Mosquito repellent properties of *Delonix elata* (L.) gamble (Family: Fabaceae) against filariasis vector, *Culex quinquefasciatus* Say. (Diptera: Culicidae)

Marimuthu Govindarajan*

Unit of Vector Biology and phytochemistry, Department of Zoology, Annamalai University, Annamalainagar–608 002, Tamilnadu, India

**Objective:** To determine the repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *Delonix elata* (*D. elata*) leaf and seed against *Culex quinquefasciatus* (*Cx. quinquefasciatus*).

**Methods:** Evaluation was carried out in a net cage (45 cm×30 cm×25 cm) containing 100 blood starved female mosquitoes of *Cx. quinquefasciatus*. Repellent activity was carried out in the laboratory conditions according to the WHO 2009 protocol. Plant crude extracts of *D. elata* were applied at 1.0, 2.5, and 5.0 mg/cm² separately in the exposed fore arm of study subjects. Ethanol was used as the sole control.

**Results:** In this study, the applied plant crude extracts were observed to protect against mosquito bites. There were no allergic reactions experienced by the study subjects. The repellent activity of the extract was dependent on the strength of the extract. Among the tested solvents, the leaf and seed methanol extract showed the maximum efficacy. The highest concentration of 5.0 mg/cm² provided over 150 min and 120 min protection, respectively.

**Conclusions:** Crude extracts of *D. elata* exhibit the potential for controlling *Cx. quinquefasciatus*, the mosquito vector of filariasis.
mosquito–borne disease caused by mosquito–transmitted filarial nematodes, including *Wuchereria bancrofti* (W. bancrofti) and *Brugia malayi*. *Culex quinquefasciatus* (Cx. quinquefasciatus) is the main vector for lymphatic filariasis. Around 120 million people infected worldwide and 44 million people have common chronic clinical manifestation. According to WHO[1], about 90 million people worldwide are infected with *W. bancrofti*, the lymphatic dwelling parasite, and ten times more people are at the risk of being infected. In India alone, 25 million people harbor microfilaria and 19 million people suffer from filarial disease manifestations[3]. Insect pests have mainly been controlled with synthetic insecticides and repellents in the last 50 years. However, one major drawback with the use of these chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment[4]. Plant products have been used traditionally by human communities in many parts of the world against vectors and many species of insects. The phytochemicals derived from plant sources can act as larvicides, insect growth regulators and repellents[5].

The authors[6] previously reported that the leaf methanol, benzene, and acetone extracts of *Cassia fistula* were studied for the larvicidal, ovicidal, and repellent activities against *Aedes aegypti* (Ae. aegypti), Baba et al.[7] have screened the phytochemicals and compared their mosquito repellent activities of essential oils from *Hyptis spicigera*, *Striga hermonthica* and *Ocimum basilicum* (Basil) against *Anopheles gambiae* and Cx. quinquefasciatus under laboratory conditions[8]. Swathi et al.[8] have evaluated ethanolic extracts of *Datura stramonium* leaves for larvicidal and mosquito repellent activities against *Ae. aegypti*, *Anopheles stephensi* (An. stephensi) and Cx. quinquefasciatus. The larvalicidal efficacy of different solvent leaf extracts of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Anopheles subpictus* and *Culex tritaeniorhynchus*[9] has been studied. Phasomkusolsil and Soonwera[10] showed the repellency of seven essential oils to female *Ae. aegypti*, *Anopheles dirus* and Cx. quinquefasciatus. Govindarajan et al.[11] have assessed the larvicidal and ovicidal potential of the crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts from the medicinal plant *Delonix elata* (D. elata) against two medically important vectors. They have further reported on the toxicity of mosquito larvicidal activity of leaf essential oil and their major chemical constituents from *Mentha spicata* against Cx. quinquefasciatus, *Ae. aegypti*, and *An. stephensi*[12]. As far as the author’s literature survey could ascertain, no information was available on the repellent activity of the experimental plant species given here against Cx. quinquefasciatus. Therefore, the aim of this study was to investigate the mosquito repellent activity of the different solvent extracts of *D. elata* plant species from Tamilnadu, India. This is the first report on the mosquito repellent activity of the solvent extracts of selected plant.

