Assessing Larval Toxicity of Sphaeranthus Indicus Linn Essential Oil Against Dengue and Filarial Vectors

ABSTRACT. This study focuses on a crucial objective: eradicating the formidable dengue and filarial vectors through larval toxicity using a novel essential oil derived from Sphaeranthus indicus Linn. The research encompasses several key methodologies, including pest rearing, homology modeling, and molecular docking. The G Power value is taken as 0.8, with two groups and each group carrying 5 sample sizes. Si-CVO is toxic to Aedes aegypti larvae, and it causes dose-dependent mortality in the II, III, and IV instar larvae, respectively. Maximum mortality rates for all instars were 500 ppm: 91.3% (II instar), 87.4% (III instar), and 83.2% (IV instar). While the lowest dosage of 100 ppm had a lower death rate throughout all larval instars.

Keywords: Mosquito, Sphaeranthus indicus Linn, Ae. aegypti, Mortality, Toxicity, Docking, Novel Essential Oils, Protein.

1 Introduction
In recent years, more than a thousand articles have appeared in Google Scholar and Elsevier Springer. The article with the highest number of citations is referenced as [4]. Mosquito species exhibit resistance characteristics that are developed through chemical exposure. This encompasses the application of EOs for repelling, larvicidal, ovicidal, and adulticidal purposes [5,6]. The use of novel essential oils derived from plants can play a significant role in diminishing human-vector contact, thereby lowering the risk of disease transmission. Additionally, the larvicidal properties of these plant-based essential oils are effective in decreasing the larval population in the environment. Furthermore, their ovicidal activity contributes to reducing the quantity of eggs present in the environment [7]. The effectiveness of new essential oils obtained from plants in reducing the population of adult mosquitoes in the environment is notable due to their insect-killing capabilities.

Sphaeranthus indicus Linn, a fragrant herb belonging to the Asteraceae family, grows to a height of 1-2 feet and is commonly found throughout the plains of India and in subtropical regions. Traditionally, this herb has been employed in several types of native medicinal practices in some parts of India for treating humans. Known as Koṭṭaikkarantai in Tamil, this plant has a wide range of medicinal applications. It is commonly used for alleviating skin conditions, managing gastric disorders with bleeding, reducing glandular swellings, and providing relief from pain and nervous depression. Additionally, it possesses antibiotic and diuretic properties. Preparations made from this plant have shown effectiveness in treating various ailments, including asthma, leucoderma, jaundice, bronchitis, and scabies.

The extraction of essential oils from fragrant herbs is commonly achieved through the process of steam distillation, a method well-documented in various research studies. These oils have garnered significant attention from the global scientific community due to their biological effects. In recent years, a major goal of cutting-edge research has been to effectively manage mosquito populations, known carriers of disease-causing pathogens, highlighting the necessity of this field of study. This study focuses on extracting and identifying key chemical components from the novel essential oils obtained from the fresh leaves of Sphaeranthus indicus Linn (Si-EO). It also includes a toxicological assessment of Si-EO against two significant mosquito species, C. quinquefasciatus and Ae. aegypti, along with an analysis of its effects on natural mosquito predators in comparison to conventional pesticides. The creation of this type of vector control agent represents a potential milestone in public health, offering a secure and efficient method for safeguarding people against diseases transmitted by mosquitoes.

2 Materials and Methods

This research was conducted in a controlled laboratory environment at the Department of Bioinformatics, Saveetha School of Engineering, SIMATS. The study encompassed several procedures, including the collection of plant materials, rearing of pests, conducting bioassays, and engaging in homology modelling and molecular docking. To determine the appropriate sample size for the study, the $g$-power tool was utilised, which suggested a sample size of 10 with a $g$-power value of 0.8.

2.1 Plant Harvesting and Essential Oil Extraction

Known as Koṭṭaikkarantai in Tamil, this plant has a wide range of medicinal applications. It is commonly used for alleviating skin conditions, managing gastric disorders with bleeding, reducing glandular swellings, and providing relief from pain and nervous depression. Additionally, it possesses antibiotic and diuretic properties. Preparations made from this plant have shown effectiveness in treating various ailments, including asthma, leucoderma, jaundice, bronchitis, and scabies.

