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Mosquito larvicidal and ovicidal properties of *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae) against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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PEER REVIEW

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Comments
The present resurgence of these diseases is due to the higher number of breeding places in today's throwaway society. Further, the indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance and toxic hazards to humans. The secondary metabolites synthesized by plant extracts demonstrate a broad spectrum of bioactivity against mosquitoes.
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ABSTRACT

Objective: To assess the larvicidal and ovicidal potential of the crude hexane, benzene, chloroform, ethyl acetate and methanol solvent extracts from the medicinal plant, *Pithecellobium dulce* (*P. dulce*) against filariasis vector mosquito, *Culex quinquefasciatus* (*Cx. quinquefasciatus*).
Methods: Twenty five early third instar larvae of *Cx. quinquefasciatus* were exposed to various concentrations and were assayed in the laboratory by using the protocol of WHO (2005). The larval mortality was observed after 24 h of treatment. The ovicidal activity was determined against *Cx. quinquefasciatus* mosquito eggs to various concentrations ranging from 100–750 mg/L under the laboratory conditions.
Results: The methanol extract of the leaves and seed of *P. dulce* was the most effective against the larvae with LC_{50} and LC_{90} values 164.12 mg/L, 214.29 mg/L, 289.34 mg/L and 410.18 mg/L being observed after 24 h of exposure. The efficacy of methanol was followed by that of the ethyl acetate, chloroform, benzene and hexane extracts. The mean percent hatchability of the egg rafts were observed after 48 h of treatment. About 100% mortality was observed at 500 mg/L for leaf and 750 mg/L for seed methanol extracts of *P. dulce*.
Conclusions: From the results, it can be concluded that the larvicidal and ovicidal effect of *P. dulce* against *Cx. quinquefasciatus* make this plant product promising as an alternative to synthetic insecticide in mosquito control programs.

KEYWORDS
Culex quinquefasciatus, *Pithecellobium dulce*, Larvicidal activity, Ovicidal activity, Leaf and seed

1. Introduction

Blood–feeding female mosquitoes are responsible for the intolerable biting nuisance and the transmission of a large number of diseases, such as malaria, yellow fever, dengue, filariasis, chikungunya and encephalitis, causing serious health problems to humans and obstacles to socioeconomic development of developing countries, particularly in the tropical region[1]. Especially, lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by mosquito *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is found to be more endemic in the Indian subcontinent. Synthetic insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance and harm to mammals. Hence, there is a constant need for developing biologically active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimising the residue accumulation in the environment. Natural products are generally preferred because of their less harmful nature

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to non-target organisms and their innate biodegradability[2]. In the search for environmentally safe and relatively inexpensive methods for controlling mosquitoes, plant extracts received much interest as potential bioactive agents against mosquito egg and larvae.

Most of the mosquito control programmes target the egg and larval stage in their breeding sites with ovicides and larvicides because the adulticides may only reduce the adult population temporarily[3,4]. Therefore, a more efficient way to reduce mosquito population is to target the eggs and larvae. In the last few years, there was an increase of public concern on the safety of many chemical products that instigated a renewed interest on the use of natural products from plant origin for vector management. New botanical natural products are effective, environment-friendly, easily biodegradable, inexpensive, and readily available in many areas of the world, being no ill effect on non-target organisms and having novel modes of action[5].

Samidurai *et al*. observed that the leaf extracts of *Pemphis acidula* were evaluated for larvicidal[6], ovicidal and repellent activities against *Cx. quinquefasciatus* and *Aedes aegypti* (*Ae. aegypti*). Govindarajan investigated the larvicidal efficacy of different extracts of *Ficus benghalensis* against *Cx. quinquefasciatus*, *Ae. aegypti* and *Anopheles stephensi* (*An. stephensi*)[7]. The larvicidal activity of *Sida acuta* was evaluated against 3rd instar larvae of *Anopheles subpictus* and *Cx. tritaeniorhynchus*[8]. The larvicidal activity of methanol extracts of *Cassia obtusifolia*, *Cassia tora* and *Vicia tetrasperma* were tested against early fourth stage larvae of *Ae. aegypti* and *Culex pipiens* (*Cx. pipiens*)[9]. The acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Leucas aspera* were studied against the early fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*[10]. Laboratory evaluation of a phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Cx. quinquefasciatus* (Diptera: Culicidae) and non-target organisms[11]. The ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* (*E. coronaria*) and *Caesalpinia pulcherrima* were against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*[12].

