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Larvicidal & ovicidal efficacy of *Pithecellobium dulce* (Roxb.) Benth. (*Fabaceae*) against *Anopheles stephensi* Liston & *Aedes aegypti* Linn. (Diptera: Culicidae)

M. Govindarajan, M. Rajeswary & R. Sivakumar

Department of Zoology, Annamalai University, Annamalainagar, Tamil Nadu, India

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***Background & objectives*: In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken 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* against the mosquito vectors, *Anopheles stephensi* and *Aedes aegypti* (Diptera: Culicidae).**

***Methods*: Larvicidal activity of *P. dulce* plant extracts was studied in the range of 60 to 450 mg/l against early third instar larvae of *An. stephensi* and *Ae. aegypti* in the laboratory. The larval mortality was observed after 24 h of exposure. The ovicidal activity was determined against *An. stephensi* and *Ae. aegypti* to various concentrations ranging from 100 to 750 mg/l under the laboratory conditions. Mean per cent hatchability of the eggs were observed after 48 h post treatment.**

***Results*: All leaf and seed extracts showed moderate larvicidal and ovicidal effects; however, the highest larval mortality was found in methanol extract of leaf of *P. dulce* against the larvae of *An. stephensi* and *Ae. aegypti* with the LC_{50} and LC_{90} values 145.43, 155.78 mg/l and 251.23, 279.73 mg/l, respectively. The per cent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Zero hatchability was observed at 400 mg/l for leaf methanol extract and 625 mg/l for seed methanol extract of *P. dulce* against *An. stephensi* and *Ae. aegypti,* respectively. Compared to leaf extracts, seed extracts have low potency against the two mosquitoes.**

***Interpretation & conclusions*: The present results suggest that the leaf and seed extracts of *P. dulce* have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes.**

Key words *Aedes aegypti* - *Anopheles stephensi* - larvicidal-ovicidal - *Pithecellobium dulce*

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, chikungunya, filariasis, schistosomiasis, and Japanese encephalitis. Mosquitoes also cause allergic responses that include local skin and systemic reactions such as angioedema in humans[1]. *Aedes aegypti* (L.) is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas, and also for yellow fever in Central and South America and West

Africa[2]. In the past decade, chikungunya - a virus transmitted by *Aedes* spp mosquitoes - has re-emerged in Africa, southern and southeastern Asia, and the Indian Ocean Islands as the cause of large outbreaks of human disease[3]. Malaria is a protozoan infection of erythrocytes caused in human beings by five species of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale, P. malariae*, and *P. knowlesi*). In most cases, malaria is transmitted via the bite of an infected female anopheline mosquito, but congenital malaria and acquisition through infected blood transfusion are well described[4]. More than 40 per cent of the world's population - approximately 3 billion people - are exposed to malaria in 108 endemic countries[5]. About one million cases of malaria are reported in India every year. In 2010 an estimated 219 million (range 154-289 million) cases occurred worldwide and 660,000 people died (range 610 000-971 000), mostly in children under five years of age[6]. Presently, organochlorine, organophosphate, and synthetic pyrethroid insecticides are being used for mosquito control. Successive changes in the insecticides result in multiple insecticide resistant malaria vectors. Malaria vectors in India are resistant to several insecticides (DDT, HCH, malathion, and deltamethrin)[7].

There has been an increasing interest in anti-mosquito products derived from natural origin because the continued applications of synthetic compounds have some drawbacks, including the widespread development of insecticide resistance[8]. Another drawback with the use of chemical insecticides is that these are non-selective and could be harmful to other organisms in the environment. The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides, and for more detailed studies of naturally-occurring insecticides[9]. It is, therefore, necessary to develop new materials for controlling mosquitoes in an environmentally safe way, using biodegradable and target-specific insecticides against them[10].