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sections for steam distillation, using a Clevenger apparatus. The essential oil extracted, known as Si-EO, was carefully gathered in sterilized glass vials. To remove any water remnants, anhydrous sodium sulphate was used, and the Si-EO samples were stored at 4 °C for subsequent research purposes.

2.2 Pest Rearing

Pest rearing, the practice of cultivating and managing pest populations such as insects or rodents, is essential for research and various practical purposes. This technique involves creating optimal environmental conditions, regulating the size of the population, and ensuring adequate food supply. In some cases, it also includes genetic modifications, like the induction of mutations. Essential for a range of research initiatives, pest rearing is also instrumental in controlling pest numbers in environments like farms, cities, and natural habitats. At the Environmental Toxicology Laboratory (BET Lab), the cultivation of Cx. quinquefasciatus and Ae. aegypti has been effectively sustained.

2.3 Larvicidal Bioassay

To evaluate larvicidal activity, a modified version of the World Health Organization's 1981 procedure, as adapted by [11], was employed. The procedure involved using 250 ml glass beakers, each containing four different concentrations of Si-EO (62.5, 125, 250, and 500 ppm). In each beaker, 20 early fourth instar larvae of both C. quinquefasciatus and A. aegypti were introduced. Acetone was used as a control substance separate from EO. Throughout the experiment, the larvae were deprived of food, and their mortality was recorded after 24 hours. This process was repeated five times, with each set including a control group. Further analysis was conducted using Finney's (1971) probit method, and adjustments for control group mortality were made following Abbott's (1925) formula when necessary.

2.4 Statistical Analysis

The accuracy (%) of insect mortality comparison, utilizing the decision tree algorithm and the advanced AdaBoost algorithm, underwent comprehensive scrutiny using IBM-SPSS 29.0.0.0 (241). The primary variable of interest in this study was the accuracy of mortality identification. For the mortality experiment data, variance analysis was conducted (ANOVA of arcsine-square root-transformed percentages, logarithmic). The data presented encompassed mean replicates derived from five assessments. Significant distinctions between insects and plant extracts were ascertained using Tukey’s multiple range test (with significance set at P < 0.05) facilitated by the Minitab®17 software. Additionally, Minitab®17 was employed to perform probit analysis, accompanied by a 95% confidence interval, to compute the lethal concentrations (LC50) required for a 50% larval mortality rate within a 24-hour timeframe.

2.5 Homology Modelling

In homology modelling, we infer the protein's 3-D structure from its primary sequence. This approach predicts the 3-D shape of the target protein by aligning its sequence with those of known proteins, termed homologs, using specialized algorithms. The structure of a template protein guides this process, helping to pinpoint areas of similarity between the target and template. This alignment paves the way for crafting a detailed model of the target protein's 3-D structure. The crystallographic data for the Glutathione S-transferase (GST-Aedes aegypti) protein isn't available within the Protein Data Bank (PDB). Consequently, the SWISS MODEL workspace resorted to homology modelling, leveraging existing templates to predict the protein's structure [11]. Subsequently, this structure underwent refinement via GROMOS and was subsequently subjected to validation through the meta server provided by the structural validation platform.
SAVES software serves a crucial role in evaluating the protein structure's quality, furnishing scores as per the PROCHECK, 3D Verify, and ERRAT algorithms. This entails a comprehensive scrutiny of stereochemical aspects, encompassing rotation, torsion angle, and bond angle stretch, gauging the percentage of residues occupying favourable regions, encompassing non-proline, glycine, and other residues. Moreover, the structure's quality was further assessed through the QMEAN approach, a valuable tool for evaluating the advancement and enhancement of crystallographic models.

2.6 Structural Modelling and Refinement

The study utilized template ID 7rhp.1.A with a sequence identity of 44.13% to conduct homology modelling, aiming to unveil the intricate structure of the glutathione S-transferase protein, as depicted in Figure 2. Subsequently, the model was subjected to rigorous scrutiny to ensure its structural validity. This assessment encompassed the utilization of PROCHECK, VERIFY 3D, and ERRAT. The PROCHECK analysis yielded significant insights, revealing that an impressive 91.5% of residues occupied the most preferred region within the Ramachandran plot. Furthermore, 7.4% were located in the extra permitted region, 1.1% in the generously allowed region, and a remarkable 0.0% ventured into the banned regions. These findings, presented in Figure 3, underscored the structural soundness of the model and its conformity to established standards.

