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Evaluation of Larvicidal Effect of Lantana Camara Linn Against Mosquito Species *Aedes aegypti* and *Culex quinquefasciatus*

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Abstract: Mosquito larvicidal activity and phytochemical screening of methanol and ethanol extract of leaves and flowers of *Lantana camara Linn* belongs to the family of Verbanaceae have been evaluated in the present study. Larvicidal effect on 3rd and 4th instar larvae of mosquito species *Aedes aegypti* and *Culex quinquefasciatus* have been investigated in a dose dependent manner for 24 h. With 1.0 mg/ml concentration of extracts of *Lantana camara* maximum mortality was observed in *Aedes aegypti* exposed for 24 h. In the case of *Culex quinquefasciatus* the mortality was seen maximised when the concentration increased to 3.0mg/ml. Presence of saponin, flavonoids, terpenoids and cardiac glycosides have also been observed and GC/MS analysis was carried out on methanol flower and leaf extract to find out the components.

Key words: Lantana camara L · *Aedes aegypti* · Culex quinquefasciatus · Ethanol extract · Methanol extract · Phytochemicals

INTRODUCTION

Prevalence of Mosquito borne diseases are one of the world's most health hazardous problems. Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue and dengue haemorrhagic fever, yellow fever and chickungunya [1]. Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito borne disease is by killing mosquito at larval stage. The current mosquito control approach is based on synthetic insecticides. Even though they are effective they created many problems like insecticide resistance [2], pollution, toxic side effect on human beings [3]. This has necessitated the need for a research and development of environmentally safe, biodegradable indigenous method for vector control. Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides [1]. The effects of botanical derivatives against mosquito have been reviewed by Sukumar *et al.* [4]. Extracts from leaves, flowers and roots of plants and oils were found to have mosquito larvicidal activity [5, 6]. In this study, mosquito larvicidal activity was investigated using *Lantana camara Linn* (Verbanaceae) a large scrambling evergreen shrub which is commonly called as wild sage and lantana weed. In Tamil language the shrub is called as 'Arisimalar' and 'Unnichedi'. The plant is said to have carmitative, antispasmodic and antirheumatic uses in traditional medicines [7]. The plant has antibacterial, antifungal [8], antioxidant [9], insecticidal [10] and nematicidal activity [11]. Dua *et al*. [12] found that *Lantana camara Linn* flowers extract in coconut oil provides protection from *Aedes* mosquitoes. The present investigation assessed the larvicidal effect of *Lantana camara* extracts against early 3rd and 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus.*

MATERIALS AND METHODS

Plant Material: The leaves and flowers of *Lantana camara* were collected from Chennai, Tamilnadu, India and authenticated by Prof. P. Jayaraman, Director of Plant Anatomy Research Centre (PARC), Chennai- 600 045, Tamilnadu, India. Following the identification a voucher specimen of the plant was deposited in the herbarium of Plant Anatomy Research centre (PARC/2008/36).

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The leaves and flowers of the plant material were shade dried (28±2ºC), ground and sieved to get fine powder from which the extracts were prepared.

Methanol extract of the plant was obtained by taking 50 g of dried leaf and flower powder in a separate container. With this 250 ml of methanol was added and kept for 24 h with periodic shaking, then filtered and the filtrate was collected. This procedure was repeated three times with fresh volume of methanol. The filtrates were pooled. Ethanol extract of the plant material was also prepared in a similar manner with that of methanol.

The pooled ethanol and methanol extracts were concentrated separately by rotary vaccum evaporator at 40ºC and evaporated to dryness and stored at 4ºC in an air tight bottle [13].

Mosquito Species: Eggs of *Aedes aegypti* and larvae of *Culex quinquefasciatus* were obtained from Department of Entomology, Loyola College, Chennai-600 035, Tamilnadu, India. *Aedes aegypti* was obtained as egg rafts on a filter paper and were reared in trays containing tap water and maintained at 28±2°C. When the eggs were hatched out into first instar larvae; they were fed with yeast powder and glucose. On the third day after hatching the first instar larvae moulted into second instar larvae. On the fifth day, third instar larvae observed, which moulted into fourth instar larvae on the seventh day [14].

Culex quinquefasciatus third and fourth instar larvae were obtained as sample dispersed in water. The third and fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were experimented for the present study.

Larvicidal Assay: In the larvicidal assay, third and fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were exposed to test concentrations of 25, 50, 75, 100, 150, 200, 300 mg of methanol and ethanol extracts of leaf and flower of *Lantana camara* in 100ml of water.

