Screening of a Few traditionally used Medicinal Plants for their Larvicidal Efficacy against *Aedes aegypti* Linn (Diptera: Culicidae), a Dengue Fever Vector

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**Abstract**

Mosquitoes are the significant group of insects as they cause morbidity and mortality to human beings by severe diseases, such as zika, malaria, filariasis, dengue fever, Japanese encephalitis, West Nile virus and chikungunya. According to World Health Organization draft on global vector control response, 2017 more than 80 percentage of global human populations were significantly infected by at least one vector-borne disease. This vector - borne diseases may induce social and economic burden over the globe. Herbal products with larvicidal properties have been used as natural insecticides for eco-friendly vector control programme. The larvicidal efficacy of nine medicinal plants collected from Wayanad district of Kerala, a part of Western Ghats, India was assessed against fourth instar larvae of *Aedes aegypti*. The larvicidal bio assay had been carried out according to WHO standard protocol with acetone, methanol, petroleum ether and water as extracts. Out of nine traditionally used medicinal plants screened, *Persicaria hydropiper* and *Plectranthus hadiensis* have significant larvicidal efficacy with LC\(_{90}\) 489.278 Mg/ L and LC\(_{90}\) 411.746 Mg/ L respectively against the fourth instar larvae of this dengue fever vector. Hence the aforesaid plants can be used as a potential natural insecticide against mosquitoes thus, to control mosquito borne diseases.

**Keywords**: *Aedes aegypti*; Vector mosquitoes; medicinal plant; larvicidal efficacy; plant extracts

**Introduction**

Mosquitoes (Diptera: Culicidae) have significant impact on human health, principally because they act as vectors for ruinous pathogens and parasites causing many severe infectious diseases such as dengue, chikungunya, malaria, West Nile virus and filariasis [1- 3]. Dengue fever recognized as the most severe mosquito-borne disease, responsible for medical and economic burden together with defeat in commercial [4-11]. The World Health Organization in 2017 reported that roughly 65 to 136 million people were infected by dengue fever in every year [12]. The genus *Aedes* are liable to transmit various arboviral diseases including dengue fever all over the world [13]. *Aedes aegypti* has been reported as the principal vector of chikungunya and dengue in the United States and other regions of tropical and sub tropical countries [14]. There are only a small number of vaccines are available to treat the pathogens transmitted by mosquitoes and the scientific community is yet to discover the vaccines for severe mosquito borne diseases including dengue [15]. However, fight against mosquito transmitting diseases is a challenging problem to public health [3,16].

Mosquito control – targeting its larvae remains the most effective approach to prevent various mosquito borne diseases [17]. Control of such ailments is ending up progressively troublesome be-reason for expanding resistance in mosquitoes to synthetic insecticides [18]. Plant derived products are safer than synthetic insecticides [19].

Several people use either synthetic or plant based repellents to protect them from mosquito transmitting diseases. Bed nets treated with insecticide and indoor residual spraying may also be used for preventing mosquito-borne diseases [3,17,20-23]. Active, frequent use of synthetic insecticides in farming and health programs may leads to various harmful results such as ecosystem destabilization, environmental pollution, hazardous effects to human beings and non target organisms [24,25].

Use of plant based insecticides against mosquitoes becoming a significant approach for the prevention of various mosquito transmitted diseases because of a number of advantages rather than artificial repellents [26,27].

Plant derived products with insecticidal activity have been used in the recent years to control different types of vectors [28].Various methods have been implicated to control mosquito population. One of the methods to prevent mosquito transmitting disease is by killing its larvae at larval stage. The modern mosquito control method is based on artificial insecticides [29].

World Health Organization in 2008 reported that 80 percentage of population of some countries in Asia and Africa may use traditional medicines to cure various diseases due to monetary and environmental constrain. Traditional medicines
are used to maintain health by preventing various severe diseases based on knowledge, experience and practice [30].

Extracts of plants constitute various bioactive phyto compounds; hence they can be used as alternative approach to mosquito larval control. Many scientific studies have proven that the plant extracts or plant derived products can be used as an alternate approach to control mosquito population [31-33].

Various vector borne diseases can be prevented by means of traditionally used medicinal plants. Hence, the demand for traditional medicines is enhancing as they are usually recognized to be bio degradable, natural, safer than synthetic drugs [34]. Thus, searching for natural insecticides is of greatest significance in vector control. This study focused on screening of few traditionally used medicinal plants for their larvicidal efficacy against dengue fever vector Aedes aegypti to develop an efficient, natural, biodegradable insecticide of plant origin.

### Methods

#### Plant Material

Fresh leaves of nine traditionally used medicinal plants were collected from different regions of Thirunelly (11°53’N, 76°0’E), Wayanad a part of Western Ghats, Kerala, India. The collection was performed during their dynamic growing season, June to September (monsoon season).

