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Mosquito repellent potential of *Pithecellobium dulce* leaf and seed against malaria vector *Anopheles stephensi* (Diptera: Culicidae)

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ABSTRACT

Objective: To determine the repellent properties of hexane, benzene, ethyl acetate, chloroform and methanol extract of *Pithecellobium dulce* (*P. dulce*) leaf and seed against *Anopheles stephensi* (*An. stephensi*).

Methods: Repellent activity assay was carried out in a net cage (45 cm × 30 cm × 25 cm) containing 100 blood starved female mosquitoes of *An. stephensi*. This assay was carried out in the laboratory conditions according to the WHO 2009 protocol. Plant crude extracts of *P. dulce* 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 concentration 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 leaf and seed methanol extract of *P. dulce* provided over 180 min and 150 min protection, respectively.

Conclusions: Crude extracts of *P. dulce* exhibit the potential for controlling malaria vector mosquito *An. stephensi*.

1. Introduction

Mosquitoes are the most important group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year. *Anopheles stephensi* (*An. stephensi*) Liston is the primary vector of malaria in India and other West Asian countries. Malaria remains one of the most prevalent diseases in the tropical world with 200 million to 450 million infections annually worldwide; it causes up to 2.7 million deaths[1]. The disease remains endemic in more than 100

developing tropical countries, and its control is a major goal for improved worldwide health. Chemical insecticides have been used to control these disease vectors. In addition to application of general toxicants against mosquitoes, phytochemicals may also have potential uses as repellents and deterrents, and growth and reproduction inhibitors[2]. The repellency activity of hexane, ethyl acetate, benzene, chloroform, and methanol extract of *Delonix elata* leaf and seed against *Culex quinquefasciatus* (*Cx. quinquefasciatus*) has been reported[3]. Essential oil of *Cinnamomum zeylanicum* showed oviposition-deterrent and repellent activities, and the essential oils of *Zingiber officinale* and *Rosmarinus officinalis* also showed both ovicidal and repellent activities against *An. stephensi*, *Aedes aegypti* (*Ae. aegypti*), and *Cx. quinquefasciatus*[4]. The leaf methanol, benzene, and acetone extracts of *Cassia fistula* were studied for the larvicidal, ovicidal, and repellent activities against *Ae. aegypti*[5].

The essential oil extracted from the fruits of *Coriandrum sativum* (Apiaceae) was evaluated for the first time for its

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repellent activity against the most invasive mosquito worldwide, *Aedes albopictus*[6]. Singha *et al.*[7] evaluated the smoke repellent potentiality and mosquito larvicidal activity of *Mesua ferra* leaves against filarial vector *Cx. quinquefasciatus*. The *Zanthoxylum armatum*, *Zanthoxylum alatum* (Rutaceae), *Azadirachta indica* (Meliaceae), and *Curcuma aromatica* (Zingiberaceae) possess repellent properties against mosquitoes[8]. The essential oil of *Zingiber officinalis* is used as a mosquito larvicidal and repellent agent against the filarial vector *Cx. quinquefasciatus*[9]. The larvicidal and repellent potential of the methanolic extracts from *Moringa oleifera* plant seeds against malarial vector *An. stephensi* at different concentrations (20, 40, 60, 80 and 100 mg/L) has been reported[10]. Phasomkusolsil and Soonwera[11] reported the repellent properties of seven essential oils against mosquitoes, *Ae. aegypti*, *Anopheles dirus* and *Cx. quinquefasciatus* using dose-response study to determine effective dosages of essential oils and arm in cage method to estimate protection time and biting rate under laboratory conditions. Repellent properties of essential oils extracted from *Acantholippia salsoides*, *Aloysia catamarcensis*, *Aphanamixis polystachya*, *Lippia integrifolia*, *Lippia junelliana* (Verbenaceae), *Baccharis salicifolia*, *Eupatorium buniifolium*, and *Tagetes filifolia* (Asteraceae) were assessed against *Ae. aegypti*[12]. The aim of this study is to screen the mosquito repellent activity of crude extracts from *P. dulce* against *An. stephensi* under the laboratory conditions.

