**INTRODUCTION**

Many mosquito species are medically important vectors that transmit dreadful diseases such as malaria, Japanese encephalitis, Yellow fever, Dengue, Chikungunya, Lymphatic Filariasis, Zika virus etc. Vector-borne diseases have been a major problem to humans in tropical and subtropical regions. Therefore, WHO has declared the mosquitoes as “Public enemy number one” (WHO, 1996). The latest addition being Zika viral infection transmitted by *Aedes* mosquitoes in part of Africa and Caribbean islands. As such a major part of the national health, budget is spent on the control of vector-borne diseases especially in tropics. Thus it is imperative to control mosquitoes in order to improve the public health (Appadurai et al., 2015). Vector control should be the priority scheme as there are no effective vaccines or treatment against many of these diseases. As chemical insecticides are otherwise harmful to human and his environment alternative methods such as biological control and phytochemicals are explored. Among mosquito borne diseases in India, Lymphatic filariasis is a disease affecting humans caused by nematode parasites *Wuchereria bancrofti* and *Brugia malayi*. Seventeen states and six Union Territories were identified to be endemic with about 553 million people exposed to the risk of infection; and of them, about 146 million live in urban and the remaining in rural areas (Sabeson et al., 2010). Filariasis is estimated that around 20% of the world populations in more than 83 countries are at risk of acquiring infection which is 1.1 billion people (WHO, 2014). About 31 million people are estimated to be the carriers of mf and over 23 million suffer from filarial disease manifestations in India (ICMR, 2017). 2,245 newly diagnosed cases of lymphatic filariasis were recorded in 2016, including 132 cases of chronic filariasis with lymphedema. It is estimated to be endemic in over 250 districts in 20 states, putting 650 million people at risk. *Cx.quinquefasciatus* is the solitary vector of bancroftian filariasis in India. Mosquito control programs have suffered a setback, primarily because mosquito vectors have developed resistance to synthetic chemical insecticides. The use of synthetic insecticides, in the long run, produces negative effects such as biomagnification, soil and water pollution which have created many public health problems. Further, excessive mortality and reduced reproductive potential in birds, fish, and other organisms are reported (Elango et al., 2009). Thus there is an obvious need for the development of alternative products to complement or even replace existing mosquito control strategies. In this regard potential botanicals are recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, ovicidal, pupicidal and adulthood properties with no known hazard to
the environment and to human health (Elango et al., 2009). Plant extracts, essential oils, secondary metabolites and lectins from several plant species have been proved to function as a general toxicant, growth and reproductive inhibitor, insect repellent, larvicidal, ovicidal, oviposition deterrent against mosquito vectors (Khandagle et al., 2011). In line with this trend, the present study was undertaken to investigate the larvicidal, ovicidal and oviposition deterrent activities of different solvent extracts of two local plants Dalbergia oliveri and Heracleum rigens against the filarial vector Culex quinquefasciatus.

MATERIALS AND METHODS

A few plant species were collected from Hassan District, Karnataka and identified those with larvicidal potential after preliminary experiments. As leaves of D. oliveri and seeds of H. rigens were found to be effective, these were dried under shade for 8-10 days at room temperature, powdered mechanically with the help of a laboratory hand blender. This powder was subjected for extraction with different solvents such as petroleum ether, ethyl acetate, chloroform, methanol and acetone using Soxhlet extractor to obtain the crude form. The extracts were allowed to dry and used for conducting preliminary larval bioassay. Larvae were procured from the colony maintained at Vector Biology Research Lab, Department of Zoology, University of Mysore, Mysuru.

Bioassay for larvicidal efficacy

The larvicidal efficacy of the two plant extract was evaluated as per the method of World Health Organization (WHO, 2005). Different concentrations of the extracts were prepared by serial dilutions of stock solution using acetone as solvent. Group of 25 early 4th instar larvae were released into the glass beakers containing 249ml dechlorinated tap water and 1ml of extract. The toxicity of each extract was determined with five different concentrations. The beakers contained 249ml dechlorinated tap water with 25 larvae and 1ml of acetone served as control. Control and test beakers were maintained at same conditions at 25±2°C, 14:10 light and dark regime. No food was provided to the larvae till the mortality was monitored. All treatments were repeated four times. The larvae were considered as dead or moribund, if they were not responsive to gentle prodding with a fine needle.

