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Ovicidal activity *of Pithecellobium dulce* (Family: Fabaceae) leaf and seed extracts against filariasis vector mosquito *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

Mosquito-borne diseases with an economic impact create a loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates. Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. In view of recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, in the present study ovicidal efficacy of different solvent leaf and seed extract of *Pithecellobium dulce* against filariasis vector *C. quinquefasciatus*. The ovicidal efficacy of the crude leaf extracts of *P. dulce* with five different solvents, *such as* benzene, hexane, ethyl acetate, methanol, and chloroform, was tested against *C. quinquefasciatus*. Ovicidal activity was determined against *C. quinquefasciatus* mosquito to various concentrations ranging from 100 to 600 mg/L under the laboratory conditions. Among five solvent tested the maximum efficacy was observed in the leaf and seed methanol crude extract was found to be most effective for ovicidal activity against vector mosquito. The extract of methanol exerted 100% mortality at 500-600 mg/L against *C. quinquefasciatus*. From the result, it can be concluded the crude extract of *P. dulce* was a potential candidate to develop newer and safer ovicides against *C. quinquefasciatus*.

KEY WORDS: *Culex quinquefasciatus*, eco-friendly control, filariasis vector mosquito, ovicides, plant-borne extract

INTRODUCTION

Mosquitoes not only cause nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue, and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang *et al*., 2002; Benelli, 2015a). Lymphatic filariasis is a mosquito-borne disease caused by mosquito-transmitted filarial nematodes, including *Wuchereria bancrofti* and *Brugia malayi*. The infected people carry the nocturnally periodic *W. bancrofti,* which has *Culex quinquefasciatus* as the main mosquito vector. *C. quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide, and 44 million people have common chronic manifestations (Bernhard *et al*., 2003). In India alone, 25 million people harbor microfilaria, and 19 million people suffer from filarial disease manifestations (Govindarajan *et al*., 2011). The major problems associated with the use of chemicals for the control of pests including mosquitoes include: The development of resistance to the chemicals, issues around the residues in animals and the environment, and their undesirable side effects (Peter *et al*., 2005). Extracts from plants may be alternative sources of mosquito control agents since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Plant extracts, in general, have been recognized as an important natural resource of insecticides (Benelli, 2015b; Benelli *et al*., 2015).

Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne

diseases at the individual as well as at the community level (Govindarajan *et al.*, 2008). For instance, the ovicidal activity of neem-borne azadirachtin against the mosquitoes *Culex tarsalis* and *C. quinquefasciatus* (Su and Mulla, 1998). The leaf extract of *Cassia fistula* with different solvents, such as methanol, benzene, and acetone was studied for the larvicidal, ovicidal, and repellent activity against *Aedes aegypti* (Govindarajan, 2009). The toxicity of dichloromethane, petroleum ether, and methanol extracts from *Vitex negundo* seed and leaf to the second- and fourth-instar larvae showed oviposition-deterrent effects on *Plutella xylostella* (Yuan *et al.*, 2006). The active components dymalol and triterpenes isolated from the extract of *Dysoxylum malabaricum* act as an oviposition repellent and/or deterrent to *Anopheles stephensi* (Govindachari, 1999). The leaf extracts of *Pemphis acidula* were evaluated for larvicidal, ovicidal, and repellent activities against *C. quinquefasciatus* and *A. Aegypti* (Samidurai *et al.*, 2009).

Furthermore, 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 (Govindarajan *et al.*, 2011). Larvicidal, ovicidal, and repellent activities of marine sponge *Cliona celata* (Grant) extracts against *C. quinquefasciatus* say and *A. aegypti* (Reegan *et al.*, 2013). Govindarajan and Karupannan, (2011) investigated the larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Eclipta alba* against dengue vector, *A. aegypti*. The larvicidal, pupicidal, ovicidal and ovipositional deterrent activity of methanol leaves extract of *Spathodea campanulata* against *A. aegypti* (Karthika Devi *et al.*, 2013). In this research, we evaluated the ovicidal potential of *P. dulce* leaf and seed extracts against the filariasis vector *C. quinquefasciatus*.

MATERIALS AND METHODS

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. Voucher specimens were deposited at the Herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University.

Plant Extracts

The leaves and seeds were washed with tap water, shade-dried, and finely ground. The finely ground plant leaf powder (0.8 ***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 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 ovicidal bioassays.

Mosquito Rearing

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 the 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 photoperiod of 14 h light, 10 h dark.

Ovicidal Activity

The ovicidal activity, slightly modified method of Su and Mulla (1998) was performed. The eggs of *C. quinquefasciatus* were collected from vector control laboratory, Annamalai University. The leaf and seed extracts were diluted in the ethanol to achieve various concentrations ranging from 100 to 600 mg/L. Eggs of these mosquito species (100 numbers of 12-18 h old eggs) 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 the following formula.

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

RESULTS AND DISCUSSION

The ovicidal activity of crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf, and seed of *P. dulce* against the vector mosquito *C. quinquefasciatus* are presented in Table 1. Among extracts tested for ovicidal activity against *C. quinquefasciatus* the leaf and seed methanol extract of *P. dulce* exerted 100% mortality (zero hatchability) at 500 and 600 mg/L, respectively. The leaf extract of *P. dulce* was found to be most effective than seed against eggs of vector mosquito. Control eggs showed the 100% hatchability.

