Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Mosquitocidal properties of *Solanum trilobatum* L. (Solanaceae) leaf extracts against three important human vector mosquitoes (Diptera: Culicidae)

Selvaraj Premalatha[1], Kuppusamy Elumalai[2], Alagarmalai Jeyasankar[1*]

[1]*Department of Zoology & Botany, Arignar Anna Government Arts College, Musiri-621 211 Tamil Nadu, India*
[2]*Unit of Entomotoxicity, Department of Advanced Zoology & Biotechnology, Govt. Arts College Nandanam, Chennai - 600 035, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 8 July 2012
Received in revised form 28 August 2013
Accepted 15 September 2013
Available online 20 November 2013

Keywords:
Solanum trilobatum leaf extracts
Aedes aegypti
Culex quinquefasciatus
Anopheles stephensi
Larvicidal

ABSTRACT

Objective: To determine the larvicidal and pupicidal activites of *Solanum trilobatum* (*S. trilobatum*) leaf extracts against *Aedes aegypti* (*Ae. aegypti*), *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and *Anopheles stephensi* (*An. stephensi*). **Methods:** The larvicidal and pupucidal was determined at five different concentrations of 50, 100, 150, 200 and 250 ppm. Percentage of larval mortality was assessed after 48 h. **Results:** Methanol extracts of S. trilobatum was found to be more susceptible against the larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* at 250 ppm with a LC_{50} value of 125.43, 127.77 and 116.64 ppm respectively. Leaf methanol extracts of *S. trilobatum* also exhibited pupicidal and adult emergence properties. **Conclusions:** These results suggested that the leaf extracts of *S. trilobatum* showed potential to be used as an ideal ecofriendly approach for the control of the *Ae. Aegypti*, *Cx. quinquefasciatus* and *An. stephensi*.

1. Introduction

Mosquitoes are the important single 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 death every year[1]. Over and injudicious use of synthetic insecticides in vector control has resulted in environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms[2]. Plant derived materials are comparatively safer to humans and ecosystem and easily biodegradable[3]. Phytochemicals extracted from various plant species have been tested for their larvicidal activity against mosquitoes[4].

Solanum trilobatum (*S. trilobatum*), a thorny creeper with bluish violet flower, more commonly available in Southern India has been used traditionally in Siddha system of medicines to treat various diseases[5]. It has been widely used to treat respiratory disorders, especially bronchial asthma[6,7]. It was reported that antioxidant activity, hepatoprotective activity[8] and protects UV induced damage and radiation induced toxicity in mice[9]. Sobatum, the partially purified petroleum ether extract of *S. trilobatum* was reported to be very effective in tumor reduction[10]. The leaf extracts of *S. trilobatum* posseses ovicidal activity against *Culex quinquesfasciatus* (*Cx. quinquefasciatus*) and *Culex tritaeniorhnchux (Cx. tritaeniorhnchux)*[11], and oviposition deterent and skin repellent activity against *Anopheles stephensi* (*An. stephensi*)[12]. Furthermore, aqueous methanol and n-butanol extracts of *S. trilobatum* showed

*Corresponding author: Dr. A. Jeyasankar, Assistant Professor of Zoology, Arignar Anna Govt. Arts College, Musiri, Trichy-621 211, Tamil Nadu, India.
Tel: +91 90800 57099
E-mail: sankar.alagarmalai@gmail.com

potential antimicrobial activity against Gram (+) and Gram (−) bacteria[13]. Various chemical constituents are reported to be isolated from Solanum species, which includes alkaloids, phenolics, flavanoides, sterols saponins and their glycosides[14]. Alkaloides such as soladunalinidine and tomatidine were isolated from leaf and stem of Solanum species. Therefore the present investigation was carried out to determine the mosquitocidal activity of *S. trilobatum* leaf extracts against three important vector, *Aedes aegypti* (*Ae. aegypti*), *Cx. quinquefasciatus* and *An. stephensi*.