Ovicidal activity assay

For ovicidal activity assay, the freshly laid eggs were collected by providing ovitraps in mosquito cages. Two days after the female mosquitoes were given a blood meal. The egg rafts were carefully removed from the piece of filter paper with a brush and exposed for 48h to different concentrations of test solution. Distilled water mixed with acetone served as control. A minimum of 100 eggs were used for each treatment, and the experiment was replicated four times. After treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cups filled with dechlorinated water for hatching assessment after counting the eggs under a stereomicroscope. The percent effective repellency for each treatment as assayed by providing repellents to oviposition experiments were expressed as a mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula (Rajkumar and Jebanesan, 2009).

\[
\text{NC} - \text{NT} = \frac{\text{ER} \times 100}{\text{NC}}
\]

Where,

\[
\text{ER} = \text{Effective repellency},
\]

\[
\text{NC} = \text{Number of eggs in control},
\]

\[
\text{NT} = \text{Number of eggs in treatment}
\]

The oviposition experiments were expressed as a mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula.

\[
\text{NT} - \text{NS} = \frac{\text{OAI}}{\text{NT} + \text{NS}}
\]

Where,

\[
\text{NT} = \text{Total number of eggs in the test solution and NS} = \text{Total number of eggs in the control solution}
\]

Oviposition active index of +0.3 and above are considered as attractants while those with −0.3 and below are considered as repellents (Kramer and Mulla, 1979). Positive values indicate that more eggs were deposited in the test cups than in the control cups and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control cups than in the test cups and that the test solutions were a deterrent.

Data analysis

The analysis of larval mortality, egg hatchability, effective repellency data were subjected to Probit analysis for calculating LC_{90}, LC_{95}, at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), and chi-square values. The mean values and standard deviations were calculated from replicate data.
The larvicidal activity in *D. oliveri* leaves are presented in Table 1 and Figure 1 and 2. The larvicidal activity in terms of LC<sub>50</sub> by petroleum ether, ethyl acetate, chloroform, methanol and acetone extracts of *D. oliveri* leaves are 36.28, 94.56, 104.13, 156.73, and 214.5 ± 2.02 ppm respectively. Likewise, the LC<sub>50</sub> values of petroleum ether, ethyl acetate, chloroform, methanol and acetone extracts of *H. rigens* are 36.28, 94.56, 104.13, 156.73, and 214.5 ± 2.02 ppm respectively. The highest larvicidal activity was observed in petroleum ether extract of *D. oliveri* with LC<sub>50</sub> and LC<sub>90</sub> values of 36.28 ppm and 60.61 ppm respectively. The larvicidal activity was found to be significantly different between the treatments (P < 0.05).

The extracts of *D. oliveri* leaf and *H. rigens* seed were tested for ovicidal activity at different concentrations. The percentage of egg hatchability of the vector in various extracts is presented in Table 2 and 2.1. The petroleum ether extracts of both *D. oliveri* and *H. rigens* exerted 100% mortality at 100 ppm and 125 ppm respectively. The LC<sub>50</sub> value for *D. oliveri* was 24.98 ± 0.00 ppm as against 34.83 ppm in *H. rigens*. In all treatments, the ovicidal activity was concentration dependent. Results of oviposition deterrent activity with different solvent extracts of *D. oliveri* and *H. rigens* against *Cx. quinquefasciatus* is given in Table 3 and 3.1. Here too petroleum ether extract significantly deterred oviposition by *C. Quinquefasciatus* gravid female at all the concentration tested as they preferred to lay eggs in a control medium compared to the treated solution (P < 0.05). Strong deterrent (100%) was found at a concentration of 50 ppm for *D. oliveri* extract and 125 ppm for *H. rigens* extract.

### Table 1: Larvicidal activity of different solvent extracts of *Dalbgeria oliveri* leaf and *Harelanda rigens* seed against *Culex quinquefasciatus*

