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# Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae)

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ABSTRACT

**Objective:** To determine the ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* (*E. coronaria*) and *Caesalpinia pulcherrima* (*C. pulcherrima*) against *Culex quinquefasciatus* (*Cx. quinquefasciatus*), *Aedes aegypti* (*Ae. aegypti*) and *Anopheles stephensi* (*An. stephensi*). **Methods:** The ovicidal activity was determined against three mosquito species at various concentrations ranging from 50–450 ppm under the laboratory conditions. The hatch rates were assessed 48 h after treatment. The repellent efficacy was determined against three mosquito species at three concentrations *viz.*, 1.0, 2.5 and 5.0 mg/cm$^2$ under the laboratory conditions. **Results:** The crude extract of *E. coronaria* exerted zero hatchability (100% mortality) at 250, 200 and 150 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The crude extract of *C. pulcherrima* exerted zero hatchability (100% mortality) at 375, 300 and 225 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. Stephensi*, respectively. The methanol extract of *E. coronaria* found to be more repellenct than *C. pulcherrima* extract. A higher concentration of 5.0 mg/cm$^2$ provided 100% protection up to 150, 180 and 210 min against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The results clearly showed that repellent activity was dose dependent. **Conclusions:** From the results it can be concluded the crude extracts of *E. coronaria* and *C. pulcherrima* are an excellent potential for controlling *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* mosquitoes.

## 1. Introduction

Mosquito-borne diseases, like malaria, yellow and dengue fevers, are a major threat to over 2 billions people in the tropics[1]. An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides[2], and for more detailed studies of naturally-occurring insecticides[3]. Plants may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals. They are active against a limited number of species including specific target insects, and are bio-degradable. They are potentially suitable for use in integrated pest management programs[4]. The leaf and seed extract of plant *Agave americana*[5] had mosquito larvicidal properties, and the extract of *Tagetes minuta* flowers had mosquito larvicidal activity against *Aedes aegypti* (*Ae. aegypti*)[6]. The methanolic fraction of leaves of *Mentha piperita, Phyllanthus niruri, Leucas aspera* and *Vitex negundo* were against larvae of *Culex quinquefasciatus* (*Cx. quinquefasciatus*)[7]. The methanolic extracts of *Solanum suratense, Azadirachta indica* and *Hydrocotyle javanica* exhibited larvicidal activity against *Cx. quinquefasciatus*[8].

More than 1 005 plant species are found to possess insecticidal properties, 384 contain antifeedants, 297 contain repellents, and 27 contain attractants and possess growth inhibitors. All these indicate that the plant kingdom is a vast storehouse of potentially useful chemicals for pest control. It is believed that insect resistance is less likely to occur because many botanicals contain multiactive principles. The pest control principles include properties of insecticide, antifeedant, repellent, chemosterilant,

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attractant, juvenile and anti-juvenile hormone, moulting and antimoulting hormone, nematicide, rodenticide, fungicide and bactericide[9-11]. Sivagnaname and Kalyanasundaram[12] evaluated the methanolic extracts of the leaves of *Atlanta monophylla* (Rutaceae) for mosquitocidal activity against immature stages of three mosquito species, *Cx. quinquefasciatus*, *Anopheles stephensi* (*An. stephensi*) and *Ae. aegypti* in the laboratory. Vasudevan *et al*[13] noted that ovicidal activity of castor oil extracted from castor seeds against mosquitoes *An. stephensi, Culex fatigans* and *Ae. aegypti*. Prakash[14] stated that ovicidal action of certain chitin synthesis inhibitors diflubenzuron, penfluron and bay SIR 8514 against mosquitoes, *Cx. quinquefasciatus*, *Ae. aegypti*, *An. stephensi* and *Anopheles culicifacies* (*An. culicifacies*). Ovicidal effect of the seed extract of *Atriplex canescens* was reported against *Cx. quinquefasciatus*[15]. Su and Mulla[16] reported that the ovicidal activity of neem products *Azadirachtin* against mosquitoes *Culex tarsalis* (*Cx. tarsalis*) and *Cx. quinquefasciatus*. Rajkumar and Jebanesan[17] studied that ovicidal activity of *Solanum trilobatum* leaf extract against *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*.

