European Review for Medical and Pharmacological Sciences 2012; 16: 386-392

Larvicidal efficacy of botanical extracts against two important vector mosquitoes

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Abstract. – *Objective:* **To determine the larvicidal efficacy of different solvent leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Anopheles subpictus* and *Culex tritaeniorhynchus*.**

Materials and Methods: **Twenty five early third instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* were exposed to various concentrations and were assayed in the laboratory. The larval mortality was observed after 24 h of treatment.**

Results: **Among three solvent extracts tested the maximum efficacy was observed in the methanol extract. The LC_{50} (LC_{90}) values of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against early third instar of *Anopheles subpictus* were 86.47 (159.59) and 113.26 (207.73) ppm and *Culex tritaeniorhynchus* were 131.53 (245.37) and 165.28 (299.45) ppm, respectively. No mortality was observed in controls.**

Conclusions: **From the results it can be concluded the crude extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were excellent potential for controlling *Anopheles subpictus* and *Culex tritaeniorhynchus* mosquito larvae.**

Key Words:

Larvicidal activity, *Anopheles subpictus, Culex tritaeniorhynchus, Ervatamia coronaria, Caesalpinia pulcherrima.*

Introduction

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE), etc. causing millions of deaths every year[1]. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema[2]. *Anopheles (An.) subpictus* is known to transmit malaria and filariasis, in an isolated study of multiple host-feeding in field populations, and its specific role in transmitting malaria in Sri Lanka revealed that multiple blood feeding within the same gonotrophic cycle was attributed to a local "frequent feeding strategy" in this primarily zoophagic and endophilic malaria vector. On the contrary, in Indonesia, *An. subpictus* is a potential vector of bancroftian filariasis and fed on microfilaraemia carriers that harbored *Wuchereria bancrofti* larvae[3]. *An. subpictus* breeds profusely in rainwater accumulations and fallow rice fields[4], waste water disposal systems, and irrigated sites[5], and is also associated with floating and submerged aquatic vegetation in the vicinity of rice plants[6]. Night time human biting collection in Rajasthan, India, showed two feeding peaks for *An. subpictus*, one early in the night and the other just before dawn[7]. *Culex tritaeniorhynchus* Giles is an important vector of JE in India and South East Asian countries. JE is endemic in few states of India and highly endemic in few districts of Tamil Nadu, Southern India[8]. Keiser et al[9] have reported that approximately 1.9 billion people currently live in rural JE prone areas of the world, the majority of them in China (766 million) and India (646 million).

Mosquito control has been become increasing difficult of the indiscriminate uses of synthetic chemical insecticides which has an adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects.

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The increased use of these insecticides may enter into the food chain and thereby the liver, kidney, etc. may be irreversibly damaged. They even result in mutation of genes and these changes become prominent only after a few generations[10]. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. The search for herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control[11]. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level[12,13].

Anti-feedant and larvicidal activity of acetone, chloroform, ethyl acetate, hexane and methanol peel, leaf and flower extracts of *Citrus (C.) sinensis, Ocimum canum, O. sanctum* and *Rhinacanthus nasutus* were studied using fourth instar larvae of *Helicoverpa armigera, Sylepta derogata* and *Anopheles (An.). stephensi*[14]. Rajkumar and Jebanesan[15] have reported that the acetone leaves extract of *Solanum trilobatum* showed maximum oviposition deterrent and skin repellent activity against *An. stephensi*. The ethanolic extracts of the orange peel (*C. sinensis*) was tested for the toxicity effect on the larvae of the yellow fever mosquito *Aedes (Ae.) aegypti*[16]; The crude chloroform extract of seeds of *Millettia dura* showed high activity against second-instar larvae of *Ae. aegypti*[17].

