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Repellent properties of *Pithecellobium dulce* (Roxb.) Benth. (Family: Fabaceae) against filariasis vector, *Culex quinquefasciatus* Say (Diptera: Culicidae)

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ABSTRACT

To determine the repellent activity of hexane, ethyl acetate, benzene, chloroform, and methanol extract of *Pithecellobium dulce* leaf and seed against filariasis vector, *Culex quinquefasciatus*. Evaluation was carried out in a net cage (45 cm^2 × 30 cm^2 × 25 cm^2) containing 100 blood starved female mosquitoes of three mosquito species and were assayed in the laboratory condition by using the protocol of WHO: The plant leaf crude extracts of *P. dulce* was applied at 1.0, 2.5, and 5.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. 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. Among the tested solvents, both the leaf and seed methanol extracts showed maximum efficacy. The highest concentration of 5.0 mg/cm^2 provided over 120 min and 90 min protection for the leaf and seed extracts, respectively. From the results, it can be concluded the crude extract of *P. dulce* was potential for controlling filariasis vector, *C. quinquefasciatus* mosquito.

KEY WORDS: Filariasis, *Culex quinquefasciatus*, leaf and seed, *Pithecellobium dulce*, repellency

INTRODUCTION

Mosquitoes are ecologically important components of the aquatic and terrestrial food chain; then they are the most important group of insects in terms of public health importance, and thus, appropriate control programs are justified. Until a few years ago, only the adults were sprayed, but now, it is well known that a more efficient way to reduce mosquito populations is to target the larvae (Tennyson *et al*., 2012). Mosquito-borne diseases such as malaria, filariasis, dengue, and viral encephalitis contribute to a larger proportion of health problems of developing countries. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It also resulted in the development of resistance, undesirable effects on non-target organisms, and fostered environmental and human health concern (Govindarajan, 2011). Most of the mosquito repellent formulations available in the market are mainly prepared with active ingredients of synthetic origin. Long-term exposure of newborn babies and children to parathyroid based mosquito repellents is known to cause clinical, biochemical and neurological changes. Thus, there is a need to find new, effective and safe, mosquito repelling agents. It has been already shown that certain phytochemicals, produced by those species that are already traditionally used in treating numerous diseases, exhibit a significant mosquito repellent activity against variety of *Anopheles* and *Culex* species (Mathivanan *et al*., 2010).

Mandal (2011) reported that repellent activity of *Eucalyptus* and *Azadirachta indica* seed oil against the filarial mosquito *Culex quinquefasciatus*. Tennyson *et al*. (2012) evaluated the repellent activity of *Ageratum houstonianum* leaf extracts against *Anopheles stephensi*, *Aedes aegypti,* and *C. quinquefasciatus*. Larvicidal and repellent activities of ethanolic extract of *Datura stramonium*

leaves against mosquitoes, respectively (Swathi *et al*., 2012). Govindarajan and Sivakumar, (2011) tested the repellent activities of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of leaf of *Eclipta alba* and *Andrographis paniculata* at three different concentrations of 1.0, 2.5, and 5.0 mg/cm^2 against important vector mosquito *A. aegypti*. Methanolic extracts of leaves and seeds from, *Tribulus terrestris* (Zygophyllaceae) was tested against 3rd instar larvae and adults of mosquito, *Anopheles arabiensis* under laboratory condition (Tarek *et al*., 2012).

Repellent activity of hexane, ethyl acetate, benzene, chloroform, and methanol extract of *Cardiospermum halicacabum* was evaluated against *C. quinquefasciatus*, *A. Aegypti,* and *A. stephensi* (Govindarajan and Sivakumar, 2012). Murugan *et al*. (2012) evaluated the larvicidal, pupicidal, repellent, and adulticidal activity of *Citrus sinensis* orange peel extract against *A. stephensi, A. aegypti* and *C. quinquefasciatus*. The larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogan citrates*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis,* and *Zingiber officinale* against *Culex tritaeniorhynchus* and *Anopheles subpictus* (Govindarajan, 2011). The larvicidal, ovicidal, and repellent activities of crude benzene and ethyl acetate extracts of leaf of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were assayed for their toxicity against three important vector mosquitoes, *viz*., *A. stephensi, A. aegypti*, and *C. quinquefasciatus* (Govindarajan *et al*., 2011). In Argentina, *Tagetes minuta* EO composed mainly of limonene (66%) and (E) ocimenone (19%) deterred *A. aegypti* from biting for 90 min at a 25% concentration (Gillij *et al*., 2008). In the present study, an attempt has been made to evaluate the repellent efficacy of leaf and seed extracts of *Pithecellobium dulce* against *C. quinquefasciatus* under laboratory conditions. This is the first report on the mosquito repellent activity of the solvent extracts of leaf and seed of the selected plant.

MATERIALS AND METHODS

Plant Collection

Fully developed leaves and seeds of the *P. dulce* were collected from Thanjavur District (Between 9°50' and 11°25' North latitude and 78°45' and 70°25' East longitude), Tamil Nadu, India. The collected specimens were 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.