Larvicidal efficacy of the crude leaf extract of *Ficus benghalensis* with three different solvents like methanol, benzene and acetone was tested against the early second, third, fourth instar larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. Stephensi*[18]. The acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Acalypha indica, Achyranthes aspera, Leucas aspera, Morinda tinctoria* and *Ocimum sanctum* were studied against the early fourth-instar larvae of *Ae. aegypti* L and *Cx. quinquefasciatus* Say[19]. Larvicidal activities of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis, Coccinia indica, Cucumis sativus, Momordica charantia*, and *Trichosanthes anguina*, were tested against the early fourth instar larvae of *Ae. aegypti* L. and *Cx. quinquefasciatus*[20]. The benzene and methanol extracts of *Artemisia vulgaris* have repellent activity against *Ae. aegypti*[21]. The *Zanthoxylum armatus, Zanthoxylum alatum* (Rutaceae), *Azadirachta indica* (Mailiaceae) and *Curcuma aromatica* (Zingiberaceae) possess repellent properties against mosquitoes[22]. The repellent activity of active compound octacosane was from *Moschosma polystachyum* against the vector *Cx. quinquefasciatus*[23]. The essential oil of *Zingiber officinalis* was used as a mosquito larvicidal and repellent agent against the filarial vector *Cx. quinquefasciatus*[24]. The present study was carried out to determine the ovicidal and repellent efficacy of *Ervatamia coronaria* (*E. coronaria*) and *Caesalpinia pulcherrima* (*C. pulcherrima*) plant leaves extract against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

## 2. Materials and methods

### 2.1. Plant collection

Fully developed leaves of the *E. coronaria* and *C. pulcherrima* were collected from in and around Annamalai University Campus, Annamalainagar, Tamil Nadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen was deposited at the Herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University.

### 2.2. Extraction

The dried leaves (1.0 kg) were extracted with methanol (3.0 L) in a soxhlet apparatus and the extract was evaporated in a rotary vacuum evaporator to yield a dark greenish mass. Standard stock solutions were prepared at 1% by dissolving the residues in methanol, which was used for the bioassays.

### 2.3. Mosquitoes

The mosquitoes, *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28±2)℃, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

### 2.4. Ovicidal activity

Ovicidal activity was assessed by the slightly modified method of Su and Mulla[16]. The egg raft/eggs of *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* were collected from Vector Control Laboratory, Annamalai University. The different leaf extract was diluted in the appropriate solvent to achieve various concentrations ranging from 50 to 450 ppm. Eggs of these mosquito species (100 nos.) were exposed to each concentrations of leaf extract. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h after treatment by the following formula.

$$\%\ \text{of egg mortality} = \frac{\text{No. of hatched larvae}}{\text{Total No. of eggs}} \times 100$$

### 2.5. Repellent activity

The repellent study was following the method of WHO[25]. Three-day-old blood-starved female *Cx. quinquefasciatus* and *An. stephensi* mosquitoes (100) were kept in a net cage (45 cm × 30 cm × 45 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of volunteer, only 25 $cm^2$ dorsal side of the skin on each arms was exposed and the remaining area

covered by rubber gloves. The crude extract was applied at 1.0, 2.5 and 5.0 mg/cm$^2$, separately in the exposed area of the forearm. Only ethanol served as control. The time of the test depended on whether the target mosquitoes day- or night biters. *Ae. aegypti* was tested during the day time from 7:00 to 17:00, while *Cx. quinquefasciatus* and *An. stephensi* were tested during the night from 19:00 to 5:00. The control and treated arms were introduced simultaneously into the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arms into the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood; making out a 5 min protection. The percentage of repellency was calculated by the following formula.

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where $T_a$ is the number of mosquitoes in the control group and $T_b$ is the number of mosquitoes in the treated group.

