Research Article

MOSQUITO LARVICIDAL PROPERTIES OF Ocimum sanctum Linn. (Lamiaceae) AGAINST Aedes aegypti (Linn.), Anopheles stephensi (Liston), Culex quinquefasciatus (Say)

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Abstract

The use of botanicals as an alternative to the chemical compounds is gaining tremendous momentum because of its multifarious advantages. In view of its increasing interest, an attempt was made in the present study to assess the larvicidal potential of important plant like Ocimum sanctum against three mosquito species. The third instar larvae were exposed to different concentrations (i.e. 50, 100, 150, 200 and 250 ppm) of methanol, ethyl acetate and hexane extracts of O. sanctum plant. The mortality was recorded after 24 hrs exposure and LC50 and LC90 were determined. The present investigation revealed that the LC50 and LC90 values methanol, ethyl acetate and hexane extracts of O. sanctum against Culex quinquefasciatus larvae were 101.32, 112.18 and 120.87 mg/L; 182.32, 193.96 and 202.61 mg/L, respectively. The results clearly show that larvicidal activity was dose reliant. The highest larvicidal activity against Culex quinquefasciatus was obtained with methanol extract of O. sanctum.

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1. Introduction

Mosquitoes represent a significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide (WHO, 2010). Several species belonging to genera Aedes, Anopheles and Culex are vectors for the pathogen of various diseases like dengue fever, dengue hemorrhagic fever, malaria, Japanese encephalitis and filariasis (Borah et al., 2010; Rahuman et al., 2009; Samuel, 2010). Ae. aegypti is known to carry dengue and yellow fever; malaria is carried by An. stephensi; and filarial disease by Cx. quinquefasciatus. The dengue fever incidence has increased fourfold since 1970 and nearly half the world’s population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50% (Hales et al., 2002). An outbreak of Chikungunya virus disease emerged in the southwest Indian Ocean islands in 2005, spread out to India, and resulted in an ongoing outbreak that has involved >1.5 million patients, including travelers who have visited these areas (Taubitz et al., 2007). An.
stephensi are major malaria vectors in India. With an annual incidence of 300-500 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world’s population live in areas where malaria is endemic (Werndorfer, 2003). Culex quinquefasciatus, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard et al., 2003). The present proliferation of these diseases is not only due to higher number of breeding places in urban agglomeration, but also due to increasing resistance of mosquitoes to current commercial insecticides such as organochlorides, organophosphates, carbamates and also to biological insecticides (Goettel et al., 1992; Das and Amalraj, 1997; Yadav et al., 1997).

The native people have exploited a variety of herbal medicines for effective curing of various diseases. The plants used and preparation and administration of drugs varies from area to area. Although the knowledge of herbal medicine is gradually vanishing, some of the traditional healers and aged tribals are still practicing plants as a herbal medicine. Modernization has exposed the human race to increased risk of bronchitis, asthma, lung cancer and various skin diseases. The faster pace of life and the need for rapid cure led to the proliferation of synthetic drugs. However, the use of synthetic drugs leads to the problems of side effects, ill effects, and complications. This has revived the herbal treatments for a large number of diseases. In India, about 2,500 plant species are used for medicinal purpose by traditional healers (Aniwal et al., 2006; Bussmann and Glenn, 2010; Chandel et al., 1996; Sankar et al., 2007). Among them Holy Basil, Ocimum sanctum has been well documented for its therapeutic potential (Prakash and Gupta, 2005). Tulsi is a fragrant bushy perennial growing up to 1.5 m in height with profusions of white blooms and slightly purple tinted foliage. This herb has been known from as the vedic period and is held sacred by the Hindus and is often planted around temples and used in rosaries. It is native to India, reached Western Europe in the 16th century. In several ancient system of medicine including Ayurveda, Greek, Roman, Siddha and Unani, O. sanctum has vast number of therapeutic applications such as in cardiopathy, haemopathy, leucoderma, asthma, bronchitis, catarrhal fever, otalgia, hepatopathy, vomiting, lumbago, hiccups, ophthalmia, gastropathy, genitourinary disorders, ringworm, verminosis and skin diseases, etc. It is commonly used in cough, cold, mild indigestion, diminished appetite and malaise. The only side effect reported is constipation (Subir Kumar Das and Vasudevan, 2005). In this context, the purpose of the present investigation is to explore the larvicidal properties of O. sanctum leaf extract and against Chikungunya vector, Ae. aegypti, An. stephensi and Cx. quinquefasciatus under the laboratory conditions.

2. Material and Methods

2.1. Collection and Identification of Plant material

The medicinal plant, O. sanctum was collected from Sirkazhi, Nagapattinam District, in Tamil Nadu, India. Bulk samples were air-dried in the shade. After drying, these were ground to fine powder. At the time of collection, voucher herbarium specimens were prepared and identified with the help of Plant Taxonomist, Department of Botany, Poompuhar College, Melaiyur.

