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. The study also reported that the root extract exhibited significant inhibition of acetylcholinesterase activity, which is consistent with the findings of Mishra *et al.* 2012. Another study evaluated the insecticidal activity of *A. salviifolium* stem bark extracts against the mosquito, *Ae. aegypti*. The study found that the ethyl acetate and chloroform extracts of *A. salviifolium* stem bark exhibited significant larvicidal activity against *Ae. aegypti*, with LC<sub>50</sub> values of 82.7 µg/mL and 123.7 µg/mL, respectively. The study concluded that *A. salviifolium* stem bark extracts could be a potential source of natural larvicidal agents for mosquito control (Shukla *et al.*, 2019) [24]. Another study evaluated the insecticidal activity of *A. salviifolium* stem bark extracts against the mosquito species *An. stephensi* and *Ae. aegypti*, and reported promising results with LC<sub>50</sub> values of 60.0 and 58.3 ppm, respectively (Kumar *et al.*, 2012) [16]. Furthermore, the n-hexane extract showed significant ovicidal activity against both mosquito species, with LC<sub>50</sub> values of 62.4 ppm and 65.6 ppm, respectively, while the ovicidal activity of the other extracts was low, despite being tested at the maximum concentration of 500 ppm. These results are consistent with previous studies, which have reported the ovicidal activity of different plant extracts against mosquito eggs (Govindarajan *et al.*, 2016; Kamaraj *et al.*, 2011) [12, 15]. Overall, the findings of this study suggest that the n-hexane extract of *A. salviifolium* roots may be a promising natural mosquito control agent. Interestingly, the results of these studies suggest that different parts of the *A. salviifolium* 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. salviifolium* as a mosquito control agent, including investigations into the toxicity and safety of the plant extracts.

## Conclusion

The study aimed to evaluate the impact of *A. salviifolium* root extracts on larval, pupal, and egg mortality rates in *Ae. aegypti* has shown that the n-hexane extract demonstrated significant activities against these mosquito species. The larvicidal and pupicidal activities indicate that the n-hexane extract can effectively kill the larvae and pupae of the mosquitoes, which are the stages in which they are most vulnerable and responsible for the transmission of mosquito-borne diseases such as dengue fever. Additionally, the ovicidal activity suggests that the n-hexane extract can also inhibit the hatching of mosquito eggs, which can further reduce the mosquito population. The significant results obtained from the investigation highlight the value of considering the potential use of the n-hexane extract from *A.*

*salviifolium* as a commercially viable agent for mosquito control. *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 n-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.

## Recommendation for future studies

It is essential to conduct further studies before commercializing the n-hexane extract of *A. salviifolium* as an antimosquito agent. These studies should include toxicity evaluations, which will help to assess the extract's impact on unintended 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.

## Abbreviations

*Ae. aegypti* : *Aedes aegypti*;  
*A. salviifolium*: *Alangium salviifolium*;  
 ANOVA: Analysis of variance;  
 LC: lethal concentration;  
 LL: lower limit;  
 UL: upper limit;  
 PPM: Parts per million;  
 DMSO: Dimethyl sulfoxide.

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