2. Materials and methods

2.1. Plant collection and extraction

Leaves of *S. trilobatum* was collected in and around Tiruchirapalli district, Tamil Nadu, India and brought to the laboratory at PG and Research Department of Zoology, Arignar Anna Government Arts College, Musiri, Tiruchirapalli, Tamil Nadu, India; shade dried under room temperature and powdered using an electric blender. A total of 1 kg of dried and powdered leaves was subjected to sequential extraction using 3 L of acetone, chloroform and methanol for a period of 48 h to obtain the crude extracts using rotary vacuum evaporator. The extract was concentrated under reduced pressure 22−26 mmHg at 45 ℃ by 'Rotavapour' and the residue obtained was stored at 4 ℃ until testing for subsequent bioassays.

2.2. Test organisms

All tests were carried out against laboratory reared vector mosquitoes *viz*., *Ae. aegypti*, *Cx. quinquefasciatus* and *An. Stephensi* free of exposure to insecticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 25−29 ℃ and 80%−90% relative humidity in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio of 3:1) and adult mosquitoes on 10% glucose solution.

2.3. Larvicidal activity

Standard WHO protocol with slight modifications was adopted for the study[15]. From the stock solution, concentrations of 50, 100, 150, 200 and 250 ppm were prepared. Twenty five early third instar larvae were introduced in 250 mL beaker containing 200 mL of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 48 h. A total of three trials were carried out with five replicates per trial against vector mosquitoes. However, when the control mortality ranged from 5−20 per cent, the observed percentage mortality was corrected by Abbott's formula[16],

Pupicidal assay: Batches of ten pupae were introduced into 500 mL of the test medium containing particular concentration of the crude extract in a plastic cups in five replications. In control, the same number of pupae was maintained in 500 mL of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature (28±2) ℃ with naturally prevailing photoperiod (12 h: 12 h/L: D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded after 48 of exposure to the extract.

2.4. Statistical analysis

SPSS 11.5 version package was used for determination of LC_{50} and LC_{90}[17]. Data from mortality and effect of concentrations were subjected to analysis of variance. The percentage data obtained was angular transformed. Difference between the treatments was determined by Tukey's test ($P<0.05$).

3. Results

The larvicidal activity of leaf extracts of *S. trilobatum* against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* reported in the present study exhibit the mosquitocidal properties in the plant leaf extracts suggesting their use in mosquito population control (Tables 1&2). The different solvent crude extracts of *S. trilobatum* showed promising larval mortality against important mosquito species. According to the data, larvae of *Cx. quinquefasciatus* were more susceptible than *Ae. aegypti* and followed by *An. stephensi*. The data pertaining to the methanol extract of *S. trilobatum* against the fourth instar larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* are shown in Table 1. The larval mortality of the *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* was more prominent as evidenced from the Table 1, which showed 100% mortality in all species at 250 ppm concentration with the LC_{50} of 125.43 (LCL=115.70; UCL=134.68), LC_{50} of 122.77 ppm (LCL=113.43; UCL=204.49) and LC_{50}=116.64 (LCL=98.22; UCL=136.62) respectively. Similar trend of larval toxicity was also observed in chloroform extract of *S. trilobatum* against *Cx. quinquefasciatus* with the LC_{50} 121.06 (LCL=111.78; UCL=129.90). Thus, the methanol extract of *S. trilobatum*

exhibited the promising larvicidal activity against the larvae of mosquito species are needed to be explored.

The pupal mortality and adult emergence inhibition rates for *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* due to the exposure of different solvent extracts of *S. trilobatum* at 250 ppm concentration are presented Table 3. In the present study, *Cx. quinquefasciatus* showed more susceptible to the methanol extracts than other mosquito species. About 29.23 (97.47%) pupae were found dead with 2.57% adult emergence when it was found on *Cx. quinquefasciatus* treated with 250 ppm concentration of methanol extract of *S. trilobatum*. Similarly, (27.18±2.52) (n=30; 90.60%) on *Ae. aegypti* and (28.21±1.90) (94.03%) on *An. stephensi* pupal mortality was recorded from the experimental pupae treated with methanol extracts at the same concentration. Percentage of adult emergence was significantly reduced on pupae were treated methanol extracts on *Cx. quinquefasciatus* (2.57%), *An. stephensi* (5.97%) and *Ae. aegypti* (9.40%). Whereas the other solvent extracts did not showed significant results. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices.