The larvicidal activity of crude acetone, hexane, ethyl acetate, methanol, and petroleum ether extracts of the leaf of *Centella asiatica, Datura metal, Mukia scabrella, Toddalia asiatica*, extracts of whole plant of *Citrullus colocynthis* and *Sphaeranthus indicus*[11]; the leaf benzene, chloroform, ethyl acetate, and methanol extracts of *Acalypha indica*[12]; the methanol extracts of leaves of *Dysoxylum malabaricum* have been tested against mature and immature stages of *An. stephensi* under laboratory conditions[13]; root bark extracts of *Turraea wakefieldii* and *Turraea floribunda* against third-instar larvae[14] and extracts of *Pelargonium citrosa* leaf have been tested for their biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency, and biting deterrency[15] against *An. stephensi*. Hexane extract obtained from leaves of *Eucalyptus citriodora* was tested against larvae of *An. stephensi, Culex quinquefasciatus*, and *Ae. aegypti* to assess its toxicity and growth-inhibiting activity[16].

Pithecellobium 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 (*Vilayati babul*). The presence of steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides has been reported in the seeds. The bark contains 37 per cent of tannins of catechol type. Quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves[17]. The plant has been shown to have potential in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders. However, no information was available on the ovicidal and larvicidal activities of this plant species against *An. stephensi* and *Ae. aegypti*. Therefore, the aim of this study was to investigate the mosquito ovicidal and larvicidal activities of the different solvent extracts of *P. dulce*.

Material & Methods

Collection of plants: Fully developed leaves and seeds of the *P. dulce* were collected from Thanjavur district, Tamil Nadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University, Annamalainagar. A voucher specimen was deposited at the herbarium of plant phytochemistry division, Department of Zoology, Annamalai University.

Extraction: The healthy 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 per cent by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal and ovicidal bioassays.

Test organisms: The laboratory-bred pathogen free strains of mosquitoes were 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. At the time of adult feeding, these mosquitoes were 3-4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time 500 mosquitoes per cage were fed on blood using a feeding unit fitted with parafilm as membrane for 4 h. *Ae. aegypti* feeding was done from 1200 to 1600 h, and *An. stephensi* were fed during 1800 to 2200 h. A membrane feeder with the bottom end fitted with parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C were maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28 ± 2°C, 70-85 per cent relative humidity, with a photo period of 12-h light and 12-h dark.

Larvicidal bioassay: The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization[18]. Batches of 25 third instar larvae were transferred to a small disposable paper 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 (60-450 mg/l). 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} (lethal concentration that kills 50 per cent of the exposed larvae) and LC_{90} (lethal concentration that kills 90 per cent of the exposed larvae) values were calculated after 24 h by probit analysis[19].

Ovicidal activity: For ovicidal activity, slightly modified method of Su and Mulla[20] was performed. *An. stephensi* and *Ae. aegypti* eggs were collected from vector control laboratory, Department of Zoology, Annamalai University. The leaf and seed extracts were 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 24 h 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:

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

Statistical analysis: The average larval 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) values were calculated using the SPSS12.0 (Statistical Package of Social Sciences Inc., USA) software.

Results

The results of the larvicidal activity of crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent leaf and seed extracts of *P. dulce* against the larvae of two important vector mosquitoes, *viz*. *An. stephensi* and *Ae. aegypti* are presented in Table I. Among the extracts tested, the highest larvicidal activity was observed in leaf methanol extract against *An. stephensi* followed by against *Ae. aegypti* with the LC_{50} and LC_{90} values were 145.43, 155.78 mg/l and 251.23, 279.73 mg/l, respectively. Compared to leaf extracts seed have low potency against two mosquitoes. The 95% confidence limits LC_{50} (LCL-UCL) and LC_{90} (LCL-UCL) were also calculated. The mean per cent egg hatchabilities of *An. stephensi* and *Ae. aegypti* were tested with five different solvents at different concentrations of *P. dulce* leaves and seed extracts, and the results are listed in Table II. The per cent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Zero hatchability of *An. stephensi* (*Ae. aegypti*) eggs was attained at the concentration of 400 mg/l (400 mg/l) for leaf extract and 500 mg/l (625 mg/l) for seed extract of *P. dulce*. Control eggs showed the 100 per cent hatchability. The leaf extract of *P. dulce* was found to be most effective than seed against larvae and eggs of the two vector mosquitoes.

