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Adulticidal activity of *Pithecellobium dulce* (Roxb.) Benth. against *Culex quinquefasciatus* (Say)

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ABSTRACT

Objective: To determine the adulticidal efficacy of different solvent leaf and seed extracts of *Pithecellobium dulce* (*P. dulce*) against *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** Sucrose-fed and blood starved mosquitoes were released into the tube, and the mortality effects of the extracts were observed every 10 min for 3 h exposure period. At the end of 1, 2, and 3 h exposure periods, the mosquitoes were placed in the holding tube. Cotton pads soaked in 10% sugar solution with vitamin B complex were placed in the tube during the holding period of 24 h. Mortality of the mosquitoes was recorded after 24 h. **Results:** Among five solvent extracts tested, the maximum efficacy was observed in the methanol extract of leaves of *P. dulce*. The LC_{50} and LC_{90} values of *P. dulce* leaves and seeds against *Cx. quinquefasciatus* were 234.97, 309.24 ppm and 464.86, 570.80 ppm, respectively. No mortality was observed in controls. The χ^2 -square values were significant at $P < 0.05$ level. **Conclusions:** From the results it can be concluded that the crude extract of *P. dulce* has excellent potential for controlling filariasis vector mosquito, *Cx. quinquefasciatus*. This is the first report on the mosquito adulticidal activity of the reported *P. dulce* plant.

1. Introduction

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[1]. Personal protection is one of the approaches to preventing mosquito bites. Apart from mosquito nets, the repellent plays an important role in protection against arthropods, because they can be used anywhere and anytime. When properly used, they are reported to reduce disease transmission[2]. Current control

involves mainly the use of synthetic repellents, which has a potential toxic effect on public health and the environment. Repeated use of synthetic repellents has disrupted natural biological systems and often resulted in the development of resistance[3].

The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants, having deterrent activities observed by different researchers[4–6]. The leaf extracts of *Acalypha indica* with different solvents *viz*, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal, ovicidal activity and oviposition attractancy against *Anopheles stephensi* (*An. stephensi*)[7]. The leaf extracts of *Cassia fistula* with different solvents *viz*, methanol, benzene and acetone were studied for the larvicidal, ovicidal and repellent activity against *Aedes aegypti* (*Ae. aegypti*)[8]. The larvicidal and repellent activities of *Sida acuta* (*S. acuta*) extract were tested against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*[9]. The larvicidal activity of alkaloid from *S. acuta* was evaluated against the 3rd instar larvae

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of *An. subpictus* and *Cx. tritaeniorhynchus*[5]. The mosquito larvicidal activity of leaf essential oil and their chemical constituents from *Clausena anisata* were against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*[10].

Govindarajan and Karupannan[11] investigated the larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *E. alba* against dengue vector, *Ae. aegypti*. The larvicidal and ovicidal efficacy of different extracts of *Cardiospermum halicacabum* (*C. halicacabum*) were against *Cx. quinquefasciatus* and *Ae. aegypti*[12]. The larvicidal and ovicidal activity of extracts of the leaf of three plants, *E. alba*, *C. halicacabum*, and *Andrographis paniculata* (*A. paniculata*), were tested against the early third-instar larvae of *An. stephensi*[13]. The larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogon citrates*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis* and *Zingiber officinale* against *Cx. tritaeniorhynchus* and *An. subpictus*[3]. 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., *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*[14]. The larvicidal and ovicidal efficacy of different extracts of *A. paniculata* and *Ficus benghalensis* was against *Cx. quinquefasciatus* and *Ae. aegypti*[15,16].

Pithecellobium dulce (*P. dulce*) Benth (Fabaceae) is a small to medium sized, evergreen, spiny tree, up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as 'Vilayati babul' in Hindi and 'Kodukkapuli' in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also used in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifacient and antidiabetic properties. The presences of steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported in the seeds. The bark contains 37% of tannins of catechol type. Quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves[17]. Roots have been reported to possess estrogenic activity[18]. It is evident that the plant has great potentials in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders[19]. As far as our literature survey could ascertain, no information was available on the adulticidal activity of the experimental plant species given here against *Cx. quinquefasciatus*. Therefore, the aim of this study was to investigate the mosquito adulticidal activity of the different solvent extracts of *P. dulce* plant species from Tamil Nadu, India. This is the first report on the mosquito adulticidal activity of the solvent extracts of the selected plant.

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), Tamil Nadu, 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 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 adulticidal 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 chick for blood meal. Mosquitoes were held at (28±2) °C, 70–85% relative humidity, with a photo period of 14-h light and 10-h dark.