Table 1

Larvicidal activity of leaf extracts of *S. trilobatum* against 4th instar larvae of mosquitoes vector.

Concentration (ppm)		Larval mortality* (%)		
		Acetone	Chloroform	Methanol
Ae. aegypti	Control	0.0±0.0^a	1.1±1.2^a	0.0±0.0^a
	50	17.4±2.3^b	18.2±3.1^b	16.5±2.1^b
	100	38.3±3.4^c	32.1±2.5^c	38.7±3.4^c
	150	53.4±2.5^d	56.7±3.8^d	56.4±3.8^d
	200	87.2±1.8^e	86.3±2.6^e	82.3±2.7^e
	250	96.9±2.4^f	99.8±2.8^f	100.0±2.8^f
Cx. quinquefasciatus	Control	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	50	18.4±2.3^b	18.4±3.2^b	15.2±3.4^b
	100	35.3±2.5^c	36.3±2.9^c	37.1±2.7^c
	150	56.4±2.7^d	58.6±2.7^d	59.2±3.5^d
	200	88.6±2.4^e	89.2±2.5^e	88.7±2.4^e
	250	98.9±1.2^f	100.0±3.2^f	100.0±3.2^f
An. stephensi	Control	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	50	15.3±2.3^b	16.4±3.2^b	18.5±2.4^b
	100	30.4±2.3^c	39.5±2.0^c	36.3±2.7^c
	150	49.6±1.6^d	52.6±2.3^d	54.5±2.5^d
	200	78.3±2.4^e	80.4±2.1^e	84.7±2.4^e
	250	95.9±1.3^f	92.3±2.2^f	100.0±1.2^f

Value represents mean±SD of five replications. *Number of pupae subjected to the experiment. **Mortality of the pupae observed after 7 d of exposure period. Values in the column with a different superscript alphabet are significantly different at P<0.05 level DMRT test.

Table 2

Larvicidal activity of leaf extracts of *S. trilobatum* against 4th instar larvae of mosquitoes vector.

Mosquitoes	Extracts	LC_{50} (ppm)	95% Confidence limits(ppm)		LC_{90} (ppm)	95% Confidence limits (ppm)		χ^2value (df=4)
			LCL	UCL		LCL	UCL	
Ae. aegypti	Acetone	125.67	115.68	135.17	219.83	205.36	238.53	5.551
	Chloroform	125.87	98.30	150.62	212.50	181.72	274.14	8.000
	Methanol	125.43	115.73	134.68	216.33	202.29	234.44	7.743
Cx. quinquefasciatus	Acetone	123.50	114.01	132.56	211.40	197.87	228.77	6.575
	Chloroform	121.06	111.78	129.90	205.80	192.72	222.54	7.385
	Methanol	122.77	113.79	131.39	204.49	191.82	220.63	5.386
An. stephensi	Acetone	121.99	108.37	137.32	245.24	210.55	284.25	5.847
	Chloroform	119.52	105.38	135.50	347.24	324.12	379.21	6.014
	Methanol	116.64	98.22	136.62	312.85	252.05	395.78	6.254

Table 3
Pupicidal activity of leaf extract of *S. trilobatum* at 250 ppm concentrations tested against the pupae of mosquitoes vectors (n=30).

