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Exploring the potential of *Sphaeranthus Indicus* flower extracts as natural mosquito larvicides

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Abstract
Extensive insecticide use for mosquito-borne disease control has led to resistant mosquito strains, undermining effectiveness. This study assessed the efficacy of *Sphaeranthus Indicus* flower extracts against *Aedes Aegypti* and *Culex quinquefasciatus* mosquitoes, major vectors of dengue, chikungunya, lymphatic filariasis and malaria etc. Extracts were evaluated for larvicidal and pupicidal effects, and LC_{50} values were calculated. The hexane and methanol extracts demonstrated significant larvicidal activity with LC_{50} values of 134.18 ppm and 116.54 ppm for *Aedes Aegypti*, and 115.96 ppm and 114.91 ppm for *Culex quinquefasciatus*, respectively. The methanol extract also exhibited effective pupicidal activity with LC_{50} values of 172.90 ppm (*Aedes Aegypti*) and 126.98 ppm (*Culex quinquefasciatus*). Ovicidal activity was not observed. These findings suggest the potential of *Sphaeranthus Indicus* extracts as natural alternatives to chemical insecticides for controlling disease-carrying mosquitoes. Further research should consider safety, environmental impact, and cost-effectiveness before integrating these extracts into vector control programs, as alternatives to conventional insecticides.

Keywords: *Sphaeranthus Indicus* flowers, *Aedes Aegypti, Culex quinquefasciatus,* extract

1. Introduction

Vector-borne diseases, such as mosquito-borne diseases, are caused by pathogens transmitted to humans or animals through the bite of infected arthropods, particularly mosquitoes. Mosquitoes are the most important vectors for transmitting various diseases, including malaria, dengue fever, chikungunya, Zika virus, filariasis, Japanese encephalitis and yellow fever, among others [1]. The proliferation of mosquitoes is influenced by various factors, including climate, availability of suitable breeding sites, and human activities such as urbanization, deforestation, and water storage practices [2]. There are approximately 3,500 known species of mosquitoes worldwide, belonging to the family Culicidae. However, not all species of mosquitoes are vectors of human diseases [3]. An estimated 175 to 200 species of mosquitoes are known to be capable of transmitting human diseases, with different species transmitting specific pathogens in different regions of the world [4]. In India, there are about 422 species of mosquitoes, of which around 100 species are known to be vectors of human diseases. Some of the most common mosquito species found in India include *Aedes Aegypti* (vector of dengue, chikungunya, and Zika virus), *Anopheles stephensi* (vector of malaria), and *Culex quinquefasciatus* (vector of filariasis) [5-7]. Vector-borne diseases, particularly mosquito-borne diseases, continue to be a significant public health concern worldwide and millions of people are infected with mosquito-borne diseases each year, resulting in hundreds of thousands of deaths globally [8]. In India, vector-borne diseases are a major health burden, with malaria, dengue, and chikungunya being the most common mosquito-borne diseases. The National Vector Borne Disease Control Programme (NVBDCP) in India has been implementing various strategies, including the use of insecticide-treated bed nets, indoor residual spraying, and community mobilization, to control mosquito-borne diseases [9]. Chemical insecticides have been widely used for mosquito control, including the use of insecticide-treated bed nets, indoor residual spraying, and fogging. These methods have been effective in reducing mosquito populations and controlling vector-borne diseases. However, the overreliance on chemical insecticides has led to the development of insecticide resistance in mosquitoes, which poses a challenge in mosquito control efforts [10, 11].

In recent years, there has been growing interest in the use of plant-derived products as alternative methods for mosquito control. Plant-based insecticides, such as neem oil, citronella oil, and pyrethrum, have shown promising results in controlling mosquitoes without causing harm to the environment or developing resistance. These plant-derived products can be used in various forms, including sprays, coils, and candles, and are considered safer options for mosquito control [12-15].