2.4. Adulticidal activity

Five to six day old sugar-fed adult female mosquitoes were used. The plant leaf and seed extracts were diluted with ethanol to make different concentrations. The diluted plant extracts were impregnated on filter papers (140×120 mm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring (125×44 mm) following the WHO method[20]. One tube served to expose the mosquitoes to the plant extracts and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated

papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16 mesh size wire screen. Sucrose-fed and blood starved mosquitoes (20) were released into the tube, and the mortality effects of the extracts were observed every 10 min for 3 h exposure period. At the end of 1, 2, and 3 h exposure periods, the mosquitoes were placed in the holding tube. Cotton pads soaked in 10% sugar solution with vitamin B complex were placed in the tube during the holding period of 24 h. Mortality of the mosquitoes was recorded after 24 h. The above procedure was carried out in triplicate for each solvent plant crude extracts concentration.

2.5. Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC_{50} , LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and *Chi*-square values were calculated using the SPSS 12.0 version software. Results with $P < 0.05$ were considered to be statistically significant.

3. Results

The results of the adulticidal activity of hexane, ethyl acetate, benzene, chloroform and methanol leaf and seed extract of *P. dulce* against the adult of filariasis vector mosquito, *Cx. quinquefasciatus* are presented in Table 1.

Among five solvent extracts tested, the highest adulticidal activity was observed in methanol leaf extract of *P. dulce* against *Cx. quinquefasciatus*. The rates of mortality increased with the concentration (Table 1). At higher concentrations the adult showed restless movement for some times with abnormal wagging and died.

Table 1

Mortality of different solvent leaf and seed extracts of *P. dulce* against *Cx. quinquefasciatus* (mean \pm SD).

Solvents	Concentration (ppm)		Mortality (%)	
	leaf	seed	leaf	seed
Hexane	120	140	21.2 \pm 1.6	20.3 \pm 1.6
	240	280	38.3 \pm 1.2	33.1 \pm 1.8
	360	420	52.9 \pm 2.0	51.2 \pm 2.0
	480	560	65.7 \pm 0.8	66.3 \pm 1.2
	600	700	82.7 \pm 1.4	87.1 \pm 0.8
Ethyl acetate	120	140	24.5 \pm 1.6	23.4 \pm 0.8
	240	280	43.3 \pm 1.8	37.6 \pm 1.6
	360	420	57.6 \pm 1.2	53.5 \pm 1.2
	480	560	69.7 \pm 2.0	70.1 \pm 1.0
	600	700	89.2 \pm 0.8	89.0 \pm 0.8
Benzene	120	140	27.2 \pm 1.6	25.7 \pm 1.6
	240	280	47.8 \pm 1.8	62.6 \pm 2.0
	360	420	40.2 \pm 2.0	57.6 \pm 1.2
	480	560	74.9 \pm 0.8	76.8 \pm 1.0
	600	700	92.5 \pm 1.6	92.4 \pm 1.4
Chloroform	120	140	31.3 \pm 0.8	28.3 \pm 1.2
	240	280	49.5 \pm 1.6	44.2 \pm 1.8
	360	420	64.7 \pm 1.8	61.3 \pm 1.6
	480	560	83.8 \pm 2.0	82.5 \pm 0.8
	600	700	96.5 \pm 1.2	95.4 \pm 2.0
Methanol	120	140	38.7 \pm 1.0	29.8 \pm 1.6
	240	280	57.4 \pm 1.6	48.4 \pm 1.2
	360	420	71.8 \pm 2.0	67.5 \pm 0.8
	480	560	87.2 \pm 1.4	85.1 \pm 2.0
	600	700	98.9 \pm 1.8	98.6 \pm 1.4

The LC_{50} and LC_{90} values of leaf and seed methanol extracts of *P. dulce* against *Cx. quinquefasciatus* were 234.97, 309.24 ppm and 464.86, 570.80 ppm, respectively. The *Chi*-square values were significant at $P < 0.05$ level. The *Chi*-square values in the bioassays indicated probably the heterogeneity of the test population. The 95% confidence limits [LC_{50} (LCL–

Table 2

LC_{50} and LC_{90} and other statistical analysis of different solvent leaf and seed extracts of *P. dulce* against *Cx. quinquefasciatus*.

