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RESEARCH ARTICLE

LARVICIDAL ACTIVITY OF ALKALOID FROM *Sida acuta* AGAINST *Anopheles subpictus* and *Culex tritaeniorhynchus*

Niraimathi Selvam[1], Balaji Nagarajan[2], Venkataramanan Nagarajan[3] and Govindarajan Marimuthu[4*]

[1]PG & Research Department of Biochemistry, RVS College of Arts & Science, Karaikal-609 609, Tamilnadu, India.
[2]Watson Pharma Pvt. Ltd. Ambarnath, Mumbai, India.
[3]Lupin laboratories Ltd., Bhopal, India.
[4]Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Tamilnadu, India.

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ABSTRACT

The larvicidal activity of *Sida acuta* was evaluated against 3rd instars larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus*. The crude leaf extract and active compound cryptolepine showed negligible mortality against early third instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*. The 24 h LC_{50} value was observed at 38.68, 50.81 mg/l and 9.98, 12.69 mg/l for crude leaf extract and active compound cryptolepine, respectively. The structure was elucidated from infrared, ultraviolet, ^{1}H-Nuclear Magnetic Resonance, ^{13}C-NMR, and mass spectral data.

INTRODUCTION

Mosquito-borne diseases, like malaria, yellow and dengue fevers, are a major threat to over 2 billion people in the tropics (Service, 1993). An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. 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 toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides (Macedo *et al*., 1997), and for more detailed studies of naturally-occurring insecticides (Govindarajan *et al*., 2008a). 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 bio-degradable. They are potentially suitable for use in integrated pest management programs (Alkofahi *et al*., 1989). The mosquito larvicidal properties of leaf and seed extract of plant *Agave Americana* (Dharmshaktu *et al*., 1987). The mosquito larvicidal activity in the extract of

***Corresponding author:** *drgovind1979@rediffmail.com.*

Tagetes minuta flowers against *Aedes aegypti* (Green *et al*., 1991). The methanolic fraction of leaves of *Mentha piperita, Phyllanthus niruri, Leucas aspera* and *Vitex negundo* against larvae of *Cx. quinquefasciatus* (Pandian *et al*., 1994). The methanolic extracts of *Solanum suratense*, *Azadirachta indica* and *Hydrocotyle javanica* exhibited larvicidal activity against *Cx. quinquefasciatus* (Muthukrishnan *et al*., 1997). 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 *Cx. quinquefasciatus, Ae. aegypti* and *An. Stephensi* (Govindarajan , 2010).

The acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Acalypha indica*, *Achyranthes aspera*, *Leucas aspera*, *Morinda tinctoria* and *Ocimum sanctum* were studied against the early fourth-instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* Say (Bagavan *et al*., 2008). Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis, Coccinia indica, Cucumis sativus, Momordica charantia,* and *Trichosanthes anguina*, were tested against the early fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* (Abdul Rahuman *et al*., 2008). The benzene and methanol extracts of *Artemisia vulgaris* has been repellent activity against *Ae. Aegypti* (Yit *et al*., 1985). The *Zanthoxylum armatus*, *Z.alatum* (Rutaceae), *Azadirachta indica* (Mailiaceae) and *Curcuma aromatica* (Zingiberaceae) were possess repellent properties against mosquitoes (Das *et al*., 2000). The repellent activity of active compound *Octacosane* from *Moschosma polystachyum* against the vector *Cx.quinquefasciatus* (Rajkumar *et al*., 2004). The essential oil of *Zingiber officinalis* as a mosquito larvicidal and repellent agent against the filarial vector *Cx. quinquefasciatus* (Pushpanathan *et al*., 2008).

Sida acuta Burm F. (Malvaceae), locally known as "arivalmukku pachilai" is an erect, branched small perennial herb or small shrub which grows abundantly on cultivated fields, waste areas, roadsides and open clearing in Tamilnadu, India. The bark is smooth, greenish, the root is thin, long, cylindrical and very rough; leaves are lanceolate, the flowers are yellow, solitary or in pairs; seeds are smooth and black. In Indian traditional medicine, the root of *S. acuta* is extensively used as a stomachic, diaphoretic and antipyretic. It is regarded as cooling, astringent, tonic and useful in treating nervous and urinary diseases and also disorders of the blood, bile and liver (Khare *et al.,* 2002). In the present study, we report the mosquito larvicidal properties of the leaves of *S. acuta* against *An. subpictus* and *Cx. tritaeniorhynchus*.