Sphaeranthus Indicus Linn belongs to the Asteraceae family, also known as the sunflower family. It is commonly known as East Indian globe thistle or simply globe thistle. It has several synonyms, including *Sphaeranthus hirtus*, *Sphaeranthus mollis*, and *Sphaeranthus* senegalensis [16]. It is native to India and Southeast Asia, but it is also found in other tropical and subtropical regions around the world, such as Africa, the Caribbean, and South America. It has been traditionally used in Ayurvedic medicine for various medicinal purposes. It has been used to treat fever, digestive problems, skin diseases, respiratory disorders, and inflammation. Recent studies have also investigated its potential therapeutic effects in various health conditions, such as inflammation, hyperlipidemia, and microbial infections. It contains various bioactive compounds, including sesquiterpene lactones, flavonoids, phenolic acids, and alkaloids. Some of the major chemical constituents identified in the plant include sphaerantholide, dehydrosphaerantholide, 7-hydroxycoumarin, kaempferol, and quercetin. Several pharmacological activities have been attributed to *S. Indicus*, including anti-inflammatory, antimicrobial, antihyperlipidemic, antihyperlipidemic, antioxidant, and analgesic activities [17-22]. There are several eco-friendly and cost-effective vector control tools that have shown promise in mitigating the problem of insecticide resistance. These include the use of natural products, such as plant extracts and phytoconstituents, as demonstrated in the study on *S. Indicus* flower extracts. These natural products often have insecticidal properties and can be used as alternatives to chemical insecticides. The objective of this study is to evaluate the larvicidal, pupicidal, and ovicidal effects of different extracts (hexane, chloroform, and methanol) obtained from the flowers of *S. Indicus* against *Ae. Aegepti* and *Cx. quinquefasciatus* mosquitoes. Overall, the development and implementation of eco-friendly and cost-effective vector control tools are essential and helping to reduce the spread of mosquito-borne diseases while minimizing environmental and health risks.

2. Materials and Methods

2.1 Collection of the plant material

During April 2022, *S. Indicus* flowers were gathered in Palayamkottai, Tamil Nadu, and India. Dr. KN Sunilkumar, a Research Officer at the Department of Pharmacognosy in Siddha Central Research Institute, Chennai, verified and authenticated the plant material to ensure its precision. A voucher specimen was consigned to the institute's herbarium, and an Authentication Code No: S240123011 was allocated for accurate identification and traceability of the utilized plant material in the study.

2.2 Extraction of the plant material

To procure flower extracts, 1 kg of shade-dried and coarsely powdered material underwent successive extraction via n-hexane, chloroform, and methanol within a Soxhlet apparatus. Following each extraction, the solution obtained was filtrated and concentrated using vacuum rotary evaporation. The resultant dry extracts were securely stored in airtight containers at 4 °C. Yields of hexane, chloroform, and methanol extracts were 32.7 g, 16.5 g, and 65.2 g, correspondingly. This method is called sequential solvent extraction and is a widely used technique in natural product chemistry to obtain different classes of compounds from plant material, with the selection of solvent (s) depending on the desired compound (s) for extraction.

2.3 Insect Rearing

Third instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus* were sourced from Loyola College's Entomology Research Institute in Chennai. These larvae were cultivated under controlled conditions with a temperature of 27±2 ºC, relative humidity of 75-85%, and a 13:11 light-to-dark photoperiod. For larval feeding, a mixture of dog biscuits and Brewer's yeast in a ratio of 3:2 was provided, along with chlorine-free tap water [23].

2.4 Larvicidal and Pupicidal Assays

We gauged the effectiveness of diverse extracts in their larvicidal and pupicidal roles, adhering to protocols outlined by the World Health Organization [24]. Four concentrations (500, 250, 125, and 62.5 parts per million) of the extracts were examined, with each concentration tested in five replicates for all three activities. Solutions were formulated as emulsions in 1% aqueous DMSO. In individual trials, 20 larvae or pupae were introduced to 100 ml of the solution within a 150 ml plastic receptacle. A negative control of 1% aqueous DMSO and a positive control of Temephos were employed. After 24 hours of incubation, immobility upon gentle contact indicated mortality in larvae or pupae. The ensuing percentage mortality and corrected percentage mortality were calculated employing designated formulas. [25].