Pithecellobium dulce (*P. dulce*) (Roxb.) Benth. (Family: Fabaceae) is a small to medium sized, evergreen, spiny tree, native of tropical America and cultivated throughout the plains of India. It is known as 'Vilayati babul' in Hindi and 'Kodukkapuli' in Tamil. The leaves have been reported to possess astringent, emollient, abortifacient and antidiabetic properties. Quercetin, kaempferol, dulcitol and afezilin have been reported from the leaves. 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[13]. To date, there is no report about the *P. dulce* extract against the mosquitoes. We decided to investigate the potential use of this plant extract against *Cx. quinquefasciatus*, since this plant is very common across India. In the present paper, an attempt has been made to a step forward to observe rational impact of *P. dulce* leaf and seed extracts on *Cx. quinquefasciatus* due to their larvicidal and ovicidal activity. To best of our knowledge, this is the first report on mosquito larvicidal and ovicidal activities of the plant *P. dulce*.

2. Materials and methods

2.1. Collection of plants

Fully developed leaves and seeds of the plant, *P. dulce* (Roxb.) Benth. (Family: Fabaceae) (Common name: Sweet tamarind) were collected from Thanjavur District (Between 9°50′ and 11°25′ of the north latitude; 78°45′ and 70°25′ of the east longitude), Tamil Nadu, India. A voucher specimen is 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 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 residue of 7.8, 8.6, 9.1, 9.7, 10.2% and 7.2, 7.9, 8.2, 8.8, 9.7%, respectively. 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 larvicidal and ovicidal bioassays.

2.3. Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization[14]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 mL of water. The appropriate volume of dilution was added to 200 mL water in the cups to obtain the desired target dosage, starting with the lowest concentration. Four replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. To this, 1 mL of ethanol was added. The LC_{50} value was calculated after 24 h by probit analysis[15].

2.4. Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla was performed[16]. The eggs of *Cx. quinquefasciatus* were collected from vector control laboratory, Annamalai University. The leaf and seed extracts diluted in the ethanol to achieve various concentrations ranging from 100 to 750 mg/L. Eggs of these mosquito species (100) were exposed to each concentration of leaf and seed extracts. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

$$\% \text{ of egg hatchability} = \frac{\text{No. of hatched larvae}}{\text{Total No. of eggs}} \times 100$$

2.5. Statistical analysis

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

3. Results

The larvicidal activity of leaf and seed extract of *P. dulce* against filariasis vector mosquito, *Cx. quinquefasciatus* are presented in Table 1. The leaf extracts were found to be more effective than the seed extracts. The LC_{50} values of leaf of *P. dulce*, with five different solvents *viz.*, hexane, benzene, chloroform, ethyl acetate and methanol against *Cx. quinquefasciatus* were 197.23, 184.96, 175.38, 170.66 and 164.12 mg/L respectively and the LC_{90} values were 348.34, 331.42, 309.07, 301.91 and 289.34 mg/L respectively. The 95 percent confidence limits LCL, UCL LC_{50} were ranged from 159.70 to 126.71 mg/L and 234.51 to 200.35 mg/L respectively. The LCL, UCL LC_{90} were ranged from 300.21 to 244.86 mg/L and 431.37 to 372.14 mg/L respectively. The *Chi*-square values were significant at $P<0.05$ level. Among the five solvents tested against *Cx. quinquefasciatus*, the methanol extract was found to be more effective than the other four solvent extracts. The percentage of egg hatchability of *Cx. quinquefasciatus* was tested with five different solvents at different concentrations of leaf and seed of *P. dulce* extracts and the results are listed in Tables 2 and 3. The leaf extracts were found to be more effective than the seed extracts. The methanol extract was more toxic to the egg rafts of *Cx. quinquefasciatus* and exhibited 100 percent mortality at 500 mg/L, followed by ethyl acetate, chloroform and benzene extracts exerting 100 percent mortality at the concentration of 600 mg/L. The control eggs showed 100 percent hatchability. The rate of hatchability was higher in lower concentrations and when the concentrations of the extracts increased, the hatchability rate decreased. These results clearly revealed that the toxicity of leaf and seed extracts was dependent on its concentration which would determine the egg hatchability.

Table 2

Ovicidal activity of *P. dulce* plant leaf extracts against *Cx. quinquefasciatus*.

Solvent used	Percentage of egg hatch ability						
	Control	100 mg/L	200 mg/L	300 mg/L	400 mg/L	500 mg/L	600 mg/L
Hexane	100.0±0.0	81.8±1.6	71.9±1.1	58.6±1.5	37.8±1.3	28.4±1.4	16.8±1.3
Benzene	100.0±0.0	78.2±1.2	63.4±1.3	48.7±1.3	31.1±1.4	24.3±1.2	NH
Chloroform	100.0±0.0	70.4±1.8	54.6±1.5	42.4±1.8	28.6±1.2	20.2±1.4	NH
Ethyl acetate	100.0±0.0	65.3±1.1	49.5±1.6	38.7±1.4	25.4±1.9	18.5±1.1	NH
Methanol	100.0±0.0	56.1±2.0	41.8±1.4	29.8±2.0	19.1±1.0	NH	NH

Each value (mean±SD) represents the average of six values; NH–No hatch ability (100% mortality).

