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Phytochemical screening and ovicidal activity of *Scutellaria violacea* (Lamiaceae) leaf extract against vector mosquitoes (Diptera: Culicidae)

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Abstract

The present investigation was aimed to determine the preliminary phytochemical analysis and ovicidal activity of *S.violacea* leaf extracts against three important mosquito vectors, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus.* The ovicidal activity was determined against three mosquito species at the concentrations of 50, 100, 150, 200 and 250 ppm and mortality was assessed after 48 hours. Highest percentage of ocividal activity was recorded in ethanol extract on *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*, respectively. Preliminary phytochemical analsysis showed the preence of Flavonoids, Tannins, Saponins, Alkaloids, coumarins, sterols, steroids and Starch in ethnol extract. These results suggested that the leaf extracts of *S.violacea* showed possible to be used as an ideal environmental approach for the control of the *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*

Keywords: *Aedes aegypti, Anopheles stephensi*, *Culex quinquefasciatus, Scutellaria violacea* and ovicidal activity.

Introduction

Mosquitoes are at a standstill instead of the world's number one vector of human and household animals. Comprising about 3500 genus, mosquitoes are initiate further than the tropical and subtropical regions of the world with which they are typically connected. Mainly right for the chief genera which vector human disease causing pathogens Anopheles (malaria, filariasis), Aedes (yellow fever, dengue, chikungunya), and Culex (West Nile, Japanese encephalitis, filariasis) mosquitoes are thin globally and most female mosquitoes take blood meals from vertebrates to obtain the necessary nutrition to create their eggs, injecting saliva (which possibly will control pathogens) into the host animal. While many mosquitoes are distinctly selective feeders, restricted to one or a few closely related species, some feed in a less restrictive manner, varying among the mammals, birds, and reptiles. Mosquitoes breed in water, infrequently deposit eggs straight on water, but generally using a range of moist surfaces, tree holes,

and containers (Reiter, 2001; White, 2004; WHO, 2009; 2010*)*. The use of chemical insecticides in controlling mosquitoes has been encountered by a lot of problems due to harmful hazards of organic artificial pesticides to human, domestic animals, wildlife and the environment (Matsumura, 1975). Vector control represents an significant approach for prevention of disease diffusion and epidemic outbreaks. In the present time, more than use of chemical insecticides leads mosquitoes to increase the struggle towards chemical insecticides. To defeat this problem scientists have initiated the search for other control events. Thus, investigate is listening carefully on result newer insecticides of plant origin with high power, safety and easy availability at low cost (Brogdon and McAllister, 1998; Hemingway and Ranson, 2000). Recently, Subhashini *et al.*, (2017) reported that larvicidal activity of ethanol extracts of *S.violacea* showed significant mortality on *A. aegypti*, *A. stephensi* and *C. quinquefasciatus.*

In view of the recently improved interest in developing plant origin insecticides as an different to chemical insecticide, this study was undertaken to review the ovicidal potential of the different solvent crude extracts from the *S.violacea* against the medically important mosquito vectors, *A. aegypti*, *A. stephensi* and *C. quinquefasciatus.*

Materials and Methods

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 Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India and The Voucher specimen (IPH-45) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

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Extraction method

The dried leaves (1000g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, chloroform and ethanol (500ml, Ranchem), in a soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22-26mm hg at 45º C by 'Rotavapour' and the residue obtained was stored at 4ºC in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4ºC.

Vector rearing

The mosquito larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were collected from National centre for disease control, Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, 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.

Ovicidal activity

For ovicidal activity, slightly modified method of (Su and Mulla1998) was performed. *A.aegypti*, *A. stephensi* and *C. quinquefasciatus* eggs were collected from Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, India. The leaf extracts were used in the Hexane, Chloroform and Ethanol extract to achieve various concentrations ranging from 50 to 250ppm. Eggs of these mosquito species (100) were exposed to each concentration of leaf extracts. After 48 h treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated five times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula:

$$\% \text{ hatchability} = \frac{\text{No. of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

Preliminary phytochemical screening

It involves testing of different extracts of *S.violacea* for various phytochemicals by qualitative analysis tests to give general idea regarding the nature of constituents present in crude extracts. The qualitative chemical tests for various phytoconstituents (Earnsworth et al., 1974; Bruneton J, 1999; Trease and Evans, 1987 and Paris and Moyse, 1969) were carried out for three different solvent of extracts of *S.violacea* as explained below.

1-Test for the phenolic compounds:

Flavonoids:

The ethanol extract (5 ml) was added to a concentra ted sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 min) indic ates the presence of flavonoids.

Tannins:

2 ml of the aqueous extract was added to 2 ml of water, a 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins

2-Test for saponins:

To 1 ml of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

3-Test for alkaloids:

15 ml of the aqueous extract was added 2 ml of NH4 OH à 10% (ph=7). The alkaloid was extracted 3 times with 10 ml chloroform. The chloroform layer was hed 3 times with 2 ml of HCL (10%). This was divided into two portions. Mayer's reagent was added to one portion and Wagner's reagent to the other. The formation of a brown or white precipitate was regarded as positive for the presence of alkaloids sels.

