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An Indigenous Medicinal Plant *Pterolobium hexapetalum* with Potential Mosquitocidal Properties

Samundeeswari A[1]*, Poovarasan.S[2]
[1, 2]Post-Graduate Department of Plant Biology and Plant Biotechnology, Government Arts College for Men (Autonomous), Nandanam, Chennai -600 035, India

Abstract**—** *The present study was undertaken to assess the ovicidal, larvicidal, and the repellent activity of Pterolobium hexapetalum an indigenous medicinal plant extracts of pentane, ethyl acetate and methanol against the selected vector Mosquitoes namely Aedes aegypti, Anopheles stephensi and, Culex quinquefasciatus. The undertaken results of Ovicidal activity of methanol extract of P. hexapetalumwas tested against the eggs of Aedes aegypti, 32.8%; Anopheles stephensi, 38.4%; Culex quinquefasciatus79.2% showed mortality of the eggs was increased with increased concentration of the extract respectively.. Methanol extract of P. hexapetalum also showed 96.82% insecticidal activity against the larvae of Aedes aegypti, 100% larvicidal activity against the larvae of Anopheles stephensi, and Culex quinquefasciatus at 25mg/l concentration of the extract. The repellent activity of methyl acetate extract against Aedes aegypti showed that 60.0±0.12%, 65.0±4.84% and 87.5±4.51% repellency was noted against the mosquitoes of An. stephensi and C. quinquefasciatus respectively.*

Keywords**—** *Ovicidal, larvicidal, Repellent, Insecticide.*

I. INTRODUCTION

Chemicals derived from plants offer promise in future mosquito control programs. In addition to application as general toxicants against various life stages of mosquitoes, phytochemicals also have potential uses as growth and reproduction inhibitors, repellents and as oviposition deterrents. Research on the use of phytochemicals against mosquitoes should consider such factors as mosquito species, life stage specificity to a compound, the plant parts and solvent used for extraction, phototoxic activity and the geographical origin of a plant compound. Phytochemicals offer not only effective mosquito control agents, but also are biorational alternatives to organic synthetic pesticides[1]. The fact these chemicals are from natural sources, with a high degree of biodegradation, makes them environmentally sound control agents. The present investigation is designed to assess the bioefficacy of *Pterolobium hexapetalum* (leaf) against a human malarial vector mosquitoes, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus.*

Plant Description:

Pterolobium hexapetalum (Roth).

The Indian Redwing, Camp Siege or Bhoca[2] (*Pterolobium hexapetalum*) is a flowering plant in the legume family, Fabaceae. Indian Redwing is an extensive straggling shrub, with long, arching branches. Leaves are double-compound, with leaflets oblong-oblanceolate. Indian Redwing is indigenously named as Bhoca in the Nilgiris by the Irula community. Flowers are borne in racemes in leaf axils or at the end of branches, of a yellowish white colour. Pod is a samara, oblong, with red wings. Seeds are solitary at base, obovoid. It is a characteristic species of dry deciduous forest in parts of South India. In south India, it is a major bee-forage plant. Flowering: March-April.

Taxonavigation:
Subfamilia: Caesalpinioideae
Family caesaipinaceae
Tribus: Caesalpinieae
Genus: *Pterolobium*
Species: *Pterolobium hexapetalum*

II. MATERIALS AND METHOD

Plant Material

Plant sampling was carried out during the growing season (March- April) of 2010 from different places of Javadhu Hills, Vellore district of the Tamilnadu. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder.

Extraction Method

The dried leaf (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with pentane, ethyl acetate and methanol (500 mL, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mmHg at 45^{0}C by "Rotavapour" and the residue obtained was stored at 4 ^{0}C.

Mosquito Rearing

The mosquitoes, *Ae.aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, were reared[3] in the Entomology extension unit, Department of Zoology, Government Arts College, Chennai. The larvae were fed on dog biscuits and yeast power in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28± 2), 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

Ovicidal Activity

The method of Su and Mulla[4] was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of selected mosquitoes were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment,

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the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

$$\%\text{Ovicidal Activity} = \frac{\text{No.of eggs hatched}}{\text{Total no.of eggs treated}} \text{ X100}$$

Repellent Activity

The repellent study was following the methods of WHO.[5] 3-4 days old blood-starved female selected mosquitoes (100) were kept in a net cage (45cmX 45cmX 40cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arm only 25 cm2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The A. bracteata leaf extract at 1.5, 3.0 and 6.0 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every 30 min. The experiment was conducted five times. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula.

