LARVICIDAL POTENTIAL OF SOME INDIAN MEDICINAL PLANT EXTRACTS AGAINST AEDES AEGYPTI (L.)

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ABSTRACT

The larvicidal potential of different solvent crude (hexane, chloroform, ethyl acetate, acetone and methanol) leaf extracts of four plants (Blepharis maderaspatensis, Elaeagnus indica, Maesa indica, Phyllanthus wightianus and Memecylon edule) was tested against the fourth-instar larvae of Aedes aegypti. Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24-h exposure. All the tested extracts showed moderate to good larvicidal activities. However, the maximum larval mortality was detected in acetone extract of E. indica (LC50 90.89, LC90 217.21 and LC99 441.88 ppm) followed by M. indica acetone extract (LC50 173.21, LC90 289.36 and LC99 441.04 ppm). These results revealed that larvicial properties of the selected plants and encourages further effort to investigate the bioactive compounds in those extracts that might possess good larvicidal properties when it will be isolated in pure form.

Keywords: Blepharis maderaspatensis, Elaeagnus indica, Maesa indica, Phyllanthus wightianus, Memecylon edule, dengue vector mosquito.

INTRODUCTION

Mosquitoes are vector for various disease including malaria, yellow fever, filariasis Japanese encephalitis and chikungunya. Among these mosquito borne diseases dengue fever dengue haemorrhagic fever, yellow fever and chikungunya are endemic in Southeast Asia and Africa[1]. It is transmitted by Aedes aegypti (Linn.). One of the methods available for controlling the mosquitoes is use of synthetic insecticides. Mosquitoes develop genetic resistance to synthetic insecticides[2] and even to biopesticides such as Bacillus sphearricus[3]. Also synthetic insecticides adversely affect the environment by contaminating air, water, and soil. There is a urgent need to find alternatives to the synthetic insecticides which is more potent and low-cost.

Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target-insects and are eco-friendly[4]. Traditionally plant based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemical may act as insecticides, antifeedants, moulting hormones, oviposition deterents, repellents, juvenile hormone mimics, growth inhibitors, antimioulting hormones as well as attractants. Plant based pesticides are less toxic, delay the development of resistance because of its new structure and easily biodegradable[5].

Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities[6]. About 2000 species of terrestrial plants have been reported for their insecticidal properties [7]. Search for eco-safe, low cost and a highly potential insecticide for the control of mosquitoes needs the preliminary screening of plants to evaluate their insecticidal activities.

Plant based products does not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponins[8], sterols[9][10], isoflavonoids[11], essential oils[12], alkaloids and tannins[13] has potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitoes[14].

The present investigation was carried out to validate the larvicidal potential of different solvent extracts of four (Blepharis maderaspatensis (L.) B. Heyne ex Roth., Elaeagnus indica Servett., Maesa indica (Roxb.) DC, Phyllanthus wightianus Müll.Arg., and Memecylon edule Roxb.) medicinal plants against fourth instar Ae. aegypti larvae. All the plants were selected based on their ethnobotanical literatures and least explored. This is first hand report on larvicidal activity of all the selected plants against Ae. aegypti larvae.

MATERIALS AND METHODS

Plant material

Healthy leaves of B. maderaspatensis (Acanthaceae), E. indica (Elaeagnaceae), M. indica (Myrsinaceae) P. wightianus (Phyllanthaceae) and M. edule (Melastomataceae) were collected from various regions of Eastern Ghats of Tamil Nadu, India. The plants were identified with the references of standard books and herbariums from the Natural Drug Research Laboratory (NDRL), Department of Biotechnology, Periyar University, Salem, India. The plant materials were cleaned, air-dried at room temperature for two weeks and coarsely powdered.

Preparation of extracts

Powdered plant materials were extracted successively by using different solvents of increasing polarity (hexane, chloroform, ethyl acetate, acetone and methanol) in soxhlet apparatus for 18 h and the extractives were filtered through Whatman filter paper No. 4 then the extracts were concentrated at 40ºC in vacuum and stored at 4ºC for this investigations.

Test insects

Ae. aegypti larvae was obtained from National Centre for Disease Control (NCDC) Coonoor, Tamil Nadu and maintained at Department of Biotechnology, Periyar University Salem. Larvae were fed a diet of Brewer’s yeast and powdered dog biscuits in the ratio of 3:1, kept at 27 ± 2ºC and 75% - 85% relative humidity (RH) with a photoperiod of 14:10 LD for the larval growth. Late third instars to early fourth instars larvae were used for larval bioassay which obtained from the stock culture maintained at Department of Biotechnology, Periyar University, Salem.
Larvicidal bioassay

The larvicidal activity of crude extracts of the selected plants was assessed by the protocol of WHO[15] with some modifications and as per the method of Rahuman et al[16]. For the bioassay in a container 25 fourth instar larvae were kept in 249 ml of distilled water with 1 ml of extracts (400 ppm) in DMSO. Tween-80 was used as an emulsifier at concentration of 0.02% (v/v). The chamber containing the control larvae received 1 ml of DMSO served as negative control. After 24 hours exposures the dead larvae were counted and corrected by using Abbott’s[17] formula and the percentage mortality was recorded from the average of six replicates.

