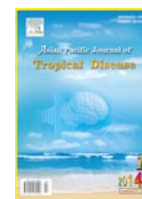




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Adulticidal properties of *Pithecellobium dulce* (Roxb.) Benth. (Family: Fabaceae) against dengue vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae)

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PEER REVIEW

ABSTRACT

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Comments

The research work is very much important to the society to control morbidity and other defects caused by mosquitoes and also other insect vectors. This work reports a novel approach for the control of vector mosquitoes. The study is interesting and important in its field. It has scientific value and will be of help for the mankind.

Details on Page S452

Objective: To examine the toxicity of mosquito adulticidal activity of different solvent leaf and seed extracts of *Pithecellobium dulce* (*P. dulce*) against dengue vector, *Aedes aegypti* (*Ae. aegypti*).

Methods: Adulticidal efficacy of the crude leaf and seed extracts of *P. dulce* with five different solvents like benzene, hexane, ethyl acetate, methanol and chloroform was tested against the five to six day old adult female mosquitoes of *Ae. aegypti*. The adult mortality was observed after 24 h under the laboratory conditions.

Results: Among the tested solvents the maximum efficacy was observed in the leaf and seed methanol extract. The LC₅₀ and LC₉₀ values of *P. dulce* leaf and seed extract against adults of *Ae. aegypti* were 218.64, 257.99 mg/L and 426.05, 507.73 mg/L, respectively. No mortality was observed in controls. The Chi-square values were significant at $P < 0.05$ level.

Conclusions: From the results it can be concluded the crude extract of *P. dulce* leaf and seed was an excellent potential for controlling dengue vector mosquito, *Ae. aegypti*.

KEYWORDS

Adulticidal activity, *Pithecellobium dulce*, Leaf, Seed, *Aedes aegypti*, Dengue vector**1. Introduction**

The occurrence of dengue has grown dramatically around the world in recent decades. Some 2.5 billion people—two fifths of the world's population—are now at risk from dengue. WHO currently estimates there may be 50 million dengue infections worldwide every year^[1]. In the Indian scenario, almost the entire country is endemic to the mosquito-borne diseases due to favorable ecological conditions. *Aedes aegypti* (*Ae. aegypti*), a vector of dengue that carries the arbovirus responsible for this

disease, is widely distributed in the tropical and subtropical zones. The only way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes. Mosquito control is being strengthened in many areas, but there are significant challenges, including an increasing mosquito resistance to insecticides and a lack of alternative, cost-effective, and safe insecticides. The use of phytochemicals is one such strategy that may be suitable for mosquito control. Thus, attempts to develop novel materials as mosquitocide are still necessary. Biologically active plant materials have attracted considerable

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interest in mosquito control programs in the recent times. Many studies on plant extracts and their active constituent compounds against mosquitos have been conducted around the world^[2].

The adulticidal efficacy of *Olea vera*, *Linum usitatissimum* and *Piper nigrum* were evaluate against *Anopheles stephensi* (*An. stephensi*) and *Ae. aegypti* under laboratory conditions^[3]. Ovicidal and repellent activities of methanol leaf extracts of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were evaluated against *Culex quinquefasciatus* (*Cx. quinquefasciatus*), *Ae. aegypti* and *An. stephensi*^[4]. Govindarajan and Karupannan investigated the larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extracts of *Eclipta alba* against dengue vector, *Ae. aegypti*^[5]. Maharaj et al.^[6] reported screening for adulticidal bioactivity of South African plants against *Anopheles arabiensis*. Repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extracts of *Cardiospermum halicacabum* were evaluated against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*^[7]. Choochote et al.^[8] investigated the adulticidal potential of *Piper longum*, *Piper ribesoides* and *Piper sarmentosum*, against vector *Stegomyia aegypti*.

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 '*kodukkappuli*' 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. Quercetin, kaempferol, dulcitol and afezilin have been reported from the leaves. Roots have been reported to possess estrogenic activity. 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^[9]. As far as our literature survey could ascertain, no information was available on the adulticidal activity of the experimental plant species given here against *Ae. aegypti*. 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 different solvent extracts of the selected plant against dengue vector mosquito *Ae. aegypti*.

2. Materials and methods

2.1. Plant collection

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. *Ae. aegypti*

Ae. aegypti 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 12-h light and 12-h dark.

2.4. Adulticidal activity

Five to six days old sugar-fed adult female mosquitoes were used. The different concentration of 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 freshly 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^[10]. 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 was 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 lethal concentration 50 (LC₅₀), lethal

concentration 90 (LC₉₀) and other statistics at 95% confidential 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 extracts of *P. dulce* against the dengue vector mosquito, *Ae. aegypti* adults are presented in Table 1. Among five solvent extracts tested, the highest adulticidal activity was observed in methanol leaf extract of *P. dulce* against *Ae. aegypti*. At higher concentrations the adult showed restless movement for some times with abnormal wagging and died. The LC₅₀ and LC₉₀ values of leaf and seed methanol extract of *P. dulce* against *Ae. aegypti* were 218.64, 257.99 mg/L and 426.05, 507.73 mg/L, 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 [LC₅₀ (LCL–UCL)] and [LC₉₀ (LCL–UCL)] were also calculated.

Table 1

Adulticidal activity of different solvent leaf and seed extracts of *P. dulce* against *Ae. aegypti*.