VERIFY 3D exhibited an impressive atomic coordinate compatibility of 93.2%, as depicted in Figure 4A and 4B. Additionally, the overall structural accuracy, assessed by Qmean4, yielded a score of -0.89, while ERRAT confirmed the structure's robustness with an overall protein quality factor of 95.4. This comprehensive validation process was followed by a meticulous molecular docking analysis to explore the intricate conformations and interactions governing functional activity.

2.7 Molecular Docking

In the field of molecular biology, molecular docking stands as a pivotal computational method. Its primary function lies in predicting the optimal arrangement of molecules as they unite to form a stable complex. This technique finds immense utility in forecasting the binding affinities between peptides, proteins, and small molecules and a designated target possessing a known 3D configuration. Molecular docking proves instrumental in the journey of lead compound optimization, facilitating virtual screening across extensive compound libraries, evaluating outcomes, and shedding light on the structural mechanisms through which ligands impede the target.

Within the context of this study, molecular docking was employed to scrutinize the interaction between the Glutathione S-transferase protein and piperine, functioning as a ligand. To facilitate this investigation, Pyrx, a specialized tool tailored for modelling interactions between macromolecules and ligands and for conducting virtual screenings of compound libraries, was utilized. Pyrx employs a docking algorithm to precisely position a given ligand within the binding site of the target protein. The docking process leverages Vina algorithms to orchestrate protein-ligand interactions.

3 Results

The impact of Si-CVO on Ae. aegypti larvae shows a clear relationship between dosage and mortality. At a higher concentration of 500 ppm, mortality rates were the highest, with 91.3% for second instar larvae, 87.4% for third instar larvae, and 83.2% for fourth instar larvae.
contrast, when exposed to a lower concentration of 100 ppm, all larval instars experienced lower mortality rates (see Fig. 1).

Similarly, when Si-CVO is applied to *Cx. quinquefasciatus* larvae, its toxicity varies depending on the dosage, resulting in different mortality rates for larvae in the second, third, and fourth instar larvae. The highest mortality rates, which were 89.7% for second instar, 84.3% for third instar, and 81.7% for fourth instar larvae, were observed at a concentration of 500 ppm. Conversely, at the lower dosage of 100 ppm, all larval stages showed lower mortality rates. Notably, second instar larvae of both pests were most affected by the 500ppm concentration, with a mortality rate of 97%, and this was significantly different from the other treatments. For third instar larvae, the lethal concentrations (LC50 and LC90) were calculated as 231.17 and 425.88 ppm, respectively.

Fig. 1. Bar graph comparison of Aegypti and Culex in terms of mean harmonics.

X Axis: Culex vs. Egypt. Y Axis: Mean Detection Accuracy ± 1 SD.

Fig. 2. 3D structure of protein from Swiss model.
Fig. 3. Procheck-Ramancharan plot from SAVES 6.0.

The further evaluation of the structure’s quality using QMEAN (Fig. 4 A&B).

Fig. 4(A). 3D verification of chain A - SAVES 6.0.

Fig. 4(B). 3D verification of chain B - SAVES 6.0.
In the analysis conducted, it was observed that piperine, functioning as the ligand, established connections with the Glutathione S-transferase protein. Notably, two hydrogen bonds formed during this interaction, specifically between Ser169 (OG) and O2 (Ligand) at a distance of 3.08 Å and between Ser12 (OG) and O1 at a distance of 2.70 Å, as depicted in Figures 2 and 3. Furthermore, the structural quality was rigorously assessed using QMEAN, with visual representations available in Figures 4A and 4B. The examination also revealed the presence of 11 hydrophobic interactions between the Glutathione S-transferase protein and the piperine ligand, illustrated in Figures 5 and 6. Supplementary accuracy values for *Ae. aegypti* and *Cx. quinquefasciatus* can be found in Table 1.

There were 11 hydrophobic interactions present between Glutathione S transferase protein and Piperine ligand (Fig. 5 & 6).