2. Materials and methods

2.1. Collection of plants

Fully developed leaves and seeds of the *P. dulce* 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 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

An. stephensi 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 and three days old starved female *An. stephensi* 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 were washed and cleaned with ethanol and ethanol served as control. After air drying, the each arm was exposed and the remaining area was covered by rubber gloves. The different concentrations of crude extracts with different solvents (1.0, 2.5, and 5.0 mg/cm²) were applied. *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 the sides were gently tapped 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 minute every 5 min. The mosquitoes that land on the hand were recorded and then shaken off before it imbibed any blood. The percentage of repellency was calculated by the formula:

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

Where T_a is the number of mosquitoes in the control arm, and T_b is the number of mosquitoes in the treated arm.

3. Results

In the present observation, the results from the skin repellent activity of hexane, benzene, chloroform, ethyl acetate, and methanol extract of *P. dulce* leaf and seed against blood starved adult female of *An. stephensi* is given in Tables 1 and 2. The present results showed that the percentage protection was related 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 *P. dulce* provided over 180 and 150 min protection

Table 1Repellency of different solvent leaf extracts of *P. dulce* against *An. stephensi*.

Solvent	Concentration (mg/cm ²)	Repellency (%)							
		15 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Methanol	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	95.2 ± 1.5	82.3 ± 1.1	69.0 ± 1.0
	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	94.5 ± 1.5	81.3 ± 1.5
	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	96.7 ± 1.6
Ethyl acetate	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.2 ± 2.4	78.6 ± 1.4	65.4 ± 1.8
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.6 ± 2.1	81.6 ± 1.9	68.5 ± 1.4
	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	95.3 ± 1.0	81.9 ± 2.0
Chloroform	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.4 ± 1.8	82.4 ± 2.0	69.6 ± 1.6	56.3 ± 1.3
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.1 ± 1.2	79.6 ± 2.1	66.7 ± 1.9
	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	95.3 ± 1.5	80.5 ± 1.7
Benzene	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.3 ± 1.6	81.0 ± 1.5	67.6 ± 1.6	53.8 ± 2.0
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.1 ± 1.6	78.1 ± 1.3	65.4 ± 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	93.8 ± 1.9	79.4 ± 1.1
Hexane	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.7 ± 1.2	79.6 ± 1.9	63.8 ± 1.3	51.0 ± 2.0	38.5 ± 1.7
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.4 ± 2.3	82.7 ± 1.5	69.1 ± 1.5	55.7 ± 2.2
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	95.7 ± 1.9	81.4 ± 1.6	67.8 ± 1.6

Data were expressed as mean ± SD.

Table 2Repellency of different solvent seed extracts of *P. dulce* against *An. stephensi*.

Solvent	Concentration (mg/cm ²)	Repellency (%)							
		15 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Methanol	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.9 ± 1.0	81.6 ± 1.7	67.5 ± 1.8	54.1 ± 1.5
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.8 ± 1.1	79.6 ± 2.2	64.9 ± 1.8
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	95.6 ± 1.2	82.1 ± 1.4
Ethyl acetate	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	92.8 ± 1.1	80.2 ± 1.5	65.3 ± 1.7	51.8 ± 2.2
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	91.9 ± 1.8	76.7 ± 1.9	62.5 ± 2.0
	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	93.1 ± 1.0	78.6 ± 1.6
Chloroform	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	92.4 ± 1.5	77.8 ± 2.1	63.3 ± 1.5	48.5 ± 1.3
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	95.3 ± 1.6	78.4 ± 2.6	65.7 ± 2.3	51.8 ± 1.1
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.5 ± 1.5	81.6 ± 1.0	68.3 ± 1.0
Benzene	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	95.1 ± 1.9	82.3 ± 1.0	70.7 ± 2.0	56.9 ± 1.5	45.0 ± 1.8
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.4 ± 1.8	76.2 ± 1.3	62.8 ± 1.6	49.2 ± 1.9
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.2 ± 1.0	79.4 ± 1.9	67.1 ± 1.4
Hexane	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	91.8 ± 1.6	80.2 ± 2.1	67.3 ± 1.4	55.2 ± 2.1	42.7 ± 2.3
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	92.2 ± 1.0	75.4 ± 1.8	61.0 ± 2.8	47.5 ± 1.4
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	92.7 ± 1.6	78.1 ± 1.5	65.3 ± 1.2

