Mosquito larvicidal activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae) aerial extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

Murugesan Sakhthivadivel, Palani Gunasekaran, Murugesan Sivakumar, Subramanian Arivoli, Rajasingh Raveen and Samuel Tennyson

Abstract
Mosquito-borne diseases have intruded the globe since immemorial time. The present scenario for commanding the mosquitoes is aimed at application of target and stage-specific, cost-effective and biodegradable phytoproducts. Plant extracts are safer for non-target organisms including man. Plant based formulations would be more feasible environmental products with proven potential as insecticide or repellent. It can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as community level. Various reports on the natural plant products as larvicides against mosquito vectors have been published during thirties and forties including the introduction of DDT and other insecticidal chemicals were successful in apparent eradication of the disease during fifties and sixties, but last three decades suffered serious setback. Although effective, the repeated use of synthetic insecticides has disrupted natural biological control systems and sometimes resulted in widespread development of physiological resistance. These problems have warranted the need for developing alternative strategies using eco-friendly products. These steadily growing problems demand an intensive search for new products that are environment-friendly, target specific and degradable. The present scenario for commanding the mosquitoes is aimed at application of target and stage-specific, cost-effective and biodegradable phytoproducts. This has necessitated the continued effort for the search and development of environmentally safe, target specific and degradable. Plant extracts are safer for non-target organisms including man. Therefore, plant based formulations would be more feasible environmental products with proven potential as insecticide or repellent. It can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. Various reports on the natural plant products as larvicides against mosquito vectors have been published. In response to the urgent need for environment friendly mosquito control agents; a medicinal plant *Hyptis suaveolens* of the family Lamiaceae was tested for larvicidal activity against the filarial vector, *Culex quinquefasciatus* was studied. Standard WHO protocols with minor modifications was 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 hours. Amongst the solvent extracts tested, acetone exhibited highest larvicidal activity and LC₅₀ values was 485.61 followed by petroleum ether and chloroform extract which were 493.44 and 625.97 mg/L after 24 hours. In the case of 48 hours, petroleum ether extract (LC₅₀ 298.76 mg/L) was found to exhibit highest larvicidal activity followed by acetone (LC₅₀ 344.03 mg/L) and chloriform (LC₅₀ 429.50 mg/L). Further investigations are needed to explore the larvicidal activity of acetone and petroleum ether 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: *Hyptis suaveolens*, crude aerial extracts, *Culex quinquefasciatus*, larvicidal activity