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] × 100

To prevent overestimation of treatment effectiveness in experiments where the mortality rate in the control group is less than 5%, a corrected percentage mortality formula is recommended. This formula considers the mortality rate in the control group and uses the number of surviving larvae or pupae after treatment (nT) and in the control group (nC) to calculate the percentage mortality. By using this corrected formula, researchers can obtain a more accurate estimate of treatment efficacy and avoid potential biases caused by low mortality in the control group.

2.5 Ovicidal Activity

To test the ovicidal activity of the extracts, a modified version of the method described by Elango [26] was used. Distinct concentrations of extracts were applied to recently laid eggs of *Ae. Aegypti* and *Cx. quinquefasciatus*, with five repetitions per concentration, mirroring the assessment of larvicidal and pupicidal effects. Eggs were scrutinized under a compound microscope to determine their hatching capability, and the ovicidal activity percentage was ascertained 120 hours post-treatment through a specific 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.

2.6 Statistical Analysis

To determine the LC_{50} and LC_{90} values for each concentration of larvicidal, pupicidal, and ovicidal data, the corrected percentage mortality values were analyzed using probit analysis. The analysis was conducted using the US EPA probit analysis software, version 1.5. A p-value of ≤ 0.05 was considered statistically significant, indicating a significant difference between the results obtained [27].

3. Results and Discussion

Our study delved into the larvicidal and pupicidal potentials of flower-derived extracts (hexane, chloroform, and methanol) against third instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus*. The outcomes are presented comprehensively in Tables 1 and 2.

Table 1: Larval fatality threshold (in ppm) of crude *S. Indicus* flower extracts against *Ae. Aegypti* and *Cx. quinquefasciatus* larvae

Species	Extract	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ^2
			LL	UL		LL	UL			
Ae. Aegypti larvae	Hexane	134.18	114.27	155.18	528.91	415.89	743.95	2.1±0.2	0.4±0.4	0.3*
	Chloroform	387.7	340.4	455.1	1038.9	806.7	1503.9	-2.7±0.7	2.9±0.3	4.8*
	Methanol	115.96	100.57	131.66	356.49	296.75	456.55	2.6±0.2	-0.4±0.5	3.5*
Cx. quinquefasciatus larvae	Hexane	116.54	101.68	131.75	341.49	286.46	431.99	2.7±0.2	-0.6±0.5	4.8*
	Chloroform	403.6	338.5	510.1	1588.7	1087.3	2849.2	-0.6±0.5	2.1±0.2	1.6*
	Methanol	114.91	100.99	129.17	314.21	266.47	390.90	2.9±0.2	-1.0±0.5	5.6*

LC_{50} represents the lethal concentration causing mortality in 50% of larvae; LC_{90} signifies the concentration fatal to 90%; LL corresponds to lower limit (95% confidence); UL pertains to upper limit (95% confidence). *$p \leq 0.05$, denoting significance level for chi-square values.

Table 2: Pupicidal potency (in ppm) of crude *S. Indicus* flower extracts against *Ae. Aegypti* and *Cx. quinquefasciatus* pupae

Species	Extract	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ^2
			LL	UL		LL	UL			
Ae. Aegypti Pupae	Hexane	246.03	207.87	299.13	1244.58	852.77	2227.40	1.8±0.2	0.6±0.4	1.2*
	Chloroform	359.36	198.30	1573.66	1117.40	503.29	14736.47	2.6±0.3	-1.2±1.1	5.7*
	Methanol	172.90	147.33	217.72	462.63	378.34	827.63	2.9±0.8	-1.7±1.8	5.9*
Cx. quinquefasciatus Pupae	Hexane	226.01	191.07	272.72	1155.82	799.06	2037.55	1.8±0.2	0.7±0.4	1.6*
	Chloroform	304.51	175.05	1202.51	957.56	456.38	114965.58	2.5±0.4	-1.3±1.0	5.5*
	Methanol	126.98	53.45	225.75	334.92	197.66	3683.47	3.0±0.5	-1.4±1.1	5.4*

