Larvicidal activity of leaf extract of some weeds against malaria vector *Anopheles stephensi*

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Abstract

The extensive use of synthetic insecticides for mosquito control resulted in the insecticide resistance and fostered environmental deterioration. Recently, plants become alternative source of mosquito control agents. The present study assessed the larvicidal activity of leaf extracts of three invasive weeds: *Vernonia cinerea*, *Prosopis juliflora* and *Cassia tora* against third instar larvae of *Anopheles stephensi*. Larval bioassays were carried out at different concentrations (10-200 ppm) of acetone leaf extracts. The mortality was recorded after 24 hrs exposure and LC₅₀ and LC₉₀ were determined. Amongst these three plant species, *P. juliflora* showed highest activity (LC₅₀ 43.11 ppm) followed by *V. cinerea* (LC₅₀ 97.38) and *C. tora* (LC₅₀ 149.0 ppm) in 24 hrs. The results indicate that leaf extracts of these plants can be an ecofriendly larvicide for *An. stephensi*. Further studies are suggested on the screening, isolation and purification of bioactive compounds.

Keywords: Malaria, Larvicide, Weed, Leaf extract, Vector Management.

INTRODUCTION

Mosquitoes transmit various diseases such as malaria, dengue, chikungunya and elephantiasis and remain a major source of morbidity and mortality worldwide. Malaria is an important cause of death and illness in children and adults in tropical countries, transmitted by *Anopheles* mosquito. Half of the world's population is at risk from malaria. Each year almost 250 million cases occur, causing 860000 deaths (WHO, 2010). Many approaches have been developed to control the mosquitoes, in which the mosquito control at larval stage is considered as an efficient way in the integrated vector management (Rutledge et al., 2003). The current mosquito control methods are based on synthetic insecticides. Synthetic insecticides are the first line of action due to their quick action, but their continuous use may lead to the development of resistance and adverse effect on environment. These factors have created a need for search of easily biodegradable alternative insecticides. The use of plant extracts for vector control has several appealing features as they are biodegradable, less hazardous and rich stock house of chemicals of diverse biological activity.

Weeds as botanical larvicide against mosquitoes have several advantages, as weeds are resistant to microbes (not a host to plant pathogen) and insect predators, required little technical input for cultivation and procurement. A number of weeds have been studied for their insecticidal properties. Invasive weeds *Cassia uniflora* and *Synedrella nodiflora* have been reported to possess larvicial activity against *Ae. aegypti* (Ghayal et al., 2010). Leaf extract of exotic weed *Croton bonplandianum* exhibited larvicial activity against *Ae. aegypti* (Jeeshna et al., 2010).

*V. cinerea* belonging to the family Asteraceae is an annual plant widely distributed in India. It is commonly known as ‘Little Iron’ weed in English and ‘Sahdevi’ in Hindi. The plant is extensively used in indigenous medicine as stomachic
and for cold, asthma and bronchitis (Kirtikar and Basu, 2000). Further more *V. cinerea* also possesses insect antifeedent property (Tandan et al., 1999).

*P. juliflora* is a drought resistant, wide spread exotic weed in semi arid areas of India. This deciduous thorny shrub belongs to Fabaceae family commonly known as ‘Vilayati Babul’ in Hindi. Leaves of this plant have strong antifungal properties (Kaushik et al., 2002). Insecticidal property of this plant has also reported (Singh and Sharatchandra, 2005).

*Cassia tora* (Family: Cesalpinaceae), commonly known as ‘Wild Senna’, is a very common Indian herb having various medicinal properties for the treatment of different kinds of disease, viz. antifungal, wound healing, and antidiabetic agents, respectively (Nadkarni 1982; Christopher 2005). Amerasan et al., (2012) studied the adulticidal and repellent property of *Cassia tora* against mosquitoes. Hence the present study was aimed to determine the larvicidal effect of leaf extracts of three invasive weeds- *V. cinerea*, *P. juliflora* and *C. tora* against third instar larvae of *An. stephensi*.