Dose-response bioassay

Based on the preliminary screening results, in which above 90% mortality of larvae occurs alone, were subjected to dose–response larvicidal bioassay. The desired mortality percentage was observed in acetone and ethyl acetate extracts of *E. indica*, ethyl acetate extract of *B. maderaspatensis* and acetone extract of *M. indica* at 400 ppm concentration were subjected to dose dependent bioassay. Different concentrations (50-400 ppm) of the above mentioned crude extracts were tested for larvicidal activity described by WHO[15]. The average mortality percentages of six replicates were recorded and corrected by using Abbott’s formula.

Statistical analysis

Data were analyzed using one-way ANOVA. Significant differences between treatments were determined using Tukey’s multiple range tests (P ≤ 0.05). LC50, LC90 and LC99 values were calculated using probit analysis[15].

RESULTS AND DISCUSSION

The results of larvicidal efficacy of different solvent extracts of the selected plants were shown in Table 1. All the plant extracts showed good to moderate effect on fourth instar larvae of *Ae. aegypti* after 24 h of exposure at 400 ppm (0.04%) concentration. The highest mortality (100%) was observed in acetone extracts of *E. indica* and *M. indica*. Significant (p>0.05) activity was detected in ethyl acetate extracts of *E. indica* (97%) and *B. maderaspatensis* (90%) followed by *M. indica* chloroform extract (85%). Most of the extracts of *P. wightianus* exhibits considerable (45-82%) larvicidal activity and the remaining extracts of the selected plants showed least larvicidal activity. The least activity was detected in *M. edule* chloroform extract (1%).

<table>
<thead>
<tr>
<th>Plant names</th>
<th>% Mortality*</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. maderaspatensis</em></td>
<td>80±1.0</td>
<td>48±0.6</td>
<td>906±0.5</td>
<td>26.6±2.3</td>
<td>10.6±1.1</td>
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<tr>
<td><em>E. indica</em></td>
<td>22±2.0*h</td>
<td>100±0.0</td>
<td>973±0.5</td>
<td>21.3±0.5</td>
<td>34.6±1.1</td>
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<tr>
<td><em>M. indica</em></td>
<td>24±2.0*</td>
<td>100±0.0</td>
<td>14.6±1.5</td>
<td>85.3±1.5</td>
<td>6.6±3.0</td>
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<tr>
<td><em>M. edule</em></td>
<td>5.3±1.5*a</td>
<td>0.0±0.0</td>
<td>10.6±0.5</td>
<td>1.3±0.5</td>
<td>17.3±0.5</td>
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<tr>
<td><em>P. wightianus</em></td>
<td>42±6.1</td>
<td>73±3.1</td>
<td>786±2.0</td>
<td>82.6±1.1</td>
<td>70.6±2.0</td>
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</table>

Control—Nil mortality. Total no of larvae = 25, *Mean value of six replicates ± SD. Significant at p<0.05 level.

The toxicity of dose–response larvicidal bioassay was given in Table 2. According to preliminary screening results, four extracts were subjected to dose–response larvicidal bioassay which has above 90% larval mortality. Among them significant mortality rate was observed in acetone extract of *E. indica* with LC50 and LC90 values of 90, 217 and 441 ppm respectively followed by acetone extract of *M. indica* with LC50 and LC90 values of 173, 289 and 441 ppm respectively. The larvicidal activity of the different selected plant extracts were found to be dose depended. *E. indica* ethyl acetate extract showed considered mortality with LC50 and LC90 values of 151, 456 and 1121 ppm respectively.

<table>
<thead>
<tr>
<th>Plant names</th>
<th>Conc. (ppm)</th>
<th>% Mortality*</th>
<th>LC50 ± SE (LCL-UCL)</th>
<th>LC90 ± SE (LCL-UCL)</th>
<th>LC99 ± SE (LCL-UCL)</th>
<th>df</th>
<th>p * (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. maderaspatensis</em></td>
<td>100</td>
<td>18.6±0.5</td>
<td>197.6±2.0 (181.6-213.8)</td>
<td>438.0±0.3 (381.6-531.3)</td>
<td>838±3.8 (664.3-1174.3)</td>
<td>4</td>
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<tr>
<td><em>E. indica</em></td>
<td>150</td>
<td>30.6±0.5</td>
<td>200</td>
<td>626±1.5</td>
<td>490.6±2.5</td>
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<td></td>
<td>200</td>
<td>426±2.0</td>
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<td></td>
<td>300</td>
<td>773±1.5</td>
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<tr>
<td><em>E. indica</em></td>
<td>50</td>
<td>70.6±0.5</td>
<td>90.8±0.1 (80.1-101.1)</td>
<td>217.2±0.2 (191.5-254.8)</td>
<td>441.8±0.6 (358.7-587.5)</td>
<td>52</td>
<td>0.005</td>
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<tr>
<td><em>M. indica</em></td>
<td>100</td>
<td>18.6±1.5</td>
<td>151.2±0.4 (93.3-224.2)</td>
<td>456.1±0.5 (284.6-500.5)</td>
<td>1121.7±1.2 (527.0-16044.2)</td>
<td>20</td>
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<td>150</td>
<td>26.6±1.1</td>
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<tr>
<td><em>M. edule</em></td>
<td>100</td>
<td>160±1.0</td>
<td>173.2±0.7 (135.9-206.9)</td>
<td>289.8±0.9 (237.2-448.8)</td>
<td>441.0±2.2 (325.6-968.0)</td>
<td>17.8</td>
<td>0.005</td>
</tr>
<tr>
<td><em>M. edule</em></td>
<td>150</td>
<td>30.6±1.5</td>
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<td></td>
<td>400</td>
<td>968±0.5</td>
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</table>