LC_{50} represents the lethal concentration causing mortality in 50% of larvae; LC_{90} signifies the concentration fatal to 90%; LL corresponds to lower limit (95% confidence); UL pertains to upper limit (95% confidence). *$p \leq 0.05$, denoting significance level for chi-square values.

The results of our study indicate that the methanol extract from the flowers of the studied plant exhibited the highest larvicidal activity against both *Ae. Aegypti* and *Cx. quinquefasciatus* mosquitoes, with LC_{50} values of 115.96 ppm and 114.91 ppm, respectively. The n-hexane extract also showed moderate larvicidal activity, with LC_{50} values of 134.18 ppm and 116.54 ppm, and was more active than the chloroform extract. Additionally, the methanol extract was found to be more effective in controlling pupae than the hexane and chloroform extracts, with LC_{50} values of 172.90 ppm and 126.98 ppm for *Ae. Aegypti* and *Cx. quinquefasciatus*, respectively. However, none of the extracts showed significant ovicidal activity against either mosquito species, with the methanol extract showing the least activity of 13.6% to 16.0% against both *Ae. Aegypti* and *Cx. quinquefasciatus*. The chloroform extract showed a similar level of ovicidal activity as the methanol extract. These results were consistent with the graphical representation depicted in Figures 1 and 2, which compared the activity of the three extracts against *Ae. Aegypti* and *Cx. quinquefasciatus* eggs, respectively. The figures demonstrated that the methanol extract was the most effective larvicidal and pupicidal agent against both mosquito species, while none of the extracts showed significant activity against mosquito eggs. Overall, our study highlights the potential of the methanol extract from the flowers of the studied plant as a natural insecticide for controlling *Ae. Aegypti* and *Cx. quinquefasciatus* populations. However, further research is needed to identify and isolate the active compounds responsible for these biological activities.

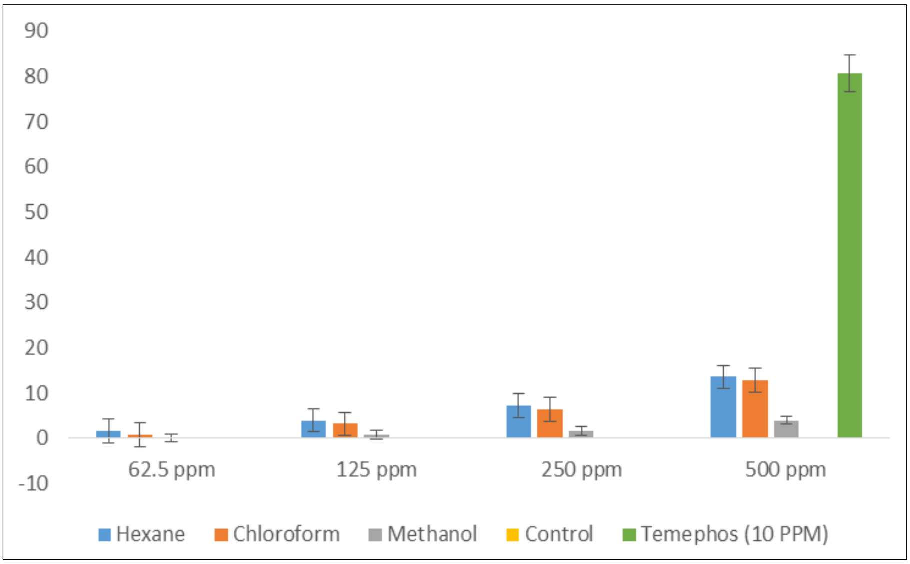

Fig 1: Ovicidal effectiveness percentage of crude *S. Indicus* flower extracts on *Ae. Aegypti* eggs.