2.2. Extraction method

The dried leaves (100 g) were powdered mechanically using commercial stainless steel Blender and extracted sequentially with methanol, ethyl acetate and hexane (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22 - 26 mm Hg at 45°C by ‘Rotavapour’ and the residue obtained was stored at 4°C in an amber vial. Then, the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4°C.

2.3. Larvicidal activity

The larvicidal activity of crude extract was evaluated as per the protocol previously described by WHO (2005). From the stock solution, six different test concentrations (50, 100, 150, 200 and 250 mg/L) were prepared and tested against
the freshly molted (0 – 6 hrs) III instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The test medium (500 ml plastic cups) was prepared by adding 1 ml of appropriate dilution of test concentrations and mixed with 249 ml of dechlorinated water to make up 250 ml of test solution. The larvae were fed with dry yeast powder on the water surface (50 mg/l). The control (without plant extracts) experiments were also run parallel with each replicate. For each experiment, four replicates were maintained at a time. A minimum of 25 larvae per concentration was used for all the experiments. The larval mortality was observed and recorded after 24 hrs post-treatment. Per cent mortality was corrected for control mortality using Abbott’s formula (Abbott, 1925).

2.4. Statistical analysis

The larval mortality data were subjected to probit analysis (Finney, 1971) for calculating LC$_{50}$ and LC$_{90}$ and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit values were calculated using the SPSS software package 16.0 (2007). Results with $p \leq 0.05$ were considered to be statistically significant.

### 3. Results

The result of the larvicidal activity of crude methanol, ethyl acetate and hexane solvent extracts of *O. sanctum* against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The methanol extract of *O. sanctum* reported in the present study showed the larvicidal properties in the plant signifying their use in mosquito population control (Figure 1, 2 and 3). The data are presented in Table 1. The methanol extracts of *O. sanctum* showed larval mortality. *Cx. quinquefasciatus* was more vulnerable followed by *An. stephensi* and *Ae. aegypti*. The methanol extract of *O. sanctum* exhibited the maximum larvicidal activity with LC$_{50}$ and LC$_{90}$ values of 101.32 and 182.32 mg/L against the larvae of *Cx. quinquefasciatus*; The LC$_{50}$ and LC$_{90}$ values of methanol, ethyl acetate and hexane extract of *O. sanctum* against *An. stephensi* were 115.32, 122.97 and 144.18 mg/L; 209.25, 212.37 and 249.41 mg/L, respectively. The LC$_{50}$ and LC$_{90}$ values of hexane extract of *O. sanctum* against *Ae. aegypti* were 134.73, 153.39 and 175.12 mg/L; 237.97, 265.67 and 290.07 mg/L, respectively (Table 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Extract</th>
<th>LC$_{50}$ (mg/L)</th>
<th>95% Confidence Limits</th>
<th>LC$_{90}$ (mg/L)</th>
<th>95% Confidence Limits</th>
<th>$x^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ae. Aegypti</em></td>
<td>Methanol</td>
<td>134.73</td>
<td>124.23 - 144.85</td>
<td>237.97</td>
<td>221.60 - 259.49</td>
<td>4.165</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>153.39</td>
<td>142.53 - 164.37</td>
<td>265.67</td>
<td>246.47 - 291.44</td>
<td>0.489</td>
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<tr>
<td></td>
<td>Hexane</td>
<td>175.12</td>
<td>164.03 - 187.12</td>
<td>290.07</td>
<td>268.41 - 319.66</td>
<td>0.087</td>
</tr>
<tr>
<td><em>An. stephensi</em></td>
<td>Methanol</td>
<td>115.32</td>
<td>104.91 - 124.92</td>
<td>209.25</td>
<td>195.07 - 227.63</td>
<td>5.761</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>122.97</td>
<td>113.30 - 132.14</td>
<td>212.37</td>
<td>198.66 - 230.02</td>
<td>6.233</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>Methanol</td>
<td>144.18</td>
<td>133.72 - 154.47</td>
<td>249.41</td>
<td>232.27 - 272.02</td>
<td>1.283</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>101.32</td>
<td>91.57 - 110.21</td>
<td>182.32</td>
<td>169.98 - 198.24</td>
<td>4.317</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>120.87</td>
<td>111.82 - 129.51</td>
<td>202.61</td>
<td>190.01 - 218.66</td>
<td>3.631</td>
</tr>
</tbody>
</table>

LC$_{50}$= Lethal concentration that kills 50% of the exposed parasite, LC$_{90}$= Lethal concentration that kills 90% of the exposed parasite. LCL- Lower Confident Limit, UCL- Upper Confident Limit.
4. Discussion