#### Preparation of Extracts

Fresh leaves of nine medicinal plants were collected and shade dried (Table 1). The dried plant materials were ground to fine powder using a mechanical grinder and proceeded for soxhlet extraction using different solvents such as petroleum ether, methanol acetone and water. The extracts obtained were evaporated and used for further study.

#### Mosquito’s Culture

*Aedes aegypti* were reared in the Communicable Disease Research Laboratory, Department of Zoology, St Josephs College, Irinjalakuda. The larvae were maintained and fed with dog biscuit and yeast in the ratio 3:1. Adult mosquitoes were provided with 10% sucrose solution and young chick was kept within the cage to offer blood meal. Mosquitoes were held at 27 ± 2°C and 75–85% relative humidity, with 12:12 Light and dark photoperiod cycle.

#### Larvicidal Bioassay

Larvicidal bioassay of plant extracts were tested against fourth instar larvae of *Aedes aegypti*. The tests were conducted in glass beakers with WHO standard protocol. Larvicidal efficacy was tested against fourth instar larvae of *Aedes aegypti* using petroleum ether, methanol, acetone and water extracts of the plant material (1000 mg/L). A set of control groups were included for each test. 1 ml of petroleum ether, acetone, methanol and water was mixed separately with 249ml of distilled water for control groups. Twenty five healthy larvae were released in each glass beaker and mortality was observed after 24 hours of exposure. The dead larvae in replicates were pooled and percentage of larval mortality was calculated. The larvicidal bioassay was performed at 27 ± 2°C and 75–85% relative humidity, with 12:12 Light and dark photoperiod cycle.

#### Statistical Analysis

Larval mortality was calculated in percentage and if the control mortality was ranged from 5-20%, it was corrected using Abbott’s formula [35].

\[
\text{corrected mortality} = \frac{\text{test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

### Table 1: List of plants collected for bioassay from Thirunelly, Western Ghats, Kerala, India

<table>
<thead>
<tr>
<th>S No.</th>
<th>Plant Name</th>
<th>Family</th>
<th>Local Name</th>
<th>Parts used</th>
<th>GPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sphaeranthus indicus</td>
<td>Asteraceae</td>
<td>Adakkamaniyan</td>
<td>Seed</td>
<td>11°53'29.20&quot;N 76° 0'59.00&quot;E</td>
</tr>
<tr>
<td>2</td>
<td>Hydrocotyle javanica</td>
<td>Apiaceae</td>
<td>Eranga</td>
<td>Leaf</td>
<td>11°53'29.50&quot;N 76° 0'34.20&quot;E</td>
</tr>
<tr>
<td>3</td>
<td>Deris trifoliatae Lour</td>
<td>Fabaceae</td>
<td>Thuduthudupankayi</td>
<td>Leaf</td>
<td>11°53'29.40&quot;N 76° 0'34.20&quot;E</td>
</tr>
<tr>
<td>4</td>
<td>Persicaria hydropiper</td>
<td>Polygonaceae</td>
<td>Kovanenji</td>
<td>Leaf</td>
<td>1°53'25.00&quot;N 76° 0'50.40&quot;E</td>
</tr>
<tr>
<td>5</td>
<td>Acanthospermum hispidum</td>
<td>Asteraceae</td>
<td>Nherinjil</td>
<td>Leaf</td>
<td>11°53'27.80&quot;N 76° 0'47.80&quot;E</td>
</tr>
<tr>
<td>6</td>
<td>Drymaria cordata</td>
<td>Caryophyllaceae</td>
<td>Odhally</td>
<td>Leaf</td>
<td>11°53'21.14&quot;N 76° 0'59.64&quot;E</td>
</tr>
<tr>
<td>7</td>
<td>Toddelia asiatica</td>
<td>Rutaceae</td>
<td>Narinarakam</td>
<td>Leaf</td>
<td>11°53'37.30&quot;N 76° 0'38.90&quot;E</td>
</tr>
<tr>
<td>8</td>
<td>Plectranthus hadiensis</td>
<td>Lamiaceae</td>
<td>Bhaya</td>
<td>Leaf</td>
<td>11°53'23.00&quot;N 76° 1'1.90&quot;E</td>
</tr>
<tr>
<td>9</td>
<td>Triumfetta rhomboidea</td>
<td>Malvaceae</td>
<td>Kodithoova</td>
<td>Leaf</td>
<td>11°53'27.64&quot;N 76° 0'47.59&quot;E</td>
</tr>
</tbody>
</table>
Results