Extraction

The leaves and seeds were washed with tap water, shade-dried, and finely ground. The finely ground plant leaf and seed powder (1.0 kg/solvent) was loaded in Soxhlet extraction apparatus and was extracted with five different solvents, namely, hexane, benzene, chloroform, ethyl acetate, and methanol, individually. The solvents from the extracts were removed 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.

Test Organisms

C. 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 chick for blood meal. Mosquitoes were held at 28°C ± 2°C, 70-85% relative humidity, with a photo period of 12 h light and 12 h dark.

Repellent Activity

The repellency was evaluated using the percentage of protection in relation to dose method (WHO, 2009). 100, 3-day-old starved female *C. 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 were washed and cleaned with ethanol. Ethanol served as control. After air drying the arms of the volunteer, only 25 cm^2 of skin on the dorsal side of each arm was exposed. The remaining area was covered by rubber gloves. The different concentrations of crude extracts were applied. *C. quinquefasciatus* mosquitoes were tested during the night from 19.00 h to 05.00 h. The control and treated arm were introduced simultaneously into the mosquito cage, and the mosquitoes were activated by gently tapping the sides of the experimental cages. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every 30 min. The experiment was conducted five times. It was observed that there was no skin irritation from the plant extract. 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 group, and T_b is the number of mosquitoes in the treated group.

RESULTS

In the present observation, the results from the skin repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extracts of *P. dulce* leaf and seed against blood starved adult female of *C. quinquefasciatus* were given in Tables 1 and 2. The present result shows that the percentage protection in relation to dose and time (minutes). Among the tested solvents the maximum efficacy was observed in both the leaf and seed methanol extracts. The highest concentrations of 5.0 mg/cm^2 leaf and seed methanol extract of *P. dulce* provided over 120 and 90 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 volunteer. Furthermore, the repellent activity was dependent on the strength of the plant extracts. The tested plant crude extracts of leaf and seed show promising repellent activity against all the three mosquitoes.

DISCUSSION

Our results showed that crude extract of *P. dulce* leaf and seed have significant repellent activity against *C. quinquefasciatus* mosquito. This result is also comparable to earlier reports of Mullai *et al*. (2008) have also reported that the skin repellent test at 1.0, 2.5, and 5.0 mg cm^{-2} concentration gave the mean complete protection time ranged from 119.17 to 387.83 min against *A. stephensi* with the benzene, petroleum ether, ethyl acetate, and methanol extracts of *Citrullus vulgaris* tested. The methanol extract of *E. coronaria* was found to be more repellent than *C. pulcherrima* extract. A higher

Table 1: Repellency of different solvent leaf extracts of *P. dulce* against *C. quinquefasciatus*

Solvent	Concentration (mg/cm^2)	Repellency % ±SD							
		Time of post application (min)							
		15	30	60	90	120	150	180	210
Methanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	93.4±1.5	79.6±1.8	65.4±1.8	52.6±1.9
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	95.6±2.0	82.4±1.4	70.2±1.4	57.4±1.4
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.3±1.0	81.8±1.9	68.9±1.7
Ethyl acetate	1.0	100±0.0	100±0.0	91.4±1.5	79.6±1.5	66.8±2.1	53.2±1.6	41.2±1.5	29.3±1.0
	2.5	100±0.0	100±0.0	100±0.0	93.5±1.2	81.3±1.6	67.1±1.2	54.7±1.8	42.6±1.8
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	95.7±1.4	82.4±2.0	68.2±1.4	55.3±2.2
Chloroform	1.0	100±0.0	100±0.0	90.3±1.4	77.5±1.3	65.1±1.0	51.2±1.5	39.3±1.6	25.6±2.1
	2.5	100±0.0	100±0.0	100±0.0	91.6±1.0	78.6±1.9	66.4±1.7	52.3±1.2	40.3±1.3
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	93.0±1.4	81.2±1.3	67.1±2.0	53.7±1.8
Benzene	1.0	100±0.0	100±0.0	90.3±1.6	78.6±2.0	64.2±1.0	51.4±1.2	38.9±1.9	24.3±1.5
	2.5	100±0.0	100±0.0	92.6±1.3	80.3±1.2	66.3±1.3	53.5±1.0	42.6±1.8	31.0±1.0
	5.0	100±0.0	100±0.0	100±0.0	94.2±1.9	81.6±2.1	68.4±2.0	55.3±2.1	43.2±2.4
Hexane	1.0	100±0.0	100±0.0	89.5±1.0	75.6±1.1	61.7±2.3	49.3±1.6	35.2±1.0	21.0±1.0
	2.5	100±0.0	100±0.0	91.2±2.0	78.2±2.4	64.5±1.7	51.6±1.8	39.3±1.7	26.2±1.1
	5.0	100±0.0	100±0.0	100±0.0	92.2±1.3	79.4±1.4	65.6±1.1	52.7±1.6	39.2±1.7

SD: Standard deviation, *P. dulce: Pithecellobium dulce, C. quinquefasciatus: Culex quinquefasciatus*

Table 2: Repellency of different solvent seed extracts of *P. dulce* against *C. quinquefasciatus*