### 2.6. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating $LC_{50}$, $LC_{90}$ and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and *Chi*-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

## 3. Results

The methanol extracts of *E. coronaria* and *C. pulcherrima* have been studied for use as natural insecticides instead of organic phosphorous materials or other synthetic agents. Results on the ovicidal and repellent effects of leaf extract were reported in the present study and confirmed their potential for control of the mosquito populations (Table 1-4). The crude extract of *E. coronaria* exerted zero hatchability (100% mortality) at 250, 200 and 150 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*,

**Table 1**
Ovicidal activity of methanol extract of *E. coronaria* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

| Mosquito | Percentage of egg hatch ability | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 50 ppm | 100 ppm | 150 ppm | 200 ppm | 250 ppm | 300 ppm |
| *Cx. quinquefasciatus* | 100.0±0.0 | 66.8±1.9 | 45.6±1.7 | 31.2±1.9 | 23.8±1.3 | NH | NH |
| *Ae. aegypti* | 100.0±0.0 | 57.9±1.5 | 38.2±0.8 | 26.4±1.9 | NH | NH | NH |
| *An. stephensi* | 100.0±0.0 | 51.2±1.8 | 34.6±1.5 | NH | NH | NH | NH |

Each value ( $\chi$ ±SD) represents the mean of six values; NH: No hatchability (100% mortality).

**Table 2**
Ovicidal activity of methanol extract of *C. pulcherrima* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

| Mosquito | Percentage of egg hatch ability | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 75 ppm | 150 ppm | 225 ppm | 300 ppm | 375 ppm | 450 ppm |
| *Cx. quinquefasciatus* | 100.0±0.0 | 66.4±1.5 | 49.8±1.2 | 32.6±1.6 | 23.4±1.2 | NH | NH |
| *Ae. aegypti* | 100.0±0.0 | 59.6±1.2 | 37.4±1.6 | 23.8±1.7 | NH | NH | NH |
| *An. stephensi* | 100.0±.0.0 | 54.6±1.5 | 28.8±1.6 | NH | NH | NH | NH |

Each value ( $\chi$ ±SD) represents the average of six values; NH: No hatchability (100% mortality).

**Table 3**
Repellent activity of crude methanol extract of *E. coronaria* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

| Mosquitoes | Concentration (mg/cm$^2$) | % of repellency | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 30 min | 60 min | 90 min | 120 min | 150 min | 180 min | 210 min | 240 min |
| *Cx. quinquefasciatus* | 1.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 83.2±1.2 | 72.6±1.9 | 64.3±2.2 | 56.5±1.6 | 41.6±1.6 |
| | 2.5 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 84.3±0.0 | 79.2±1.8 | 68.2±1.4 | 59.4±1.2 |
| | 5.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 88.7±1.2 | 88.9±1.9 | 74.3±1.8 |
| *Ae. aegypti* | 1.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 79.2±1.8 | 71.3±0.8 | 61.7±1.7 | 52.0±1.9 |
| | 2.5 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 82.2±1.7 | 73.5±1.2 | 64.2±1.3 |
| | 5.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 89.7±0.8 | 76.8±1.4 |
| *An. stephensi* | 1.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 78.0±1.5 | 69.2±1.9 | 57.7±1.2 |
| | 2.5 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 86.4±1.4 | 78.2±0.8 |
| | 5.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 87.4±1.4 |

Each value ( $\chi$ ± SD) represents average of six values.

**Table 4**
Repellent activity of crude methanol extract of *C. pulcherrima* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

| Mosquitoes | Concentration (mg/cm$^2$) | % of repellency | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 60 min | 90 min | 120 min | 150 min | 180 min | 210 min |
| *Cx. quinquefasciatus* | 1.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 78.2±0.8 | 68.8±1.5 | 53.1±1.8 | 36.5±1.2 | 31.2±0.9 |
| | 2.5 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 76.3±1.2 | 66.4±1.5 | 47.4±1.5 | 38.9±1.8 |
| | 5.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 78.9±1.3 | 69.9±1.4 | 58.4±1.9 |
| *Ae. aegypti* | 1.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 80.8±1.8 | 63.7±1.6 | 61.7±1.6 | 56.6±1.6 |
| | 2.5 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 81.6±1.4 | 72.3±1.2 | 66.0±1.8 |
| | 5.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 83.5±2.1 | 73.5±1.3 |
| *An. stephensi* | 1.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 79.3±1.5 | 68.4±1.6 | 59.0±1.4 |
| | 2.5 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 83.3±1.5 | 75.5±1.8 |
| | 5.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 82.1±2.2 |