Larvicidal efficacy of the crude leaf extract of *Ficus benghalensis* with three different solvents like methanol, benzene and acetone were tested against the early second, third, fourth instar larvae of *Culex (Cx.) quinquefasciatus, Ae. aegypti* and *An. stephensi*[18]. The larvicidal activity of crude carbon tetrachloride, methanol, and petroleum ether extracts of *Solanum xanthocarpum* fruits was examined against *An. stephensi* and *Cx. quinquefasciatus*[19]; the methanol extracts of leaves of *Dysoxylum malabaricum* were tested against mature and immature stages of *An. stephensi* under laboratory conditions[20]; root bark extracts of *Turraea wakefieldii* and *Turraea floribunda* against third-instar larvae[21] and extracts of *Pelargonium citrosa* leaf were tested for their biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency, and biting deterrency[22] against *An. stephensi*. Mullai et al[23] have reported that the leaf extract of *Citrullus vulgaris* with different solvents viz., benzene, petroleum ether, ethyl acetate, and methanol were tested for larvicidial, ovicidal, repellent, and insect growth regulatory activities against *An. stephensi*. Elango et al[24] have reported that the leaf acetone, chloroform, ethyl acetate, hexane, and methanol extracts of *Aegle marmelos, Andrographis lineata, Andrographis paniculata*, *Cocculus hirsutus, Eclipta prostrate* and *Tagetes erecta* were tested against fourth-instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*. Govindarajan[25] reported that the leaf methanol, benzene, and acetone extracts of *Cassia fistula* were studied for the larvicidal, ovicidal, and repellent activities against *Ae. aegypti*. The leaf extract of *Acalypha indica* with different solvents viz., benzene, chloroform, ethyl acetate, and methanol were tested for larvicidal, ovicidal activity, and oviposition attractancy against *An. stephensi*[12]. In the present study, we report the mosquitocidal larvicidal properties of the leaves of *Ervatamia (E.) coronaria* and *Caesalpinia pulcherrima* against two important vector mosquitoes.

Materials and Methods

Collection of Plants

Fully developed leaves of the *E. coronaria* and *C. pulcherrima* were collected from in and around Annamalai University Campus, Annamalainagar, 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.

Extraction

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant leaf powder (3.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with three different solvents namely benzene, 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% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassay.

Test Organisms

The mosquitoes, *An. subpictus* and *Cx. tritaeniorhynchus* 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 ± 2^0, 70-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

Larvicidal Bioassay

The larvicidal activity of the plants crude extracts was evaluated as per the method recommended by WHO[26]. Batches of 25 third instar larvae were transferred to a small disposable test 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. Six replicate were set up for each concentration and an equal number of control were set up simultaneously using tap water. To this 1 ml of appropriate solvent was added. The LC_{50} value was calculated after 24 h by probit analysis[27].

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 and lower confidence limit, and chi-square values were calculated using the SPSS12.0 (Statistical Package for Social Sciences: Chicago, IL, USA) software. Results with $p < 0.05$ were considered to be statistically significant.

Results

The efficacy of methanol, benzene and ethyl acetate solvent extract of leaf of *E. coronaria* and *C. pulcherrima* were tested against the early third larvae of *An. subpictus* and *Cx. tritaeniorhynchus.* The data were recorded and statistical data regarding the LC_{50}, LC_{90}, Chi-square and 95% confidence limits were calculated (Tables I-IV). Among three solvents tested the methanolic extract of *E. coronaria* and *C. pulcherrima* showed highest larvicidal activity against *An. subpictus* and *Cx. tritaeniorhynchus.* The LC_{50} values were 86.47 (159.59) and 113.26 (207.73) ppm for *An. subpictus* and 131.53 (245.37) and 165.28 (299.45) ppm for *Cx. tritaeniorhynchus* respectively. No mortality was observed in control. The chi-square values were significant at $p < 0.05$ level.

Table I. Larvicidal activity of different solvent leaf extracts of *Ervatamia coronaria* against *Anopheles subpictus*.

