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...
The leaves and seeds 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:

\[\% \text{ Repellency} = \left(\frac{Ta - Tb}{Ta}\right) \times 100\]

Where Ta is the number of mosquitoes in the control arm, and Tb 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...
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 larvical activity of crude extract of *Sida acuta* against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* with LC$_{50}$ 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 *Cq. 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/cm$^2$ 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

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### Table 1

| Solvent   | Concentration (mg/cm$^2$) | 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 | 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 | 94.5 ± 1.5 | 81.3 ± 1.5 |         |
| Ethyl acetate | 1.0                       | 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 | 96.6 ± 2.1 | 81.6 ± 1.9 | 68.5 ± 1.4 |
| Chloroform | 1.0                       | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 95.3 ± 1.0 | 81.9 ± 2.0 |         |
|           | 2.5                       | 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 |
| Benzene   | 1.0                       | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 95.3 ± 1.5 | 80.5 ± 1.7 |         |
|           | 2.5                       | 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 |
| Hexane    | 1.0                       | 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 |
|           | 2.5                       | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 93.8 ± 1.9 | 79.4 ± 1.1 |         |
|           | 5.0                       | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 94.2 ± 1.8 | 81.6 ± 1.5 | 67.5 ± 1.6 |

Data were expressed as mean ± SD.

### Table 2

| Solvent   | Concentration (mg/cm$^2$) | 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 | 93.8 ± 1.1 | 79.6 ± 2.2 | 64.9 ± 1.8 |         |
| 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 | 91.9 ± 1.8 | 76.7 ± 1.9 | 62.5 ± 2.0 |         |
| Chloroform | 1.0                       | 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 |         |
|           | 2.5                       | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 93.8 ± 1.9 | 79.4 ± 1.1 |         |         |
| Benzene   | 1.0                       | 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 |
|           | 2.5                       | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 95.3 ± 1.5 | 78.4 ± 2.1 | 63.3 ± 1.5 | 48.5 ± 1.3 |
| Hexane    | 1.0                       | 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 |         |
|           | 2.5                       | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 95.3 ± 1.9 | 78.4 ± 2.0 | 65.7 ± 2.3 |         |
|           | 5.0                       | 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 |         |

Data were expressed as mean ± SD.
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 *A. marmelos*, *A. lineata*, and *Cocculus hirsutus* 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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