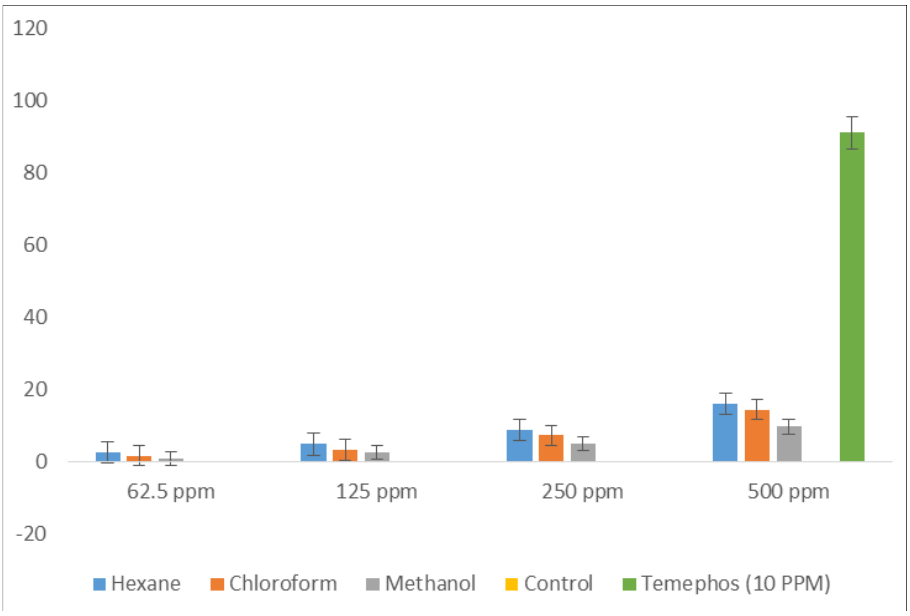

Fig 2: Ovicidal effectiveness percentage of crude *S. Indicus* flower extracts on *Cx. quinquefasciatus* eggs.

4. Discussion

The extensive body of research explored in this study underscores the multifaceted potential of *S. Indicus* as a natural resource for mosquito control. Across various studies, the larvicidal, pupicidal, and ovicidal effects of crude extracts derived from *S. Indicus* flowers have been thoroughly investigated against prominent mosquito species. These studies have demonstrated the efficacy of *S. Indicus* extracts in disrupting various stages of mosquito development, offering a promising avenue for integrated vector management. The research conducted by Murugan *et al.* [28] and Kamaraj *et al.* [29] unveils the larvicidal potency of *S. Indicus* extracts against *Ae. Aegypti* and *An. stephensi*, respectively. These findings, in line with previous studies [30], underscore the capacity of these extracts to impede mosquito larval growth and development, ultimately curbing the expansion of mosquito populations. The presence of bioactive compounds such as flavonoids, alkaloids, terpenoids, and phenolic compounds [31] contributes to the larvicidal effect, rendering *S. Indicus* a valuable natural alternative to synthetic insecticides. Remarkably, the pupicidal potential of S. Indicus extracts stands out as a noteworthy revelation. The work by Arivoli *et al.* [32], Ramkumar *et al.* [33], and Jayaseelan *et al.*[34] collectively emphasizes the efficacy of *S. Indicus* extracts in targeting pupal stages. This novel approach addresses a crucial developmental phase in mosquito life cycles, thereby reducing the emergence of adult vectors [35]. This pupicidal effect is attributed to phytochemical interference with pupal development. Intriguingly, the ovicidal activities reported in various studies present a complex scenario. The work of Surendra *et al.* [36] and Jayaseelan *et al.* [37]. Contrasts with the findings of the current study, where no significant ovicidal activity was observed. This discrepancy underscores the need for further investigation into the specific conditions or compounds responsible for ovicidal properties [38]. However, the persistent larvicidal and pupicidal effects underscore the potential of *S. Indicus* extracts for population control. Particularly intriguing is the investigation into the essential oil composition of *S. Indicus* [39]. The identification of key compounds, notably alpha-pinene and beta-caryophyllene, showcases the plant's potential for applications beyond mosquito control, expanding its utility to the perfume and flavour industries. This multidimensional application adds an intriguing layer of novelty to the study. The results of the current investigation align with previous studies highlighting the larvicidal activities of methanol extracts against mosquito larvae [40]. Furthermore, the solvent-dependent nature of larvicidal activity, as highlighted by the n-hexane extracts' moderate effects [41], reveals the complexity of the interactions between different solvent extracts and mosquito larvae.