The results of present study are comparable with earlier reports Dhanasekaran et al., (2013) have that the LC$_{50}$ of ethanol crude extracts of selected indigenous medicinal plants are G. ula extract in the experimental larvae of An. stephensi (LC$_{50}$ = 82.86ppm), followed by S. hispida (LC$_{50}$ = 89.45ppm). Baluselvakumar et al. (2012) reported that the LC$_{50}$ and LC$_{90}$ values of methanol O. esculentum leaf extract against An. stephensi were 63.84 and 122.48 ppm, respectively. Krishnappa et al. (2012) reported that the LC$_{50}$ and LC$_{90}$ values of methanol extract against An. stephensi were 78.18 and 155.42 mg/l, and A. digitata exerted 100% up to 150 min at 4 and 6 mg/cm$^2$. Cent percent repellency of An. stephensi was noticed up to 180 min with chloroform extract at concentrations. But, hexane extract showed 100% repellency up to 120 min, whereas methanol extract showed strong repellency up to 210 min except the minimal concentration (2 mg/cm$^2$). Baranitharan and Dhanasekaran (2014) reported that the evident larvicidal activity of ethyl acetate extract followed by hexane, chloroform and acetone extracts of C. caudata showed LC$_{50}$ values of Ae. aegypti are 97.19, 112.85, 99.17 and 109.67 mg/L; An. stephensi are 96.04, 104.16, 97.13 and 106.53 mg/L; Cx. quinquefasciatus are 94.76, 102.95, 95.98 and 105.09 mg/L, respectively. Among two plant solvents tested, C. quadrangularis extracts were found to be most significant ovicidal activity 100% eggs mortality observed at 50 ppm and 350 ppm for C. quadrangularis. Gokulakrishnan et al. (2012) reported that the larvicidal and ovicidal efficacy of different solvent leaf extract of A. indica against An. stephensi. The hatch rates were assessed 48 h after treatment. The LC$_{50}$ and LC$_{90}$ values of acetone, benzene, chloroform, hexane and methanol extracts of A. indica against An. stephensi larvae in 24 hrs were 76.29, 58.82, 53.59, 65.84, 51.78 and 205.85, 193.23, 185.16, 196.72 and 181.00 ppm, respectively. The larvicidal activity of different solvents extracts of C. sparciflorus were...
tested against the three vector mosquitoes. Among the different solvents the maximum larvicidal activity was observed in ethyl acetate. The LC\textsubscript{50} and LC\textsubscript{90} values of \textit{C. sparciflorus} against three vector mosquitoes of \textit{A. aegypti}, \textit{An. stephensi} and \textit{Cx. quinquefasciatus} were 34.02, 28.88 and 36.22 ppm, 74.57, 65.35 and 79.89 ppm, respectively (Baranitharan \textit{et al.}, 2014).

Baluselvakumar \textit{et al.} (2012) reported that the methanol plant extract of \textit{M. maderaspatana} had ovicidal and repellency against \textit{Ae. aegypti} with the methanol extract of \textit{M. maderaspatana} exerted 100% egg mortality at 120, 160, 200 and 240 ppm for \textit{Ae. aegypti}, and a higher concentration of 3.0 mg/cm\textsuperscript{2} methanol extract of \textit{M. maderaspatana} provided 100% protection up to 80, 100,120 and 140 min. Baranitharan and Dhanasekaran (2014) reported that the larvicidal activity of diethyl ether followed by hexane, benzene and acetone extracts of \textit{C. aromaticus} showed 73.49, 85.93, 76.03 and 80.56 mg/L, respectively. Elumalai \textit{et al.} (2012) reported that the \textit{E. roseum} acetone and methanol extracts of LC\textsubscript{50} values of 121.65 and 139.86 ppm, it was that 100% mortality was noted from the acetone and methanol extracts of 100 ppm. The leaf extract of \textit{C. vulgaris} with different solvents were tested for repellent activities against \textit{An. stephensi} and showed that Skin repellent test at 1.0, 2.5 and 5.0 mg per cm\textsuperscript{2} concentration gave the mean complete protection time ranged from 119.17 to 387.83 minutes with the four different extracts tested (Mullai \textit{et al.}, 2008). Baranitharan and Dhanasekaran (2014) results that the LC\textsubscript{50} and LC\textsubscript{90} values of ethyl acetate followed by hexane, chloroform and acetone of \textit{A. adenophora} against \textit{Cx. quinquefasciatus} larvae I-instar in 24 h were 136.75, 145.69, 139.49 and 143.64 mg/L; 149.07, 158.24, 151.95 and 156.14 mg/L, respectively. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

5. Conclusion

In general, it could be concluded that diethyl ether extracts of \textit{O. sanctum} used in the present study act as larvicidal inhibiting against the mosquito vectors, \textit{Ae. aegypti}, \textit{An. stephensi} and \textit{Cx. quinquefasciatus}. The high larvicidal activity of methanol extract of \textit{O. sanctum} and the abundant availability of these plants in tropical and subtropical countries may make them economical for field use in mosquito control programs.

Acknowledgements

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6. Reference

7) Baranitharan M and Dhanasekaran S (2014). Mosquitocidal efficacies of medicinal plant of \textit{Colesus aromaticus} Benth (Lamiaceae) leaf extracts Chikungunya vector, \textit{Aedes aegypti} © 2015 Published by JPS Scientific Publications Ltd. All rights reserved