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \text{ x } 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

Larvicidal Activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO[6]. From the stock solution, six different test concentrations (*viz*. 5, 10, 15, 20 and 25mg/l) were prepared and they were tested against the freshly moulted (0 - 6 h) third instar larvae of selected mosquitoes. The larvae of test species (25) were introduced in 500-mL plastic cups containing 250 mL of aqueous medium (249 mL of dechlorinated water + 1mL of emulsifier) and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} value was calculated by using probit analysis (Abbot's 1965).[7]

Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, LC90 and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and chi-square value were calculated using the SPSS software package 23.0. Results with $P<0.05$ were considered to be statically significant.

III. Results and Discussion

Ovicidal activity of **pentane extract of *P. hexapetalum*** was tested against the *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus* and the data obtained from the experiments are shown in **(Table I)**. The mean per cent mortality of the eggs was increased with increased concentration of the extract and also the experiments. It is apparent from the above data that, 12.0, 14.8, 16.8, 22.0 and 28.0% ovicidal activities respectively were recorded from the experimental batches treated with 5, 10, 15, 20 and 25ppm treated eggs of *Ae. aegypti*. Similarly, 17.6, 20.0, 27.6, 29.6 and 56.0% ovicidal activities respectively were recorded from the experimental batches *A. stephensi* eggs treated with 5, 10, 15, 20 and 25ppm of the pentane extract. In the same way, 5, 10, 15, 20 and 25ppm of the extract showed 24.0, 28.4, 37.6, 42.0 and 64.0% ovicidal activities respectively were recorded from the experimental batch eggs of *Cx. quinquefasciatus.*

TABLE I. Ovicidal activity of pentane extracts of *P.hexapetalum* tested against the eggs of selected mosquitoes .

Concentrations tested (mg/l)	Mosquito eggs tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	12.0	17.6	24.0
10	14.8	20.0	28.4
15	16.8	27.6	37.6
20	22.0	29.6	42.0
25	28.0	56.0	64.0

Values represent the mean of three replications.

Ovicidal activity of **ethyl acetate extract of *P. hexapetalum*** was tested against the *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus* and the data obtained from the experiments are shown in **(Table II)**. The mean percent mortality of the eggs was increased with increased concentration of the extract and also the experiments. It is apparent from the above data that, 8.0, 8.0, 20.8, 26.8 and 32.8% ovicidal activities respectively were recorded from the experimental batch eggs of *Ae. aegypti* treated with 5, 10, 15, 20 and 25ppm concentration of the extract. Similarly, 18.4, 23.2, 26.4, 31.2 and 36.4% ovicidal activities respectively were recorded from the experimental batch of *An. Stephensi* treated with 5, 10, 15, 20 and 25ppm concentration of the extract. In the same way, 5, 10, 15, 20 and 25ppm of the extract showed 26.8, 34.0, 39.6, 47.2 and 76.4% ovicidal activities respectively were recorded from the experimental group eggs of *Cx. quinquefasciatus.*

TABLE II. Ovicidal activity of ethyl acetate extract of *P. hexapetalum* tested against mosquitoes.

Concentrations tested (mg/l)	Mosquito eggs tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	8.0	18.4	26.8
10	8.0	23.2	34.0
15	20.8	26.4	39.6
20	26.8	31.2	47.2
25	32.8	36.4	76.4

Values represent the mean of three replications.

TABLE III. Ovicidal activity of methanol extract of *P. hexapetalum* tested against mosquitoes.

Concentrations tested (mg/l)	Mosquito eggs tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	12.4	19.6	31.2
10	18.0	24.4	33.6
15	24.4	31.2	38.4
20	31.2	33.6	48.4
25	32.8	38.4	79.2

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Values represent the mean of three replications. Different superscript alphabets in the column differs significantly from each other ($p<0.005$%, Tukey test)

Ovicidal activity of **methanol extract of *P. hexapetalum*** was tested against the eggs of *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus* and the data obtained from the experiments are shown in **(Table III).** The mean per cent mortality of the eggs was increased with increased concentration of the extract and also the experiments. It is apparent from the above data that, 12.4, 18.0, 24.4, 31.2 and 32.8%; 19.6, 24.4, 31.2, 33.6 and 38.4%; 31.2, 33.6, 38.4, 48.4 and 79.2% against 5, 10, 15, 20 and 25ppm on the eggs of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* respectively (**Table III**).

TABLE IV. Repellent activity of pentane extract of *P. hexapetalum* tested against mosquitoes.