Mosquitoes	Extracts	Mortality**		Adult emergence	
		Pupal mortality	Mortality (%)	Adult	Emergence (%)
Ae. aegypti	Acetone	17.23±2.42[b]	57.43	12.77±1.60[b]	42.57
	Chloroform	21.42±3.43[b]	71.40	8.58±2.26[a]	28.60
	Methanol	27.18±2.52[c]	90.60	2.82±1.43[a]	9.40
	Control	2.12±1.28[a]	7.07	27.88±2.31[c]	92.93
Cx. quinquefasciatus	Acetone	21.21±1.87[b]	70.70	8.79±2.67[b]	29.30
	Chloroform	25.01±2.98[b]	83.37	4.99±1.53[a]	16.63
	Methanol	29.23±1.40[c]	97.43	0.77±1.29[a]	2.57
	Control	1.42±1.23[a]	4.73	28.58±2.21[c]	95.27
An. stephensi	Acetone	18.11±1.23[b]	60.37	11.89±0.37[b]	39.63
	Chloroform	20.76±2.38[b]	69.20	9.24±1.53[a]	30.80
	Methanol	28.21±1.90[c]	94.03	1.79±1.23[a]	5.97
	Control	1.00±0.99[a]	3.33	29.00±2.83[c]	96.67

Value represents mean±SD of five replications. *Number of pupae subjected to the experiment. **Mortality of the pupae observed after 7 d of exposure period. Values in the column with a different superscript alphabet are significantly different at P<0.05 level DMRT test.

4. Discussion

The results of present study are comparable with similar reports of earlier workers. Sharma *et al*[18] 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[19]. 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[20-22]. Jang *et al*[23] have reported that the methanol extracts of *Cecropia obtusifolia, Cassia tora* and *Vicia tetrasperma* exhibited more than 90% larval mortality at 200 ppm on *Ae. aegypti* and *Culex pipiens*. The larvicidal activity of petroleum ether, ethanolic, aqueous extracts of dried leaves and fixed oil from the seeds of *Caesalpinia bonduc* (Family: Caesalpiniaceae) showed 100% mortality in 1% concentration of petroleum ether and ethanolic extract of leaf, whereas it was 55.0% in 2.5% concentration of aqueous extract and 92.6% in 2.5% concentration of fixed oil against the fourth instar larvae of *Cx. quinquefasciatus*[24]; the petroleum ether extract of *Solanum xanthocarpum* was observed to be the most toxic with LC_{50} of 1.41 and 0.93 ppm and LC_{90} of 16.94 and 8.48 ppm at 24 and 48 h after application, respectively against *An. stephensi*[25]. Venkatachalam and Jebanesan[26] have also reported that the repellent activity of methanol extract of Ferronia elephantum leaves against *Ae. aegypti* activity at 1.0 mg/cm^2 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.

The findings of the present investigation revealed that the leaf extracts of *S. trilobatum* possess larvicidal and pupicidal activities against vector mosquitoes. It may concluded that plant origin chemicals from the *S. trilobatum* leaf extracts showed insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/ compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *S. trilobatum* leaf extracts to control the immature stages of vector mosquitoes. In conclusion, an attempt has been made to evaluate the role of *S. trilobatum* against an alternative approach to combat with the important human vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interests.

Acknowledgements

The authors are grateful to Principal and HOD of Zoology

Department, Arignar Anna Govt. Arts College, Musiri, Tamil Nadu, India for their help and suggestion. This work was conducted in laboratory which is financially supported by University Grant Commission (UGC) X plan (UGC/TN/GAC -2008) for college teachers.

References

[1] Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J Vect Borne Dis* 2007; **44**: 145-148.

[2] Bansal SK, Singh KV, Sharma S, Sherwani MRK. Comparative larvicidal potential of different plant parts of *Withania somnifera* against vector mosquitoes in the semi-arid region of Rajasthan. *J Environ Biol* 2011; **32**(1): 71-75.

[3] Kalu IJ, Ofoegbu U, Eroegbusi J, Nwachukwu CU, Ibeh B. Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. *J Med Plant Res* 2010; **4**(6): 496-498.

[4] Pavela R. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Parasitol Res* 2008; **102**: 555-559.

[5] Mohanan PV, Rao JM, Kutti MAS, Devi KS. Cytotoxicity of extracts of *Solanum trilobatum* and anticarcinogenic activity of sobatum. *Biomedicine* 1998; **18**: 106-111.