MATERIALS AND METHODS

Plant collection and extraction

Fully developed leaves of *S. acuta* were collected from the adjoining area of R.V.S College. Voucher specimen was deposited at the Department of Biochemistry, R.V.S College, Karaikal, India. Leaves were washed with dechlorinated water, shade dried and powdered using a mechanical grinder. Powdered leaves (1 kg) extracted with ethanol (3.0 l) in a soxhlet apparatus for 8 h and the extract was concentrated in a rotary vacuum evaporator to yield 82 g of a dark greenish material, which was used for the bioassay.

Gas chromatography analysis

Analysis was carried on a varian-gas chromatograph equipped with a flame ionization detector and a BPI (100 % dimethyl polysiloxane) capillary column. Helium at a flow rate of 1.0 ml min^{-1} and 8 psi inlet pressure was employed as a carrier gas. Temperature was programmed from 60 to 220^{o}C at 5 ^{o}c min^{-1} with a final hold time of 6 min .The injector and detector temperatures were maintained at 250 and 300^{o}C , respectively. The sample (0.2 µl) was injected with 1:20 split ratio.

Gas chromatography –mass spectrometry analysis

Gas chromatography –mass spectrometry (GC-MS) analysis was performed on an Agilent 6890 GC equipped with 5973 N mass selective detector and an HP-5(5% phynyl methylpolysiloxane) capillary column. The oven temperature was programmed from 50 to 280^{o}c at the rate of 4^{o}c min^{-}1and held at

this temperature for 5 min. The inlet and interface temperatures were 250 and 280°C, respectively. The carrier gas was helium at a flow rate of 1.0 ml min^{-1} (constant flow).The sample (0.2µl) was injected with a split of 20:1. Electron impact mass spectrometry was carried out at 70 eV. Ion source and quadrupole temperatures ware maintained at 230 and 150 °C respectively.

Mosquitoes

A laboratory colony of *An. subpictus* and *Cx. tritaeniorhynchus*, originally collected from Vector Control Research Center, Pondicherry, India, and colonized continuously for over 20 generations in a laboratory, free of exposure to pathogens and insecticides. The larvae were fed with dog biscuits and yeast powder in the ratio 3:1. Adults were provided with 10% sucrose solution and 1-week chick for blood meal. Mosquitoes were held at 28±2°C, 75±5% RH, and a photo regime of 10:14 h (dark/light).

Larvicidal activity

The larvicidal activity of crude extract and cryptolepine were assessed by using the WHO standard method (WHO 1996). For experimental treatment, One gram of crude extract was first dissolved in 100 ml of ethanol (stock solution), Crude extracts concentration ranging from 20 to 100 mg/l and cryptolepine concentration ranging from 5 to 25 mg/l were tested. For bio assay test, 25 larvae were taken in four batches in 250 ml of water. The control was set up with ethanol. The larval mortality was calculated after 24 h of the exposure period and the percentage mortality was reported from the average of four replicates. The data were subjected to probit analysis in order to estimate the LC_{50}, and LC_{90} values (Finney, 1971).

Statistical Analysis

Statistical evaluation was done using Statistical Package of Social Sciences (SPSS) 14.0 for windows, significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

A literature survey of the plant-isolated compound revealed that the compound under investigation could be cryptolepine (Fig. 1). The compound was confirmed by comparison with the reference spectrum (McLafferty and Staufer, 1989; Cheng *et al.,* 2001). The larvicidal activity of the of crude methanolic leaf extract of *Sida acuta* against *An. subpictus* and *Cx. tritaeniorhynchus* are given in Table 1. The LC_{50} and LC_{90}values of the crude extract on *An. subpictus* and *Cx. tritaeniorhynchus* larvae in 24 h were 38.68, 50.81 mg/l and 74.48, 91.36 mg/l, respectively. The larvicidal activity of the compound cryptolepine was more active than the crude extract (Table 2). The LC_{50} and LC_{90} values of the compound cryptolepine on *An. subpictus* and *Cx. tritaeniorhynchus* larvae in 24 h were 9.98, 12.69 mg/l and 18.94, 22.94 mg/l, respectively.