**MATERIAL AND METHODS**

**Plant material**

Leaves of *Vernonia cinerea*, *Cassia tora* and *Prosopis juliflora* were collected from wasteland areas of Gwalior, Madhya Pradesh India. Leaves were air dried in shaded place for 10 days at room temperature. Dried materials were powdered by using an electric blender. Powdered plant material (100 g) was soaked in acetone (500 ml) in airtight wide mouth bottle (1000 ml) and kept for 7 days with periodic shaking. After that, the cold extracts were filtered using Whatman filter paper and kept in Petri dishes for drying at room temperature (Kongkathip, 1994). Dried extracts were used for the preparation of stock solution (2 %).

**Mosquito**

*Anopheles stephensi* mosquito colony was maintained in our laboratory at 27±2°C temperature, 12:12 light dark photoperiod and 70±5 % relative humidity. Larvae were maintained in aluminum bowls (2.5 L) by providing yeast powder as larval food. Adult mosquitoes were reared in wooden cages (30 x 30 x 30 inches) and were provided cotton soaked with 10% sugar solution. *An. stephensi* females were offered blood, once in a week.

**Larval Bioassay**

Larval bioassay was carried out as per WHO (1981) procedure in four replications for each concentration. Twenty, early third instar larvae of *An. stephensi* were inoculated in glass beakers (250 ml) containing 100 ml tap water. Different doses of leaf extracts ranging from 10, 20, 50, 100 and 200 ppm in acetone were prepared. Larvae were treated with 1 ml of test solution for each concentration and controls were treated with acetone. Larval mortality was recorded after 24 and 48 hrs of exposure. To determine the lethal concentration (*LC*$_{50}$ and *LC*$_{90}$) for each plant species, data were analyzed by Probit analysis (Finney, 1971) using POLO PC software.

**RESULTS**

Larval susceptibility to acetone leaf extracts of three plant species *V. cinerea*, *P. juliflora* and *C. tora* are presented in Table 1 and Figure 1. *Anopheles* larvae were found to be more susceptible to *P. juliflora* (>90 % larval mortality) followed by *V. cinerea* (76 %) and *C. tora* (65 %) at highest concentration of 200 mg/L after 48 hrs exposure. Control showed < 2 % larval mortality after 48 hrs. The *LC*$_{50}$ and *LC*$_{90}$ for *P. juliflora* leaf extract were determined to be 37.55, 514.35 and 31.28, 267.80 mg/L after 24 and 48 hrs respectively (Table 2). *V. cinerea* and *C. tora* were showed effective larval mortality after 48 hrs exposures with *LC*$_{50}$, *LC*$_{90}$ values as 69.46, 629.73 and 93.00, 934.64 respectively.

**DISCUSSION**

The results of the study suggest that the acetone leaf extract of *V. cinerea*, *P. juliflora* and *C. tora* exhibited larvicidal properties against *Anopheles* larvae. In the present study malaria vector *An. stephensi* larvae showed higher susceptibility to *P. juliflora* as compared to other tested plant extracts. In earlier studies Sakhthivadivel and Daniel (2008) have been reported that petroleum ether extracts of flowers of *P. juliflora* were also quite effective against Cx.
The mode of action and side effects of larvicidal phytochemicals may be due to plant part used, geographical location of the plant, photosensitivity of some of the compounds in the extract, the solvent of extraction, the process of extraction and finally the plant part used. It was observed in the present study that V. cinerea extract was also shown effective larvicidal activity. In a study Jang et al. (2002) found that the methanolic seed extract of C. tora showed larvicidal activity against Ae. aegypti and Cx. quinquefasciatus. It is notable that the larvicidal activities of P. juliflora extract are varying in different studies. Reason of this variation probably due to plant part used, geographical location of the plant, photosensitivity of some of the compounds in the plant extract, the solvent of extraction, the process of extraction and finally the mosquito species responses to the extracts (Sukumar et al., 1991). However the susceptibility of mosquito strain and the larval stage taken may also affect the results. Furthermore, the effect of larval mortality was depended on the concentration of leaf extract.

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Plant extract might have complex mixture of biocidal active compounds, including phenolics, terpenoides, flavonoids and alkaloids which may jointly or independently contribute to mortality and delayed growth of larvae; this may be the cause of larvicidal activity in the present study. Other study reported that acetone extracts maximum amount of phenols and flavonoids, while methanol extracts flavones, terpinoids, tannins and polyphenols (Tiwari et al., 2011), which contains larvicidal activity. The mode of action and site of effect for larvicidal phytochemicals has received little attention. Ray et al. (1999) and David et al. (2000) found that botanical derivatives primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae.