Solvents	LC_{50} (LCL–UCL) (ppm)		LC_{90} (LCL–UCL) (ppm)		χ^2	
	leaf	seed	leaf	seed	leaf	seed
Hexane	356.57 (285.64–436.72)	416.79 (346.99–494.05)	664.31 (554.29–888.47)	746.55 (638.38–944.23)	12.654*	10.446*
Ethyl acetate	323.21 (246.09–403.21)	392.58 (315.24–475.10)	609.73 (505.06–829.25)	720.17 (608.14–934.94)	15.458*	12.487*
Benzene	296.06 (215.84–373.69)	362.77 (287.00–439.18)	566.21 (467.45–771.69)	667.44 (565.98–855.57)	16.951*	12.817*
Chloroform	269.41 (193.90–339.54)	334.50 (256.45–409.92)	511.16 (424.37–682.62)	618.74 (522.25–798.40)	16.907*	14.205*
Methanol	234.97 (138.60–316.53)	309.24 (229.01–384.40)	464.86 (371.46–677.32)	570.80 (478.41–745.70)	23.914*	15.894*

*: $P < 0.05$; LCL: Lower confidence limit; UCL: Upper confidence limit; LCL–UCL: 95% confidence limits;

UCL)] and [LC₉₀ (LCL– UCL)] were also calculated. (Table 2)

4. Discussion

Today, the environmental safety of an insecticide is considered paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable. Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector managing agents. Phytoextracts are emerging as potential mosquito control agents, with low–cost, easy–to–administer, and risk–free properties[21]. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. Different parts of plants contain a complex of chemicals with unique biological activity which is thought to be due to toxins and secondary metabolites, which act as mosquitocidal agent[22]. Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors[23]. Simple crude extracts from plants have been used as insecticides in many countries for centuries.

The results of the present study are compared with earlier reports. Methanol leaf extract of *C. fistula* was found to be more lethal to the larvae of *An. stephensi* than *Cx. quinquefasciatus* with LC₅₀ values of 17.97 and 20.57 mg/L, respectively[24]. Larvicidal activity of *Ocimum basilicum*(*O. basilicum*), *Thymus vulgaris*(*T. vulgaris*), *Cymbopogon citatus*(*C. citatus*), *Mentha arvensis*(*M. arvensis*) and *Pelargonium graveolens*(*P. graveolens*) essential oils were tested against the late third instar of mosquito, *Cx. quinquefasciatus*. The LC₅₀ values of *O. basilicum*, *T. vulgaris*, *C. citatus*, *M. arvensis* and *P. graveolens* were 29.98, 30.31, 165.70, 178.04 and 226.52 ppm, respectively[25]. Essential oil from *Z. officinalis* was evaluated for larvicidal and repellent activity against the filarial mosquito *Cx. quinquefasciatus*. The LC₅₀ value was 50.78 ppm. Skin repellent test at 1.0, 2.0, 3.0, and 4.0 mg/cm² concentration of *Z. officinalis* gave 100% protection up to 15, 30, 60, and 120 min[26]. The LC₅₀ values of *F. benghalensis* against early second, third and fourth larvae of *Cx. quinquefasciatus*, *Ae. Aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm, respectively[27]. The crude extract of *E. coronaria* and *C. pulcherrima* exerted zero hatchability (100% mortality) at 250, 200, 150 ppm and 375, 300 and 225 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The methanol extract of *E. coronaria* was found to be more repellent than *C. pulcherrima*[28]. Ansari et al[29] suggested that the peppermint oil (*Mentha piperita*) showed strong repellent activity against adult mosquitoes when

applied on the human skin. The protection obtained against *An. annularis*, *An. culicifacies*, and *Cx. quinquefasciatus* was 100.0%, 92.3%, and 84.5%, respectively.

The root extract of *Valeriana jatamansi* which exhibited adulticidal activity of 90% lethal concentration against adult *An. stephensi*, *An. culicifacies*, *Ae. aegypti*, *An. albopictus*, and *Cx. quinquefasciatus* were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm², respectively[30]. Nathan et al[31] considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity against *An. stephensi* and the larval mortality was dose–dependent with the highest dose of 1 ppm azadirachtin, evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *An. stephensi*. The larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts *Eucalyptus globulus*, *Cymbopogon citratus*, *Artemisia annua*, *Justicia gendarussa*, *Myristica fragrans*, *Annona squamosa*, and *Centella asiatica* were tested against *An. stephensi*, and the most effective between 80% and 100% was observed in all extracts[32]. From these results it was concluded that the plant *P. dulce* exhibits adulticidal activity against *Cx. quinquefasciatus*. [32] Further analysis to isolate the active compound for adult control is under way in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

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