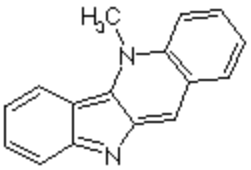

Fig. 1. Cryptolepine

The dark greenish methanolic leaf extract of *Sida acuta* was subjected to column chromatography and thin layer chromatograpy and the active compound cryptolepine was isolated. The entomotoxicity of the crude leaf extract and the compound cryptolepine results were also comparable with earlier reports. Methanolic leaf extract of *Cassia fistula* was tested for larvicidal activity *against Cx. quinquefasciatus* and *An. stephensi* (Govindarajan *et al*., 2008a). 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 *Anopheles stephensi.* The larval mortality was observed after 24 h exposure. The LC_{50} values are 19.25, 27.76, 23.26 and 15.03 ppm, respectively (Govindarajan *et al*., 2008b). The extract was found to be more lethal to the larvae of *An. stephensi* than *Cx.quinquefasciatus* with LC_{50} values of 17.97 and 20.57 mg/ l, respectively. The extracts of *Quercus iusitania* var. *infectoria* galls (Oliv.) showed larvicidal activity and their possible use in biological control of *Culex pipines* (Redwane *et al*., 2002). The LC_{50} values are 335 and 373 ppm for the 2nd and 4th instar larvae. The

Table 1. Larvicidal activity of methanol extract of *Sida acuta* against *Culex tritaeniorhynchus* and *Anopheles subpictus*

Mosquito	Concentration (mg/l)	Mortality (%)	LC_{50} (mg/l)	95%Confidence Limits(mg/l)		LC_{90} (mg/l)	χ^2
				Lower	Upper		
Culex tritaeniorhynchus	20	26.4±1.5	50.81±1.40	39.26±1.65	62.43±1.40	91.36±0.71	15.703*
	40	38.6±1.7					
	60	56.0±2.4					
	80	79.2±2.1					
	100	96.2±1.3					
	Control	Nil					
Anopheles subpictus	20	36.2±2.1	38.68±1.60	23.98±1.80	51.36±0.86	74.48±1.20	22.772*
	40	58.4±0.8					
	60	74.0±1.4					
	80	88.4±1.1					
	100	100.0±0.00					
	Control	Nil					

Each value (X ± S.D.) represents mean of six values
*Significant at P<0.05 level.

Table 2. Larvicidal activity of cryptolepine from *Sida acuta* against *Culex tritaeniorhynchus* and *Anopheles subpictus*

	Concentration (mg/l)	24 h mortality (%)	LC_{50} (mg/l)	95%Confidence Limits(mg/l)		LC_{90} (mg/l)	χ^2
				Lower	Upper		
Culex tritaeniorhynchus	5	21.0±1.2	12.69	9.57	15.78	22.94	17.383*
	10	46.2±1.2					
	15	61.4±1.8					
	20	73.0±1.4					
	25	96.2±1.4					
	control	Nil					
Anopheles subpictus	5	32.2±0.8	9.98	6.37	13.13	18.94	22.453*
	10	59.2±1.2					
	15	73.2±1.8					
	20	86.6±1.4					
	25	100.0±0.0					
	control	Nil					

Each value (X ± S.D.) represents mean of four values
*Significant at P<0.05 level.

leaf extract of *Cassia fistula* with different solvens viz, methanol, benzene and acetone were studied for the larvicidal, ovicidal and repellent activity against *Aedes aegypti*. The 24 h LC_{50} concentration of the extract against *Aedes aegypti* were observed at 10.69, 18.27 and 23.95 mg/l respectively (Govindarajan, 2009). The mosquito larvicidal properties of the leaf extract of a herbaceous plant, *Ocimum canum* against *Aedes aegypti*. The LC_{50} values for 2nd, 3rd and 4th instar larvae were 177.82, 229.08 and 331.13 ppm respectively (Singh *et al*., 2003). Phytochemicals obtained from huge diversity of plant species are an important source of safe and biodegradable chemicals which could be screened for mosquito repellent and insecticidal activities. Repellents of plant origin do not pose hazards of toxicity to human and domestic animal and are easily biodegradable. Natural products are safe for human when compared to synthetic compounds (Sharma and Ansari, 1994).

CONCLUSION

This study reveals that the *S.acuta* has remarkable larvicidal properties against *An. subpictus* and *Cx. tritaeniorhynchus*. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of

mosquito and other pests. 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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