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M. Deepa
Department of Zoology, Bharathiar University, Coimbatore – 641 046, Tamilnadu, India.

K. Palanisamy
Department of Botany, Annamalai University, Annamalainagar – 608 002, Tamilnadu, India

K. Krishnappa
Department of Advanced Zoology and Biotechnology, Govt. Arts College (Autonomous), Nandanam, Chennai-600035, Tamilnadu, India.

K. Elumalai
Department of Advanced Zoology and Biotechnology, Govt. Arts College (Autonomous), Nandanam, Chennai-600035, Tamilnadu, India.

For Correspondence:
K. Elumalai
Department of Advanced Zoology and Biotechnology, Govt. Arts College (Autonomous), Nandanam, Chennai-600035, Tamilnadu, India.

Mosquitocidal activity of *Polygala arvensis* Willd against *Aedes aegypti* (Linn.), *Anopheles stephensi* (Liston.) and *Culex quinquefasciatus* (Say.) (Diptera: Culicidae)

M. Deepa, K. Palanisamy, K. Krishnappa and K. Elumalai

Abstract
To determine the larvicidal, ovicidal and repellent activities of benzene and methanol extract of *Polygala arvensis* against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* Twenty five 3rd instar larvae of selected mosquitoes species were exposed to various concentrations (60-300 ppm) and were assayed in the laboratory by using the protocol of WHO 2005; the 24 h LC_{50} values of the *P. Arvensis* leaf extract was determined following Probit analysis. The ovicidal activity was determined against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* exposed to various concentrations were tested under laboratory conditions and the hatch rates were assessed 120hrs post treatment. The repellent efficacy was determined against selected mosquitoes at three concentrations *viz.*, 1.0, 2.0 and 3.0 mg/cm^2 under the laboratory conditions. The LC_{50} and LC_{90} values of benzene and methanol extract of *P. arvensis* against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* larvae in 24 h were 75.32, 88.26, 82.46, 58.21, 46.37, 42.68 and 260.48, 275.26, 251.39, 208.45, 189.82 and 130.44 ppm, respectively. It has been noticed that the higher concentrations of *P. arvensis* extracts possesses strong ovicidal activity at 200 ppm concentration against *Ae. aegypti, An. stephensi* and *C. quinquefasciatus*, no egg hatchability was recorded. In the same way, methanol extracts showed maximum ovicidal activity followed by benzene extract against selected vector mosquitoes. In repellent activity, among two extracts tested *P. arvensis* methanol extract had strong repellent action against selected mosquitoes as it provided 100% protection against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* for 280min. From the results it can be concluded the *P. arvensis* extract was an excellent potential for controlling *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes.

Keywords: Larvicidal activity, Ovicidal activity, Repellent activity, *Polygala arvensis, Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus.*

1. Introduction
Mosquitoes are nuisance pests and a major vector for the transmission of several life threatening diseases such asmalaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis, 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]. *Ae. aegypti* is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas [3]. *An. stephensi* is the primary vector of malaria in India and other West Asian countries, Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths [1]. *C. quinquefasciatus* acts as a vector for filariasis in India. Human filariasis is a major public health hazard and remains a challenging socioeconomic, problem in many of the tropical countries [4]. Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have 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 [5].
Plants may be alternative sources of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the

effectiveness of plant extracts or essential oils against mosquito larvae [6-8]. Phytochemicals are advantageous due to their eco-safety, target-specificity, non-development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide [9-12]. This study was undertaken to assess the larvicidal, ovicidal, and repellent potential of *Polygala arvensis* against the medically important mosquito vectors, *Ae. aegypti, An. stephensi* and *C. quinquefasciatus*.

2. Materials and methods

2.1 Plants collection and solvent extraction

Fully developed leaves of *Polygala arvensis* was collected from in and around Yercaud hill station (11.77940N, 78.20340E) Salem Districts of the Tamilnadu India. The leaves were collected during the June 2012- January 2013 and brought to the laboratory. The leaves were washed with tap water, shade-dried, and finely ground with the help of electrical blender. The finely ground plant leaf powder (1.0 kg) was loaded and extracted in Soxhlet apparatus. The solvents from the extracts were removed using a rotary vacuum evaporator (Rota vapour, Systronics India Ltd., Chennai, India) 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, ovicidal, and repellent bioassays.