4-Test for coumarins:

Evaporate 5 ml of ethanolic solution, dissolve the residue in 1-2 ml of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 ml 10% NH4OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.

5-Test for sterols and steroids:

Sterols and steroids were sought by the reaction of Liebermann. Ten (10 ml) ml of ethanolic extract was evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride; we added 0.5 ml of the filtrate chloroforme.Treated with the reagent of Libermann Burchardt. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.

Results and Discussion

The results of phytochemical characterization of *S.violacea* are presented in Table 1. The preliminary phytochemical screening revealed the strong occurrence of Tannins, Saponins, Alkaloids, coumarins, sterols, steroids and Starch in ethanol extract. The efficacy of leaf crude extracts such as hexane, chloroform and ethanol extracts of *S.violacea* evaluated for ovicidal (eggs) activity against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* and results are presented in table 2. Generally, as the concentration increases the rate of eggs mortality are also increases. It has been noticed that the higher concentrations of *S.violacea* extracts of ethanol extract possesses strong ovicidal activity at 250ppm concentration against *A. aegypti* , *A. stephensi* and *C. quinquefasciatus* (100%) no egg hatchability was recorded in Table 2. Results of this study show that the *S.violacea* evaluvated selected extracts may be a potent source of natural ovicidal activity against selected important vector mosquitoes.

Table 1: Preliminary phytochemical analysis of different solvent extract of *S.violacea*

Constituents	Test for indicates the presence of compound	Hexane extract	Chloroform extract	Ethanol extract
Flavonoids	Red coloration	-	-	-
Tannins	Dark green	+	+	+
Saponins	Stable persistent	-	+	+
Alkaloids	Brown coloration	-	-	+
coumarins	Intense fluorescence	+	-	+
sterols	Ring of blue-green	+	+	-
steroids	Ring of blue-green	-	-	+

+ Presence of compound - Absence of compound

Table 2: Ovicidal activity of different solvent extract of *S.violacea* against mosquito vectors

Concentrations (ppm)	Different solvent		
	Hexane extract	Chloroform extract	Ethanol extract
A. Aegypti			
Control	100 ±0	100 ±0	100 ±0
50	50.2 ±2.4	48.2±2.4	26.2±3.0
100	42.4 ±2.6	40.3±3.2	16.6±2.6
150	30.2±3.1	30.4±2.4	10.5±2.6
200	16.4±2.8	12.4±2.3	4.5±3.2
250	8.5±3.2	5.2±4.2	NH
A. stephensi			
Control	100 ±0	100 ±0	100 ±0
50	54.2±2.3	35.6±2.3	30.2±1.6
100	41.2±2.5	24.5±1.6	22.3±2.4
150	38.4±3.8	14.5±2.3	11.3±2.4
200	17.5±3.0	10.3±2.4	5.5±1.4
250	9.2±2.3	4.6±3.8	NH
C. quinquefasciatus			
Control	100 ±0	100 ±0	100 ±0
50	48.5±3.2	42.4±2.9	28.4 ±1.7
100	32.4 ±2.6	33.4±2.2	17.5 ±2.1
150	19.1±2.7	21.2 ±3.3	10.2 ±3.2
200	12.4±2.9	10.2±2.1	2.2±3.0
250	6.3 ±3.9	4.2 ±3.3	NH

Values in a row with a different superscript are significantly different at p <0.05% level. Each value (M± SD) represents the mean of six values. NH = No hatchability (100% mortality)

Vector control programme is a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting counter measures such as developmental of novel insecticides (Chandre *et al* 1998). Vector control has experienced a paradigm shift over time as public health officials have come to better appreciate the potential applications of natural products in the mission of disease control (Hardin et al 2009). Indian flora comprises a rich storehouse of phytochemicals/ botanical insecticides which serve as suitable alternatives to synthetic insecticides (Vatandoost *et al* 2012) as they are relatively safe, degradable, and are readily available in many areas of the world. Secondary metabolites present in plant act as key candidate with insecticidal properties and can be explored to develop the natural compounds to control mosquito population (Kumar *et al* 2012). In the present study, *S.violacea* crude extracts were tested for their ovicidal activity against three important mosquitoes. Among the solvent extracts of *S.violacea* tested, the ethanol extract showed better ovicidal activity against tested mosquito eggs.