Solvent	Concentration (mg/cm^2)	Repellency % ±SD							
		Time of post application (minutes)							
		15	30	60	90	120	150	180	210
Methanol	1.0	100±0.0	100±0.0	100±0.0	90.4±1.0	75.2±1.6	61.3±1.3	48.6±2.0	36.5±2.1
	2.5	100±0.0	100±0.0	100±0.0	92.5±1.7	78.6±2.1	66.2±1.2	51.3±1.4	40.8±1.8
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	94.2±1.4	81.3±1.9	67.5±1.3	54.2±1.6
Ethyl acetate	1.0	100±0.0	100±0.0	92.7±1.9	79.4±1.4	65.3±1.9	52.4±2.1	39.3±2.2	27.6±2.2
	2.5	100±0.0	100±0.0	94.5±1.5	81.2±1.6	68.4±1.3	55.2±1.0	42.7±1.3	30.1±1.5
	5.0	100±0.0	100±0.0	100±0.0	96.1±1.0	83.5±1.2	70.3±1.6	56.6±1.8	42.6±1.4
Chloroform	1.0	100±0.0	100±0.0	91.4±1.9	76.3±1.9	62.6±2.0	48.9±1.7	35.6±1.7	22.0±1.8
	2.5	100±0.0	100±0.0	93.2±2.0	79.6±1.8	66.5±1.2	53.7±2.1	41.1±1.8	28.3±1.3
	5.0	100±0.0	100±0.0	100±0.0	93.4±2.1	81.4±1.8	68.3±1.6	54.1±1.2	41.0±1.8
Benzene	1.0	100±0.0	95.9±1.6	82.6±1.1	69.4±2.0	55.8±1.6	43.2±1.8	28.3±1.0	12.7±1.6
	2.5	100±0.0	100±0.0	92.6±1.8	77.3±1.6	65.1±1.7	51.4±1.0	35.6±1.6	24.1±2.0
	5.0	100±0.0	100±0.0	100±0.0	92.1±1.3	80.2±1.3	65.6±1.3	52.7±2.0	39.4±1.9
Hexane	1.0	100±0.0	94.2±1.4	81.0±1.4	67.5±1.7	54.2±1.0	32.7±2.1	21.4±1.3	12.2±1.0
	2.5	100±0.0	100±0.0	88.9±1.6	75.6±2.0	62.7±1.5	49.8±2.4	33.2±1.5	21.3±1.4
	5.0	100±0.0	100±0.0	100±0.0	91.0±1.6	79.6±1.6	64.1±1.3	50.5±1.7	36.9±1.5

SD: Standard deviation, *P. dulce: Pithecellobium dulce, C. quinquefasciatus: Culex quinquefasciatus*

concentration of 5.0 mg/cm^2 provided 100% protection up to 150, 180, and 210 min against *C. quinquefasciatus, A. aegypti,* and *A. stephensi*, respectively (Govindarajan *et al*., 2011). The larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with lethal concentration 50% (LC_{50}) values ranging between 38 and 48 mg/L. The crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *A. stephensi* for 180 min followed by *A. aegypti* (150 min) and *C. quinquefasciatus* (120 min) (Govindarajan, 2010). The LC_{50} values of benzene, hexane, ethyl acetate, methanol, and chloroform extract of *E. alba* against early third instar larvae of *A. aegypti* were 151.38, 165.10, 154.88, 127.64, and 146.28 ppm, respectively (Govindarajan and Karuppannan, 2011). Karunamoorthi *et al*. (2008) have also reported that the leaves of *Echinops* sp. (92.47%), *Ostostegia integrifolia* (90.10%), and *Olea europaea* (79.78%) were also effective and efficient to drive away mosquitoes and the roots of *Silene macroserene* (93.61%), leaves of *Echinops* sp. (92.47%), *O. integrifolia* (90.10%), and *O. europaea* (79.78%) were exhibited the significant repellency by direct burning. Aarthi *et al*. (2010) evaluated the larvicidal and mosquito repellency activity of *Spathodea campanulata*. The extracts of *S. campanulata* were found most effective with LC_{50} value of 1.343, 1.607, 1.981, 2.165, 2.432 of I, II, III, IV and pupa respectively. Pushpanathan *et al*. (2008) 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 *C. quinquefasciatus*. The benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *A. paniculata* was found to be more effective against *C. quinquefasciatus* than *A. aegypti*. The LC_{50} values were 112.19, 137.48, 118.67, 102.05, 91.20 ppm and 119.58, 146.34, 124.24, 110.12, 99.54 ppm respectively (Govindarajan, 2011). The mosquito repellency and larvicidal activity of mature leaves of *Swietenia mahagoni* against *C. quinquefasciatus* were tested. Chloroform:methanol extract of *S. mahagoni* exhibits 100% repellency up to 2 h 15 min (Adhikari *et al*., 2012). Compared with earlier reports, our results revealed that the experimental plant extracts were effective to control *C. quinquefasciatus*. From these results, it was concluded that the plant leaf and seed extracts of *P. dulce* exhibits repellent activity against filariasis vector mosquito *C. quinquefasciatus*.

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