Evaluation of *Solanum trilobatum* L. (Solanaceae) aerial extracts for mosquito larvicidal activity against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

Murugesan Sakthivadivel, Palani Gunasekaran, Murugesan Sivakumar, Arul Samraj, Subramanian Arivoli and Samuel Tennyson

Abstract

Crude petroleum ether, chloroform, and acetone aerial extracts of *Solanum trilobatum* were tested for larvicidal activity against the filarial vector, *Culex quinquefasciatus*. Standard W.H.O. protocols with minor modifications were adopted for the larvicidal bioassay. Larvicidal activity was evaluated at concentrations of 62.5, 125, 250, 500 and 1000 mg/L. Larval mortality was observed for 24 and 48 h. Amongst the solvent extracts tested, petroleum ether exhibited highest larvicidal activity and LC50 values was 203.87 and 165.04 followed by acetone and chloroform extract which were 295.87 and 186.38; 535.74 and 346.08 mg/L after 24 and 48 h respectively. Further investigations are needed to explore the larvicidal activity of the petroleum ether aerial extract of this plant against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

Keywords: *Solanum trilobatum*, crude aerial extracts, *Culex quinquefasciatus*, larvicidal activity

1. Introduction

Mosquitoes are a serious threat to public health transmitting several dangerous diseases in the tropics [1]. *Culex quinquefasciatus*, the principle vector for filariasis is one of the cosmopolitan mosquitoes which occur in human habitations in the tropics and subtropics of the world. There has been a large increase in the insecticide resistance of this vector and it has become a global problem. Insecticides residues in the environment, as a result of chemical insecticide usage, have turned the researcher’s attention towards natural products [2]. In the past years, the plant kingdom has been of great interest as a potential source of insecticidal products. Many species in the plant kingdom synthesize a variety of secondary metabolites which play a vital role in defense of plants against insects/mosquitoes. Phytochemicals obtained from plants are usually less environmentally harmful than synthetic chemicals and it has renewed the interest in the research on these compounds, considering them as an ecologically safe alternative for synthetic insecticides [3].

Crude plant extracts and inorganic larvicides were largely used as natural insecticides before the organic laboratory-synthesized insecticides became available in the 1940s [4–6]. Applications of phytochemicals in mosquito control were in use since the 1920s [7], but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities [8].

Unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes.
Thus, there is a very little chance of pests developing resistance to such substances. Identifying bioinsecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. The botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative products to fight against mosquito-borne diseases\(^9\).

Crude plant materials and botanical derivatives had primary role for pest control in agriculture, veterinary and public health in ancient era. Chemically synthesized insecticides, due to their magical success replaced the popularity at intermediate time. Over and injurious application of synthetic insecticides for long term causing various impairment of eco-system has renewed the importance of insecticides of green origin recently. Different plants contain different complex chemicals with unique biological activities. Literature provides a good volume of documents on efficacy of plant derived materials against different mosquitoes\(^8\)-\(^19\).

Solanum trilobatum grows as a climbing undershrub widely distributed in India and Sri Lanka\(^20\)-\(^21\). The plant is known as alarka in Sanskrit, alarkapatramu in Telugu, tuduvalai in Tamil and tutuvalam in Malayalam\(^21\). Solanum trilobatum is used in the field of folk medicine for treating all kinds of cough\(^21\), diabetes, vomiting with blood and leprosy\(^22\). Besides these, it is also used to increase male fertility and also counter acts diabetes, vomiting with blood and leprosy\(^22\). Further, the plant is rich in phytochemical constituent like alkaloids, hepatoprotective\(^27\)-\(^29\), antiulcer\(^30\) and antiandradru\(^31\). The plant is also rich in phytochemical constituent like alkaloids, phenolics, flavonoids, sterols, saponins and glycosides\(^30\). Further, the plant is reported for its mosquitocidal property which includes ovicidal activity against Culex quinquefasciatus and Culex tritaeniorhynchus\(^32\), larvicial activity\(^33\), oviposition deterrent and skin repellent activity against Anopheles stephensi\(^32\). Recently, Premalatha et al.\(^34\) reported the acetone, chloroform and methanol leaf extracts of Solanum trilobatum for its larvicial, pupicidal and adult emergence inhibition properties against Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi. Therefore, in the present investigation, the crude aerial extracts of Solanum trilobatum has been evaluated for its larvicial activity against Culex quinquefasciatus.

