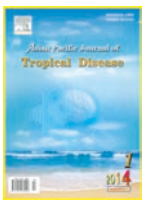
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(14)60438-2

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

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PEER REVIEW

Peer reviewer

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Comments

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.
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ABSTRACT

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/cm^2 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/cm^2 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.

KEYWORDS

Repellency, *Delonix elata*, Leaf and seed, *Culex quinquefasciatus*, Filariasis

1. Introduction

Mosquitoes are primary vectors for many dreadful and fatal diseases such as dengue, malaria, yellow fever and filariasis. They transmit diseases to more than 700 million people each year. Insect-transmitted disease remains a major source of illness and death worldwide. Diseases that are health care associated transmission of viruses to humans by mosquitoes are an expanding problem in tropical and subtropical regions. Some of them such as filariasis, dengue, malaria and West Nile virus are amongst the most prevalent diseases in the world[1]. Lymphatic filariasis is a

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Foundation Project: Supported by the University Grants Commission (UGC) [F.No.39-646/2010 (SR)], New Delhi, India.

Article history:
Received 15 Nov 2013
Received in revised form 26 Nov, 2nd revised form 4 Dec, 3rd revised form 12 Dec 2013
Accepted 17 Dec 2013
Available online 28 Jan 2014

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[2], 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 in the last 50 years. However, one major drawback with the use of they 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 author[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[7]. 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 larvicidal 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 activities 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 cm^2 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. *Cx. 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 a 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.

% repellency = $[(T_a - T_b)/T_a]\times100$

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 *Cx. 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/cm^2 leaf and seed methanol extract of *D. elata* provided over 150 and 120 min protection against *Cx. 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 leaf extracts of *D. elata* against *Cx. quinquefasciatus*.

Solvent	Concentration (mg/cm^2)	Repellency%±SD							
		Time of post application (min)							
		30	60	90	120	150	180	210	240
Methanol	1.0	100±0.0	100±0.0	100±0.0	94.5±1.5	81.7±1.4	66.5±1.2	51.2±1.7	38.9±1.5
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	93.7±1.7	79.6±1.4	65.4±1.0	51.7±1.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.2±1.9	82.0±1.2	69.3±1.2
Ethyl acetate	1.0	100±0.0	100±0.0	93.4±1.6	77.6±1.6	61.8±1.5	46.2±1.7	33.5±2.1	21.7±1.0
	2.5	100±0.0	100±0.0	100±0.0	94.0±1.9	80.4±1.6	67.5±1.0	50.3±2.5	37.6±1.9
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	96.1±1.4	82.3±2.6	69.6±1.0	53.2±2.0
Chloroform	1.0	100±0.0	100±0.0	91.4±1.9	75.2±1.1	58.9±1.8	45.2±2.4	32.1±1.4	20.2±2.5
	2.5	100±0.0	100±0.0	100±0.0	92.5±1.9	78.1±1.6	65.3±1.0	48.6±0.9	31.7±1.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	93.4±1.2	79.3±1.6	65.7±1.4	51.4±1.4
Benzene	1.0	100±0.0	100±0.0	89.0±1.9	74.1±1.6	55.2±1.5	42.8±1.4	30.6±1.5	19.3±1.3
	2.5	100±0.0	100±0.0	100±0.0	90.7±1.2	77.0±1.0	63.9±1.2	45.6±1.3	30.3±1.8
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	91.6±2.5	78.4±1.3	64.1±1.0	49.2±1.0
Hexane	1.0	100±0.0	100±0.0	88.2±1.5	72.6±1.9	52.7±1.9	40.5±2.0	29.2±1.6	15.3±1.5
	2.5	100±0.0	100±0.0	92.7±1.6	78.4±1.4	62.6±1.4	47.2±1.8	33.2±1.9	20.8±1.4
	5.0	100±0.0	100±0.0	100±0.0	93.5±1.0	79.8±2.0	63.7±1.4	49.5±2.3	35.0±1.0

Table 2
Repellency of different solvent seed extracts of *D. elata* against *Cx. quinquefasciatus*.

solvent	Concentration (mg/cm^2)	Repellency%±SD							
		Time of post application (minutes)							
		30	60	90	120	150	180	210	240
Methanol	1.0	100±0.0	100±0.0	100±0.0	92.8±2.3	79.8±1.2	65.9±1.5	52.3±2.1	39.5±2.0
	2.5	100±0.0	100±0.0	100±0.0	94.2±1.7	80.5±1.6	68.2±2.0	55.4±1.3	41.8±1.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	96.8±2.3	82.1±1.6	69.5±1.8	56.1±1.8
Ethyl acetate	1.0	100±0.0	100±0.0	94.1±1.1	80.0±1.5	66.2±1.5	53.1±1.7	38.1±1.1	25.6±2.5
	2.5	100±0.0	100±0.0	95.2±1.9	82.1±1.6	68.9±1.8	54.7±1.3	41.9±1.7	30.3±1.9
	5.0	100±0.0	100±0.0	100±0.0	96.2±2.0	81.3±1.8	68.3±2.1	54.1±2.3	41.0±2.4
Chloroform	1.0	100±0.0	100±0.0	92.5±2.1	78.1±1.8	64.2±1.6	51.0±2.6	36.2±1.9	23.8±1.6
	2.5	100±0.0	100±0.0	94.2±1.2	79.8±1.3	67.0±1.2	53.0±1.8	40.2±2.0	28.5±1.8
	5.0	100±0.0	100±0.0	100±0.0	94.3±1.4	80.7±1.0	66.2±1.9	51.9±1.6	40.2±1.4
Benzene	1.0	100±0.0	100±0.0	90.7±2.0	76.8±2.1	62.0±2.3	49.8±1.2	34.3±1.8	21.9±1.0
	2.5	100±0.0	100±0.0	92.7±1.1	78.5±1.6	66.1±1.5	51.8±1.0	38.1±1.5	26.2±1.7
	5.0	100±0.0	100±0.0	100±0.0	93.2±1.8	79.3±2.0	65.1±1.4	49.2±1.6	37.5±1.3
Hexane	1.0	100±0.0	95.6±0.8	82.1±1.4	69.4±2.1	55.3±1.6	43.7±2.4	31.1±1.9	18.9±1.0
	2.5	100±0.0	100±0.0	88.4±1.7	75.3±2.3	61.7±1.7	48.2±2.3	35.3±1.8	24.2±1.5
	5.0	100±0.0	100±0.0	100±0.0	91.4±1.0	78.0±1.0	63.8±1.0	47.6±1.2	36.2±2.0

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 *Cx. 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 *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Amersan *et al.*[15] have shown that hexane, chloroform benzene, acetone, and methanol extracts of *Cassia tora* provided significant repellent activity against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. These researchers saw the highest repellency for 210 min with methanol extract against *An. stephensi*. The methanol extract repelled *An. stephensi* the most followed by *Ae. aegypti* and *Cx. 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 *An. 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/cm^2 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 LC_{50} and LC_{90} values (186.00 and 346.06 mg/L respectively). In contrast, the LC_{50} and LC_{90} 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 *Ervatamia coronaria* was found to be more repellent than *Caesalpinia 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[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 LC_{50} 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/cm^2 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.

Conflict 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. bancrofti*, 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.

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