Each value ( $\chi \pm$SD) represents average of six values.

respectively. The crude extract of *C. pulcherrima* exerted zero hatchability (100% mortality) at 375, 300 and 225 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The methanol extract of *E. coronaria* was found to be more repellent than *C. pulcherrima* extract. A higher concentration of 5.0 mg/cm$^2$ provided 100% protection up to 150, 180 and 210 min against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The repellent activity was very high at the initial stage of exposure. Increase in the exposure period showed reduction in repellent activity and it depends upon the concentration of the extract and density of mosquito. The results clearly showed that repellent activity was dose dependent.

## 4. Discussion

The results of entomotoxicity of *E. coronaria* and *C. pulcherrima* crude leaf extract were also comparable with earlier reports. Larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform and methanol extracts of the leaf of three plants, *Eclipta alba* (*E. alba*), *Cardiospermum halicacabum* (*C. halcacabum*), and *Andrographis paniculata* (*A. paniculata*), were tested against the early third instar larvae of *An. stephensi*, the highest larval mortality was found in methanol extract of *A. paniculata*, *E. alba* and *C. halicacabum* against the larvae of *An. stephensi* ($LC_{50}$=79.68, 112.56 and 133.01 ppm; $LC_{90}$=154.66, 220.68 and 270.72 ppm), respectively. The percent hatchability was inversely proportional to the concentration of extract. Mortality of 100% with methanol and ethyl acetate extract of *A. paniculata* and methanol extract of *E. alba* was exerted at 200 ppm and methanol and benzene extract of *C. halicacabum* exerted at 150 ppm[26]. Methanolic leaf extract of *Cassia fistula* was tested for larvicidal activity against *Cx. quinquefasciatus* and *An. stephensi*[27]. The leaf extract of *Acalypha indica* with different solvents *viz*, benzene, chloroform, ethyl acetate and methanol was tested for larvicidal, ovicidal activity and oviposition attractancy against *An. stephensi*. The larval mortality was observed after 24 h exposure. The $LC_{50}$ values were 19.25, 27.76, 23.26 and 15.03 ppm, respectively[28]. The extract was found to be more lethal to the larvae of *An. stephensi* than *Cx. quinquefasciatus* with $LC_{50}$ values of 17.97 and 20.57 mg/L, respectively. The extracts of *Quercus iusitania* var. infectoria galls (Oliv.) showed larvicidal activity and their possible use in biological control of *Culex pipines*[29]. The $LC_{50}$ values are 335 and 373 ppm for the 2nd and 4th instar larvae. The leaf extract of *Cassia fistula* with different solvents *viz*, methanol, benzene and acetone was studied for the larvicidal, ovicidal and repellent activity against *Ae. aegypti*. The 24 h $LC_{50}$ concentration of the extract against *Ae. aegypti* were observed at 10.69, 18.27 and 23.95 mg/L, respectively[30]. The mosquito larvicidal properties of the leaf extract of a herbaceous plant, *Ocimum canum* against *Ae. aegypti* was observed. The $LC_{50}$ values for 2nd, 3rd and 4th instar larvae were 177.82, 229.08 and 331.13 ppm, respectively[31]. Phytochemicals obtained from huge diversity of plant species are an important source of safe and biodegradable chemicals which could be screened for mosquito repellent and insecticidal activities. Repellents of plant origin do not pose hazards of toxicity to human and domestic animal and are easily biodegradable. Natural products are safe for human when compared with synthetic compounds[32].