[6] Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. *J Ethnopharmacol* 1999; **66**: 205-210.

[7] Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. Further studies on the clinical efficacy of *Solanum trilobatum* in bronchial asthma. *Phytotherapy Res* 2004; **18**: 805-809.

[8] Shahjahan M, Sabitha KE, Mallika Devi R, Shyamala CS. Effect of medicinal plants on tumourogenesis. *Ind J Med Res* 2004; **123**(5-8): 23-27.

[9] Mohanan PV, Devi KS. Toxological evaluation of Sobatum. *Cancer Lett* 1998; **127**(1-2): 135-140.

[10]Mohanan PV, Devi KS. Cytotoxic potential of the preparation fromn *Solanum trilobatum* and the effect sobatum on tumour reduction in mice. *Cancer Lett* 1996; **110**: 71-76.

[11]Rajkumar S, Jebanesan A. Ovicidal activity of *Solanum trilobatum* Linn (Solanaceae) leaf extract against *Culex quinquefasciatus* Say and *Culex triaeniorhynchus* Gile (Diptera: Culicidae). *Intl J Trop Sci* 2004; **24**: 340-342.

[12]Rajkumar S, Jebanesan A. Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi*. *J Ins Sci* 2005; **5**: 1-3.

[13]Latha PS, Kannabiran K. Antimicrobial activity and phytochemicals of *Solanum trilobatum* Linn. *Afr J Biotech* 2006; **5**(23): 2402-2404.

[14]Amir M, Kumar S. Possible Industrial application of genus *Solanum* in twentyfirst century- A review. *J Sci Ind Res* 2004; **63**: 116-124.

[15]WHO. *Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96. 1.* Geneva: Control of Tropical Diseases Division; 1996.

[16]Abbott WS. A method of computing the effectiveness of an insecticide. *J Eco Entomol* 1925; **18**: 265-267.

[17]SPSS. *SPSS for windows, version 11.5*. Chicago, IL: SPSS; 2007.

[18]Sharma P, Mohan L, Srivastava CN. Anti-juvenile activity of Azadirachta indica extract on the development and morphometry of filaria vector, *Culex quinquefasciatus* (Diptera: Culicidae) Say. *Parasitol Res* 2009; **105**: 1193-1203.

[19]Prabakar K, Jebanesan A. Larvicidal efficacy of some cucurbitaceous plant leaf extracts against *Culex quinquefasciatus* (Say). *Bioresour Technol* 2004; 95: 113-114.

[20]Arivoli S, Samuel T. Larvicidal and adult emergence inhibition of *Abutilon indicum* (Linn.) (Malvaceae) leaf extracts against vector mosquitoes. *J Biopest* 2011; **4**(1): 27-35.

[21]Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Curr Res* 2011; **3**(4): 296-304.

[22]Arivoli S, Samuel T. Mosquitocidal activity of *Hyptis suaveolens* (L) Poit (Lamiaceae) extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Rec Sci Res* 2011; **2**(5): 143-149.

[23]Jang YS, Baek BR, Yang YC, Kim MK, Lee HS. Larvicidal activity of leguminous seeds and grains against *Aedes aegypti* and *Culex pipiens* pallens. *J Am Mosq Control Assoc* 2002; **18**(3): 210-213.

[24]Saravanan KS, Periyanayagam K, Ismail M. Mosquito larvicidal properties of various extract of leaves and fixed oil from the seeds of *Caesalpinia bonduc* (L) Roxb. *J Commun Dis* 2007; **39**(3): 153-157.

[25]Mohan L, Sharma P, Srivastava CN. Comparative efficacy of *Solanum xanthocarpum* extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector, *Anopheles stephensi*. *Southeast Asian J Trop Med Public Health* 2007; **38**(2): 256-260.

[26]Venketachalam MR, Jebanesan A. Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extract against *Aedes aegypti*. *Biores Technol* 2001; **76**(3): 287-288.