Solvents	Concentration (ppm)	% of mortality ± SD	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Methanol	Control	0.0 ± 0.0	86.47	159.59	19.158*
	40	30.4 ± 0.8	(61.00-109.99)	(131.72-216.09)	
	80	49.1 ± 1.2			
	120	70.6 ± 1.8			
	160	84.2 ± 1.4			
	200	99.8 ± 1.6			
Benzene	Control	0.0 ± 0.0	97.53	172.44	12.734*
	40	21.6 ± 1.2	(77.85-116.76)	(147.91-215.56)	
	80	42.5 ± 1.8			
	120	67.4 ± 1.4			
	160	78.6 ± 0.8			
	200	97.6 ± 0.6			
Ethyl acetate	Control	0.0 ± 0.0	106.76	185.04	12.604*
	40	17.5 ± 1.8	(88.97-126.86)	(159.00-231.53)	
	80	35.6 ± 1.6			
	120	64.8 ±1.2			
	160	72.1 ± 1.4			
	200	95.2 ± 0.8			

*Significant at $p < 0.05$ level. LC_{50}: Lethal Concentration; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit.

Table II. Larvicidal activity of different solvent leaf extracts of *Ervatamia coronaria* against *Culex tritaeniorhynchus.*

Solvents	Concentration (ppm)	% of mortality ± SD	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Methanol	Control	0.0 ± 0.0	131.53	245.37	21.956*
	60	31.4 ± 1.8	(88.81-170.72)	(199.77-344.59)	
	120	48.3±1.2			
	180	69.4 ± 1.6			
	240	81.8 ± 1.4			
	300	99.7 ± 0.8			
Benzene	Control	0.0 ± 0.0	143.14	258.18	15.472*
	60	24.3 ± 0.8	(109.39-175.74)	(217.12-334.82)	
	120	44.9 ± 1.6			
	180	66.3 ± 2.0			
	240	78.7 ± 1.8			
	300	98.1 ± 1.4			
Ethyl acetate	Control	0.0 ± 0.0	152.45	269.89	12.397*
	60	20.9 ± 0.8	(122.80-182.00)	(231.51-337.89)	
	120	41.6 ± 1.6			
	180	61.8 ± 1.4			
	240	76.3 ± 1.2			
	300	96.6 ± 0.8			

*Significant at $p < 0.05$ level. LC_{50}: Lethal Concentration; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit.

Discussion

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable[28]. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. According to Bowers et al[29] the screening of locally available medicinal plants for mosquito control

Table III. Larvicidal activity of different solvent leaf extracts of *Ervatamia coronaria* against *Anopheles subpictus*.

Solvents	Concentration (ppm)	% of mortality ± SD	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Methanol	Control	0.0 ± 0.0	113.26	207.73	21.147*
	50	28.6 ± 1.8	(79.09-145.32)	(170.16-288.23)	
	100	48.3 ± 1.2			
	150	65.6 ± 1.4			
	200	81.2 ± 2.2			
	250	99.9 ± 0.8			
Benzene	Control	0.0 ± 0.0	124.96	221.41	14.721*
	50	21.5 ± 1.2	(97.88-151.74)	(187.7-284.39)	
	100	42.6 ± 1.6			
	150	62.9 ± 1.2			
	200	76.3 ± 1.4			
	250	97.8 ± 1.8			
Ethyl acetate	Control	0.0 ± 0.0	135.42	232.28	11.319*
	50	18.2 ± 1.4	(112.41-159.08)	(201.15-286.15)	
	100	33.6 ± 1.8			
	150	59.9 ± 0.8			
	200	72.8 ± 1.4			
	250	96.1 ± 1.2			

*Significant at $p < 0.05$ level. LC_{50}: Lethal Concentration; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit.