Importantly, the methanol extract's potent pupicidal activity, surpassing the effects of n-hexane and chloroform extracts, provides novel insights into effective pupal stage targeting [42]. This finding has significant implications for the design of mosquito control strategies, as pupal stages represent vulnerable yet often overlooked targets for control efforts. Nonetheless, the absence of ovicidal activity in the current study echoes the observations of limited ovicidal potential in plant extracts [43]. This reinforces the need for thorough investigations into the mechanisms underlying ovicidal effects and the specific conditions required for their manifestation. The holistic advantages of *S. Indicus* as a natural mosquito control agent are further highlighted. Its affordability, ease of preparation, and safety for non-target organisms position it as an environmentally friendly alternative to synthetic insecticides. This aligns with the global push for sustainable vector management strategies.

In conclusion, the comprehensive examination of *S. Indicus* extracts' effects on different mosquito life stages yields valuable insights into its potential for integrated mosquito control. By adopting a novel strategy of targeting pupal phases and pinpointing pivotal compounds within essential oils, and the observed larvicidal and pupicidal activities underscore *S. Indicus's* multifaceted utility. Further research is essential to uncover the mechanisms behind these effects, optimize application methods, and evaluate the extracts' effectiveness in real-world conditions.

5. Conclusions

In summary, the methanol extract derived from the plant's blossoms demonstrated the most potent larvicidal and pupicidal efficacy against *Ae. Aegypti* and *Cx. quinquefasciatus* mosquitoes, while the hexane extract displayed moderate activity. Nonetheless, ovicidal activity was not prominent in any of the extracts against both mosquito species. These outcomes imply the methanol extract's potential as a natural insecticide for managing populations of *Ae. Aegypti* and *Cx. quinquefasciatus*.

6. Recommendation for future studies

Based on the conclusion of the study, here are some recommendations for future studies: Extraction and identification of active components: The investigation revealed that the methanol flower extract displays robust larvicidal and pupicidal effects on *Ae. Aegypti* and *Cx. quinquefasciatus* mosquitoes. However, it is unclear which compounds are responsible for this activity. Therefore, future studies could focus on isolating and characterizing the active compounds in the methanol extract. Formulation of the extract as an insecticide: The findings suggest that the methanol extract could serve as a viable natural insecticide for managing populations of *Ae. Aegypti* and *Cx. quinquefasciatus* mosquitoes. Therefore, future studies could focus on formulating the extract into a more stable and effective insecticide that can be easily applied in the field. Further evaluation of the safety and efficacy of the extract: Before the methanol extract can be used as an insecticide, further studies are needed to evaluate its safety and efficacy. This could involve testing the extract on non-target organisms to determine its potential toxicity and conducting field trials to determine its effectiveness in controlling mosquito populations. Evaluation of the extract against other mosquito species: The research has centered on evaluating the larvicidal, pupicidal, and ovicidal effects of the extract on both *Ae. Aegypti* and *Cx. quinquefasciatus* mosquitoes. Future studies could evaluate the extract against other mosquito species to determine its potential as a broad-spectrum insecticide.

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8. Competing Interests

The authors have no conflicting interests to declare.

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