Table 3

Ovicidal activity of *P. dulce* plant seed extracts against *Cx. quinquefasciatus*.

Solvent used	Percentage of egg hatch ability						
	Control	125 mg/L	250 mg/L	375 mg/L	500 mg/L	625 mg/L	750 mg/L
Hexane	100.0±0.0	92.6±1.6	78.5±1.9	65.9±1.2	50.7±1.4	33.6±1.6	26.8±1.1
Benzene	100.0±0.0	88.9±1.1	67.2±1.2	56.5±1.8	41.3±1.6	28.7±1.4	20.6±0.9
Chloroform	100.0±0.0	81.6±1.0	61.4±1.4	49.7±1.4	35.1±1.2	24.2±1.8	18.2±1.3
Ethyl acetate	100.0±0.0	73.7±1.7	57.6±1.3	44.1±0.9	30.4±1.9	19.3±0.9	NH
Methanol	100.0±0.0	62.5±2.0	51.6±1.9	39.3±1.4	23.5±1.7	17.8±1.2	NH

Each value (mean±SD) represents the average of six values; NH–No hatch ability (100% mortality).

4. Discussion

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. Phytoextracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties. Our result showed that the crude hexane, benzene, chloroform, ethyl acetate and methanol solvent extracts of leaf and seed of *P. dulce* had significant larvicidal and ovicidal properties against filariasis vector mosquito, *Cx. quinquefasciatus*, with the LC_{50} values less than 200 mg/L for leaf and 350 mg/L for seed. When the larvae were treated with different solvent extracts, the larvae were at first restless then they were sluggish and coiled and finally death occured. Compared with other results, Mathivanan *et al.* determined that the LC_{50} and LC_{90} values of crude methanol extract of leaves of *E. coronaria* on *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* larvae in 24

Table 1

Larvicidal activity of different solvent leaf and seed extracts of *P. dulce* against *Cx. quinquefasciatus*.

Solvents	LC_{50} (LCL–UCL) (mg/L)		LC_{90} (LCL–UCL) (mg/L)		χ^2	
	L	S	L	S	L	S
Methanol	164.12 (126.71–200.35)	214.29 (135.07–285.46)	289.34 (244.86–372.14)	410.18 (328.71–600.15)	15.382*	24.510*
Ethyl acetate	170.66 (131.79–207.97)	240.37 (166.31–310.90)	301.91 (256.08–385.74)	467.04 (379.78–661.37)	14.782*	19.565*
Chloroform	175.38 (134.51–214.99)	274.74 (200.77–354.32)	309.07 (260.78–400.32)	526.81 (426.35–764.05)	15.975*	18.469*
Benzene	184.96 (137.13–230.79)	303.22 (241.97–374.72)	331.42 (276.37–440.60)	565.86 (468.81–770.71)	17.945*	13.171*
Hexane	197.23 (159.70–234.51)	322.80 (268.09–389.37)	348.34 (300.21–431.37)	587.89 (494.24–772.90)	11.773*	10.620*

*: Significant at $P<0.05$; L: Leaf; S: Seed; LCL: Lower confidence limits; UCL: Upper confidence limits; χ^2: *Chi*-square.

h were 72.41, 65.67, and 62.08 and 136.55, 127.24, and 120.86 mg/L, respectively[17]. The larvicidal, ovicidal, and repellent activities of crude benzene and ethyl acetate extracts of leaf of *E. coronaria* and *Caesalpinia pulcherrima* were assayed for their toxicity against three important vector mosquitoes, *viz.*, *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. All extracts showed moderate larvicidal effects. However, the highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* with the LC_{50} and LC_{90} values 79.08, 89.59, and 96.15 mg/L and 150.47, 166.04, and 174.10 mg/L, respectively[5].