*Fig. 5. ERRAT- SAVES 6.0*
Program: ERRAT2
File: 7rhp.1.A.pdb
Chain#:A
Overall quality factor**: 94.045

*On the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value.

**Expressed as the percentage of the protein for which the calculated error value falls below the 99% rejection limit. Good high-resolution structures generally produce values around 99% or higher. For lower resolutions (2.5 to 3.0 Å) the average overall quality factor is around 91%.

*Fig. 6. 2D structural analysis using Ligplot*
Accuracy values of *Ae. aegypti* and *Culex* are mentioned (Table 1).

**Table 1.** Accuracy values of *Ae. aegypti* and *Culex* are mentioned in the below table.

<table>
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<tr>
<th>Sl.No</th>
<th>Accuracy in %</th>
<th>Aegypti</th>
<th>Culex</th>
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**Table 2.** Group statistical analysis of *Aegypti* and *Culex* by taking each of 5 variables.

<table>
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<tr>
<th>Group Statistics</th>
<th>II Instar</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
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<tr>
<td>Accuracy</td>
<td>Aegypti</td>
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<td></td>
<td>Culex</td>
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**Table 3.** Independent sample test for significance and standard error determination.

<table>
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<tr>
<th>Accuracy</th>
<th>Levene's Test for Equality of Variances</th>
<th>T-test of Equality of Means</th>
<th>95% of the confidence interval of the Difference</th>
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<td>Equal variances assumed</td>
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<td>Equal variances not assumed</td>
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4 Discussion

The persistent global threat posed by mosquito-borne diseases, with dengue and filariasis standing out as formidable adversaries, continues to challenge public health on a global scale. This pressing challenge necessitates the pursuit of innovative solutions that not only safeguard human well-being but also place a strong emphasis on environmental sustainability. In this context, our research embarks on an exploration of the comparative larval toxicity of the plant-derived essential oil extracted from Sphaeranthus Indicus Linn (Si-EO), a subject of study that holds great promise and relevance.

Our investigation ventures into the intricate realm of mosquito control, with a specific focus on two medically significant vectors: Culex quinquefasciatus and Aedes aegypti. These vectors have gained notoriety for their pivotal role in transmitting diseases like dengue and filariasis, afflictions that annually affect millions of individuals worldwide. Si-EO, sourced from the Sphaeranthus Indicus Linn plant, exhibits a remarkable array of properties, encompassing repellence against mosquitoes, larvicidal potency, ovicidal effects, and even adulticidal activity. This multifaceted approach positions Si-EO as a highly promising candidate for bio-insecticide development, potentially revolutionizing prevailing mosquito management strategies.

However, our exploration transcends the boundaries of conventional mosquito control methods. We delve into the molecular intricacies of the Glutathione-S-transferase protein and its intricate interactions with Si-EO. Employing advanced molecular docking techniques, we gain profound insights into the binding affinities between the ligand, piperine, and the protein. These groundbreaking findings hold the potential to catalyse innovative and eco-friendly insecticide development, ushering in a sustainable approach to mosquito control and disease prevention that aligns with the principles of environmental conservation.

5 Conclusion

In this study, we have illuminated the larvicidal toxicity exhibited by the novel essential oil extracted from Sphaeranthus indicus Linn against both Cx. quinquefasciatus and Ae. aegypti larvae. The comprehensive evaluation of repellence, larvicidal, ovicidal, and adulticidal activities underscores the promising potential of Si-EO as a valuable bio-insecticide for combatting medically significant mosquitoes. This promising avenue could lead to more effective and eco-friendly strategies in mosquito control.

Furthermore, our exploration ventured into the molecular realm as we successfully docked the substrate with the Glutathione S-transferase protein, complemented by a thorough 2-dimensional structural analysis utilising ligplot. This multifaceted approach not only enhances our understanding but also opens doors to innovative solutions in the field of insecticide development and vector control.

In a world grappling with the consequences of mosquito-borne diseases, this study embarks on a journey towards a more sustainable and effective approach to vector control. Si-EO emerges as a beacon of hope, offering a holistic solution that not only safeguards human health but also champions environmental well-being.

References


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