2. Materials and methods

2.1. Plant collection

Fully developed leaves and seeds of the *D. elata* were collected from Thanjavur District (Between 9°50’ and 11°25’ of the north latitude and 78°45’ and 70°25’ of the east longitude), Tamilnadu, 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 leaves and seeds were washed with tap water, shade dried, and finely ground. The finely ground leaf and seed powder (1.0 kg/solvent) was loaded in soxhlet extraction apparatus. Five different solvents, namely, hexane, benzene, chloroform, ethyl acetate and methanol were used for extraction. The solvents were removed from the extracts using a rotary vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared and these solutions were used for repellent bioassay.

2.3. Test organisms

Cx. quinquefasciatus was reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week old chicks for blood meal. Mosquitoes were held at (28±2) °C, 70%–85% relative humidity, with a photo period of 12–h light and 12–h dark.

2.4. Repellent activity

The repellency was evaluated by using the percentage of protection in relation to dose method[13]. One hundred three–day old starved female *Cx. quinquefasciatus* mosquitoes were kept on a net cage (45 cm×30 cm×45 cm). Two cages with hungry mosquitoes for test and control were kept aside. The volunteer had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The arms of the volunteer skin washed and cleaned with ethanol and ethanol served as control, respectively. After air drying, the
arms of the volunteer, only 25 cm² dorsal side of the skin on each arm was exposed and the remaining area covered by rubber gloves. The different concentrations of crude extracts were applied. *C. quinquefasciatus* were tested during the night from 19:00 to 05:00 h. The control and treated arm were introduced simultaneously into the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. The volunteer conducted their test of each concentration by inserting the treated and control arm into cages at the same time for one full minute for every 5 min. The mosquitoes that land on the hand were recorded and then shaken off before it imbibes any blood. The percentage of repellency was calculated by the formula.

\[
\%\text{ repellency} = \left(\frac{T_a - T_b}{T_a}\right)\times 100
\]

Where \(T_a\) is the number of mosquitoes in the control group, and \(T_b\) is the number of mosquitoes in the treated group.

### 3. Results

In the present observation, the results from the skin repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *D. elata* leaf and seed against blood starved adult female of *C. quinquefasciatus* is given in Tables 1 and 2. The present results show that the percentage protection in relation to dose and time (min). Among the tested solvents, the maximum efficacy was observed in the leaf and seed methanol extract. The highest concentrations of 5.0 mg/cm² leaf and seed methanol extract of *D. elata* provided over 150 and 120 min protection against *C. quinquefasciatus*, respectively. In this observation, the plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity is dependent on the strength of the plant extracts. The tested plant crude extracts have exerted promising repellent against all the three mosquitoes.

### Table 1

Repellency of different solvent seed extracts of *C. quinquefasciatus*.

| Solvent | Concentration (mg/cm²) | Repellency (%) | Time of post application (min) |
|---------|------------------------|----------------|-------------------------------|
| Methanol | 1.0                    | 99.24±0.8      | 30                            |
|         | 2.5                    | 96.42±1.7      | 60                            |
|         | 5.0                    | 92.19±1.2      | 90                            |
| Ethyl   | 1.0                    | 95.42±1.6      | 120                           |
|         | 2.5                    | 94.12±1.9      | 150                           |
|         | 5.0                    | 92.62±1.8      | 180                           |
| Chloroform | 1.0                  | 92.32±1.1      | 120                           |
|          | 2.5                    | 90.42±1.5      | 150                           |
|          | 5.0                    | 88.52±1.9      | 180                           |
| Benzene | 1.0                    | 88.23±1.5      | 30                            |
|          | 2.5                    | 86.42±1.4      | 60                            |
|          | 5.0                    | 84.62±1.4      | 90                            |
| Hexane  | 1.0                    | 83.82±1.0      | 30                            |
|          | 2.5                    | 81.92±1.4      | 60                            |
|          | 5.0                    | 79.92±1.4      | 90                            |

### 4. Discussion

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. Phytoextracts are emerging as potential mosquito control agents, with low cost, easy-to-administer, and risk-free properties. Our results showed that the crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf and seed of *D. elata* have significant repellent property against filariasis vector mosquito *C. quinquefasciatus*. This result is comparable to earlier reports by Murugan *et al.*[14] who observed maximum repellent activity were observed at 450 mg/L with ethanol extracts of *Citrus sinensis*. They also observed a mean complete protection time ranged from 150 to 180 min. The ethanol extract of *Citrus sinensis* showed 100% repellency in 150 min and showed complete protection in 90 min at 350 mg/L against *A. stephensi*, *Ae. aegypti* and *C. quinquefasciatus*, respectively. Amersan *et al.*[15] have shown that hexane, chloroform benzene, acetone, and methanol extracts of *Cassia tora* provided significant repellent activity against *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. These researchers saw the highest repellency for 210 min with methanol extract against *A. stephensi*. The methanol extract repelled *A. stephensi* the most followed by *Ae. aegypti* and *C. quinquefasciatus* in that order.