Plant extracts have been screened and studied for their ovicidal activity against mosquitoes (Samuel *et al* 2011). Ovicidal compounds are able to interrupt embryo development, impair the survival of larva inside the egg or block egg hatching. Fresh eggs from control showed embryogenesis in progress while impairment of embryo development was detected in treated eggs, reflecting ovicidal activity (Madhiyazhagan *et al* 2012; Govindarajan *et al* 201). (Thavara *et al* 2002) reported that the phytochemicals provided protection for 7 h against *Aedes aegypti*, and at least 8 h against *Culex quinquefasciatus* and *Anopheles dirus* under laboratory conditions. (Bream *et al* 2010). reported that the repellent action of the plant extracts tested varied depending on the plant parts, solvent used in extraction and the dose of the extract and further reported the petroleum ether extracts of the leaf, stem and root of *Echinochloa stagninum* at 5, 5 and 4.3 mg/cm2 to exhibit 100% repellency against mosquitoes. Yang *et al* (2004) stated that the methanol extracts from 23 aromatic medicinal plant species for their repellent activity against female blood starved *Aedes aegypti*. Skin repellency test at 1, 2.5 and 5 mg/cm2 concentrations of *Cymbopogon citratus* gave 100% protection up to

3, 4 and 5 h, respectively, while the total protection percentage of the essential oil was recorded as 49.64% at 1 mg/cm2, 62.19% at 2.5 mg/cm2 and 74.03% at 5 mg/cm2 against *Culex quinquefasciatus* for 12 h (Yang *et al* 2004). (Mullai et al 2008) reported skin repellent test at 1.0, 2.5 and 5.0 mg/cm2 concentration gave a mean complete protection time ranging from 119.17 to 387.83 min against Anopheles.

Table 3: Ovicidal activity of different solvent extract of *S.violacea* against mosquito vectors on LC_{50} and LC_{90} Calculated by spss 16.00.

Mosquito vectors	Solvent Extracts								
	Hexane			Chloroform			Ethanol		
	LC_{50} (LCL-UCL)	**LC_{90} (LCL-UCL)**	**X^2**	**LC_{50} (LCL-UCL)**	**LC_{90} (LCL-UCL)**	**X^2**	**LC_{50} (LCL-UCL)**	**LC_{90} (LCL-UCL)**	**X^2**
A. Aegypti	237.38 (215.41-269.96)	422.87 (367.17-516.42)	1.355	240.52 (220.04-370.22)	407.15 (357.91-487.17)	3.953	322.40 (285.41-390.76)	481.97 (408.19-624.35)	2.440
A. stephensi	225.60 (205.20-254.82)	410.90 (357.94-498.91)	4.307	308.64 (270.95-376.98)	509.52 (426.14-668.94)	0.271	300.25 (270.28-351.33)	450.77 (389.20-561.45)	3.051
C. quinquefasciatus	258.97 (235.11-295.49)	430.57 (375.02-523.40)	0.308	265.86 (240.96-304.76)	436.20 (379.02-533.03)	1.008	303.31 (274.08-353.40)	439.18 (380.93-545.32)	2.430

Values are mean ± S.D of fivereplication; Number of larvae =10; LC_{50}=Lethal concentration 50 and LC_{90}=Lethal concentration 95; Values with different alphabet in column are statistically significant (p<0.05 level; DMRT).

Jeyasankar and Ramar, (2015) reported that the crude ethyl acetate solvent leaf extract of *B.vitis idaea* had significant ovicidal and pupicidal properties against *Ae. aegypti* , *An. stephensi* and *Cx. quinquefasciatus* with the LC_{50} values less than 100mg/L respectively. Compare with other results, Petroleum Ether extract of *Andrographis paniculata* against mosquito vectors exhibited more than maximum percentage of ovicidal and pupicidal activity was record in petroleum ether extract 250ppm on *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi*. (Roni *et al* 2013) showed in ovicidal activity by ethyl acetate, aqueous solution, ethanol leaf extract of *Nerium oleander* against *An. stephensi* at 100, 150, 200, 250, and 300 ppm were considered. With each extract at a concentration of 100 ppm, the take of hatchability was very high and nil hatchability was recorded as the concentration of extract was better to 300 ppm in the case of aqueous and ethanol extract. (Kuppusamy *et al* 2008) reported that the ovicidal activity indicated an significant result that the larvae which hatched out of the treated eggs were succumbed to death contained by an hour or two. In the case of ovicidal activity, exposure to the newly laid eggs was more effective than that to the older eggs. (Singh and Mittal 2013) reported that the seed extract of *Solanum nigrum* significantly concentrated the amount of eggs deposited by gravid *An. stephensi* . At the highest dose (0.5%) egg lying was reduced up to 99%. Likewise, ovicidal effect of ethyl acetate extract of *S.violacea* against *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi*. For this reason of this study clearly reveals that the *S.violacea* has potency to control the mosquito *Ae. aegypti, Cx. quinquefaciatus* and *An.stephensi*. As a result of natural products establish to be an valuable approach in eco-friendly mosquito management and control.

In conclusion, the results of the present investigation revealed that the ethanol extract of *S. violacea* to exhibit ovicidal property against tested mosquitoes when compared to the other solvents extracts which might be due to the polarity index and nature of the plant materials. Further studies are needed to elucidate the activity principles present in *S. violacea* ethanol extract which may responsible for ovicidal activity.

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