Results for screening of the nine traditionally used medicinal plants against *Aedes aegypti* fourth instar larvae were shown in Table 2. The highest mortality was observed in leaf extract of two plant species such as Persicaria hydropiper and Plectranthus hadiensis. The petroleum ether extract of Plectranthus hadiensis showed promising larvicidal efficacy with LC$_{50}$ - 411.746 mg/L against fourth instar larvae of *Aedes aegypti* (Table 3). However, methanol, acetone and water extracts of the same may also showed considerable larval mortality. Methanol extract of Persicaria hydropiper also showed significant larvicidal property with LC$_{90}$ - 489.278 mg/L against *Aedes aegypti* (Table 3). Besides, this, petroleum ether, acetone and water extracts of Persicaria hydropiper exhibit substantial larvicidal property against fourth instar larvae of *Aedes aegypti*. The medicinal plant *Sphaernthus indicus* showed less mortality in Petroleum ether, methanol, Acetone and Water extract against *Aedes aegypti*. Similarly, the other medicinal plant species such as *Sphaernthus indicus*, Hydrocotyle javanica, Deris trifoliata Lourd, Acanthospermum hispidum Drymaria cordata, Toddelia Asiatica and Triumfetta rhomboide show relatively less mortality against *Aedes aegypti* fourth instar larvae. Among the nine plants investigated, maximum larval mortality was obtained from the petroleum ether and methanol extract of Plectranthus hadiensis and Persicaria hydropiper respectively.

Table 2: Screening of plant extracts for larvicidal activity against *Aedes aegypti* at 1000 mg/L concentration

<table>
<thead>
<tr>
<th>S No.</th>
<th>Plants tested</th>
<th>Petroleum ether</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Sphaernthus indicus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Hydrocotyle javanica</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Deris trifoliata Lour.</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Persicaria hydropiper</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td><em>Acanthospermum hispidum</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>Drymaria cordata</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td><em>Toddelia asiatica</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td><em>Plectranthus hadiensis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td><em>Triumfetta rhomboide</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ above 50 percent mortality at 24 hr, - No larval mortality

Table 3: Larvicidal activity of plant extracts against fourth instar larvae of *Aedes aegypti* after 24 h exposure (mg/L)

<table>
<thead>
<tr>
<th>Plants</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Petroleum ether</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Persicaria hydropiper</strong></td>
<td><em>LC$_{50}$</em></td>
<td><em>LC$_{90}$</em></td>
<td><em>LC$_{50}$</em></td>
<td><em>LC$_{90}$</em></td>
</tr>
<tr>
<td>658.005 (422.174-1625.366)</td>
<td>5572.689 (2027.987-14392.195)</td>
<td>489.278 (316.218-962.889)</td>
<td>4121.964 (1651.772-68018.777)</td>
<td>750.326 (494.333-1763.542)</td>
</tr>
<tr>
<td><strong>Plectranthus hadiensis</strong></td>
<td><em>LC$_{50}$</em></td>
<td><em>LC$_{90}$</em></td>
<td><em>LC$_{50}$</em></td>
<td><em>LC$_{90}$</em></td>
</tr>
</tbody>
</table>

Discussion

Natural insecticides are safer than synthetic ones as they possess little chance of developing insecticide resistance [1]. Vector control at its larval stage is the significant option as they posses little chance of developing insecticide resistance [1]. However, frequent use of synthetic insecticides for mosquito control may leads to various harmful effects to human beings and non target organisms [24,25]. Hence, the demand for plant based products is enhancing as they are usually recognized to be bio degradable, natural, and safer than synthetic drugs [34].

Extracts of plants contain various active phyto compounds; hence they can be used as alternative approach to mosquito larval control. Many scientific studies have proven that plant derived products can be used as an alternate approach to control mosquito population [31,32].

The finding of the present investigation indicated that *Persicaria hydropiper* can be used as an alternative to synthetic insecticides to control mosquito transmitting diseases. The
petroleum ether, methanol, acetone and water extract of Persicaria hydropiper showed highest mortality against fourth instar larvae of Aedes aegypti. Similarly Plectranthus hadiensis showed potential mortality against fourth instar larvae of Aedes aegypti. The remaining plant species such as Sphaeranthus indicus, Hydrocotyle javanica, Deris trifoliata, Lour, Acanthospermum hispidum, Drymaria cordata, Toddelia asiatica, Triumfetta rhomboidea comparatively showed less mortality than the other two plant species.

The present investigation obviously proved that the leaf extracts of Plectranthus hadiensis and Persicaria hydropiper have remarkable larvicidal property against Aedes aegypti vector mosquitoes. The flora vegetation of India has prosperous aromatic plant diversity; hence they can be used for the development of natural insecticides for controlling mosquito population and to prevent mosquito transmitting diseases. The results from the present investigation might encourage the search for novel, natural insecticides offering an alternative to synthetic insecticides from traditionally used medicinal plants. The leaf extracts of Plectranthus hadiensis and Persicaria hydropiper have the potential to be used as an ideal approach for the mosquito control programmes.

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