Data were expressed as mean ± SD.

against *An. stephensi*, 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 concentrations of the plant extracts. The tested plant crude extracts have exerted promising repellent activity against the malaria vector mosquito *An. stephensi*.

4. Discussion

Mosquito control represents an important strategy for prevention of disease transmission and epidemic outbreaks. However, a high level of insecticide resistance has developed through chemical control of the vector and pests, threatening the control strategies. To overcome these problems, it is necessary to search for alternative methods of vector control. The failure of chemical insecticides to control the insect and growing public concern for safe food and a healthy environment have catalyzed the search for more environmentally benign control methods for the management of the vectors. Our result showed that the crude

hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf and seed of *P. dulce* have significant repellent properties against *An. stephensi*. This result is also comparable to earlier report of Govindarajan[14] which reported that the larvicidal activity of crude extract of *Sida acuta* against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* with LC₅₀ values ranging between 38 and 48 mg/L. The seed acetone extract of *Tribulus terrestris* showed strong repellent activity against *Anopheles culicifacies* species, 100% repellency in 1 and 6 h, 100% repellency in 0, 4, and 6 h against *An. stephensi* and 100% repellency in 0, 2, and 4 h against *Cx. quinquefasciatus*, at 10% concentration, respectively[15].

In another study, the highest repellency was observed in *Zingiber officinale* extract, a higher concentration of 5.0 mg/cm² provided 100% protection up to 150 and 180 min against *Culex tritaeniorhynchus* and *Anopheles subpictus*, respectively[16]. The leaf extract of *Artemisia nilagirica* (*A. nilagirica*) have significant repellent activity against *An. stephensi* and *Ae. aegypti* mosquitoes. The highest concentrations of 450 mg/L provided over 150 and 90 min protection in methanol

extracts of *A. nilagirica* against *An. stephensi* and *Ae. aegypti*, respectively[17]. The maximum repellent activity was observed at 500 mg/L in methanol extracts of *Aegle marmelos* (*A. marmelos*) and *Acacia lineata* (*A. lineata*) and ethyl acetate extract of *Cocculus hirsutus*, and the mean complete protection time ranged from 90 to 120 min with the different extracts tested against *Anopheles subpictus*. No egg hatchability was observed with ethyl acetate extract of *A. marmelos*; the percentage of effective oviposition repellency were 92.6%, 93.04%, 95.2%, 88.26%, 92.8%, 94.01%, 95.77%, 96.93%, and 92.54% at 500 mg/L, and the lowest repellency were 47.14%, 58%, 56.52%, 64.93%, 71.09%, 66.42%, 50.62%, 57.62%, and 65.73% at 31.25 mg/L in acetone, ethyl acetate, and methanol extracts of *A. marmelos*, *A. lineata*, and *Cocculus hirsutus*, respectively[18]. In conclusion, the present study clearly proved that the efficacy of leaf and seed extracts of *P. dulce* can be suggested as a repellent agent against *An. stephensi* as target species. The results reported the repellent efficacy of natural product extracts against mosquitoes since they are considered as environmentally safe and eco-friendly approaches for the vector control programmes.

Conflict of interest statement

We declare that we have no conflict of interest.

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