Evaluation of larvicidal efficacy of *Cleome viscosa* L. (Capparaceae) aerial extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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**ABSTRACT**

**Objective:** To evaluate the larvicidal efficacy of crude petroleum ether, chloroform, acetone and aqueous aerial extracts of *Cleome viscosa* against the filarial vector, *Culex quinquefasciatus*.  

**Methods:** Standard World Health Organization protocols with minor modifications was adopted for the larvicidal bioassay. Larvicidal activity of extracts was evaluated at concentrations of 62.5, 125, 250, 500 and 1000 mg/L. Larval mortality was observed after 24 and 48 h.

**Results:** Amongst the solvent extracts tested, petroleum ether exhibited the highest larvicidal activity and LC₅₀ values was 52.62 and 43.16 mg/L followed by acetone, aqueous and chloroform extract with LC₅₀ values of 328.64 and 280.58; 493.44 and 298.76; 509.27 and 434.40 mg/L after 24 and 48 h respectively.

**Conclusions:** 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.

1. Introduction

Mosquito is the most indisputable medicinal significant arthropod vector of diseases[1]. The application of chemical insecticides for decades has met with tremendous setbacks in the light of the development of vector resistance and some attendant environmental hazards[2]. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. The body of scientific literature documenting bioactivity of plant derivatives to different pests continues to expand, yet only a handful of botanicals are currently used[3]. Natural insecticides of plant origin have been regarded important due to their ecofriendly nature and biodegradability as a substitute of synthetic insecticides for the control of vectors of public health importance. Botanical larvicides can contribute remarkably to the lowering of vector mosquito population[4–9]. Different types of phytochemicals of plant either from the whole part or from the specific parts come out with solvent during chemical extraction depending on the polarity of the solvent[10]. These phytochemicals generally act as a toxicant towards adult, as well as larval form of mosquitoes, while some interfere with the growth (growth inhibitory activity). Insecticides of botanical origin are attractive alternatives because they contain rich sources and various bioactive compounds, many of which are selective and have little or no harmful effect on non–target organisms and the environment[6]. Furthermore, the complex and variable mixtures of bioactive constituents with different modes of action may lessen the chance of resistance in mosquito populations[11].

*Cleome viscosa* L. (*C. viscosa*) (Capparaceae) is an annual, herbaceous plant present in Northern and North Eastern parts[12,13], over the plains of India, and throughout the tropics of the world[12]. The plant is also found distributed in the plains of Arabia, Egypt, Pakistan and also in Asia and Africa[14]. *C. viscosa* is commonly known as wild or dog mustard[12], tickweed, wild mustard and spider plant in English[15], nayikkadugu in Tamil and hurhuria in Bengali[15,16]. *C. viscosa* is used in Indian system of medicine for treating fever, inflammations, liver diseases,
bronchitis and diarrhoeal[17]. The whole herb is used in treatment of ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders[18]. C. viscosa is reported to possess pharmacological properties viz., antihelminthic, antipyretic, anti diarrhoeal, anti-inflammatory, hepatoprotective, antiseptic, carminative, febrifuge, sudorific, cardiac stimulant and immunomodulatory, and the phytochemical constituents/compounds including alkaloids, flavanoids, saponins, tannins and steroids and diterpenoids[19]. Very few reports are present pertaining to the insecticidal property of C. viscosa. C. viscosa leaves was found to possess insecticidal activity when tested against the larva of Culex quinquefasciatus (Cx. quinquefasciatus) [20]. Recently, Bansal et al. has reported on the larvicidal potential of crude petroleum ether, acetone and methanol seed extracts of C. viscosa against Anopheles stephensi, Aedes aegypti and Cx. quinquefasciatus[21]. Therefore, in the present investigation, the crude aerial extracts of C. viscosa has been evaluated for its larvicidal activity against Cx. quinquefasciatus.

2. Materials and methods

2.1. Plant collection and extraction

Mature aerial parts of C. viscosa 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, acetone and distilled water for a period of 96 h and filtered. The filtrate was then concentrated at reduced temperature on a rotary evaporator. The crude petroleum ether, chloroform, acetone and aqueous aerial extracts thus obtained were lyophilized and a stock solution of 100000 mg/L prepared by adding adequate volume of Tween 80 at 4 °C until testing for bioassay.

