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Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Stapf. (Family: Apocynaceae)

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Mosquitoes are the most important group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year. Chemical insecticides have been/ are being used to control these disease vectors. The greatest harm from chemical insecticides is that once introduced into the system, they may remain there forever or for a very long duration. Thus, they pose a threat to life and help insects to develop resistance against them. This is the reason that there has always been a need for such an insecticide which is more powerful, with lesser side effects and degrading after sometime, reducing the change to develop resistance against it. These problems have renewed interest in exploiting the pest control potential of plants. In addition to application as general toxicants against mosquitoes, phytochemicals may also have potential uses as larvicides, repellents, ovicides and oviposition deterrents, and growth and reproduction inhibitors[1,2].

Ervatamia coronaria Stapf (Synonym: *Tabernaemontana divaricata*) belonging to the family Apocynaceae, is a glabrous, evergreen tree indigenous to India and is cultivated in gardens for its ornamental and fragrant flowers. This species has been extensively investigated and a number of chemical constituents such as alkaloids[3], triterpenoids[4], steroids[4], flavonoids[5], phenyl propanoids[5] and phenolic acids were isolated from leaves, roots and stems of the plant. In Indian traditional system of medicine, this plant material is widely used as a purgative, tonic to the brain, the spleen and the liver; in the treatment of cancer, wounds and inflammations[6]. The plant extract was also found to possess analgesic, antipyretic and anti-inflammatory properties[3]. Furthermore, mosquitocidal properties of *E. coronaria* has not yet reported. Therefore, the present study was carried out to determine the larvicidal efficacy of *E. coronaria* leaves extract against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae).

The leaves of *E. coronaria* were collected from in and around Vittaloor, Thanjavur 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, Annamalai University, India.

Culex 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 one week old chick for blood meal. Mosquitoes were held at 28 ± 2°C, 70–85% relative humidity (RH), with a photo period of 14 h light: 10 h dark. The dried leaves (3 kg) were extracted with methanol (5.5 L) by a soxhlet apparatus method and the extract was evaporated in a rotary vacuum evaporator to yield a dark greenish mass (295 g). Standard stock solutions were prepared at 1% by dissolving the residues in methanol, which was used for the bioassays. Qualitative analyses of the phytochemicals present were carried out using methods described by Harbone[7].

Table 1. Phytochemicals in methanolic leaf extract of *E. coronaria*

Phytochemical components	*E. coronaria* leaf extracts
Alkaloids	+
Saponins	+
Tannins	+
Anthroquinones	–
Steroids	+
Flavonoids	+
Terbinoids	–

+ = Present; – = Absent.

The larvicidal activity of crude extract was evaluated as per the protocol previously described[8]. Early III instar larvae (25) 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 25 to 150 mg/l was tested. Each test was repeated six times. The larval mortality data were subjected to probit analysis[9] 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 *E. coronaria* showed the presence of alkaloids, saponins, tannins, flavonoids and steroids (Table 1). The LC_{50} and LC_{90} values of crude methanol extract of leaves of *E. coronaria* on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae in 24 h were 72.41, 65.67, 62.08 and 136.55, 127.24 and 120.86 mg/l, respectively (Table 2).

In our previous study, we have reported the methanol extract of *Cassia fistula* exhibited LC_{50} values of 17.97 and 20.57 mg/l against *An. stephensi* and *Cx.*

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

Mosquito species	Concentration (mg/l)	24 h mortality (%)	LC_{50} in mg/l (95% confidence limits)	LC_{90} (mg/l)	χ^2(df)
Cx. quinquefasciatus	25	22.2±1.6[a]	72.41(60.98–83.59)	136.55	11.606*(5)
	50	38.6±1.2[b]			
	75	54.8±1.4[c]			
	100	70.2±1.4[d]			
	125	83.6±1.8[e]			
	150	94.8±1.2[f]			
	Control	0.8±1.2[g]			
Ae. aegypti	25	26.2±1.0[a]	65.67(53.90–76.77)	127.24	12.325*(5)
	50	42.2±1.4[b]			
	75	61.4±1.2[c]			
	100	72.2±1.6[d]			
	125	87.4±1.4[e]			
	150	96.0±1.6[f]			
	Control	1.2±1.2[g]			
An. stephensi	25	29.2±1.4[a]	62.08(47.29–75.64)	120.86	19.181*(5)
	50	46.8±1.2[b]			
	75	59.4±1.2[c]			
	100	76.6±0.8[d]			
	125	87.0±0.6[e]			
	150	99.8±1.4[f]			
	Control	1.2±1.2[g]			

Values in a column with a different superscript are significantly different at $p < 0.05$ level (DMRT test); Each value (mean ± S.D.) represents mean of six values; *Significant at $p < 0.05$ level.

quinquefasciatus, respectively[2]. The crude leaf extract of *Acalypha indica* with different solvents, viz. benzene, chloroform, ethyl acetate and methanol were tested for larvicidal activity against *An. stephensi*. The LC_{50} values were 19.25, 27.76, 23.26 and 15.03 ppm, respectively[1]. The LC_{50} of leaf extract of *C. fistula* with different solvents, viz. methanol, benzene and acetone against *Ae. aegypti* were 10.69, 18.27 and 23.95 mg/l respectively[10].

The present result is also comparable to earlier reports of Vasudevan *et al*[11] who observed the larvicidal effect of crude extracts of dried ripened fruids of *Piper nigrum* against *Cx. quinquefasciatus* larval instars. LC_{50} and LC_{90} values as observed for early IV larval instar of *Cx. quinquefasciatus* were 29.11 and 62.37 mg/l and 63.82 and 108.90 mg/l for aqueous and ethanol extracts respectively. A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *Cx. pipiens*[12]. The current investigation revealed that the leaf extract of *E. coronaria* possesses remarkable larvicidal activity against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi.* This is the first report on the mosquito larvicidal activity of the methanol extract of *E. coronaria* plant. Further purification and characterization of the bioactive fraction of *E. coronaria* are underway in our laboratory.

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