Discussion

Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as these 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 agents. Furthermore, the crude extracts may be more effective

Table I. Larvicidal activity of different solvent leaf and seed extracts of *P. dulce* against *Ae. aegypti* and *An. stephensi*

Parts used	Mosquito species	Solvent used	LC_{50} (mg/l)	(LCL-UCL)	LC_{90} (mg/l)	(LCL-UCL)
Leaf	*Ae. aegypti*	Methanol	155.78	(116.63-192.95)	279.73	(234.74-365.38)
		Ethyl acetate	162.36	(127.49-196.27)	283.43	(241.58-358.92)
		Chloroform	169.08	(133.66-203.99)	293.17	(249.91-371.95)
		Benzene	176.02	(136.96-214.04)	308.88	(262.08-395.10)
		Hexane	185.14	(149.43-220.67)	316.46	(271.94-395.26)
	An. stephensi	Methanol	145.43	(115.52-174.88)	251.23	(214.64-317.30)
		Ethyl acetate	153.41	(115.69-189.01)	275.87	(232.52-356.37)
		Chloroform	160.71	(125.77-194.47)	282.35	(240.62-357.36)
		Benzene	166.96	(132.89-201.27)	290.40	(247.81-367.26)
		Hexane	172.82	(130.53-213.54)	307.50	(258.16-402.33)
Seed	*Ae. aegypti*	Methanol	193.66	(117.71-260.84)	377.39	(300.46-562.98)
		Ethyl acetate	215.63	(148.99-279.03)	416.51	(338.65-590.18)
		Chloroform	240.39	(173.39-310.29)	461.28	(373.63-666.47)
		Benzene	259.42	(199.20-326.90)	489.41	(401.19-685.47)
		Hexane	281.18	(229.64-342.45)	516.33	(431.68-687.87)
	An. stephensi	Methanol	168.32	(120.15-212.55)	315.42	(262.00-419.11)
		Ethyl acetate	185.19	(130.42-235.81)	362.12	(298.36-493.05)
		Chloroform	199.39	(149.28-248.18)	381.10	(317.56-507.21)
		Benzene	241.19	(191.96-273.69)	444.77	(368.54-606.45)
		Hexane	263.09	(217.68-320.01)	478.47	(399.73-639.21)

LC_{50}, lethal concentration that kills 50 per cent of the exposed larvae; LC_{90}, lethal concentration that kills 90 per cent of the exposed larvae; UCL, upper confidence limit; LCL, lower confidence limit; Table shows that all mean significantly differ from other means (Tukey's test)

compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors. Our results showed that the crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf and seed of *P. dulce* were effective against the eggs and larvae of two important vector mosquitoes, *viz. An. stephensi* and *Ae. aegypti*. Chowdhury *et al*[21] have reported that the chloroform and methanol extracts of mature leaves of *Solanum villosum* showed the LC_{50} value for all instars between 24.20 and 33.73 mg/l after 24 h and between 23.47 and 30.63 mg/l after 48 h of exposure period against *An. subpictus*. The efficacy of 11 commonly available medicinal plants and compare its efficacy in relation to larvicidal activity against of *An. stephensi*. All the medicinal plants and the mixture were effective against larvae of *A. stephensi* as evidenced by low lethal concentration and lethal time[22]. The n-hexane, ethyl acetate and methanol extracts of *Combretum nigricans, Jatropha curcas* and *Datura innoxia, Strophantus hispidus*, *Securidaca longepedunculata* and *Sapium grahamii* exhibited 100 per cent mortality at 250 µg/ml concentrations against fourth instar larvae of *Ochlerotatus triseriatus*[23].

Mullai and Jebanesan[24] have reported that the ethyl acetate, petroleum ether and methanol leaf extracts of *C. colocynthis* and *Cucurbita maxima* had LC_{50} values 47.58, 66.92 and 118.74 mg/l and 75.91, 117.73 and 171.64 mg/l, respectively, against *Cx. quinquefasciatus* larvae. Govindarajan[25] evaluated larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 and 48 mg/l. The crude extract had strong repellent action against the three species of mosquitoes as it provided 100 per cent protection against *An. stephensi* for 180 min followed by *Ae. aegypti* (150 min) and *Cx. quinquefasciatus* (120 min). The methanol extracts of

Table II. Ovicidal activity of *P. dulce* plant leaf and seed extracts against *An. stephensi* and *Ae. aegypti*