The repellent activity of plant extract of *Artemisia nilagirica* plants at five different concentrations of 50, 150, 250, 350 and 450 mg/L have also been tested[16]. The highest repellency of 180 min was observed in methanol extract of *Artemisia nilagirica* against *A. stephensi* followed by *Ae. aegypti*, respectively[16]. Previously, the author[17] has demonstrated that the methanol extract of *Coccinia indica* is more repellent than the other extracts tested. A higher concentration of 5.0 mg/cm² provided 100% protection up to...
270 min against Cx. quinquefasciatus and 210 min against Ae. aegypti and An. stephensi, respectively. With respect to mortality, Govindarajan and Sivakumar[18] have observed the highest mortality in methanol extract of Cardiospermum halicacabum against three important vector mosquito species. Among the three species, An. stephensi produce the highest LC50 and LC90 values (186.00 and 346.06 mg/L respectively). In contrast, the LC50 and LC90 values of Cx. quinquefasciatus and Ae. aegypti were 211.78, 227.33 mg/L and 395.28, 423.33 mg/L, respectively.

The methanol extract of Eruatamia coronaria was found to be more repellent than Caesalpinia pulcherrima extract. A higher concentration of 5.0 mg/cm2 provided 100% protection up to 150, 180 and 210 min against Cx. quinquefasciatus, Ae. aegypti and An. stephensi, respectively[19]. The author[20] has also evaluated the larvicidal and repellent activities of crude extract of Sida acuta against the above noted important mosquitoes with LC50 values ranging between 38 to 48 mg/L. The crude extract Sida acuta proved to have strong repellent action as it provided 100% protection against An. stephensi for 180 min followed by Ae. aegypti (150 min) and Cx. quinquefasciatus (120 min). Pushpanathan et al.[21] reported that the essential oil of Zingiber officinalis showed repellent activity at 4.0 mg/cm2 and provided 100% protection of up to 120 min against Cx. quinquefasciatus. Compared with earlier reports, our results revealed that the experimental plant extracts were effective to control Cx. quinquefasciatus. From these results, it was concluded that the plant D. elata exhibits repellent activity against important vector mosquito. The flora of India has rich biodiversity of aromatic plants with potential for developing natural insecticides to control mosquito vectors and possibly other pests. These results of this study are encouraging and could assist in the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

Conflicts of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to the University Grants Commission (UGC) (F.No.39–646/2010 (SR)), New Delhi, India for providing financial assistance for the present investigation and thankful to the Professor and Head, Department of Zoology, Annamalai University for the laboratory facilities provided. We acknowledge the staff members of the VCRC (ICMR), Pondicherry for their cooperation.

Comments

Background

Diseases that are health care associated transmission of viruses to human from mosquitoes are an expanding problem in tropical and subtropical regions. The infected people carry the nocturnally periodic W. bancroftii, which has Cx. quinquefasciatus as the main mosquito vector. The literature reports a higher interest for a vector caused by lymphatic filariasis. However, also determine the repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of D. elata leaf and seed against Cx. quinquefasciatus.

Research frontiers

Studies are being performed in order to determine which are the significant reservoir of this result can be concluded the crude extract of D. elata was potential for controlling filariasis vector mosquito, Cx. quinquefasciatus.

Related reports

Govindarajan et al. (2012) reported larvicidal and ovicidal potential of the crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts from the medicinal plant D. elata against the medically important mosquito vectors, An. stephensi and Ae. aegypti. Swathi et al. (2012) evaluate the ethanolic extracts of leaves of Datura stramonium were evaluated for larvicidal and mosquito repellent activities against Ae. aegypti, An. stephensi and Cx. quinquefasciatus.

Innovations & breakthroughs

D. elata exhibits repellent activity against important vector mosquito. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. This study has showed that could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

Applications

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. Phytoextracts are emerging as potential mosquito control agents, with low–cost, easy–to–administer, and risk–free properties. The result of the present study repellency was evaluated by using the percentage of
protection time.

**Peer review**

This is a good study in which the authors evaluated the mosquito repellent activity of *D. elata* against medically important filariasis vector, *Cx. quinquefasciatus*. The results are interesting and suggested that plant derived *D. elata* are present especially in repellent activity. This study provides the first report on the mosquito repellent activity of the different solvent extracts of *D. elata* plant.