2.2 Mosquito Rearing

The mosquitoes, *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus,* were reared in the Department of Zoology, Govt. Arts College, Nandanam. 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}C, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.3 Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by World Health Organization (2005) [13]. From the stock solution, four different test concentrations (50, 100, 150 and 200 ppm) were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of *Ae. aegypti, An. stephensi* and *C. quinquefasciatus.* 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; DMSO) 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} and LC_{90} values were calculated by using probit analysis [14].

2.4 Ovicidal activity

The method of Su and Mulla (1998) [15] was slightly modified and used to test the ovicidal activity. The various concentrations (50, 100, 150 and 200 ppm) as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs/eggs raft of *Ae. aegypti, An. stephensi* and *C. quinquefasciatus* 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, 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.

2.5 Repellent activity

The repellent study was following the methods of World Health Organization (2009) **[16]**. 3–4 days old blood-starved female *Ae. aegypti, An. stephensi* and *C. quinquefasciatus* mosquitoes (100) was kept in a net cage (45×45× 40 cm). 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 cm^2 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 selected medicinal plant leaf extract at 1, 2 and 3 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 40 min and the experiment was conducted five times. It was observed that there was no skin irritation from the plant extract.

2.6 Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression value, slope, and chi-square values were calculated using the SPSS17.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

3. Results

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide must not cause high mortality in target organisms in order to be acceptable many researchers. The results of the present study clearly have shown in table 1, 2 & 3. Data of the larvicidal activity of *P. arvensis* leaf extracts against selected mosquitoes are presented in (table 1). The LC_{50} and LC_{90} values of benzene and methanol extract of *P. arvensis* against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* larvae in 24 h were 75.32, 88.26, 82.46, 58.21, 46.37, 42.68 and 260.48, 275.26, 251.39, 208.45, 189.82 and 130.44ppm, respectively. It has been noticed that the higher concentrations of *P. arvensis* extracts possesses strong ovicidal activity at 200ppm concentration against *Ae. aegypti, An. stephensi* and *C. quinquefasciatus,* no egg hatchability was recorded. In the same way, methanol extracts showed maximum ovicidal activity followed by benzene extract against selected vector mosquitoes (table 2). In repellent activity, among two extracts tested *P. arvensis* methanol extract had strong repellent action against selected mosquitoes as it provided 100% protection against *Aedes aegypti, Anopheles stephensi* and *Culex*

quinquefasciatus for 280 min (table 3). From the results it can be concluded the *P. arvensis* extract was an excellent potential for controlling *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes.

Table 1: Larvicidal activity of *Polygala arvensis* extracts against 4th instar larvae of selected mosquitoes

Solvent tested	Mosquitoes	LC_{50} (ppm)	95% Fiducial Limit (ppm)		LC_{90} (ppm)	95% Fiducial Limit (ppm)		Slope	Chi-square
			LCL	UCL		LCL	UCL		
Benzene extract	*Aedes aegypti*	75.32	48.58	110.54	260.48	222.39	328.64	5.670	15.934
	Anopheles stephensi	88.26	61.26	124.60	275.26	231.40	341.27	4.062	15.570
	Culex quinquefasciatus	82.46	57.64	115.24	251.39	216.93	337.51	4.213	14.831
Methanol extract	*Aedes aegypti*	58.21	38.59	88.52	208.45	176.84	264.27	4.295	16.748
	Anopheles stephensi	46.37	36.54	75.44	189.82	152.56	238.58	3.408	14.310
	Culex quinquefasciatus	42.68	31.51	68.65	130.44	119.31	164.45	3.927	13.472

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower; Confidence Limit; UCL = Upper Confidence Limit; Slope; Chi-square.

Table 2: Ovicidal activity of *Polygala arvensis* extracts against freshly laid eggs of selected mosquitoes

Extracts tested	Mosquito species and their Percentage of egg hatch ability			
	Concentrations (ppm)	*Aedes aegypti*	*Anopheles stephensi*	*Culex quinquefasciatus*
Benzene	50	56.84±2.71	42.38±2.57	48.37±2.30
	100	38.27±2.36	32.45±2.22	34.72±2.56
	150	24.34±1.25	18.53±1.77	21.56±1.42
	200	NH	NH	NH
Methanol	50	32.79±2.45	36.62±2.37	38.22±1.61
	100	18.37±1.76	21.46±2.52	25.43±2.50
	150	NH	NH	NH
	200	NH	NH	NH
Control	00	100.0±0.0	100.0±0.00	100.0±0.00

Values represent mean ± S.D of five replications.