Rahuman and Venkatesan[33] reported the petroleum ether extract of *Citrullus colocynthis*, methanol extracts of *Cannabis indica, Cannabis sativus, Momordica charantia* and acetone extract of *Trichosanthes anguina* against the larvae of *Ae. aegypti* ($LC_{50}$=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *Cx. quinquefasciatus* ($LC_{50}$=88.24, 377.69, 623.80, 207.61 and 842.34 ppm), respectively. Larvicidal efficacies of methanol extracts of *Momordica charantia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera* and *Citrullus vulgaris* tested with $LC_{50}$ values were 465.85, 567.81, 839.81, 1 189.30 and 1 636.04 ppm, respectively, against the late third larval age group of *Cx. quinquefasciatus*[34]. The ethanol extracts of the aerial parts from five *Labiatae* species, *Teucrium divaricatum* was the most toxic, followed by *Mentha longifolia, Melissa officinalis, Salvia sclarea* and *Mentha pulegium* against the third- and fourth-instar larvae of *Culex pipiens* with $LC_{50}$

values of 18.6, 26.8, 39.1, 62.7 and 81.0 ppm, respectively[35]. Extract from *Lavandula stoechas* (Labiatae) showed $LC_{50}$ values of 89 mg/L against fourth instar larvae of *Culex pipiens* molestus[36]. Mean median lethal concentration values of the aqueous extract from the roots of *Hibiscus abelmoschus* (Malvaceae) against the larvae of *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* were 52.3, 52.6 and 43.8 ppm, respectively[37]. Larvicidal efficacy of leaf extracts of *Pavonia zeylanica* and *Acacia ferruginea (Malvaceae)* were tested against the late third-instar larvae of *Cx. quinquefasciatus*, and their $LC_{50}$ values were 2 214.7 and 5 362.6 ppm, respectively[38].

Mullai and Jebanesan[39] have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *Citrullus colocynthis* and *Cucurbita maxima* showed $LC_{50}$ values of 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae. Rahuman *et al*[40] have reported that the $LC_{50}$ value of petroleum ether extracts of *Jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta*, and *Euphorbia tirucalli* were 8.79, 55.26, 90.92, 272.36 and 4.25 ppm, respectively, against *Ae. aegypti* and 11.34, 76.61, 113.40, 424.94 and 5.52 ppm, respectively, against *Cx. quinquefasciatus*. Karunamoorthi *et al*[41] reported that the petroleum ether (60-80 ℃) extracts of the leaves of *V. negundo* were evaluated for larvicidal activity against larval stages of *Cx. tritaeniorhynchus* in the laboratory with $LC_{50}$ and $LC_{90}$ values of 2.488 3 and 5.188 3 mg/L, respectively. The methanol leaf extracts of *V. negundo*, *Vitex trifolia* (*V. trifolia*), *Vitex peduncularis* (*V. peduncularis*) and *Vitex altissima* (*V. altissima*) possessed varying levels of larvicidal activity on *Cx. quinquefasciatus* and *An. stephensi* and found with $LC_{50}$ value of 212.57, 41.41, 76.28 and 128.04 ppm, respectively[42]. The peel methanol extract of *Citrus sinensis* and the leaf and flower ethyl acetate extracts of *Ocimum canum* were tested against the larvae of *An. stephensi* ($LC_{50}$=95.74, 101.53, 28.96, $LC_{90}$=303.20, 492.43 and 168.05 ppm), respectively[43]. The larvicidal effect of ten plants corresponds to different botanical families on *An. stephensi* and *Cx. quinquefasciatus*. The highest larval mortality was found in leaf acetone and methanol of *Canna indica* ($LC_{50}$= 29.62 and 40.77 ppm; $LC_{90}$=148.55 and 165.00 ppm) against second instar larvae ($LC_{50}$=121.88 and 69.76 ppm; $LC_{90}$=624.35 and 304.27 ppm) and against fourth instar larvae of methanol and petroleum ether extracts of *Ipomoea carnea* ($LC_{50}$= 41.82 and 39.32 ppm; $LC_{90}$=423.76 and 176.39 ppm) against second instar larvae ($LC_{50}$=163.81 and 41.75 ppm; $LC_{90}$= 627.38 and 162.63 ppm) and against fourth instar larvae of *Cx. quinquefasciatus*, respectively[44]. This study reveals that the *E. coronaria* and *C. pulcherrima has* remarkable ovicidal and repellent properties against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* mosquitoes. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## References

[1] Service MW. Mosquitoes (Culicidae). In: Lane RP, Crosskey RW. (eds.) *Medical insects and arachnids*. London: Chapman & Hall; 1993, p. 723-725.