2.2. Larvicidal bioassay

Bioassay was carried out against laboratory reared vector mosquitoes with free exposure to insecticides. Standard World Health Organization protocol with slight modifications was adopted for the study[22]. The tests were conducted in glass beakers. Mosquito immature particularly third instar larvae were obtained from laboratory colonized mosquitoes of F1 generation. From the stock solutions, concentrations of 62.5, 125, 250, 500 and 1000 mg/L was prepared. Twenty healthy larvae were released into each 250 mL glass beaker containing 200 mL of water and extracts with test concentrations. Larval mortality was observed for 24 and 48 h after treatment. Larvae were considered dead if 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 water. Distilled water served as untreated control. The larval per cent mortality was calculated and when control mortality ranged from 5%-20% it was corrected using Abbott’s formula[23]. SPSS 11.5 version package was used for determination of LC50 and LC90 values. One way ANOVA followed by Duncan multiple range test (DMRT) was performed to determine the difference in larval mortality between concentrations.

### Table 1
Larval mortality of Cx. quinquefasciatus by crude aerial extracts of C. viscosa at 24 h.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>62.5 (mg/L)</th>
<th>125 (mg/L)</th>
<th>250 (mg/L)</th>
<th>500 (mg/L)</th>
<th>1000 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>7.66±0.57%</td>
<td>9.66±0.57%</td>
<td>10.00±0.00%</td>
<td>10.00±0.00%</td>
<td>10.00±0.00%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.00±0.00%</td>
<td>0.00±0.00%</td>
<td>4.33±1.53%</td>
<td>4.66±1.15%</td>
<td>9.66±0.57%</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.00±0.00%</td>
<td>3.00±1.06%</td>
<td>5.33±0.57%</td>
<td>6.66±1.15%</td>
<td>9.66±0.57%</td>
</tr>
<tr>
<td>Aqueous</td>
<td>0.33±0.57%</td>
<td>2.00±1.00%</td>
<td>4.00±1.00%</td>
<td>5.33±1.53%</td>
<td>9.66±0.57%</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.

### Table 2
Larval mortality of Cx. quinquefasciatus against crude aerial extracts of C. viscosa at 48 h.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>62.5 (mg/L)</th>
<th>125 (mg/L)</th>
<th>250 (mg/L)</th>
<th>500 (mg/L)</th>
<th>1000 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>9.66±0.57%</td>
<td>10.00±0.00%</td>
<td>10.00±0.00%</td>
<td>10.00±0.00%</td>
<td>10.00±0.00%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.00±0.00%</td>
<td>1.00±1.00%</td>
<td>4.66±0.57%</td>
<td>5.66±1.15%</td>
<td>9.66±0.57%</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.66±0.57%</td>
<td>3.66±1.15%</td>
<td>6.33±0.57%</td>
<td>7.33±1.15%</td>
<td>10.00±0.00%</td>
</tr>
<tr>
<td>Aqueous</td>
<td>0.66±0.57%</td>
<td>2.33±0.57%</td>
<td>4.00±1.00%</td>
<td>6.33±1.15%</td>
<td>10.00±0.00%</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.
aceton, aqueous and chloroform extract which were 328.64 and 280.58 mg/L; 493.44 and 298.76 mg/L; 509.27 and 434.40 mg/L after 24 and 48 h respectively (Table 3).

