MOSQUITO LARVICIDAL EFFICACY OF THE ACETONE LEAF EXTRACT OF SOLANUM TRIBOLATUM AGAINST CULEX QUINQUEFASCIATUS AND AEDES AEGYPTI

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INTRODUCTION

Mosquito-borne disease is a major health problem in worldwide for both human and veterinary sectors. Mosquitoes are a serious threat to the public and also health transmitting several dangerous diseases in tropics [1]. Diseases transmitted by mosquitoes include malaria, dengue hemorrhagic fever, Japanese encephalitis, yellow fever, and filariasis [2]. Among the 13 genera of the family Culicidae, Anopheles species, which includes alkaloids, phenolics, flavonoids, sterols, saponins, and their glycosides [13]. Alkaloids such as soladunalinidine and tomatidine were isolated from leaf and stem of Solanum species. Therefore, the present investigation was carried out to determine the mosquito larvicidal activity of S. trilobatum leaf against two mosquito vectors C. quinquefasciatus and Aedes aegypti.

METHODS

Plant collection and extraction

Healthy, disease free, mature leaves of S. trilobatum were collected from the region of Annur, Coimbatore District, Tamil Nadu. The collected plant material was identified and authenticated in Tamil Nadu Agricultural University (TNAU), Tamil Nadu. Leaves of S. trilobatum were thoroughly washed with running tap water. The leaves were again washed with sterile distilled water to remove the dirt before drying process. Then, the leaves were dried in shade room temperature for a week to find out the presence of various phytochemical constituents.

Phytochemical analysis of S. trilobatum

Freshly prepared leaf extracts of S. trilobatum were subjected to standard phytochemical analysis according to Trease and Evans [14] to find out the potential larvicidal activity of S. trilobatum. It is one among the major disease burden in developing countries. There are lots of researches carried out using the various plant extracts against larvicidal activities of the Culex quinquefasciatus and Aedes aegypti. The present investigation was aimed to investigate the phytochemical analysis and mosquito larvicidal activities of Solanum trilobatum in acetone extract against the second instar larva of C. quinquefasciatus and A. aegypti.

Methods: The leaf extract of S. trilobatum was subjected to phytochemical analysis and Gas Chromatography–Mass Spectrometry analysis. The mortality rates of the second instar larvae were recorded after 24, 48, 72, and 96 h of exposure. The lethal concentration (LC50) and LC90 were determined followed by probit analysis.

Results: The LC50 values for C. quinquefasciatus were found to be 265.69 ppm, 227.59 ppm, 212.42 ppm, and 189.47 ppm at various time intervals, and the LC90 values were 558.27 ppm, 504.92 ppm, 479.09 ppm, and 444.28 ppm. Similarly, LC50 values for A. aegypti were noticed at 30.19 ppm, 25.60 ppm, 209.75 ppm, and 167.44 ppm and the LC90 values were 582.34 ppm, 477.52 ppm, 419.40 ppm, and 371.84 ppm for the time interval of 24 h, 48 h, 72 h, and 96 h.

Conclusion: The result of the current work revealed that the leaf extract of the S. trilobatum has the potential to be acted as an alternative for the controlling of the mosquitoes.

Keywords: Gas Chromatography–Mass Spectrometry, Phytochemical analysis, Probit analysis, Mortality rate.

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**Gas Chromatography–Mass Spectrum (GC–MS) analysis**

GC–MS analysis is a common confirmation test to separate all the compounds in a sample and provides a representative spectral output. The sample is injected into the injection port of the GC device. The GC instruments vaporize the sample and then separate and analyze the various compounds ideally produce a specific spectral peak that may be recorded on a paper chart or electronically. The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. An aliquot of 2 μl of sample was injected with temperature at 28ºC. GC oven temperature started at 70ºC and holding for 8 min, and it was raised to 220ºC holding for 7 min at the rate of 10ºC/min. Iron source temperature was maintained at 300ºC. The detector was operated scan mode fit with interval of 0.5 s.