Table 1: Ovicidal activity of *P. dulce* plant leaf and seed extracts against the filariasis vector *C. quinquefasciatus*

Plant part	Solvent	Egg hatchability (%)						
		Control	100 mg/L	200 mg/L	300 mg/L	400 mg/L	500 mg/L	600 mg/L
Leaf	Hexane	100±0.0[a]	81.8±1.6[b]	71.9±1.1[c]	58.6±1.5[d]	37.8±1.3[e]	28.4±1.4[f]	16.8±1.3[g]
	Benzene	100±0.0[a]	78.2±1.2[b]	63.4±1.3[c]	48.7±1.3[d]	31.1±1.4[e]	24.3±1.2[f]	NH
	Chloroform	100±0.0[a]	70.4±1.8[b]	54.6±1.5[c]	42.4±1.8[d]	28.6±1.2[e]	20.2±1.4[f]	NH
	Ethyl acetate	100±0.0[a]	65.3±1.1[b]	49.5±1.6[c]	38.7±1.4[d]	25.4±1.9[e]	18.5±1.1[f]	NH
	Methanol	100±0.0[a]	56.1±2.0[b]	41.8±1.4[c]	29.8±2.0[d]	19.1±1.0[e]	NH	NH
Seed	Hexane	100±0.0[a]	92.6±1.6[b]	78.5±1.9[c]	65.9±1.2[d]	50.7±1.4[e]	33.6±1.6[f]	26.8±1.1[g]
	Benzene	100±0.0[a]	88.9±1.1[b]	67.2±1.2[c]	56.5±1.8[d]	41.3±1.6[e]	28.7±1.4[f]	20.6±0.9[g]
	Chloroform	100±0.0[a]	81.6±1.0[b]	61.4±1.4[c]	49.7±1.4[d]	35.1±1.2[e]	24.2±1.8[f]	18.2±1.3[g]
	Ethyl acetate	100±0.0[a]	73.7±1.7[b]	57.6±1.3[c]	44.1±0.9[d]	30.4±1.9[e]	19.3±0.9[f]	NH
	Methanol	100±0.0[a]	62.5±2.0[b]	51.6±1.9[c]	39.3±1.4[d]	23.5±1.7[e]	17.8±1.2[f]	NH

Values are means±SD, Within each row, different letters indicate significant differences (Tukey's HSD, $P<0.05$), NH: No hatchability, SD: Standard deviation, HSD: Honestly standard deviation, *C. quinquefasciatus: Culex quinquefasciatus, P. dulce: Pithecellobium dulce*

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 findings are comparable to previous research by Al-Doghairi *et al*. (2004), which reported that the ovicidal effect of *Solenostemma argel* was low. However, concentrations of 0.05% and 0.1% exhibited significant effects, producing 65% and 75%; 62.9%, and 62.9%, respectively, on the 1st and 2nd day after treatment, respectively, the 0.1% concentration reduced egg hatch by 33.7%, compared with the control and 100% mortality values were evident in concentrations as low as 0.025% at 2 days post-hatching against *C. pipiens*. The oviposition deterrent effectiveness (76-100% repellency) against *A. albopictus* of 21 commercial insect repellent products (at 0.1% concentration), including 12 botanical, six deet-based, and three synthetic organics (Xue *et al*., 2006). All essential oil showed moderate ovicidal effects. However, the leaf ethanolic extract of *Cassia obtusifolia* had significant larvicidal effect with LC_{50} and LC_{90} values of 52.2 and 108.7 mg/l, respectively, at the higher concentration (400 mg/l) that showed 92.5% effective repellency against late third instar larvae of *A. stephensi* (Rajkumar and Jebanesan, 2009). The benzene extracts of *Citrullus vulgaris* exerted 100% mortality (zero hatchability) at 250 ppm, a very low hatchability (11.8%) at 200 ppm, and complete ovicidal activity at 300 ppm. The ovicidal effect of *S. argel* was low; however, concentrations of 0.05% and 0.1% exhibited significant effects ($P < 0.05$), producing 65% and 75% and 62.9% and 62.9% on the 1st and 2nd day after treatment, respectively.

The essential oil from leaves and stems of *Piper marginatum* exhibited an oviposition deterrent effect against *A. aegypti* at 50 and 100 ***ppm*** in that significantly lower numbers of eggs (<50%) were laid in glass vessels containing the test solutions compared with the control solution (Autran *et al*., 2009). The benzene extracts of *C. vulgaris* exerted 100% mortality (zero hatchability) at 250 ppm and at 200 ppm a very low hatchability (11.8%), complete ovicidal activity at 300 ppm and the Fraction I at 80 ppm exerted a very low hatchability rate of 3.2% followed by Fraction II (6.9%), Fraction III, and IV afforded 4.9 and 5.3% hatchability recorded against *A. stephensi* and *A. Aegypti*, respectively, (Mullai *et al*., 2008). The leaf methanol extract of *C. fistula* was tested for larvicidal and ovicidal activity of against *C. quinquefasciatus* and *A. stephensi* with the LC_{50} values of 17.97 and 20.57 mg/l, respectively, (Govindarajan *et al*., 2008). The complete ovicidal activity (100% mortality) was attained at 300 ppm for methanol, benzene, petroleum ether, and ethyl acetate extracts of *Citrullus pubescens* against *C. quinquefasciatus* (Mullai and Jebanesan, 2006).

CONCLUSION

Our findings highlighted that the plant *P. dulce* exhibits moderate ovicidal activity against filariasis vector mosquito *C. quinquefasciatus*. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plant. *P. dulce* extracts may contribute to reduce environmental pollution and to an overall reduction in the population density of mosquito vectors of medical and veterinary importance.

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