Table 3
Probit analysis of larvicidal efficacy of crude aerial extracts of C. viscosa against Cx. quinquefasciatus.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$L_{C50}$ (mg/L)</th>
<th>$L_{C90}$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>52.62</td>
<td>43.16</td>
</tr>
<tr>
<td>Chloroform</td>
<td>509.27</td>
<td>434.40</td>
</tr>
<tr>
<td>Acetone</td>
<td>328.64</td>
<td>280.58</td>
</tr>
<tr>
<td>Aqueous</td>
<td>493.44</td>
<td>298.76</td>
</tr>
</tbody>
</table>

4. Discussion

Mosquitoes are a serious threat to public health, transmitting several dangerous diseases for over two billion people in the tropics. Vector–borne diseases caused by Cx. quinquefasciatus and other mosquitoes have become a global health problem.[24] Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and may act as suitable alternative product to fight against mosquito–borne diseases.[6] In the past years, the plant kingdom has been of great interest as a potential source of insecticidal products. In addition, natural insecticides can provide core structures from which new insecticidal agents can be synthesized.[25] Many species in the plant kingdom synthesize a variety of secondary metabolites which play an active 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.[26] 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. Plant products can be used as an insecticide for killing mosquito larvae.[27] Products of secondary plant metabolisms may be responsible for the chemical communication between plants and insects. Allelochemicals have been considered as potential natural insecticides and can be used for insect/mosquito management in integrated control. Reviews on botanical phytochemicals with mosquitocidal potential published by Shaalan et al. and Ghosh et al. demonstrated identification of novel effective mosquitocidal agents from botanicals containing active phytochemicals.[6,28] The review gives current state of knowledge on larvicidal plant species, extraction processes, residual capacity, effects on non–target organisms, resistance, screening methodologies, and discusses promising advances made in phytochemical research for vector control. Plants have been screened for their larvicidal activity against Cx. quinquefasciatus.[8] The results of the present study indicated that petroleum ether extract was found to be effective and $L_{C50}$ value was 52.62 and 43.16 mg/L after 24 and 48 h followed by those of acetone, aqueous and chloroform which were 328.64 and 280.58, 493.44 and 298.76, 509.27 and 434.40 mg/L respectively. The results of the present study were comparable with the reports of Bansal et al., who found that the crude petroleum ether, acetone and methanolic seed extracts of C. viscosa exhibited larvicidal activity against Anopheles stephensi, Aedes aegypti and Cx. quinquefasciatus and $L_{C90}$ values was 166.4, 106.3, 144.1, and 51.4, 138.9, 99.5 and 301.9, 118.5, 127.1 mg/L respectively by 24 h exposure.[23] Pavela et al. reported that the methanolic stem extract of Artemisia campestris exhibited larvicidal activity against Cx. quinquefasciatus and $L_{C50}$ value was 23 mg/L.[29] Besides these, the methanolic extracts of Gloeosmis coronarium flowers ($L_{C50}$ 53 mg/L), Sonchus arvensis stem ($L_{C90}$ 68 mg/L), Matricaria maritima flowers ($L_{C50}$ 72 mg/L),[30] Tagetes erecta petroleum ether leaf extract ($L_{C50}$ 100 mg/L) were found to exhibit larvicidal activity against Cx. quinquefasciatus.[31] Crude extracts of plants which showed larvicidal activity with $L_{C90}$ value above 100 mg/L against Cx. quinquefasciatus were Achillea millefolium methanolic stem extract ($L_{C50}$ 120 mg/L), Tanacetum vulgare methanolic flower extract ($L_{C50}$ 178 mg/L) and methanolic stem extract of Onanthus maritimus ($L_{C90}$ 195 mg/L).[29]

Insecticidal effects of plant extracts vary not only according to plant species, geographical distribution and parts used, but also the extraction methodology adopted and the polarity of the solvents used during extraction. It has been shown that the extraction of active biochemicals from plant depends upon the polarity of solvents used. In most of the studies solvent with minimum (hexane or petroleum ether) and maximum polarity (aqueous) was used.[6,28,32] Therefore, most effective alternative approaches under the biological control program are to explore the floral biodiversity and use safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Biopesticides may serve as suitable alternative to chemical insecticides in future as they are relatively safe and are mostly directed against larvae since larval treatment is more localized in time and space resulting in less–dangerous outcomes. Larval control can be effective due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are man–made and can be easily identified.[33] The findings of the present investigation revealed that the crude petroleum ether aerial extract of C. viscosa possess larvicidal activity against Cx. quinquefasciatus. It may concluded that natural products like extracts from parts of plants with insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. 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 C. viscosa petroleum ether aerial extracts can be used to control the immature stages of vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.
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References


