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Larvicidal activity of *Scutellaria violacea* (Lamiaceae) leaf extracts against three important human vector mosquitoes: *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae) from Tamil Nadu, India

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Abstract
The present study was conducted to determine the larvicidal activity of *Scutellaria violacea* leaf extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The larvicidal activity was determined against three vector mosquito species at the concentrations of 50, 100, 150, 200 and 250 ppm. Larval mortality was assessed after 24 hours. The leaf extracts of *S. violacea* was found to be more susceptible against the larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* respectively. Insecticidal susceptibility tests were carried out using standard method and the mortality was observed after 24-h exposure. All the tested crude extract showed moderate to good larvicidal and pupicidal activities. However, the maximum larval mortality was detected in ethanol extract against *A. stephensi* and *C. quinquefasciatus* (LC_{50} 47.6 and LC_{95} 225.3) and *C. quinquefasciatus* (LC_{50}51.8 and LC_{95} 218.4). These results suggested that the leaf extracts of *S. violacea* showed potential to be used as an ideal ecofriendly approach for the control of the *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*

Keywords: larvicidal, *Scutellaria violacea*, Leaf extract, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

1. Introduction
Mosquitoes are the most important single group of insects known for their public importance, since they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, chikungunya, malaria, filariasis and encephalitis of different types including, Japanese encephalitis [1]. Mosquitoes cause the most hazard to public health as of their capability to act as vectors of pathogens causing malaria, dengue, yellow fever, encephalitis and Filariasis [2]. Mosquito-borne diseases contri- bute radically to disease trouble, loss, shortage, and social weakness all greater than the world, mostly in tropical countries. Among these diseases, malaria residue the most serious vector-borne disease affects a number of 300 – 500million people and 1.4 to 2.6 million deaths annually during the world. More than 40% of the world's populations live in malarious areas [3]. In India about 20,000 medicinal plants have been recorded newly, but advance than 500 established communities use about 800 plant species for remedial different diseases [4].Therefore the present study was carried out to determine the larvicidal activity of *S. violacea* leaf extracts against important vectors *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*.

2. Materials and Methods
2.1. Plant material
The leaves of *S. violacea* were collected from Puliyansolai hills, Thuraiyur via. Tiruchirapalli District, Tamil Nadu, India. Collected plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India. The voucher specimens (IPH-45) were deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

2.2. Extraction method
The dried leaf (500g) was powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, chloroform and ethanol (2500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mmHg at 45 °C by 'Rota-vapour' and the residue obtained was stored at 4 °C.

2.3. Vector rearing
Eggs and larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were collected from National Centre for disease control (Communicable Diseases), Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Coimbatore, Tamil Nadu, India. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

2.4. Larvicidal bioassay
The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO [5]. From the stock solution, five different test concentrations (*viz.*, 50, 100, 150,200 and 250ppm for crude extracts and 25,50 and 75ppm for fractions was prepared and tested against the freshly moulted (0 – 6 hrs) 4th instar larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Twin20 (emulsifier) in water was treated as control. The larvae of these mosquito species (25 larvae) was introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier) 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 percentage of mortality was calculated by using Abbott's formula [6].

2.5. Statistical analysis
The LC_{50}, LC_{90}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom was calculated by using probit analysis with Statistical Package for Social Sciences (SPSS) 16.0 Version in MS-Excel, 2007.

3. Results
As discussed in materials and methods, the results of relative toxicity of *S. violacea* against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* after 24 hours of treatment are presented in table 1. In the present study larvicidal activity of crude extracts of *S. violacea* tested against larvae of mosquito species. It was evident from table 1 that all the tested crude extracts demonstrated significant larvicidal activity against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Present results reaveld that ethanol extract of *S. violacea* showed more susceptible to *A. stephensi* and followed by *C. quinquefasciatus*, *A. aegypti*. The highest larval mortality was found in ethanol extract of *S. violacea* 100% (LC_{50} 47.6 and LC_{95} 225.3) on *A. stephensi* and 100% (LC_{50}51.8 and LC_{95}218.4) observed in chloroform extract on *C. quinquefasciatus* and followed by hexane extract 57.8% (LC_{50}205.3 and LC_{95} 598.8), 90.5% (LC_{50}49.5 and LC_{95} 306.5), 91.4% (LC_{50}52.4 and LC_{95} 299.4) chloroform extract 60.8% (LC_{50}179.6 and LC_{95} 605.1), 83.5% (LC_{50}82.9 and LC_{95} 392.6) and 87.6% (LC_{50}93.9 and LC_{95} 334.7) respectively (Table 1). Chi- square value was significant at $p<0.05$ level.

Table 1: Larvicidal activity of different crude extracts of *S. violacea* against 4th instar larvae of mosquitoes.