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Solvents</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt;-SE (ppm LCL-UCL)</th>
<th>LC&lt;sub&gt;90&lt;/sub&gt;-SE (ppm LCL-UCL)</th>
<th>Regression equation</th>
<th>X&lt;sup&gt;2&lt;/sup&gt; (df)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. oliveri</em></td>
<td>Petroleum ether *</td>
<td>36.28±1.47 (28.06-46.42)</td>
<td>60.61±1.47 (54.35-66.87)</td>
<td>Y=5.7514X ±3.9706</td>
<td>15.21(3)</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>94.56±0.065 (89.79-99.94)</td>
<td>155.74±0.065 (150.15-161.32)</td>
<td>144.05-172.28</td>
<td>5.9144 X ±6.8652</td>
<td>2.11(3)</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>104.13±0.066 (99.12-108.78)</td>
<td>159.62±0.066 (154.15-172.63)</td>
<td>144.05-172.28</td>
<td>6.9088 X ±8.9391</td>
<td>4.95(3)</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>156.73±0.064 (151.42-161.71)</td>
<td>214.52±0.064 (208.84-227.82)</td>
<td>144.05-172.28</td>
<td>9.4021 X ±15.6391</td>
<td>1.81(3)</td>
</tr>
<tr>
<td><em>H. rigens</em></td>
<td>Acetone</td>
<td>165.90±0.100 (154.25-185.34)</td>
<td>231.82±0.100 (226.70-239.72)</td>
<td>144.05-172.28</td>
<td>9.4587 X ±16.0989</td>
<td>8.38(3)</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether*</td>
<td>36.28 ± 0.161 (31.04-86.34)</td>
<td>91.61±0.161 (89.64-235.53)</td>
<td>144.05-172.28</td>
<td>5.0207 ± 4.8740</td>
<td>17.91(3)</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>92.61±0.103 (77.52-108.69)</td>
<td>116.69±0.103 (114.87-267.98)</td>
<td>144.05-172.28</td>
<td>5.0207 ± 4.8740</td>
<td>8.02(3)</td>
</tr>
</tbody>
</table>

LC<sub>50</sub>=Median lethal concentration, LC<sub>90</sub>=90% lethal concentration, LCL=Lower confidence limit, UCL=Upper confidence limit df = degree of freedom * The difference in LC<sub>50</sub> is significant based on the non overlapping of 95% Fiducial limit (P<0.05)

### Table 2: Ovicidal activity of different solvent extracts of *Dalbgeria oliveri* leaf against *Culex quinquefasciatus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Egg Hatchability at different Concentrations (Means(SE))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>20 ppm</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>57.7±1.25</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>68.75±4.04</td>
</tr>
<tr>
<td>Chloroform</td>
<td>83.50±4.73</td>
</tr>
<tr>
<td>Methanol</td>
<td>88.75±6.63</td>
</tr>
<tr>
<td>Acetone</td>
<td>90.00±6.33</td>
</tr>
<tr>
<td>Control</td>
<td>100.0±0.00</td>
</tr>
</tbody>
</table>

### Table 2.1: Ovicidal activity of different solvent extracts of *Dalbgeria oliveri* leaf against *Culex quinquefasciatus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Egg Hatchability at different Concentrations (Means(SE))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>25 ppm</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>56.75±2.78</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>74.50±5.33</td>
</tr>
<tr>
<td>Chloroform</td>
<td>77.25±4.02</td>
</tr>
<tr>
<td>Methanol</td>
<td>81.75±5.34</td>
</tr>
<tr>
<td>Acetone</td>
<td>89.75±3.98</td>
</tr>
<tr>
<td>Control</td>
<td>100.0±0.00</td>
</tr>
</tbody>
</table>

Mean±standard error (SE) of four replicates. Means are separated by Tukey’s test of multiple comparison, one-way analysis of variance (ANOVA). ppm = parts per million. P<0.05, level of significance.
Table 3. Oviposition deterrent activity of different solvent extracts of Dalbergia oliveri leaf against Culex quinquefasciatus

<table>
<thead>
<tr>
<th>Solvents</th>
<th>10ppm</th>
<th>20ppm</th>
<th>30ppm</th>
<th>40ppm</th>
<th>50ppm</th>
<th>Control</th>
<th>LC50±SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>702.3±3.74</td>
<td>36.48</td>
<td>-0.22</td>
<td>511.3±3.28</td>
<td>71.13</td>
<td>-0.55</td>
<td>101.3±2.6</td>
<td>90.84</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>661.3±3.73</td>
<td>18.48</td>
<td>-1.10</td>
<td>442.0±1.82</td>
<td>54.60</td>
<td>-0.37</td>
<td>221.3±9.1</td>
<td>72.72</td>
</tr>
<tr>
<td>Chloroform</td>
<td>712±2.85</td>
<td>18.03</td>
<td>-0.90</td>
<td>535.0±2.12</td>
<td>56.83</td>
<td>-0.39</td>
<td>227.3±8.1</td>
<td>73.82</td>
</tr>
<tr>
<td>Methanol</td>
<td>758.3±3.01</td>
<td>18.60</td>
<td>-0.01</td>
<td>534.0±2.56</td>
<td>53.20</td>
<td>-0.27</td>
<td>436.0±2.07</td>
<td>71.41</td>
</tr>
<tr>
<td>Acetone</td>
<td>788.6±3.37</td>
<td>16.69</td>
<td>-0.09</td>
<td>404.4±1.6</td>
<td>57.32</td>
<td>-0.40</td>
<td>317.0±1.39</td>
<td>88.20</td>
</tr>
</tbody>
</table>

Mean±standard error (SE) of four replicates. Means are separated by Tukey’s test of multiple comparison, one-way analysis of variance (ANOVA). ppm = parts per million.