1. Introduction
Mosquito-borne diseases have intruded the globe since immemorial time. Technical innovations during thirties and forties including the introduction of DDT and other insecticidal chemicals were successful in apparent eradication of the disease during fifties and sixties, but last three decades suffered serious setback. Although effective, the repeated use of synthetic insecticides has disrupted natural biological control systems and sometimes resulted in widespread development of physiological resistance. These problems have warranted the need for developing alternative strategies using eco-friendly products. These steadily growing problems demand an intensive search for new products that are environment-friendly, target specific and degradable. The present scenario for commanding the mosquitoes is aimed at application of target and stage-specific, cost-effective and biodegradable phytoproducts. This has necessitated the continued effort for the search and development of environmentally safe, target specific and degradable. Plant extracts are safer for non-target organisms including man. Therefore, plant based formulations would be more feasible environmental products with proven potential as insecticide or repellent. It can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. Various reports on the natural plant products as larvicides against mosquito vectors have been published. In response to the urgent need for environment friendly mosquito control agents; a medicinal plant *Hyptis suaveolens* of the family Lamiaceae was tested for mosquito larvicidal activity against the filarial vector, *Culex quinquefasciatus*. *Hyptis suaveolens* (L.) Poit (Figure 1) is a potent medicinal herb commonly called as ‘American mint’ in English is found in West Africa especially Northern Nigeria, Philippines and Tropical America. The plant is also found in Deccan Peninsula, North East India and Andaman and Nicobar islands. It is called as ‘wilaiti’ in Hindi, ‘bhustrena’ in Sanskrit and...
‘wilayati tulsi’ and ‘kattu thumbai’ in Tamil [22, 23]. The herb is used as a stimulant, carminative and as a wound healing agent [24]. The leaves are used to treat skin diseases and bronchial disorders [25]. The plant is used for treating wounds, uterine infection, parasitic skin diseases [21], headache and stomachache [26]. Pharmacological properties of this plant includes antioxidant [27], antimicrobial [28], antibacterial [29], anticancer [30], antiinflammatory, antiseptic [31] and antimalarial [12]. Phytochemical constituents of *Hyptis suaveolens* include alkaloids, terpenes, phenols, tannins and steroids [32]. The plant gained increasing attention when it exhibited insecticidal properties [14]. The plant showed feeding deterrent [35] and insect repellent activity [36]. *Hyptis* extracts were used effectively to control cow pea borer, *Maruca testulalis* [37] and *Trogoderma granarium* [38]. The plant is reported to possess repellent activity against insect pests of stored grains [39, 40] and mosquitoes [30, 41]. Raja et al. [42] reported the ethyl acetate leaf extract of *Hyptis suaveolens* to possess insecticidal activity against *Spodoptera litura* and *Helicoverpa armigera*. The essential oil of *Hyptis suaveolens* exhibited repellent activity against *Sitophilus granarius* [43]. Olotua [44] pointed out that methanolic extract of *Hyptis suaveolens* possessed insecticidal activity against *Sitophilus oryzae*, *Sitophilus zeamais* and *Callosobruchus maculatus*. In East and West Africa, plants of the *Hyptis* genus are commonly utilized against mosquitoes. In Guinea Bissau (West Africa), it was demonstrated that smoldering leaves of *Hyptis suaveolens* showed repellent activity beyond 80%, whereas for fresh leaves the repellent activity was greater than 70% [45]. The plant also exhibits larvicidal activity against *Aedes aegypti* [46] and *Culex quinquefasciatus* [47]. Further, the plant was also screened for larval, adult emergence inhibition and ovicidal activity against vector mosquitoes viz., *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* [48]. Therefore, in the present investigation, the crude aerial extracts of *Hyptis suaveolens* has been tested for its larvicidal activity against *Culex quinquefasciatus*.

**Fig 1: Hyptis suaveolens** (L.) Poit

2. Materials and methods
2.1. Plant collection and extraction
Mature aerial parts of *Hyptis suaveolens* 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 hours 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 1,000 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 vector mosquitoes free of exposure to insecticides. Standard WHO [49] 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 F1 generation. From the stock solution, 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 test concentration. Larval mortality was observed for 24 and 48 hours 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 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 [50]. SPSS 11.5 version package was used for determination of LC50 and LC90 values [51]. Two 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 *Hyptis suaveolens* crude petroleum ether, chloroform and acetone aerial extracts are presented in Table 1 and 2; Figure 2 and 3. The results of the present study revealed that among the solvent extracts tested, acetone exhibited highest larvicidal activity and LC50 value was 485.61 followed by petroleum ether and chloroform extract which were 493.44 and 625.97 mg/L after 24 hours. In the case of 48 hours, it was petroleum ether extract (LC50 298.76 mg/L) which showed maximum larvicidal activity followed by acetone (LC50 344.03 mg/L) and chloroform (LC50 429.50 mg/L) (Table 3). Further, one hundred per cent larval mortality was observed at 1000 mg/L in petroleum ether and acetone extracts after 48 hours exposure.

**Table 1: Larval mortality of Culex quinquefasciatus against crude aerial extracts of Hyptis suaveolens at 24 hours**

<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>62.5</td>
<td>125</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0.00 ± 0.00%</td>
<td>0.00 ± 0.00%</td>
<td>0.66 ± 1.15%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.00 ± 0.00%</td>
<td>0.00 ± 0.00%</td>
<td>0.66 ± 1.15%</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.00 ± 0.00%</td>
<td>0.00 ± 0.00%</td>
<td>2.00 ± 2.00%</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 indicate statistical significant difference at P <0.05 level by two way ANOVA followed by DMRT.
mosquitoes have been reported from members of the plant families Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae. Thus, investigation into plants as potential larvicides is considered as a viable and preferred alternative in the control of the mosquito species at the community level.