Parts used	Solvents	LC ₅₀ (mg/L)	95% Confidence limits (mg/L)		LC ₉₀ (mg/L)	95% Confidence limits (mg/L)		<i>Chi</i> square
			LCL	UCL		LCL	UCL	
Leaf	Methanol	218.64	129.67	294.92	426.05	339.70	626.94	24.990*
	Ethyl acetate	237.86	171.00	299.44	448.95	373.53	594.97	16.697*
	Chloroform	259.98	198.07	319.04	486.86	410.92	627.71	13.965*
	Benzene	282.43	218.12	346.39	526.63	442.69	688.54	14.102*
	Hexane	311.07	251.07	374.93	571.55	483.89	737.84	11.978*
Seed	Methanol	257.99	150.25	349.65	507.73	403.79	751.93	25.289*
	Ethyl acetate	304.96	198.74	403.93	594.12	476.40	875.63	22.944*
	Chloroform	331.42	238.71	424.10	630.05	514.46	887.22	18.846*
	Benzene	363.48	283.74	449.28	676.85	563.05	910.93	14.435*
	Hexane	393.62	323.40	473.47	699.08	591.22	908.45	12.237*

*: Significant at $P < 0.05$ level. LCL: Lower confidence limits; UCL: Upper confidence limits.

4. Discussion

Now, 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. Plant extracts are emerging as potential mosquito control agents, with low-cost, easy to administer, and risk-free properties^[11]. In the present study, the crude leaf and seed extracts of *P. dulce* have significant adulticidal activity against *Ae. aegypti*. The results of the present study are also comparable to the earlier reports of Dua et al.^[12] in which the knock down time, KDT₅₀ and KDT₉₀ values of the *Lantana camara* were 20, 18, 15, 12 and 14

min and 35, 28, 25, 18 and 23 min against *Ae. aegypti*, *Cx. quinquefasciatus*, *Anopheles culicifacies*, *Anopheles fluviatilis* and *An. stephensi* with their per cent mortality of 93.3%, 95.2%, 100.0%, 100.0% and 100.0% respectively. Govindarajan evaluated the larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC₅₀ values ranging between 38 and 48 mg/L^[13]. The crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *An. stephensi* for 180 min followed by *Ae. aegypti* (150 min) and *Cx. quinquefasciatus* (120 min).

The hexane fraction from methanol extract of *Acorus calamus* rhizome, the methanol fraction of *Litsea elliptica* and hexane fraction of the *Piper aduncum* crude extract were displayed good adulticidal property with the LC₅₀ and LC₉₀ values of 0.04, 0.11, 0.20 mg/cm² and 0.09, 6.08, 5.32 mg/cm² respectively^[14]. The benzene, hexane, ethyl acetate, methanol and chloroform leaf extracts of *Andrographis paniculata* (*A. paniculata*) were found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti*. The LC₅₀ 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^[15]. The essential oil from the leaves of *Clausena anisata* exhibited significant larvicidal activity, with 24 h LC₅₀ values of 140.96, 130.19 and 119.59 ppm, respectively^[16]. The five different solvent extracts of *Eclipta alba* and *A. paniculata* were assayed adulticidal activity against *Cx. quinquefasciatus* and *Ae. aegypti*. The highest adult mortality was found in methanol extract of *A. paniculata* against the adults of *Cx. quinquefasciatus* and *Ae. aegypti* with the LC₅₀ and LC₉₀ values of 149.81, 172.37 ppm and 288.12, 321.01 ppm, respectively^[17]. Compared with earlier author's reports, our results revealed that the experimental plant extracts were effective to control *Ae. aegypti*. From these results it was concluded that the plant *P. dulce* exhibited adulticidal activity against *Ae. aegypti*. Further analysis to isolate the active compound for adult control is under way in our laboratory. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage 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

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Comments

Background

Mosquitoes are well known for their public health importance since they cause major health problems and diseases. Indiscriminate use of several mosquitocidal agents caused various side effects. Thus there is a need to develop an alternative strategy to control vector mosquitoes. *Ae. aegypti*, a vector of dengue, is widely distributed in tropical and subtropical zones. This study was aimed to investigate the efficacy of *P. dulce* against adult *Ae. aegypti* mosquitoes in the laboratory.

Research frontiers

This study aimed to determine the control of dengue vector mosquito, *Ae. aegypti* by the plant *P. dulce*.

Related reports

In this present experiment, they have followed standard protocols to assess the mosquito adulticidal action of selected plant. These findings are in close agreement with the earlier reports of Samidurai *et al.* (2009), Niraimathi *et al.* (2010) and Govindarajan *et al.* (2012).

Innovations & breakthroughs

Control of rural dengue vector is an important aspect. Using plant products as a natural enemy without causing any percentage of destruction to environment is very much important to the society. So far, there is no previous record of literature available about the mosquitocidal activity of selected plant.

Applications

Product development for mosquito control. Plants are always considered as vast repository of natural compounds, the exploration of research leading to their possible utilization certainly pave the way for search of new phytochemical compounds and their proper role in the near future as eco-friendly natural pesticides.

Peer review

The research work is very much important to the society to control morbidity and other defects caused by mosquitoes and also other insect vectors. This work reports a novel approach for the control of vector mosquitoes. The study is interesting and important in its field. It has scientific value and will be of help for the mankind.

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