### 2. Materials and Methods

#### 2.1. Plant collection and extraction

Mature aerial parts of Solanum trilobatum collected from places in and around Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered aerial parts (1 Kg) was macerated sequentially with 3 L of petroleum ether, chloroform and acetone for a period of 96 h and filtered. The filtrate was then concentrated at reduced temperature on a rotary vacuum evaporator. The crude petroleum ether, chloroform and acetone aerial extracts thus obtained were lyophilized and a stock solution of 1,00,000 mg/L prepared by adding adequate volume of Tween 80 was refrigerated at 4 °C until testing for bioassay.

#### 2.2. Larvicidal bioassay

Bioassay was carried out against laboratory reared Culex quinquefasciatus mosquitoes free of exposure to insecticides. Standard W.H.O.\(^35\) protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. Mosquito immature particularly third instar larvae were obtained from laboratory colonized mosquitoes of F\(_1\) generation. From the stock solutions, concentrations of 62.5, 125, 250, 500 and 1000 mg/L was prepared. Ten healthy larvae were released into each 250 mL glass beaker containing 200 mL of water and test concentration. Larval mortality was observed for 24 and 48h after treatment. Larvae were considered dead when they showed no signs of movement when provoked on their respiratory siphon by a needle. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of Tween 80 to distilled water. Distilled water served as control. The larval per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott’s formula\(^36\). SPSS 11.5 version package was used for determination of LC\(_{50}\) and LC\(_{90}\) values\(^37\). One way ANOVA followed by Duncan multiple range test (DMRT) was performed to determine the difference in larval mortality between concentrations.

### 3. Results

The results of Culex quinquefasciatus larval mortality tested against Solanum trilobatum crude petroleum ether, chloroform and acetone aerial extracts are presented in Table 1 and 2. No larval mortality was observed in treated and untreated control after 24 and 48 h. The results of the present study revealed that among the solvent extracts tested, petroleum ether exhibited highest larvicidal activity and LC\(_{50}\) values was 203.87 and 165.04 followed by acetone and chloroform extract which were 295.87 and 186.38; 535.74 and 346.08 mg/L after 24 and 48 h respectively (Table 3). Further, one hundred per cent larval mortality was observed at 1000 mg/L in all the extracts after 24 h exposure and at 500 mg/L in petroleum ether extract only after 48 h.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Concentration (mg/L)</th>
<th>Untreated control</th>
<th>Treated control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>125</td>
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<td></td>
<td></td>
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<td>500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0.00 ±0.00(^a) (0.0)</td>
<td>1.33 ±0.57(^a) (13.3)</td>
<td>4.66 ±0.57(^a) (46.6)</td>
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<tr>
<td>Chloroform</td>
<td>0.00 ±0.00(^a) (0.0)</td>
<td>1.33 ±0.57(^a) (13.3)</td>
<td>3.66 ±0.57(^a) (36.6)</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.00 ±0.00(^a) (0.0)</td>
<td>1.66 ±0.57(^a) (16.6)</td>
<td>3.66 ±0.57(^a) (36.6)</td>
</tr>
</tbody>
</table>

Values are mean of the three replicates of three trials ±standard deviation. Figures in parenthesis denote per cent larval mortality. Different superscript alphabets within same row indicate statistical significant difference at P <0.05 level by one way ANOVA followed by DMRT.