Concentrations tested (mg/l)	Mosquito tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	22.5±0.47	32.5±0.28	45±.025
10	30±±0.52	25±0.22	65±0.55
15	37.5±0.56	40±0.36	72.5±0.45
20	42.5±0.55	47.5±0.41	80±0.81
25	55.0 ±0.21	60.0 ±0.55	82.5±0.46

Values represent the mean ± S.E of three replications.

The repellent activity of pentane extract of *P. hexapetalum*was tested against the adult mosquitoes of *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus* and the data pertaining to the experiments are shown in **(Table IV).** It was observed that 55.0 ±0.21% repellent activity in 25ppm concentration against *A. aegypti*. Whereas, 60.0 ±0.55 and 82.5±0.46% repellency was noted in *An. stephensi* and *C.quinquefasciatus* respectively **(Table IV).**

The repellent activity of ethyl acetate extract of *P. hexapetalum* was tested against the adults of *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus* and the data pertaining to the experiments are shown in **(Table V)** It was observed that 45.0±0.54% repellent activity against *A. aegypti.*Whereas, 52.5±0.49 and 55.0±0.46% repellency was noted in *An. stephensi* and *C. quinquefasciatus* respectively **(Table V)**

TABLE V. Repellent activity of ethyl acetate extract of *P. hexapetalum* tested against mosquitoes.

Concentrations tested (mg/l)	Mosquito tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	20±0.18	27.5±0.46	32.5±0.21
10	27.5±0.31	22.5±0.18	25±0.12
15	35±0.29	30±0.44	37.5±0.38
20	32.5±0.31	37.5±0.24	42.5±0.54
25	45.0±0.54	52.5±0.49	55.0 ±0.46

Values represent the mean ± S.E of three replications.

TABLE VI. Repellent activity of methanol extract of *P. hexapetalum* tested against mosquitoes.

Concentration (mg/l)	Mosquito tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	35.0±2.31	30.0±2.26	47.5±2.14
10	40.0±3.21	47.5±1.45	55±2.71
15	47.5±3.73	50.0±2.8	60±6.54
20	52.5±7.48	60.0±4.77	62.5±6.69
25	60.0±0.12	65.0±4.84	87.5±4.51

Values represent the mean ± S.E of three replications.

The repellent activity of methanol extract of *P. hexapetalum*was tested against the adult mosquitoes of *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus* and the data pertaining to the experiments are shown in **(Table VI)**. It was observed that 60.0±0.12% repellent activity in *A. aegypti*. Whereas, 65.0±4.84 and 87.5±4.51% repellency was noted against the mosquitoes of *An. stephensi and C. quinquefasciatus* respectively **(Table VI)**

TABLE VII. Larvicidal activity of pentane extract of *P. hexapetalum* tested against mosquitoes.

Concentrations tested (mg/l)	Larvae tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	13.27a	19.39a	25.51a
10	35.71b	37.76a	34.69b
15	51.02b	47.96a	61.22c
20	76.53c	69.39b	81.63c
25	91.84c	93.88c	91.84c

Values expressed are mean mortality ± standard deviations of five replications (n=100). LSD=20.5. Values with different alphabet in the column shows statistical significance at P<0.01% level; LSD, DMRT.

TABLE VIII. Determined lethal concentrations values of pentane extract of *P. hexapetalum* tested against mosquitoes.

Exposure periods (days)	LC_{50}	95 % Fiducial Limit		LC_{90}	95% Fiducial limit		Regression	χ^2
		LCL	UCL		LCL	UCL		
A. aegypti.	13.93	12.87	14.97	24.57	22.86	26.83	0.923	1.559
An. stephensi.	13.81	10.44	16.88	25.87	21.59	35.72	0.986	2.426
C. quinquefasciatus.	12.24	10.99	13.38	24.10	22.25	26.59	0.968	2.406

Pentane extract of *P.hexapetalum*showed 91.84% insecticidal activity against *Aedes aegypti*, 93.88% activity against *Anopheles stephensi*and 91.84% activity against the larvae of *Culex quinquefasciatus*at 25mg/l concentration of the extract **(table VII)**. Lethal concentrations were determined for the three exposure periods and are shown in **(Table VIII)**. It was observed that LC_{50} values of 14.73mg/l, 13.98mg/l and 10.51mg/l were recorded against *Aedes aegypti, An. stephensi* and *Culex quinquefasciatus* **(Table VIII)**

TABLE IX. Larvicidal activity of ethyl acetate extract of *P. hexapetalum*tested against mosquitoes.