Solvent extract	Concentration (ppm)					LC_{50} (ppm)	95% Confidence Limit (ppm)		LC_{95} (ppm)	95% Confidence Limit (ppm)		*x*2 (*df*= 4)
	50(ppm)	100(ppm)	150(ppm)	200(ppm)	250(ppm)		LCL	UCL		LCL	UCL	
A. aegypti												
Hexane	24.2±2.2	35.4±2.1	41.4 ±3.5	47.4±1.1	57.8±3.9	205.3	176.8	251.8	598.8	472.64	881.1	0.534
Chloroform	30.2±1.4	38.4±3.0	46.4±2.6	52.2±3.5	60.8±3.6	179.6	150.8	220.0	605.18	470.5	925.8	0.101
Ethanol	44.2±3.5	52.6±2.1	61.5. ±2.5	74.2±1.6	89.4±2.6	86.6	58.2	106.8	341.2	295.8	417.3	3.051
A. stephensi												
Hexane	46.4± 3.5	69.2± 2.5	72.5 ±2.5	81.5±3.9	90.5±3.5	49.5	9.2	75.1	306.5	265.4	376.8	2.731
Chloroform	43.2±2.7	56.4±3.5	60.4±2.4	71.5±3.5	83.5±3.7	82.9	45.4	107.3	392.6	330.0	509.9	1.345
Ethanol	53.6±1.8	68.7±3.7	79.4 ±3.4	89.4± 2.6	100±1.5	47.6	19.8	66.7	225.3	201.4	261.4	5.260
C. quinquefasciatus.												
Hexane	50.3± 2.3	61.3± 3.3	74.6 ±3.1	82.6±2.7	91.4±3.3	52.4	15.0	76.6	299.4	260.5	364.7	0.267
Chloroform	40.1±2.8	51.2±2.3	62.3±2.8	75.6±3.4	87.6±1.5	93.9	69.0	112.5	334.7	292.6	403.2	0.812
Ethanol	55.6±2.4	67.2±1.6	78.2 ±2.5	92.2± 3.5	100±2.4	51.8	27.0	69.4	218.4	196.1	251.6	5.244

Values are mean ± SD for five replications. Values not sharing a common superscript differ significantly at $p < 0.05$.

4. Discussion
The results of present study are comparable with similar reports of earlier workers. The toxicity to the third instar larvae of *A. aegypti*, *C. quinquefaciatus* and *A. stephensi* by the ethyl acetate leafe extract of *Breyenia vitis –idaea* showed the LC_{50} value of 98.2, 107.79 and 115.8ppm respectively [7]. Sharma *et al* [8] reported that, petroleum ether extract of *Ageratum conyzoides* leaves exhibited larvicidal activity with LC_{50} value of 425.60 and 267.90 ppm after 24 and 48 h of exposure. The toxicity to the third instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Memordica charantia*, *Trichosanthus anguina* and *Luffa acutangula* showed the LC_{50} values of 465.85, 567.81 and 839.81 ppm respectively [9]. The toxicity to the late third instar larvae of *Ae. aegypti* by the hexane leaf extracts of *Abutilon indicum* and *Cx. quinquefasciatus* by dichloromethane whole plant extracts of *Citrullus colocynthis* and hexane extracts of aerial parts of *Hyptis suaveolens* was reported by Arivoli and Samuel [10]. *A. aegypti* larvae were showing to unreliable concentrations of aqueous extract of *C. collinus* and

synthesized silver nanoparticles for 24hours as per WHO protocols. Proportion of larval mortality was recorded. The synthesized nanoparticles exhibited significant larvicidal activity. This method is careful as a novel advance option using green nanochemistry technique to control dengue vector parasites of *C. collinus* leaf mediated synthesized silver nanoparticles. Jeyasankar *et al* [11] have reported that the ethyl acetate extract of *Phyllanthus Emblica* Linn. Exhibited more than 90% larval mortality at 250ppm on *C. quinquefaciatus.* Present investigation evidently exposed the value of hexane extracts set from the leaves and stem of *A. aspera* against early IV instars of *A. aegypti* as compared to the other four extracts. Similar results have been reported by [12]. Rajasekaran and Duraikannan [13] also reported elevated toxicity of petroleum ether and aqueous extract of *L. camara* against 4th instars of *A. aegypti* resulting in 100% mortality as compare to the chloroform extract which can effect in only 36.89% mortality after 24 hours. The toxicity to the third instar larvae of *A. aegypti*, *C. quinquefaciatus* and *A. stephensi* by the ethyl acetate leaf extract of *Andrographis paniculata* showed the LC_{50} value of 20.85 and LC_{95} 444.41ppm respectively [14]. The result of the present study showed that the leaf extract of *S. violacea* possess larvicidal activity against important vector mosquitoes. The results reported here open the risk of more analysis of efficacy on their larvicidal properties of natural product extract for the control of mosquitoes and thereby keep away from the environmental pollution.

5. References

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