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Table 2: Larval mortality of Culex quinquefasciatus against crude aerial extracts of Solanum trilobatum at 48 h

<table>
<thead>
<tr>
<th>Solvents</th>
<th>LC50 (mg/L)</th>
<th>LC90 (mg/L)</th>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>203.87</td>
<td>165.04</td>
</tr>
<tr>
<td>Chloroform</td>
<td>535.74</td>
<td>346.08</td>
</tr>
<tr>
<td>Acetone</td>
<td>295.87</td>
<td>186.38</td>
</tr>
<tr>
<td>Untreated control</td>
<td>366.49</td>
<td>571.23</td>
</tr>
<tr>
<td>Treated control</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Values are mean of three replicates of three trials ± standard deviation. Figures in parenthesis denote cent larval mortality.

4. Discussion

Vector-borne diseases caused by mosquitoes have become a global health problem. A review of botanical phytochemicals with mosquitoicidal potential has been published [8], demonstrating identification of novel effective mosquitoicidal from botanicals containing active phytochemicals. Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic potential insecticides or along with other insecticides under the integrated vector control. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito-borne diseases [9].

One of the earliest reports on the use of plant extracts against mosquito larvae is credited to Campbell et al. [38] who found the plant alkaloids like nicotine, anabasine, methyl anabasine and lupinie extract from Anabasis aphylla killed larvae of Culex pipiens, Culex territans and Culex quinquefasciatus. Screening of botanical material if attempted, the solvents for mosquito larvae is credited to Campbell [39]. Anabasis aphylla, Ocimum suaveolens, Artemisia absinthium and Trichosanthes anguina has been reported to have larvicidal activity against Culex quinquefasciatus [40]. Screening of botanical material is a critical step in the development of new larvicidal agents.

Table 3: Probit analysis of larvicidal efficacy of crude aerial extracts of Solanum trilobatum against Culex quinquefasciatus

<table>
<thead>
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</table>

Values are mean of three replicates of three trials ± standard deviation. Figures in parenthesis denote cent larval mortality.

5. Acknowledgements

Authors thank the Director, King Institute of Preventive Medicine and Research for the facilities provided.

Ramasswamy [42] reported the acetone extract of Agaritina adolphora twigs to be effective against the larvae of Culex quinquefasciatus (LC50 227.20 ppm). Arivoli and Samuel [43] reported that the ethyl acetate leaf extract of Strychnos nux-vomica was effective against the larvae of Culex quinquefasciatus and LC50 values were 222.28 and 146.99 ppm after 24 and 48 h. The dichloromethane extract of Citrullus colocynthis whole plant provided hundred per cent mortality and LC50 value was 240.36 ppm [44] and diethyl ether extract of Abutilon indicum leaf exhibited a LC50 value of 395.90 ppm [45] and the hexane extract of Hypis suaveolens aerial parts a LC50 value of 203.37 ppm [46] after 24 h of exposure against the third instar larvae of Culex quinquefasciatus. The acetonic leaf extract of Ocimum sanctum exhibited LC50 value of 592.60 ppm against Culex quinquefasciatus [47]. The toxicity to the third instar larvae of Culex quinquefasciatus by methanolic leaf extract of Momordica charantia, Trichosanthes anguina and Luffa acutangula showed LC50 values of 465.85, 567.81 and 839.81 ppm, respectively [48].

Vector control is one of the most powerful weapons in the process of managing vector populations so as to reduce/interrupt the transmission of disease. As a result, vector control remains considered to be a cornerstone in the vector-borne disease control program due to lack of reliable vaccine, drug resistance parasites and insecticide resistance of insect vectors [49]. Plants and plant parts have provided a good source of inspiration for novel drug compounds, as plant derived drugs have made large contribution to human health. The use of plant extracts, as well as other alternative forms of medical treatment, are enjoying great popularity since the late 1990s [50]. Different parts of plants contain a complex of chemicals with unique biological activity [51]. Preliminary screening is a good approach to evaluate the potential larvicidal activity of plants [17, 52] and the activity of crude plant extracts subjected further to partial purification with respective solvent washed fraction is often distributed to the complex mixture of active compounds [53]. Natural product research using botanicals are progressively going on to identify novel bio-potency compounds to replace environmentally hazardous synthetic pesticides. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use Solanum trilobatum petroleum ether aerial extracts to control the immature stages of vector mosquitoes.
6. References


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