Table 3: Repellent activity of *Polygala arvensis* extracts against selected mosquitoes

Mosquitoes	Concentration (mg/cm^2)	Percentage of repellency, Time post application of repellent(min)							
		40min	80 min	120 min	160 min	200 min	240 min	280 min	320 min
Benzene extract									
Aedes aegypti	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.2±3.5	65.9±2.6
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	84.3±3.7	71.6±2.5
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	88.5±3.4	76.4±2.9
Anopheles stephensi	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	71.6±3.7	55.9±2.4
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.4±3.8	62.8±2.7
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	85.3±3.2	72.4±2.5
Culex quinquefasciatus	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	72.4±1.2	64.5±3.5	45.6±2.4
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	85.6±1.5	75.8±3.6	53.7±2.8
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.2±1.6	83.7±3.4	61.5±2.9
Methanol extract									
Aedes aegypti	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	67.2±2.6
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	76.8±2.5
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	85.4±2.4
Anopheles stephensi	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	70.8±2.9
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	74.5±2.7
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	86.9±2.6
Culex quinquefasciatus	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	65.4±2.2
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.6±2.7
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	83.3±2.5

Each value mean± S.D represents average of five values

4. Discusion

Our results showed that, the crude ethanol extract of *Polygala arvensis* have significant larvicidal, ovicidal and repellent activity against selected human vector mosquitoes *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.* The results are in comparable with an earlier report by Karunamoorthi *et al.* [17] were evaluated the petroleum ether extracts of the leaves of *Vitex negundo* for larvicidal activity against larval stages of *C. tritaeniorhynchus* in the laboratory with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l, respectively. Sharma *et al.* [18] reported that the acetone extract of *Nerium indicum* and *Thuja orientelis* have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against third instar larvae of *An. stephensi* and *C. quinquefasciatus*. Larvicidal activity of the same extracts of *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida*, and *Trigonella foenum* was tested out using different concentrations of each plant (range, 25-900 ppm) against *Ae. aegypti* larvae [19] ; Rahuman and Venkatesan [20] reported that the petroleum ether extract of *Citrullus colocynthis; methanol extracts of Cannabis indica, Cannabis sativus,* and *Momordica charantia*; and acetone extract of *Trichosanthes anguina* against the larvae of *Ae. aegypti* (LC_{50}=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *C. quinquefasciatus* (LC_{50} = 88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. Earlier authors reported that the methanol leaf extracts of *V. negundo, V. trifolia, V. peduncularis*, and *V. altissima* were used for larvicidal assay with LC_{50} value of 212.57, 41.41, 76.28, and 128.04 ppm, respectively, against the early fourth instar larvae of *C. quinquefasciatus* [21]. The same extracts of *Euphorbia tirucalli* latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *C. quinquefasciatus* with LC_{50} values of 177.14 and 513.387 mg/l, respectively [22].

The benzene extracts of *Citrullus vulgaris* exerted 100% mortality (zero hatchability) at 250 ppm, a very low hatchability (11.8%) at 200 ppm, and complete ovicidal activity at 300 ppm. The fraction I at 80 ppm exerted a very low hatchability rate of 3.2% followed by fraction II (6.9%), and fractions III and IV afforded 4.9% and 5.3% hatchability recorded against *An. stephensi* and *Ae. aegypti*, respectively [23]. The ovicidal effect of *Solenostemma argel* was low; however, concentrations of 0.05% and 0.1% exhibited significant effects ($p<0.05$), producing 65% and 75% and 62.9% and 62.9%, respectively, on the 1st and 2nd day after treatment, respectively. The 0.1% concentration reduced egg hatch by 33.7%, compared with the control, and 100% mortality values were evident in concentrations as low as 0.025% at 2 days posthatching against *Culex pipiens* [24]. The seed extract of *Atriplex canescens* showed complete ovicidal at 1,000 ppm concentration in eggs of *C. quinquefasciatus* [25]. Venketachalam and Jebanesan [26] have also reported that the repellent activity of methanol extract of *Feronia elephantum* leaves against *Ae. aegypti* activity at 1.0 and 2.5 mg/cm^2 concentrations gave 100% protection up to 2.14±0.16 h and 4.00±0.24 h, respectively, and the total percentage protection was 45.8% at 1.0 mg/cm^2 and 59.0% at 2.5 mg/cm^2 for 10 h. Compared with earlier authors' reports, our results revealed that the experimental plant extracts were effective to control *Ae. aegypti*, *An. Stephensi* and *C. quinquefasciatus*. From these results, it was concluded that the plant *Polygala arvensis* exhibit larvicidal, ovicidal, and repellent activities against three important vector mosquitoes. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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