100ml of tap water was taken in a series of 250ml glass beakers. The measured amount of extracts was dissolved in 1ml of the solvent which was used for preparing the extract. The dissolved plant extract was added to the water in the beakers. A control was also maintained by adding 1ml of solvent to 100ml water. 25 larvae per concentration was used for all the experiments. The larvae were fed dry yeast powder on the water surface (50mg/l) [15]. The number of dead larvae at the end of 24h was recorded and the mortality percentage values were calculated. This experiment was repeated three times.

The phytochemical screening of plant extract for Saponin, flavonoids, terpenoids, tannin, cardiac glycosides and steroids were also carried out [16, 17].

Gc / Ms Analysis: GC / MS Analysis of methanol flower and leaf extract was determined by GC - MS. Shimadzu Model QP-2010 Mass Spectrometer under the following conditions: DB - Polyethylene Glycol coated fused silica capillary column (30m length x 0.25mm ID x 0.25µm film thickness) : Helium Carrier Gas (1.34 ml/minute). 250°C injector temperature; 240°C interface temperature. 200°C Ion Source Temperature. Column temperature programmed at 60°C with 10°C / minute rise to 230°C. For GC/MS detection an ionization energy of 70ev was used.

50 mg of Methanol flower and leaf extract sample were taken separately and made up to 10ml with methanol, from which 1µl of sample was injected (split mode) in the column. The components were identified based on NIST Library / Wiley Library.

RESULTS

The present study on the plant extracts expressed the presence of larvicidal activity of *Lantana camara*. Table 1 represented the dose dependent effect of methanol and ethanol extracts of flower and leaf of *Lantana camara* on the mortality percentage of 3rd and 4th instar larvae of *Aedes agypti*. 0.75 and 1.00 mg/ml concentration of extracts showed maximum effect on 3rd as well as 4th instar larvae. Comparing the effects of other extracts methanol leaf extract showed lesser activity. Ethanol flower extract at a concentration of 0.75 mg/ml showed a 100% mortality of 3rd and 4th instar larvae.

Dose dependent effect of these extracts on 3rd and 4th instar larvae of *Culex quinquefasciatus* showed larvicidal activity. Methanol flower extract showed 100% mortality at a concentration of 2.0 mg/ml in 3rd instar larvae and methanol leaf extract showed 100% mortality in 4th instar larvae at 2.0 mg/ml concentration, but the required concentration to have maximum mortality rate is at 3.0mg/ml (Table 2).

The phytochemicals present in various extract of *Lantana camara* were represented in Table 3. Flavonoids and cardiac glycosides were present in methanol extract sample of both leaf and flower where as saponin was present in leaf and terpenoid was found to be present in

Table 1: Mortality percentage of early 3rd and 4th instar larvae of *Aedes aegypti* (25 larvae) subjected for 24 h to different concentration of methanol and ethanol extracts of *Lantana camara*. Values are mean of three replicates

		Concentration (mg/ml)			
Larvae	Extract	0.25	0.50	0.75	1.00
3rd instar larvae	Methanol flower	12	16	88	96
Mortality %	Methanol leaf	0	16	52	56
	Ethanol flower	0	0	100	100
	Ethanol leaf	0	0	80	88
4th instar larvae	Methanol flower	12	12	80	88
Mortality %	Methanol leaf	0	20	40	48
	Ethanol flower	0	0	100	100
	Ethanol leaf	0	0	72	84

Table 2: Mortality percentage of early 3rd and 4th instar larvae of *Culex quinquefasciatus* (25 larvae) subjected for 24 h to different concentration of methanol and ethanol extracts of *Lantana camara*. Values are mean of three replicates.

		Concentration (mg/ml)						
Larvae	Extract	0.25	0.50	0.75	1.00	1.50	2.00	3.00
3rd instar larvae	Methanol flower	0	32	48	60	72	100	NT
Mortality %	Methanol leaf	0	24	32	48	56	56	64
	Ethanol flower	0	0	20	52	68	72	76
	Ethanol leaf	0	0	32	48	56	56	64
4th instar larvae	Methanol flower	0	28	28	52	52	48	60
Mortality %	Methanol leaf	0	36	48	60	64	100	NT
	Ethanol flower	0	8	32	68	72	72	72
	Ethanol leaf	0	12	28	48	48	68	72

NT – Not tested

Table 3: Qualitative analysis of phytochemicals of methanol and ethanol extracts of *Lantana camara linn*

	Methanol extract		Ethanol extract	
Phytochemical	Leaf	Flower	Leaf	Flower
Tannin	-	-	-	-
Saponin	+	-	+	+
Flavonoids	+	+	+	-
Steroids	-	-	-	-
Terpenoids	-	+	-	+
Cardiac glycosides	+	+	+	+

+: Present ; - : Absent ;

Table 4: Peak Report Total Ion chromatogram of methanol flower extract of *Lantana camara* by GC/MS.