**References**

[1] Wilder-Smith A, Chen LH, Massad E, Wilson ME. Threat of dengue to blood safety in dengue–endemic countries. *Emerg Infect Dis* 2009; 15: 8–11.

[2] World Health Organization. Global information system on alcohol and health. Geneva: WHO; 2010. [Online] Available from: http://www.who.int/gho/alcohol/en/. [Accessed on June 12th 2013]

[3] Govindarajan M, Sivakumar R. Mosquito adulticidal and repellent activities of botanical extracts against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; 4(12): 941–947.

[4] de Omena MC, de Navarro DMAF, de Paula JE, Lima JS, de Lima MRF, Sant’A Ana EAG. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. *Bioresource Technol* 2007; 98: 2549–2556.

[5] Govindarajan M. Chemical composition and larvicidal activity of leaf essential oil from *Cluansena anisata* (Willd.) Hook. ex Benth (Rutaceae) against three mosquito species. *Asian Pac J Trop Med* 2011; 4(311): 874–877.

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

[7] Baba G, Lawal AO, Sharif HB. Mosquito repellent activity and phytochemical characterization of essential oils from *Striga hermonthica*, *Hyptis spicigera* and *Ocimum basilicum* leaf extracts. *Br J Pharmacol Toxicol* 2012; 3(2): 43–48.

[8] Swathi S, Murugananthan G, Ghosh SK, Pradeep AS. Larvicidal and repellent activities of ethanolic extract of *Datura stramonium* leaves against mosquitoes. *Int J Pharmacogn Phytochem Res* 2012; 4(1): 25–27.

[9] Govindarajan M, Sivakumar R, Amsath A, Niraimathi S. Larvicidal efficacy of botanical extracts against two important vector mosquitoes. *Eur Rev Med Pharmacol Sci* 2012; 16: 386–392.

[10] Phasomkulsol S, Soonwera M. Comparative mosquito repellency of essential oils against *Aedes aegypti* (Linn.), *Anopheles dirus* (Peyton and Harrison) and *Culex quinquefasciatus* (Say). *Asian Pac J Trop Biomed* 2011; 1(1): 113–18.

[11] Marimuthu G, Rajamohan S, Mohan R, Krishnamoorthy Y. Larvicidal and oxicidal properties of leaf and seed extracts of *Delonix elata* (L.) Gamble (Family: Fabaceae) against malaria (*Anopheles stephensi* Liston) and dengue (*Aedes aegypti* Linn.) (Diptera: Culicidae) vector mosquitoes. *Parasitol Res* 2012; 111: 65–77.

[12] Govindarajan M, Sivakumar R, Rajeswari M, Yogalakshmi K. Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. *Parasitol Res* 2012; 110: 2023–2032.

[13] World Health Organization. *Guidelines for efficacy testing of mosquito repellents for human skins*. Geneva: WHO; 2009, p. 4–18.

[14] Murukan K, Mahesh Kumar P, Kovendan K, Amerasan D, Subramaniam J, Hwang JS. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2012; 111: 1757–1769.

[15] Amerasan D, Murukan K, Kovendan K, Mahesh Kumar P, Panneerselvam C, Subramaniam J, et al. Adulticidal and repellent properties of *Cassia tora* Linn. (Family: Caesalpinaceae) against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*. *Parasitol Res* 2012; 111: 1953–1964.

[16] Panneerselvam C, Murukan K, Kovendan K, Mahesh Kumar P. Mosquito larvicidal, pupicidal, adulticidal, and repellent activity of *Artemisia nilagirica* (Family: Compositae) against *Anopheles stephensi* and *Aedes aegypti*. *Parasitol Res* 2012; 111(6): 2241–2251.

[17] Govindarajan M. Ovicidal and repellent properties of *Coccinia indica* Wight and Arn. (Family: Cucurbitaceae) against three important vector mosquitoes. *Eur Rev Med Pharmacol Sci* 2011; 15: 1010–1019.

[18] Govindarajan M, Sivakumar R. Adulticidal properties of *Cardiospermum halicacabum* plant extracts against three important vector mosquitoes. *Eur Rev Med Pharmacol Sci* 2012; 16(Suppl 3): 95–104.

[19] Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; 1(1): 43–48.

[20] Govindarajan M. Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. *Asian Pac J of Trop Med* 2010; 3(9): 691–695.

[21] 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: 1289–1291.