[2] Macedo ME, Consoli RAGB, Grandi TSM, Anjos AMG, Oliveira AB, Mendes NM, et al. Screening of *Asteraceae* (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz* 1997; **92**: 565-570.

[3] Govindarajan M. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (willd.) Hook. F. Benth (Rutaceae) against three mosquito species. *Asian Pac J Trop Med* 2010; **3**: 691-695.

[4] Alkofahi A, Rupprecht JK, Anderson JE, Mclaughlin JL, Mikolajczak KL, Scott BA. Search for new pesticides from higher plants. In: Arnason JT, Philogene BJR, Morand P. (eds.) *Insecticides of plant origin*. Washington DC: Oxford University Press; 1989, p. 25-43.

[5] Dharmshaktu NS, Prabhakaran PK, Menon PK. Labortory study on the mosquito larvicidal properties of leaf and seed extract of plant *Agava americana*. *J Trop Med Hyg* 1987; **90**: 79-82.

[6] Green MM, singer JM, Sutherland DJ, Hibbon CR. Larvicidal activity of *Tagetes minuta* (Marigold) towards *Aedes aegypti*. *J Am Mosq Control Assoc* 1991; **7**: 282-286.

[7] Pandian RS, Abraham MG, Manoharan AC. Susceptibility of the larvae of *Culex quinquefasciatus* Say to extracts of medicinal plants. *Environ Pollut* 1994; **1**: 109-122.

[8] Muthukrishnan J, Pushpalatha E, Kasthuribhai A. Biological effect of four plant extracts on *Culex quinquefasciatus* say larval stages. *Insect Sci Appl* 1997; **7**: 389-394.

[9] Rajkumar S, Jebanesan A. Mosquitocidal activities of octacosane from *Moschosma polystachyum* Linn (Lamiaceae). *J Ethnopharmacol* 2004; **90**: 87-89.

[10] Govindarajan M. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (willd.) Hook. F. Benth (Rutaceae) against three mosquito species. *Asian Pac J Trop Med* 2010; **3**: 691-695.

[11] Mathivanan T, Govindarajan M, Elumalai K, Krishnappa K, Ananthan A. Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Stapf. (Family: Apocynaceae). *J Vector*

*Borne Dis* 2010; **47**: 178-180.

[12] Sivagnaname N, Kalyanasundaram M. Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms. *Mem Inst Oswaldo Cruz Rio de Jenerio* 2004; **99**(1): 115-118.

[13] Vasudevan P, Madan N, Sharma S. Ovicidal property of castor. *Pesticides* 1989; **2**: 36-39.

[14] Prakash A. Ovicidal action of certain chitin synthesis inhibitors in mosquitoes. *Entomon* 1992; **17**: 15-19.

[15] Ouda NAA, Al-Chalabi BM, Mohsen ZH. Extract of *Atriplex canescens* against *Culex quinquefasciatus*. *Pharm Biol* 1998; **36**(1): 69-71.

[16] Su T, Mulla S. Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *J Am Mosq Control Assoc* 1998; **14**(2): 204-209.

[17] Rajkumar S, Jebanesan A. Ovicidal activity of *Solanum trilobatum* L. (Solanaceae) leaf extract against *Culex quinquefasciatus* Say and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Int J Trop Insect Sci* 2004; **24**(4): 340-342.

[18] Govindarajan M. Larvicidal efficacy of *Ficus benghalensis* L. plant leaf extracts against *Culex quinquefasciatus* Say. *Aedes aegypti* L. and *Anopheles stephensi* L. (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci* 2010; **14**: 107-111.

[19] Bagavan A, Abdul Rahuman A, Kamaraj C, Geetha K. Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2008; **103**: 223-9.

[20] Rahuman A, Venkatesan P. Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. *Parasitol Res* 2008; **103**: 133-139.

[21] Yit Hs, Ku-Hua WV, Kumato JH, Mulla MS. Isolation and identification of mosquito repellent in *Artemesia vulgaris*. *J Chem Ecol* 1985; **11**: 1297-1306.