### Preparation of stock solution

Dried and powdered aerial parts (1 kg) were macerated sequentially with 3 L of acetone for 96 h and filtered. The crude plant extracts were evaporated to dryness in a room temperature.

### Test organisms

The 11 instar larvae of *C. quinquefasciatus* and *A. aegypti* obtained from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore, Tamil Nadu, India. The larvae were kept in enamelled trays containing tap water. The larvae were maintained at 25–29ºC and 75-85% relative humidity under 14:10 light and dark cycles.

### Larvicidal bioassay

The larvicidal bioassay was carried out according to standard the WHO protocol with minor modifications. From the stock solution, different concentrations of 100, 200, 300, 400, and 500 ppm were prepared. 25 healthy second instar larvae were introduced into beaker containing 200 ml of water with each test concentration. Larval mortality was observed at 24 h, 48 h, 72 h, and 96 h. A total of three trials were performed [15].

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\text{Percent mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100
\]

### Statistical analysis

SPSS 12 version package was used for the determination of lethal concentration (LC) values also calculated for both *C. quinquefasciatus* and *A. aegypti*. The experiment data of larval mortality and effect of concentration were subjected to analysis of variance (ANOVA) followed by probit analysis to determine the difference in larval mortality between concentrations.

### RESULTS AND DISCUSSION

**Phytochemical screening**

The preliminary phytochemical analysis of the *S. trilobatum* leaf extract of acetone solvent revealed the presence of alkaloids, tannins, steroids, flavonoids, carbonhydrates, glycosides, saponins, tannins, terpenoids, and anthraquinone. The present results of phytochemical compounds are coincident with other researchers [17-19].

### GC–MS analysis

Plant possesses immense medicinal properties. The aim of the present work was to identify the phytochemicals present in acetone extracts of the leaves of *S. trilobatum* by qualitative phytocchemical testing and to identify the compounds present in the acetone extract of the leaves by GC–MS analysis (Fig. 1). The GC–MS spectrum revealed that the acetone leaf extract of *S. trilobatum* contained major phytochemical compounds such as cyclicdeanol (12.42%), 2-tetradecyloxyiane (6.07%), betasitosterol (10.25%), 1H-imidazole, 4,5-dimethyl-imidazole (4.65%), 1-octadecyne (4.30%), and phenol (4.05%). The other compounds also identified are diisooamylene (2.70%), chloro-5-ido-benzoic acid pyridine-3-y1 methylene-hydrazide (1.70%), 4-(2-oxo-bicyclo (3.1.0) hex-6-y1) (1.57%), permentrin-1,3-phenoxbenzyl (1.36), 1H-pyrazole-5-carboxamide (2.30%), 2-cyclohexan-1-ol (1.30%), benzene,2-(tet-butylidimethylhydroy) oxy (2.63%), hydrochlorothiazide N-butylbronate (1.99%), silicic acid diethyl bis(trimethylsilyl)ester (2.83%), and isopomyltris (3.48%) (Table 1). Its presence in the plant suggests that it to be of medicinal value because tannins have shown potential antiviral and antiparasitic effects [20]. Krishnavi and Prashant [21] reported that the similar compound detected was 3,7,11,15-tetramethyl-2-hexadecen-1-ol in the present result.

### Larvicidal activity

Larvicidal activity of acetone extract of *S. trilobatum* leaves at various concentrations was tested against filarial and dengue vectors such as *C. quinquefasciatus* and *A. aegypti*. Acetone extract of *S. trilobatum* leaves exhibited potential larvalicidal activity at varying concentrations, whereas no larval death was observed in controls. The mortality rate obtained was 25.33% (24 h) and 37.3% (96 h) at a concentration of 100 ppm, whereas the maximum mortality rate was obtained at 300 ppm for 24 h (86.7%) and 96 h (97.3%). It clearly depicted that the larvicidal effect of acetone leaf extract of *S. trilobatum* was a dose dependent as well as duration of exposure. Similarly, larvicidal effect of acetone extract of *S. trilobatum* on *A. aegypti* exhibited minimum larval death rate at concentration of 100 ppm in 24 h (17.3%) and 96 h (38.67%). The maximum mortality 81.33% and 100% was observed at a concentration of 500 ppm at 24 h and 96 h, respectively. The mortality rate was recorded maximum at 96 h compared to 24, 48, and 72 h for both *C. quinquefasciatus* and *A. aegypti*.