Concentrations tested (mg/l)	Larvae tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	10.20a	20.41a	29.59a
10	29.59b	32.65a	39.80a
15	46.94c	44.90a	68.37b
20	78.57d	76.53b	84.69c
25	90.82d	92.86c	94.90c

Values expressed are mean mortality ± standard deviations of five replications (n=100).

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TABLE X. Determined lethal concentration values of ethyl acetate extract of *P. hexapetalum* tested against mosquitoes.

Exposure periods (days)	LC_{50}	95 % Fiducial Limit		LC_{90}	95% Fiducial Limit		Regression	χ^2
		LCL	UCL		LCL	UCL		
A. aegypti.	14.73	13.7	15.71	24.44	22.88	26.48	0.991	2.460
An. stephensi.	13.98	12.8	15.05	25.10	23.28	27.54	0.968	5.154
C. quinquefasciatus.	10.51	9.092	11.731	22.717	20.896	25.19	0.979	2.874

Larvicidal Activity of Ethyl Acetate Extract of P.hexapetalumtested against Mosquitoes.

Ethyl acetate extract of *P. hexapetalum*was tested for its insecticidal activity against the adult mosquitoes of *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus* under the laboratory conditions. The values obtained in the experiments are shown in **(table IX** and **X)**. It is clear from the observation, that, the insecticidal activity of the plant extract is dose dependent *i.e*., as the concentration of the extract increased the mortality of the larvae were also increased to a considerable extent **(Table IX** and **X)**. Ethyl acetate extract of *P. hexapetalum*showed 90.82%; 92.86% and 94.90% larvicidal activity (insecticidal) insecticidal activity against larvae of *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus* at 25mg/l concentration of the extract **(Table X)**.

Lethal concentrations were determined for the three mosquitoes and are shown in **(Table XI)**. It was observed that LC_{50} values of 14.73mg/l, 13.98mg/l and 10.51mg/l were recorded against larvae of *Aedes aegypti*, *An. stephensi and C.quinquefasciatus* **(Table X).**

TABLE XI. Larvicidal activity of methanol extract of *P. hexapetalum*tested against mosquitoes.

Concentrations tested (mg/l)	Larvae tested		
	Aedes aegypti	***Anopheles stephensi***	***Culex quinquefasciatus***
5	12.66^{a}	19.33^{a}	24.59^{a}
10	33.22^{b}	27.66^{a}	46.33^{a}
15	57.66^{c}	54.00^{a}	69.33^{b}
20	76.57^{d}	74.53^{b}	84.00^{c}
25	96.82^{d}	100.00^{c}	100.00^{d}

Values expressed are mean mortality ± standard deviations of five replications (n=100).

TABLE XII. Determined lethal concentration values methanol extract of *P. hexapetalum* tested against mosquitoes.

Exposure periods (days)	LC_{50}	95 % Fiducial Limit		LC_{90}	95% Fiducial Limit		Regression	χ^2
		LCL	UCL		LCL	UCL		
A. aegypti.	14.652	13.666	15.631	24.466	22.883	26.525	0.993	1.701
An. stephensi.	13.892	10.803	16.765	25.314	21.350	33.895	0.961	6.946
C. quinquefasciatus	10.896	9.591	12.043	22.426	20.694	24.224	0.986	2.32

Methanol extract of *P. hexapetalum* also showed 96.82% insecticidal activity against the fourth instar larvae of*Aedes aegypti*, *100%* larvicidal activity against the larvae of *Anopheles stephensi*, *Culex quinquefasciatus* at 25mg/l concentration of the extract **(Table XI)**. Similarly, insects exposed to 48hrs showed maximum response of 92.86% insecticidal activity at highest concentration (25mg/l). Lethal concentrations were determined for the three exposure periods and are shown in **(Table XII)**. It was observed that LC_{50} values of 14.652mg/l, 13.892mg/l and 10.896mg/l were recorded against the larvae of *A. aegypt., An. stephensi and C. quinquefasciatus***(Table XII)**.

IV. Conclusion

The overall performance of methanol extract showed that the maximum phytochemicals are present in the extract, because, high polarity nature of methanol made almost all the phytoconstituents dissolved in it. Thus, this line of study leads into a further research on the complete exploration of the plant, *P. hexapetalum* will be taken into further study in the future further explorative research on this aspect will certainly replace the chemical pesticides which are using currently to keep the mosquitoes in check condition. It is a well-known fact that the impact of phytopesticides may cause delayed result, but, they won't cause any harmful effects on humans or other non-target organisms.

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