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Mosquito larvicidal and phytochemical properties of *Ageratina adenophora* (Asteraceae) against three important mosquitoes

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Mosquitoes are the most important single group of insects well- known for their public importance, since they act as vector for many tropical and subtropical disease such as dengue fever, yellow fever, chikungunya, malaria, filariasis and encephalitis of different types including, Japanese encephalitis[1]. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides – organophosphates (e.g. temephos and fenthion), insect growth regulators (e.g. diflubenzuron and methoprene), etc. Although effective, their repeated use has disrupted natural biological control systems and sometimes resulting in the widespread development of resistance. These problems have warranted the need for developing alternative strategies using eco-friendly products[2]. These steadily growing problems demand an intensive search for new products that are environmentally safe, target specific and degradable. The above facts prompted us to undertake investigations of some plant species traditionally used as insecticidal agents, as well as other endangered plant species, with the aim of identifying lead compounds for the development of new plant based insecticidal agents[3].

Ageratina adenophora is a perennial herbaceous exotic shrub which may grow up to 1 or 2 m height. It has opposite trowell-shaped serrated leaves that are 6–10 cm long by 3–6 cm in width. The small compound flowers occur in late spring and summer, and are found in clusters at the end of branches. Each flower head is up to 0.5 cm in diam and creamy white in colour. They are followed by a small brown seed with a white feathery parachute. The mosquito larvicidal properties of *Ageratina adenophora* have not yet been reported. Therefore, the present study was carried out to determine the larvicidal efficacy of *A. adenophora* leaves extract against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae).

The leaves of *A. adenophora* were collected from hilly regions of the Nilgiris district, Tamil Nadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of Plant Phytochemistry Division, Department of Zoology, and Annamalai University, India.

Cx. quinquefasciatus, Ae. aegypti and *An. stephensi* 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. Adults were provided with 10% sucrose solution and membrane feeding on goat blood. Mosquitoes were held at 28 ± 2°C, 70– 85% relative humidity (RH), with a photoperiod of 12 h light : 12 h dark. The dried leaves (1 kg) were extracted with methanol (5 L) by a soxhlet apparatus method and the extract was evaporated in a rotary vacuum evaporator to yield a dark greenish mass (195 g). Standard stock solutions were prepared at 1% by dissolving the residues in methanol, which was used for the bioassays. Phytochemical screening of the leaves of *A. adenophora* was carried out by using the standard protocols for the presence of carbohydrates, proteins, phenolic compounds, saponins, flavonoids, alkaloids, tannins, glycosides, phytosterols, oil and fats[4].

The larvicidal activity of crude extract was evaluated as per the protocol previously described[5]. Early III instar larvae (20) were placed in 249 ml of water and 1 ml of methanol containing different experimental concentrations. The beaker containing the control larvae received 1 ml of methanol. Crude extract concentration ranging from 50 to 250 mg/l was tested. Each test was repeated five times. The larval mortality data were subjected to probit analysis[6] for calculating LC_{50} and LC_{90} and chi-square values were calculated by using SPSS 13.0 for Windows. Significance level was set at $p < 0.05$.

Results of preliminary phytochemical analysis of the leaf extract of *A. adenophora* showed the presence of alkaloids, saponins, glycosides and coumarines (Table 1). The crude methanol extract, was tested in the concentra-

Table 1. Phytochemicals in methanolic leaf extract of *A. adenophora*

Phytochemical components	*A. adenophora* leaf extracts
Alkaloids	+
Saponins	–
Tannins	–
Anthroquinones	–
Steroids	–
Flavonoids	–
Terbinoids	–
Carotenoids	–
Fatty acids	–
Polyoses	+
Polyphenols	–
Glycosides	+
Quinones	–
Coumarins	+

+ = Present; – = Absent.

tions of 50, 100, 150, 200 and 250 ppm and observed the percent larval mortality of 19.5, 30.6, 51.2, 68.9 and 92.8 against *Cx. quinquefasciatus*; 21.2, 38.3, 53.7, 73 and 98.6 against *Ae.aegypti*; and 25.3, 45.2, 66.5, 86.9 and 100 against *An. stephensi*, respectively. The LC_{50} and LC_{90} values of crude methanol extract of leaves of *A. adenophora* on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae in 24 h were 144.86, 132.82, 113.08; and 250.70, 231.12 and 198.81 mg/l, respectively (Table 2).

The present result is also comparable to earlier reports of the larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 and 48 mg/l[7]. The essential oil from the leaves of *Clausena anisata* exhibited significant larvicidal activity, with 24 h LC_{50} values of 140.96, 130.19 and 119.59 ppm, respectively[8]. The benzene, hexane, ethylacetate, methanol and chloroform leaf extract of *Andrographis paniculata* was found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti*. The LC_{50} values were 112.19, 137.48, 118.67, 102.05 and 91.2 ppm; and 119.58, 146.34, 124.24, 110.12 and 99.54 ppm respectively[9]. Mathivanan *et al*[10] to determine the LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia coronaria* on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae in 24 h were 72.41, 65.67 and 62.08; and 136.55, 127.24 and 120.86 mg/l, respectively. The LC_{50} and LC_{90} values of methanol extract of *F. benghalensis* against early III instar of *Cx. tritaeniorhynchus* and *An. subpictus* were 100.88 and 159.76 ppm; and and 56.66, and 85.84 ppm, respectively[11]. The LC_{50} (LC_{90}) values of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against early III instar larvae of *Anopheles subpictus* were 86.47 (159.59) and 113.26 (207.73) ppm; and *Cx. tritaeniorhynchus* were 131.53 (245.37) and 165.28 (299.45) ppm, respectively[12].

Compared with earlier author's reports, our results revealed that the experimental plant extract was effective to control *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi.* From these results it was concluded that the plant *A. adenophora* leaf exhibits larvicidal activity

Table 2. Larvicidal activity of crude methanol extract of *A. adenophora* against *Cx. quinquefasciatus, Ae. aegypti* and *An.stephensi*

Mosquito species	Concentration (mg/l)	24 h mortality (mean ± S.D.)	LC_{50} (mg/l) (LCL–UCL)	LC_{90} (mg/l) (LCL–UCL)	χ^2
Cx. quinquefasciatus	50	19.5 ± 1.8	144.86	250.7	11.489*
	100	30.6 ± 1.2	(120.51–171.17)	(215.41–314)	
	150	51.2 ± 1.6			
	200	68.9 ± 1.4			
	250	92.8 ± 1.2			
	Control	0 ± 0			
Ae. aegypti	50	21.2 ± 0.8	132.82	231.12	18.989*
	100	38.3 ± 1.2	(101.63–165.22)	(192.25–312.9)	
	150	53.7 ± 0.6			
	200	73.03 ± 0.9			
	250	98.6 ± 1			
	Control	0 ± 0			
An. stephensi	50	25.3 ± 1.2	113.08	198.81	13.976*
	100	45.2 ± 1.9	(88.37–136.92)	(169.4–251.48)	
	150	66.5 ± 1.6			
	200	86.9 ± 0.8			
	250	100 ± 0.7			
	Control	0 ± 0			

LCL–Lower confidence limits; UCL–Upper confidence limits; Each value (mean ± S.D.) represents mean of five values; *Significant at $p < 0.05$ level.

against three important vector mosquitoes. This is the first report on the mosquito larvicidal activity of the methanol extract of *A. adenophora* plant. Further, analysis to isolate the active compound for larval control is under way in our laboratory. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

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