[22] Das NG, Nath DR, Baruah I, Talukdar PK, Das SC. Field evaluation of herbal mosquito repellents. *J Commun Dis* 2000; **31**(4): 241-245.

[23] Rajkumar S, Jebanesan A. Mosquitocidal activities of octacosane from *Moschosma polystachyum* Linn. (Lamiaceae). *J Ethnophar*macol 2004; **90**: 87-89.

[24] Pushpanathan T, Jebanesan A, Govindarajan M. The essential oil of *Zingiber officinalis* Linn (Zingiberaceae) as a mosquito larvicidal and repellent agent against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res* 2008; **102**: 289-292.

[25] WHO. *Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES /IC /96*. Genera: WHO; 1996, p. 69.

[26] Govindarajan M. Evaluation of indigenous plant extracts against the malarial vector, *Anopheles stephensi* (Liston) (Diptera: Culicidae). *Parasitol Res* 2010. doi: 10.1007/s00436-010-2224-0.

[27] Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitol Res* 2008; **102**: 289-292.

[28] Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K. Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res* 2008; **103**(3): 691-695.

[29] Redwane A, lazrek HB, Bouallam S, Markouk M, Amarouch H, Jana M. Larvicidal activity of extracts from *Quercus lusitania* var. infetoria galls (oily). *J Ethnopharmacol* 2002; **79**(2): 261-263.

[30] Govindarajan M. Bioefficacy of *Cassia fistula* Linn. (Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci* 2009; **13**(2): 99-103.

[31] Singh NP, Kumari V, Chauhan D. Mosquito larvicidal properties, of the leaf extract of an herbaceous plant, *Ocimum canum* (Family: Labiatae). *J Commun Dis* 2003; **35**(1): 43-45.

[32] Sharma VP, Ansari MA. Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. *Indian Med Entomol* 1994; **31**(3): 505-507.

[33] Rahuman AA, Venkatesan P. Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. *Parasitol Res* 2008; **103**(1): 133-139.

[34] Prabakar K, Jebanesan A. Larvicidal efficacy of some Cucurbitacious plant leaf extracts against *Culex quinquefasciatus* (Say). *Bioresour Technol* 2004; **95**(1): 113-114.

[35] Cetin H, Cinbilgel I, Yanikoglu A, Gokceoglu M. Larvicidal activity of some Labiatae (Lamiaceae) plant extracts from Turkey. *Phytother Res* 2006; **20**(12): 1088-1090.

[36] Traboulsi AF, Taoubi K, El-Haj S, Bessiere JM, Rammal S. Insecticidal properties of essential plant oils against the mosquito *Culex pipiens* molestus (Diptera: Culicidae). *Pest Manag Sci* 2002; **58**: 491-495.

[37] Dua VK, Pandey AC, Alam ME, Dash AP. Larvicidal activity of *Hibiscus abelmoschus* Linn. (Malvaceae) against mosquitoes. *J Am Mosq Control Assoc* 2006; **22**(1):155-157.

[38] Vahitha R, Venkatachalam MR, Murugan K, Jebanesan A. Larvicidal efficacy of *Pavonia zeylanica* L. and *Acacia ferruginea* D.C. against *Culex quinquefasciatus* Say. *Bioresour Technol* 2002; **82**(2): 203-204.

[39] Mullai K, Jebanesan A. Larvicidal, ovicidal and repellent activities of the leaf extract of two cucurbitacious plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Trop Biomed* 2007; **24**(1): 1-6.

[40] Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2008; **102**: 867-873.

[41] Karunamoorthi K, Ramanujam S, Rathinasamy R. Evaluation of leaf extracts of *Vitex negundo* L (Family: Verbenaceae) against larvae of *Culex tritaeniorhynchus* and repellent activity on adult vector mosquitoes. *Parasitol Res* 2008; **103**: 545-550.

[42] Pushpalatha E, Muthukrishnan J. Larvicidal activity of a few plant extracts against *Culex quinquefasciatus* and *Anopheles stephensi*. *Indian J Malariol* 1995; **32**: 14-23.

[43] Kamaraj C, Rahuman AA, Bagavan A. Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston). *Parasitol Res* 2008; **103**: 1361-138.

[44] Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res* 2009; **104**: 1365-1372.