Peak	Retention Time	Area %	Name
1	3.606	4.09	2, 2-Dimethoxy propane
2	4.363	3.23	Diethylene Glycol diacetate
3	8.943	6.37	Glyceraldehyde
4	9.566	3.89	2- hydroxyl cyclopent-2-en-1-one
5	11.596	72.08	Glycerin
6	13.548	8.15	2,3-Dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one

Table 5: Peak Report Total Ion chromatogram of methanol leaf extract of *Lantana camara* by GC/MS.

Peak	Retention Time	Area %	Name
1	7.451	0.56	2, 3 - Dihydro - 3,5 dihydroxy - 6 -methyl - 4H - Pyran - 4 - one
2	7.611	0.93	β - Methyl sulfonyl acetyl pyridine
3	11.340	1.15	Diglycidyl ether
4	12.012	2.22	Caproic acid
5	14.621	0.43	6 - Methyl - 6 - (5- methyl furan - 2- yl) - heptan - 2 - one
6	14.682	0.71	4 butoxybutanol
7	15.308	0.43	Oleic acid
8	15.547	2.58	(-) Loliolide
9	16.212	16.84	Neophytadiene
10	16.506	0.03	N - tert - Butoxycarbonyl imidazole
11	17.076	1.08	Decanoic acid, 8 – methyl, methyl ester
12	17.393	11.53	Palmitic acid
13	17.490	0.64	N - methyl - N, N - di [2-(4-Pyridyl) ethyl] - (2-pyridyl) ethylamine
14	18.884	2.39	6(E), 9(Z), 13(E), - Penedectriene
15	19.025	30.52	Phytol
16	19.208	0.64	0 - Menth - 8 - ene
17	19.296	13.88	Cis, Cis, Cis-7, 10, 13 - Hexadecatrienal
18	27.817	2.61	Dioctyl phthalate

the methanol extract of flower. saponin and cardiac glycosides were present in ethanol extract of both leaf and flower samples. But flavonoid was seen in leaf and terpenoid in flower of ethanol extract. The GC/MS analysis of compounds in methanol flower and leaf extract of *Lantana camara* were shown in Table 4 and 5.

DISCUSSION

Mosquito borne diseases are one of the most public health problems in the developing countries. It can be controlled by preventing mosquito bite using repellent, causing larval mortality and killing mosquitos.

The effects of various extracts were studied in a dose dependent manner. The methanol and ethanol flower extract of *Lantana camara* was found to have higher rate of larvicidal rate against *Aedes aegypti*, where as in the *Culex quinquefasciatus* variety, the concentration of extracts have to be increased for better larvicidal effect. Senthilnathan [15] observed that higher larvicidal effect of *Eucalyptus tereticornis* oil (leaf extract) with increased doses on *Anopheles stephensi*. He also observed that first and second instar larvae were most susceptible to all treatments. In the present study the extracts result in maximum activity on 3rd and 4th instar larvae. Antifeeding effects of crude lantadene from *Lantana camara* on P*lutella xylostella* and S*podoptera*

litura larvae [18] and antifeeding and repellent effect of *Lantana camara* on tea mosquito bug [19] were reported. The insecticidal [10], nematicidal [11] activities have also been reported. Dua *et al* [12, 20] have already reported the repellent properties of flower extract in coconut oil and different fractions isolated from *Lantana camara* flowers using steam distillation against *Aedes* mosquito bite. In this present study the leaf and flower extracts of *Lantana camara* obtained using different solvents were found to have larvicidal activity proposing the use of leaves as well as flowers of *Lantana camara* as a mosquito control agent.

Earlier studies observed that phytochemicals have a major role in mosquito control programme [21,22]. Gopieshkhanna and Kannabiran[23] have observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extract having mosquito larvicidal activity. Pelah *et al.* [24] reported the use of commercial saponin from Quillaja saponaria bark as a natural larvicidal against *Aedes aegypti* and *Culex pipens*. Isolated triterpenoids were found to have an antibacterial activity [25,26]. Cardiac glycoside was found to have a acaricidal effect against larva and adult stages of the camel tick [27]. Phytol is a diterpene which is present in higher concentration in the methanol leaf extract of *Lantana camara*. The phytol was observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells[28]. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) was found to be present in both the methanol leaf and flower extract of *Lantana camara*. Medicinal activity DDMP was observed by Ban *et al*. [29]. We can conclude from this study that the presence of these phytochemicals in *Lantana camara* might be the reason for its larvicidal activity. The results of this experiment indicate that the shrub could be studied further in detail and its beneficial effect to the control of vector borne diseases could be utilized for healthy environments.

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