The LC50 values for *C. quinquefasciatus* were found to be 265.69 ppm, 227.59 ppm, 212.42 ppm, and 189.47 ppm for the time intervals of 24 h, 48 h, 72 h, and 96 h, respectively. Similarly, LC50 values for *A. aegypti* were noticed at 301.09 ppm, 256.01 ppm, 209.75 ppm, and 167.44 ppm at time intervals of 24 h, 48 h, 72 h, and 96 h, respectively. LC50 values also calculated for both *C. quinquefasciatus* and *A. aegypti*. The LC50 values for *C. quinquefasciatus* was 558.27 ppm, 504.92 ppm, 479.09 ppm, and 444.26 ppm for the time interval of 24 h, 48 h, 72 h, and 96 h, respectively. The LC50 values of *A. aegypti* were 582.34 ppm, 477.52 ppm, 419.40 ppm, and 371.84 ppm for the time interval of 24 h, 48 h, 72 h, and 96 h, respectively (Table 3).

Regression analysis revealed that the mortality rate (Y) was positively correlated with the concentration (X). Two-way ANOVA analysis demonstrated that the larvicidal effect exhibited by various concentrations of acetone leaf extract of *S. trilobatum* on *C. quinquefasciatus* and *A. aegypti* was statistically significant (p<0.05) at 48 and 96 h.

Rajkumar and Jebanesan [12] reported that the acetone leaf extract of *S. trilobatum* acts as oviposition deterrents. This indicates that *C. quinquefasciatus* and *A. aegypti* mosquitoes were acutely sensitive to phytochemical stimuli and responded to the odor of the acetone leaf extract produce maximum effective repellency against oviposition.

Preamalatha et al. [22] reported that the larvicidal activity methanol extract of *S. trilobatum*...
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Leaf extract of *S. trilobatum* was found to be more susceptible against the larvae of *A. aegypti*, *C. quinquefasciatus*, and *A. stephensi*. Leaf extract of *S. trilobatum* also exhibited pupicidal and adult emergence properties [23,24]. The plant extract provides the bases to act as alternative to synthetic insecticide to control program of mosquito [25]. Finally, the study revealed that the plant origin chemicals from the *S. trilobatum* leaf extracts showed that insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity.

Fig. 1: Gas chromatography–mass spectrum of acetone extract of *Solanum trilobatum* leaves

Table 2: Phytochemical compounds identified in the acetone leaf extract of *Solanum trilobatum* leaves by GC–MS analysis