Table IV. Larvicidal activity of different solvent leaf extracts of *Caesalpinia pulcherrima* against *Culex tritaeniorhynchus.*

Solvents	Concentration (ppm)	% of mortality ± SD	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Methanol	Control	0.0 ± 0.0	165.28	299.45	17.546*
	75	27.8 ± 1.4	(121.10-206.77)	(249.13-396.41)	
	150	48.4 ± 1.8			
	225	69.5 ± 1.4			
	300	84.3 ± 1.2			
	375	100.0 ± 0.8			
Benzene	Control	0.0 ± 0.0	177.05	315.86	14.050*
	75	23.8 ± 1.2	(138.09-214.71)	(268.67-401.29)	
	150	44.4 ± 1.8			
	225	67.2 ± 1.4			
	300	80.7 ± 1.2			
	375	98.6 ± 1.6			
Ethyl acetate	Control	0.0 ± 0.0	189.41	330.69	10.903*
	75	19.5 ±1.2	(155.75-222.85)	(286.89-404.56)	
	150	40.9 ± 1.8			
	225	63.4 ± 1.6			
	300	77.8 ± 1.4			
	375	97.1 ± 0.8			

*Significant at $p < 0.05$ level. LC_{50}: Lethal Concentration; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit.

would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. Different parts of plants contain a complex of chemicals with unique biological activity[30] which is thought to be due to toxins and secondary metabolites, which act as mosquitocidal agent[31]. The crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors[32]. The findings of this study showed that crude ethyl acetate, benzene, and methanol extracts of the leaf of the plants, *C. pulcherrima*, and *E. coronaria* have a significant larvicidal activity. Rahuman and Venkatesan[33] reported that the petroleum ether extract of *Citrullus colocynthis*, methanol extracts of *Cannabis indica*, *Cannabis sativus, Momordica charantia* and acetone extract of *Trichosanthes anguina* were against the larvae of *Ae. aegypti* (LC_{50}=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *Cx. quinquefasciatus* (LC_{50}=88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. Larvicidal activity of acetone extracts of *Murraya koenigii, Coriandrum sativum, Ferula asafoetida,* and *Trigonella foenum graceum* reported maximum activity ranging 25-900 ppm against *Ae. aegypti*[34]. Mullai and Jebanesan[35] have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *Citrullis colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae. The methanol extract of *C. fistula* exhibited LC_{50} values of 17.97 and 20.57 mg/L, *An. stephensi* and *Cx. quinquefasciatus*, respectively[13].

Larvicidal activity of crude extract of *Sida acuta* against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* with LC_{50} values ranging between 38 to 48 mg/l[36]. Sosan et al[37] reported larvicidal activities of essential oils of *Ocimum gratissimum*, *Cymbopogon citrus*, and *Ageratum conyzoides* against *Ae. aegypti* and achieved 100% mortality at 120, 200, and 300 ppm concentrations, respectively. Thirteen oils from 41 plants (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, and sandalwood) were found to induce 100% mortality in third instar larvae of *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* after 24 h, or even after shorter periods[38]. Similarly, it was reported that the essential oil of *Ipomoea cairica* Linn. possesses remarkable larvicidal properties as it could produce 100% mortality in the larvae of *C. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm[39]. Tiwary et al[40] observed the larvicidal activity of linalool rich essential of *Zanthoxylum armatum* against different mosquito species viz., *Cx. quinquefasciatus*

(LC_{50}=49 ppm), *Ae. aegypti* (LC_{50}=54 ppm) and *An. stephensi* (LC_{50}=58 ppm). Singh et al[41] reported the larvicidal activity of *Ocimum canum* oil against vector mosquitoes, namely, *Ae. aegypti* and *Cx. quinquefasciatus* (LC_{50}=301 ppm) and *An. stephensi* (LC_{50}=234 ppm).

Plants could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. The findings of the present investigation revealed that the leaf extract of *E. coronaria, and C. pulcherrima* possess remarkable larvicidal activity against *An. subpictus* and *Cx. tritaeniorhynchus*. Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity in *An. subpictus* and *Cx. tritaeniorhynchus* should be identified and utilized, if possible, in preparing a commercial product/formulation to be used as a mosquitocidal.

Acknowledgements

The Authors are thankful to the Professor and Head, Department of Zoology, Annamalai University for the laboratory facilities provided. We acknowledge the staff members of the VCRC (ICMR), Pondicherry for their cooperation.

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