Control—Nil mortality. Significant at p<0.01 level. *Mean value of six replicates ± SD. LC=Lethal Concentration, LCL=Lower Confidence Limit, UCL=Upper Confidence Limit, SE=Standard Error, χ²=chi-square and df=degree of freedom.
Nowadays, the control of mosquitoes at larval stage is focused with plant extracts. The advantage of targeting mosquito at the larval stage is they cannot escape from their breeding sites until the adult emerges and also to reduce the overall pesticide use to control adults by aerial application of agricultural chemicals. Bioactive crude extracts or isolated phyto-constituents from the plant could be used as alternative to the currently used synthetic insecticides. The biological activity of plant extracts might be due to various compounds, including phenolics, terpenoids, and alkaloids present in plants.[19]

Our preliminary screening for larvicidal properties of different solvent leaf extracts of 20 plants was tested, 4 extracts gave high larvicidal potency with low lethal concentrations (LC50: 197 ppb) against 4th instar larvae of Aedes aegypti. Likewise, Cavalcanti et al.[12] reported that the larvicidal activity of essential oils of Brazilian plants against Aedes aegypti and observed the LC50 to range from 60 to 533 ppm. Similarly, Rahuman and Venkatesan[27] screened the petroleum ether extracts of Citrullus colocynthis; ethyl acetate extract of Cannabis sativus, Cannabis indica and Morinda charantia; and acetone extract of Trichosanthes anguina against the larvae of Aedes aegypti the LC50 values are 74.57, 30.946, 49.273, 199.14, and 554.20 ppm respectively which supports the present results were comparably good.

The findings of present results, among the 25 tested extracts only 4 extracts has potential larvicidal activity which are comparable to earlier reports of Sakhivadivel and Daniel[21] that screened larvicidal activity of petroleum ether extracts of sixty three plants against Cu. quinquefasciatus, An. stephensi and Ae. aegypti larvae of which six found to be potential larvicides. Similarly, Paesel[22] reported the larvicidal activity methanolic extracts of thirty one Euro-Asianic plants against Cu. quinquefasciatus. Likewise, Nazar et al.[23] investigated 100 coastal plant extracts including B. maderaspatensis against the Cu. quinquefasciatus larvae of which seventeen plants were possessed larvicidal properties and also the whole plant extract of B. maderaspatensis showed no activity but, the present investigation revealed that larvicidal properties of B. maderaspatensis against Ae. aegypti. The findings of present study are quite comparable with previous reports of Vinayaka et al.[24] who have reported the larvicidal activities of different solvent leaf extracts of Elaeagnus kologa in which methanol, ethyl acetate and acetone extracts showed 100% in 15 and 20 mg/ml concentrations against Ae. aegypti. Suwanneepromsri et al.[25] investigated fourteen plant extracts, among those, only eight plants were showed 100% mortality against Ae. aegypti larvae at a concentration of 100 µg/ml with LC50 values range between 3.9-56.2 µg/ml to 100 µg/ml that supports present results.

The present result was supported by earlier reports of Singh et al.[26] that the larvicidal activity of Ocimum canum oil tested against Ae. aegypti and Cu. quinquefasciatus (LC50 301 ppm) and An. stephensi (294 ppm). Similarly, Ansari et al.[27] was observed the larvicidal activity of Piper longifolia oil against An. stephensi (LC50 112.6 ppm), Ae. aegypti (82.1 ppm) and Cu. quinquefasciatus (85.7 ppm). The results of our study is compared with the findings of Sumroiphon et al.[28] who have reported that the effect of water extract of citrus seed extract showed LC50 values of 135, 319 and 127, 411 ppm against the larvae of Ae. aegypti and Cu. quinquefasciatus respectively.

CONCLUSION
All the tested plants possessed different range of larvicidal property which may be used as a traditional mosquito control agent. On the basis of the present investigation results we conclude that acetone, ethyl acetate extract of L. indica, acetone extract M. indica and ethyl acetate extract of B. maderaspatensis contains potent larvicidal bioactive principles which may be needed further purifications to have its synthetic analogues which will be carry out in future.

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REFERENCES