| S. No | RT     | Area % | Peak Height % | Name of the compound                                      |
|-------|--------|--------|---------------|-----------------------------------------------------------|
| 1     | 15.025 | 15.58  | 46.15         | Tungsten                                                 |
| 2     | 18.051 | 4.65   | 0.73          | 1H-imidazole, 4,5-dimethyl-imidazole                      |
| 3     | 21.823 | 12.42  | 14.43         | Cyclocedanol                                             |
| 4     | 21.783 | 2.90   | 2.99          | Disoamylene                                              |
| 5     | 22.082 | 2.76   | 2.96          | 3,7,11,15-Tetramethyl-2-hexadeen-1ol                     |
| 6     | 22.204 | 1.34   | 1.45          | 1,2-Benzenedicarboxylic acid, Bis (2-methylpropyl) ester |
| 7     | 22.272 | 4.30   | 4.44          | 1-Octadecyne                                             |
| 8     | 23.193 | 4.05   | 1.81          | Phenol                                                   |
| 9     | 23.300 | 1.48   | 0.73          | Propennitrile, 3-ethoxy-2-(2-thienylm)                   |
| 10    | 24.861 | 6.48   | 5.09          | 2-Tetradecylociane                                       |
| 11    | 25.413 | 1.58   | 0.61          | Bicycle (3.2.1) octan-2-ol                               |
| 12    | 35.967 | 2.01   | 0.09          | 2-(1-bromoethyl)-4,5-dimethoxyoxycarbamid                |
| 13    | 36.192 | 2.04   | 1.06          | L-Alanine                                                |
| 14    | 36.726 | 6.07   | 2.68          | 3-Keto-isotetiol                                         |
| 15    | 38.135 | 10.25  | 4.07          | Beta-sitosterol                                          |
| 16    | 38.358 | 1.70   | 0.69          | 2-Chloro-5-iodo-benzoic acid pyridine-3-ylmethylen-hydrazide |
| 17    | 38.472 | 1.57   | 0.99          | 4-(2-oxo-bicyclo (3.1.0) hex-6-yl)                      |
| 18    | 38.815 | 2.78   | 1.29          | Rosellanic acid                                         |
| 19    | 39.463 | 1.36   | 0.78          | Permethrin-1, 3-phenoxybenzyl                            |
| 20    | 40.061 | 2.38   | 1.51          | 1H-pyrazole-5-carboxamide                                |
| 21    | 40.142 | 1.38   | 1.11          | 2-Cyclohexen-1-ol                                       |
| 22    | 41.034 | 2.63   | 0.81          | Benzene, 2-(tert-butyl)dimethylsilyl oxy                  |
| 23    | 41.543 | 1.99   | 0.66          | Hydrochlorothiazide H-butylbronate                       |
| 24    | 41.866 | 2.83   | 0.84          | Silicic acid diethyl bis (trimethylsilyl) ester          |
| 25    | 42.586 | 3.48   | 1.22          | Isopropyl tris                                          |

GC-MS: Gas chromatography–mass spectrum

Table 3: Probit analysis for the data on larval mortality of acetone extract at 95% confidence limit

| Hours | Mean % larval mortality | LC<sub>50</sub> | LC<sub>90</sub> | Regression equation | R value | Chi-square value |
|-------|------------------------|----------------|----------------|---------------------|---------|-----------------|
|       |                        | (ppm)          |                |                     |         |                 |
| 100   |                        |                |                |                     |         |                 |
| 210   |                        |                |                |                     |         |                 |
| 300   |                        |                |                |                     |         |                 |
| 400   |                        |                |                |                     |         |                 |
| 500   |                        |                |                |                     |         |                 |

*Culex quinquefasciatus*:

- 24 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74
- 48 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74
- 72 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74
- 96 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74

*Aedes aegypti*:

- 24 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74
- 48 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74
- 72 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74
- 96 hours: Mean % larval mortality = 24, LC<sub>50</sub> = 37.33, LC<sub>90</sub> = 54.67, Regression equation: Y = 15.602x + 8.128, R value = 0.99, Chi-square value = 0.74

Chi-square value significant at p<0.05 level. LC: Lethal concentration, LCL: Lower confident limit, UCL: Upper confidence limit
CONCLUSION

Results from the current study demonstrated that the acetone leaf extract of \textit{S. trilobatum} has significantly higher larvicidal activity against selected human vector mosquitoes \textit{C. quinquefasciatus} and \textit{A. aegypti}. The phytochemicals present in \textit{S. trilobatum} leaf extracts are effective mosquito vector control agents, and the plant extracts may be used for the further integrated pest management programs. In conclusion, the obtained results suggested that the effective plant crude extracts have the potential to be used as an ideal eco-friendly approach for the control of diseases vectors.

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AUTHORS’ CONTRIBUTIONS

Author 1 has planned and conducted the experiment. The experiment was guided by Author 2.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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