Plants belonging to the family Lamiaceae have been screened/studied for their larvicidal activity against mosquitoes. Plants that showed promising larvicidal activity were ethanolic aerial extracts of Teucrium divaricatum (LC50 18.6ppm), Mentha longifolia (LC50 26.8ppm), Melissa officinalis (LC50 39.1ppm), Salvia sclarea (LC50 62.7ppm) and Mentha pulegium (LC50 81.0ppm). Sakthivadivel and Daniel revealed that the petroleum ether leaf extracts of Leucas aspera and Ocimum sanctum also possessed LC50 ranging between 100 and 200ppm against the larvae of Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. Plants reported with LC50 values less than 500ppm were methanolic stem extracts of Origanum vulgare (LC50 256.0ppm), Stachys cretica (LC50 292.0ppm), Salvia verbenaca (LC50 311.0ppm), Teucrium hircanicum (LC50 316.0ppm) and Salvia verticillata (LC50 410.0ppm). The results of the above mentioned reports were comparable with the LC50 value of the present study thereby exhibiting larvicidal activity.

It has been demonstrated that the extraction of active biochemical from plants confides upon the polarity of the solvents utilized. Polar solvent will extract polar molecules. This was gained by using solvent systems ranging from hexane/petroleum ether, the most non polar (polarity index of 0.1 that mainly extracts essential oil) to that of water, the most polar (polarity index of 10.2) that extracts biochemical with higher molecular weights such as proteins, glycans, etc. Chloroform or ethyl acetate are moderately polar (polarity index of 4.1) that mainly extracts steroids, alkaloids, etc. It has been obtained that in most of the studies solvent with minimum polarity have been used such as hexane or petroleum ether or that with maximum polarity such as aqueous/steam distillation. However, those biochemical that were extracted using moderately polar solvents were also seen to give good results as reported by a few bioassay. Thus, different solvent types can significantly affect the potency of extracted plant compounds and there is difference in the chemo-profile of the plant species.

Use of plant extracts in insect/mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment. The findings of the present investigation revealed that the acetone and petroleum ether extracts of Hyptis suaveolens possesses larvicidal activity against Culex quinquefasciatus. Therefore, the results reported in the present study open the possibility of further investigations on evaluation, identification and isolation of the bioactive component(s) of

---

**Table 2: Larval mortality of Culex quinquefasciatus against crude aerial extracts of Hyptis suaveolens at 48 hours**

<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>62.5</td>
<td>125</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0.00 ±0.00</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.00 ±0.00</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.00 ±0.00</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
</tbody>
</table>

---

**Table 3: Probit analysis of larvicidal efficacy of crude aerial extracts of Hyptis suaveolens against Culex quinquefasciatus**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>LC50 (mg/L)</th>
<th>LC95 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>493.44</td>
<td>511.70</td>
</tr>
<tr>
<td>Chloroform</td>
<td>625.97</td>
<td>729.92</td>
</tr>
<tr>
<td>Acetone</td>
<td>485.61</td>
<td>474.31</td>
</tr>
</tbody>
</table>

---

**Fig 2: Per cent larval mortality of Culex quinquefasciatus against Hyptis suaveolens crude aerial extracts at 24 hours**

**Fig 3: Per cent larval mortality of Culex quinquefasciatus against Hyptis suaveolens crude aerial extracts at 48 hours**

**4. Discussion**

Plants are a rich source of biologically active chemicals and can be considered a potential source of mosquito control agents. There are reports of medicinal plants with insecticidal activity including ovicidal, larvicidal, antifeedant and repellent belonging to the plant families Annonaceae, Papilionaceae, Meliaceae, Mimosaceae and Lamiaceae. Mosquito larvae control using larvicidal agents is a major component in the control of vector-borne diseases. Larvicidal, repellent or adulticidal activities against different species of
Hypitis suaveolens extracts and its systemic effects on target mosquitoes, which may enable the application of the extract as larvicidal, in small-volume aquatic habitats or breeding sites of limited size in and around human dwellings for the effective control of vector mosquitoes. However, more concerted efforts would be needed to make these environment friendly plant extracts viable for field use and for small/large scale vector control operations.

5. Acknowledgements
Authors thank the Director, King Institute of Preventive Medicine and Research, Chennai 600032, Tamil Nadu, India for the facilities provided.

6. References
1. Park RGA. Insecticide as a major measure in the control of malaria, being an account of the methods and organizations put into force in Natal and Zululand during the past six years. Quarterly Bulletin of the Health Organization of the League of Nations 1936; 5:114-133.


29. Kavitha H, Satish S. Antibacterial potential of crude...


51. SPSS. SPSS for windows, Version 11.5. SPSS, Chicago, IL, 2007.