Table 3.1. Oviposition deterrent activity of different solvent extracts of Heracleum rigens seed against Culexquinquefasciatus

<table>
<thead>
<tr>
<th>Solvents</th>
<th>25ppm</th>
<th>50ppm</th>
<th>75ppm</th>
<th>100ppm</th>
<th>125ppm</th>
<th>Control</th>
<th>LC50±SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>299.0±1.63</td>
<td>26.17</td>
<td>-0.15</td>
<td>166.0±0.17</td>
<td>59.01</td>
<td>41.0±1.3</td>
<td>59.0±8.12</td>
<td>97.28</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>305.0±1.64</td>
<td>17.11</td>
<td>-0.09</td>
<td>184.0±0.71</td>
<td>50.00</td>
<td>33.0±0.3</td>
<td>77.0±0.80</td>
<td>79.07</td>
</tr>
<tr>
<td>Chloroform</td>
<td>292.0±1.70</td>
<td>27.90</td>
<td>-0.16</td>
<td>196.0±1.03</td>
<td>51.60</td>
<td>34.0±0.3</td>
<td>92.0±7.06</td>
<td>77.28</td>
</tr>
<tr>
<td>Methanol</td>
<td>312.0±2.02</td>
<td>24.27</td>
<td>-0.13</td>
<td>208.0±3.67</td>
<td>49.51</td>
<td>32.0±0.3</td>
<td>172.0±3.18</td>
<td>58.25</td>
</tr>
<tr>
<td>Acetone</td>
<td>330.0±2.48</td>
<td>34.26</td>
<td>-0.20</td>
<td>244.0±2.78</td>
<td>51.39</td>
<td>34.0±0.3</td>
<td>198.0±2.40</td>
<td>60.55</td>
</tr>
</tbody>
</table>

Mean±standard error (SE) of four replicates. Means are separated by Tukey’s test of multiple comparison, one-way analysis of variance (ANOVA). ppm = parts per million.

Fig. 1. Dosage mortality response of different solvent extracts of Dalbergia oliveri leaf against larvae of Culex quinquefasciatus

Fig. 2. Dosage mortality response of different solvent extracts of Heracleum rigens seed against larvae of Culex quinquefasciatus.
The LC\textsubscript{50} value of ER\% of \textit{D. oliveri} and \textit{H. rigens} against \textit{Cx. Quinquefasciatus} found to be 14.62ppm and 39.03ppm respectively. All the OAI values recorded for both species exhibit negative values that from -0.01 to -1.00, which is indicates strong repellency towards test solution. The ER\% observed among various extracts indicated significant (p<0.05) difference when compared to control.

DISCUSSION

After facing several problems due to indiscriminate application of synthetic insecticides, for a long time, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. So as a part of the search for new ecofriendly compounds from local plant species, an effort is made here to isolate and identify a few compounds. At present phytochemicals makeup to one percent of the world’s pesticide market. The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, the age of plant parts (young, mature or senescent), the solvent used during extraction as well as upon the available vector species (Bagavan et al., 2008). The existence of variations in the level of effectiveness of phytochemical compounds on target mosquito species depends on plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction (Sukumar et al., 1991). The screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products, and stimulate local efforts to enhance public health. In this regard, an earlier experiment carried out by Prathibha et al (2014) in our lab employing \textit{Eugenia jambolana}, \textit{Solidago canadensis}, \textit{Euodia ridley}, and \textit{Spilanthes mauritian}a plant species as larvicidal, ovicidal, and oviposition deterrent activity yielded a good result and thereby the methodology was standardized.