This study contribute to assess the possibility of using large biomass of weeds available in the wastelands of northern India as potential insecticides other than medicinal or cultivable plants which are facing extinction or severe genetic loss. For improving the potency and stability of the products, further studies are recommended.

Table 1. Larvicidal activity of leaf extracts of Vernonia cinerea, Prosopis juliflora and Cassia tora against third instar larvae of An. stephensi.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Vernonia cinerea</th>
<th>Prosopis juliflora</th>
<th>Cassia tora</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>24 hrs</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10.0 (2.88)</td>
<td>10.0 (2.88)</td>
<td>26.66 (1.67)</td>
</tr>
<tr>
<td>20</td>
<td>26.66 (7.27)</td>
<td>33.33 (8.33)</td>
<td>41.66 (4.41)</td>
</tr>
<tr>
<td>50</td>
<td>33.33 (3.33)</td>
<td>33.33 (3.33)</td>
<td>50.0 (2.89)</td>
</tr>
<tr>
<td>100</td>
<td>50.0 (5.77)</td>
<td>56.66 (6.67)</td>
<td>63.33 (1.67)</td>
</tr>
<tr>
<td>200</td>
<td>75.0 (8.66)</td>
<td>76.66 (7.27)</td>
<td>85.0 (2.89)</td>
</tr>
</tbody>
</table>

Table 2. Lethal concentrations of leaf extracts of Vernonia cinerea, Prosopis juliflora and Cassia tora against third instar larvae of An. stephensi.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>LC50 (Lower and Upper limit at 95% confidential limit)</th>
<th>LC90 (Lower and Upper limit at 95% confidential limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td>Vernonia cinerea</td>
<td>81.30</td>
<td>69.46</td>
</tr>
<tr>
<td></td>
<td>(51.35-160.40)</td>
<td>(36.20-190.64)</td>
</tr>
<tr>
<td>Prosopis juliflora</td>
<td>37.55</td>
<td>31.28</td>
</tr>
<tr>
<td></td>
<td>(20.188-63.65)</td>
<td>(13.84-56.12)</td>
</tr>
<tr>
<td>Cassia tora</td>
<td>140.98</td>
<td>93.00</td>
</tr>
<tr>
<td></td>
<td>(98.81-243.35)</td>
<td>(69.87-134.80)</td>
</tr>
</tbody>
</table>

quinquefasciatus followed by An. stephensi and Ae. aegypti. Senthilkumar et al., (2009) also evaluated the larvicidal efficacy of 10% leaf extract of P. juliflora and found to be quite effective (LC50 9.3 mg/lit) against larvae of An. stephensi. Recently Bansal et al., (2012) studied the methanol extract of P. juliflora and reported LC50 value as 128 ppm against Ae. aegypti larvae.

It was observed in the present study that V. cinerea extract was also showed effective larvicidal activity. In a study Arvoli et al., (2011) discovered the larvicidal property of this plant in ethyl acetate extract against Cx. quinquefasciatus larvae with an LC50 value of 1.63 g/L. V. cinerea is a member of Asteraceae family which possesses various types of phytochemical compounds (flavonoids, sesquiterpines, thiophene derivatives) those have been found to be toxic to insects including mosquito larvae (Ribeiro et al., 1994).

C. tora was found to be effective larvicidal at higher dose with LC50 values as 149.0 ppm. These results are also comparable to recent reports of Amerasan et al., (2012) who observed the adulticide activity of acetone extract of C. tora with LC50 263.35 against An. stephensi. In a study Jang et al., (2002) found that the methanolic seed extract of C. tora showed larvicidal activity against Ae. aegypti and Cx. quinquefasciatus.
Figure 1. Percent Larvicidal activity after 24 and 48 hrs of (A) *Vernonia cinerea* (B) *Prosopis juliflora* (C) *Cassia tora* leaf crude extracts against third instar larvae of *An. stephensi*

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