Parts used	Mosquito	Name of the solvent	Percentage of egg hatchability ± SD						
			Concentration (mg/l)						
			Control	100	200	300	400	500	600
Leaf	*An. stephensi*	Hexane	100±0.0	71.6±1.6	62.4±2.0	46.2±1.1	31.5±1.8	20.2±1.5	NH
		Benzene	100±0.0	66.4±1.3	52.3±1.1	37.2±1.6	23.9±2.0	NH	NH
		Chloroform	100±0.0	58.2±2.1	46.5±1.9	33.7±1.0	19.4±1.2	NH	NH
		Ethyl acetate	100±0.0	52.9±1.0	39.2±0.9	27.3±1.4	NH	NH	NH
		Methanol	100±0.0	47.8±1.4	34.6±1.7	17.8±1.2	NH	NH	NH
	Ae. aegypti	Hexane	100±0.0	78.2±1.8	65.7±1.5	50.2±1.8	34.7±1.0	25.6±1.2	NH
		Benzene	100±0.0	70.7±1.1	58.8±0.8	41.9±1.6	27.2±1.8	19.2±1.7	NH
		Chloroform	100±0.0	64.4±1.5	49.2±1.4	38.6±1.3	22.3±1.1	NH	NH
		Ethyl acetate	100±0.0	56.1±2.0	43.9±1.6	32.2±1.0	19.4±1.6	NH	NH
		Methanol	100±0.0	50.5±1.7	37.2±1.2	19.5±0.9	NH	NH	NH
			Control	125	250	375	500	625	750
Seed	*An. stephensi*	Hexane	100±0.0	79.7±1.5	66.5±1.7	51.2±1.1	35.6±1.4	27.6±1.8	NH
		Benzene	100±0.0	75.2±1.2	60.2±1.0	47.6±1.0	32.1±1.1	22.6±1.1	NH
		Chloroform	100±0.0	69.8±1.3	55.3±1.2	41.7±0.9	28.4±2.0	17.5±1.5	NH
		Ethyl acetate	100±0.0	62.1±1.8	48.2±2.0	36.4±1.6	24.7±1.6	NH	NH
		Methanol	100±0.0	56.3±2.1	42.9±1.6	31.5±2.0	NH	NH	NH
	Ae. aegypti	Hexane	100±0.0	86.4±1.4	70.3±1.8	56.8±1.4	42.8±1.5	30.4±0.9	23.7±1.5
		Benzene	100±0.0	80.8±1.0	62.7±1.7	50.3±1.8	37.5±1.6	25.2±1.6	NH
		Chloroform	100±0.0	75.6±1.9	59.6±1.1	44.7±1.3	32.6±1.1	20.7±2.0	NH
		Ethyl acetate	100±0.0	66.9±1.8	52.5±2.0	39.1±1.7	28.4±1.9	17.1±1.2	NH
		Methanol	100±0.0	59.8±1.4	48.1±1.3	34.6±2.1	21.6±1.0	NH	NH

Values are mean±SD of 6 observations. NH, no hatchability

Euphorbia tirucalli latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *Cx. quinquefasciatus* with LC_{50} values of 177.14 and 513.387 mg/l, respectively[26]. The aqueous extract of Piperretrofractum showed LC_{50} values of 5,124 and 9,681 mg/l against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively[27]. The water extract of citrus-seeds showed LC_{50} values of 135, 319.40 and 127, 411.88 mg/l against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*[28]. A crude chloroform extract of seeds of *Millettia dura* showed high activity (LC_{50}=3.5 µg/ml at 24 h) against second-instar larvae of *Ae. aegypti*[29].

In conclusion, our findings showed that the plant *P. dulce* exhibits larvicidal and ovicidal activity against two important vector mosquitoes. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants. *P. dulce* extracts may contribute greatly to save environment and to an overall reduction in the population density of two significant vectors (*An. stephensi* and *Ae. aegypti*). The results of this study also demonstrate the potential of new alternative sources of mosquito larvicides and ovicides which are generally free of adverse effects. The isolation and purification of these extracts are in progress and evaluation of these compounds will be needed to identify the active component.

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Reprint requests: Dr M. Govindarajan, Division of Vector Biology & Phytochemistry, Department of Zoology, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India
e-mail: drgovind1979@gmail.com