The LC_{50} value of methanol extract of the green berries of *Solanum villosum* on third instar larvae of *Ae. aegypti* was 11.67 mg/L[18]. The bio-efficacy of *Aloe vera* leaf extract and bacterial insecticide, *Bacillus sphaericus* larvicidal activity was assessed against the first to fourth instars larvae of *Ae. aegypti*, under the laboratory conditions. The LC_{50} of *Aloe vera* against the first to fourth instars larvae were 162.74, 201.43, 253.30 and 300.05 mg/L and the LC_{90} 442.98, 518.86, 563.18 and 612.96 mg/L, respectively[19]. Patil *et al.* evaluated larvicidal activity of extracts of medicinal plants *Plumbago zeylanica* and *Cestrum nocturnum* against *Ae. aegypti*[20]; the LC_{50} values of both the plants were less than 50 mg/L. The larvicidal stability of the extracts at five constant temperatures (19 °C, 22 °C, 25 °C, 28 °C and 31 °C) evaluated against fourth instars larvae revealed that toxicity of both plant extracts increased with increase in temperature. Larvicidal activity of crude extract of *Sida acuta* against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* with LC_{50} values ranging from 38 to 48 mg/L[21]; Mohan and Ramaswamy evaluated the efficacy of *Ageratina adenophora* against *Culex* and found that it showed an LC_{50} of 227.19 mg/L after 24 h of treatment[22].

The larvicidal activity of crude petroleum ether, ethyl acetate, and methanol extracts of the whole plants of *Phryma leptostachya* was assayed for its toxicity against the early fourth instar larvae of *Cx. pipiens pallens*. Among three solvent extracts from *Phryma leptostachya*, the petroleum ether extract exhibited the best larvicidal activity. The corresponding LC_{50} values of petroleum ether, ethyl acetate, and methanol extracts were 3.23, 5.23, and 61.86 mg/L against the early fourth instar larvae of *Cx. pipiens pallens*[23]. Thanigaivel *et al.* reported that the larvicidal activity of methanolic fractions from *Adhatoda vasica* leaf extracts were investigated against the *Bancroftian filariasis* vector *Cx. quinquefasciatus* and dengue vector *Ae. aegypti*[24]. The results indicated that the mortality rates were high at 100, 150, 200 and 250 mg/L of methanol extract of fractions III with R_f value 0.67 and methanol extract of fraction V with R_f value 0.64 of *Adhatoda vasica* against all the larval instars of *Cx. quinquefasciatus* and *Ae. aegypti*. The LC_{50} and LC_{90} values were 106.13 and 180.6 mg/L for fraction III, 110.6 and 170 mg/L for fraction V and 157.5 and 215.5 mg/L for fraction III and 120 and 243.5 mg/L for the fraction V, respectively.

Ethanol fractionate of *Eichhornia crassipes* showed the highest larvicidal and pupicidal activity against *Cx. quinquefasciatus* compared to other solvent extracts and fractionates with LC_{50} 71.43, 94.68, 120.42, 152.15 and 173.35 mg/L for I, II, III, IV and pupae, respectively[25]. These results clearly indicate that the plant-based insecticides, which are less expensive than synthetic insecticides, exert high larvicidal and ovicidal effect. Therefore, the results of this study indicate that the extract of *P. dulce* may be effectively used for the control of mosquito larvae and eggs in public health operations. In conclusions, this study demonstrated that the different solvent extracts of *P. dulce* had excellent mosquito larvicidal and ovicidal activities against *Cx. quinquefasciatus*. Thus, the methanolic extract of *P. dulce* has potential to be developed as natural larvicidal and ovicidal agents. However, this plant is subject of further evaluation to elucidate the constituents responsible for observed activities and further investigations for the insecticidal mode of action. Effects on non-target organisms and field evaluation are needed. Moreover, these results could be useful in the research for selecting newer, more selective, biodegradable and natural larvicidal and ovicidal compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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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 alternative strategies to control vector mosquitoes.

Research frontiers

Cx. quinquefasciatus, the major rural filarial vector was shown not much interest by many other scientists in Southern India, has been taken into account. Plant extracts of *P. dulce* in controlling mosquito larvae and egg is interesting.

Related reports

Numbers of scientists have worked on the plant extracts in controlling mosquito larva (Sivakumar *et al.* 2013); Samidurai *et al.* 2009). In this present investigation, they have followed standard protocols to assess the mosquito adulticidal action of selected plant extracts.

Innovations and breakthroughs

Control of rural filarial vector is an important aspect. Using plant extracts as a natural enemy of mosquitoes without causing any percentage of destruction to environment and combining plants is very much important to the society.

Applications

The exploration of research leading to their possible utilization certainly paves the way for search of new phytochemical compounds and their proper role in the near future as eco–friendly natural pesticides.

Peer review

The present resurgence of these diseases is due to the higher number of breeding places in today's throwaway society. Further the indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance and toxic hazards to humans. The secondary metabolites synthesized by plant extracts demonstrate a broad spectrum of bioactivity against mosquitoes.

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