The present study further throws light on the probable insecticidal property of solvent extracts of \textit{D. oliveri} leaves and seed extracts of \textit{H. rigens} against the different stages of \textit{Cx. Quinquefasciatus} development. The results indicate that among various solvent extracts obtained, petroleum ether extract has been found to possess significant larvicidal, ovicidal and oviposition deterrent efficacy in both the plant species under study (p<0.05). Between the two plants, \textit{D. oliveri} has exhibited more efficacy with larvicidal, ovicidal and oviposition deterrent activity against \textit{Cx. Quinquefasciatus} (Table 1, 2 and 3). In line with the present data, several species belonging to \textit{Genus Dalbergia} have been shown to possess insecticidal properties. \textit{D. soxatilis} possess insecticidal property against \textit{Aedes} mosquito species (Okwute et al., 2009). The previous research results on \textit{Apiaceae} family revealed significant mosquitoicidal efficacy (Navaneet et al., 2011). Pavela (2008) has reported larvicidal activity of \textit{Ammi visnaga} seed extracts against \textit{Cx. quinquefasciatus} and \textit{An.stephensi} mosquito. \textit{Anethum graveolons} too showed good larvicidal activity against \textit{Cx.quinquefasciatus} and \textit{Ae.aegypti} (Amer and Mehlhorn, 2006). \textit{Heracleum spondyllum} too possesses larvicidal activity against \textit{Culexpiennis} (Evergetis et al., 2009). This result is also comparable to earlier reports of Vahitha et al. (2002) who observed the larvicidal activity of leaf extracts of \textit{Pavonia zeylanica} and \textit{Acacia ferruginea} on \textit{Cx.quinquefasciatus}. The two plant species, \textit{D.oliveri} and \textit{H. rigens} tested by the author in the present investigation at Mysuru have exhibited promising ovicidal activity at 100ppm and 125ppm respectively in \textit{Cx. quinquefasciatus} respectively (Table 2 and 2.1). A similar ovicidal effect of the seed extract of \textit{A. canescens} was reported earlier against \textit{Cx. quinquefasciatus} (Oudo et al., 1998).Govindarajan et al. (2011) have also demonstrated that the crude extract of \textit{Eugoa coronaria} and \textit{Caesalpinia pulcherrima} exerted ovicidal efficacy at different concentrations against \textit{Cx. Quinquefasciatus} and \textit{Ae. Aegypti} at Tamil Nadu. \textit{Trachyspermumammi} seed extracts too exhibited ovicidal activity against \textit{An.stephensi} (Pandey, 2009). Differences in susceptibility to ovicides may be due to differential rates of uptake, penetration through the chorine, and conversion to the active inhibitor, detoxification, and failure of the toxicants to reach the target.

The efficacy to act on the embryo inside the egg shell depends on the efficient penetration of the insecticides, which in turn is influenced by the exposure period (Grosscurt, 1977). The same effect may be true for the present study as well as the current study clearly indicate that the ovicidal activity of the plant extract against egg raft may depend upon three key factors viz., a dose of the plant extract, the age of the egg raft and period of exposure. This observation is also in agreement with the work of Prathibha et al. (2014) on the same vector species with \textit{Eugenia jambolana} and three more plant species at Mysuru. Oviposition is one of the most important events in the life cycle of mosquitoes. By reducing the oviposition the mosquito life cycle can be disrupted and thereby population growth reduced (Xue et al., 2001). Mosquitoes are known to select or reject their specific oviposition sites by sensing chemical signals that are detected by sensory receptors on the antennae and legs. The present data indicate that petroleum ether extract of both the plants exhibited significant oviposition deterrent effect on the vector mosquito (p<0.05). In line with this finding \textit{Trachyspermumammi} seed extracts was found to exhibit oviposition deterrent activity against \textit{An.stephensi} (Pandey, 2009). Further, seed extracts of \textit{Pimpinellaanisum} too showed ovicidal, oviposition deterrent and repellent activity against \textit{Aedes}, \textit{Anopheles} and \textit{Culex} mosquito (Prajapati et al., 20056). The strong odour produced by concentrations of leaf extract in the present experiment might have produced repellency thereby preventing oviposition.

Conclusion

Thus, the present findings highlight the importance of \textit{D. oliveri} and \textit{H. rigens} which exhibited larvicidal, ovicidal, and oviposition deterrent activity against the vector mosquito under study. These results could encourage the search for new active ecofriendly compounds in addition to already existing plant products with insecticidal property. These plant extracts may contribute greatly to save the environment and to an overall reduction in the population density of the vector,\textit{Cx. quinquefasciatus}. Further studies on the isolation and characterization of the bioactive molecule from these plant species are in the pipeline.

Abbreviations

WHO – World Health Organisation
OAI - Oviposition Activity Index
REFERENCES


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