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Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes

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ABSTRACT

Objective: To determine the larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae)extract against *Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi*. **Methods:** Twenty five late III instar larve of three mosquito species were exposed to various concentrations (15–90 mg/L) and were assayed in the laboratory by using the protocol of WHO 2005; the 24 h LC_{50} values of the *Sida acuta* leaf extract was determined following Probit analysis. The repellent efficacy was determined against three mosquito species at three concentrations viz., 1.0, 2.5 and 5.0 mg/cm^2 under the laboratory conditions. **Results:** Results showed varying degree of larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 to 48 mg/L. The crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *Anopheles stephensi* for 180 min followed by *Aedes aegypti* (150 min) and *Culex quinquefasciatus* (120 min). **Conclusions:** From the results it can be concluded the crude extract of *Sida acuta* was an excellent potential for controlling *Culex quinquefasciatus, Aedes aegypti* and *Anopleles stephensi* mosquitoes.

1. Introduction

Mosquito-borne diseases are major threat to over 2 billion people in the tropics. Several mosquito species belonging to genera *Anopheles, Culex*, and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever, chikungunya, etc[1]. The major problems associated with the use of chemicals for the control of pests including mosquitoes include: the development of resistance to the chemicals, issues around the residues in animals and the environment and their undesirable side effects[2]. Extracts from plants may be alternative sources of mosquito control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Plant extracts in general have been recognized as an important natural resource of insecticides[3]. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level[4,5].

Many studies on plant extracts against mosquito larvae have been conducted around the world. The crude hexane extracts obtained from flower heads of *Spilanthes acmella, Spilanthes calva*, and *Spilanthes paniculata*[6]; the ethyl acetate extract of fruit mesocarp of *Balanites aegyptiaca*[7]; the root extract of *Solanum xanthocarpum*[8]; the acetone crude extract of *Fagonia indica* and *Arachis hypogaea*[9]; the methanol extracts of dried root powder of *Rhinacanthus nasutus*[10] and essential oils from *Cinnamomum camphora, Thymus serpyllum, Citrus limon, Anethum graveolens, Piper nigrum, Juniperus virginiana, and Boswellia carteri*[11] were tested against *Aedes aegypti (Ae. Aegypti), Anopheles stephensi (An. Stephensi)*, and *Culex quinquefasciatus (Cx. Quinquefasciatus)* larvae. Plants have been evaluated as sources of natural insecticides against *Ae. aegypti*, and larvicidal bioassays have been conducted using third and fourth instars[12]. Saponins and essential oils with larvicidal, repellent, or oviposition deterrent effects on *Ae. aegypti* have been described[13].

Sida acuta (S. acuta) Burm F. (Malvaceae), locally known

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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[14]. It is also used to treat gonorrhoea, elephantiasis and ulcers and is claimed to have aphrodisiac properties. Banzouzi *et al*[15] reported that *S. acuta* ethanolic extract showed significant antiplasmodial activity against the two strains of *Plasmodium falciparum* studied in their laboratory. A perusal of literature revealed that no studies are available so far for its mosquitocidal activity of *S. acuta*. In the present study, to assesses the larvicidal and repellent properties of the leaves of *S. acuta* against three important vector mosquitoes.

2. Materials and methods

2.1. Plant procurement

The leveas of *S. acuta* were collected from in and around Vittaloor, Thanjavur district, Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of plant phytochemistry division, Department of Zoology, Annamalai University.

2.2. Preparation of the extract

The dried leaves (3.0 kg) were extracted with methanol (5.5 L) in a soxhlet apparatus method and the extract was evaporated in a rotary vacuum evaporator to yield a dark greenish mass (240 g). Standard stock solutions were prepared at 1% by dissolving the residues in acetone, which was used for the bioassays.

2.3. Test organisms

The mosquitoes, *Cx.quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast power L at 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) ℃, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.4. Larvicidal bioassay

The larvicidal activity of crude extract was evaluated as per the protocol previously described by the WHO[16] . Late third instar larvae (25) were placed in 249 mL of distilled water and 1 mL of acetone containing different experimental concentration. The beaker containing the control larvae received 1 mL of acetone. Crude extracts concentration ranging from 15 to 90 mg/L was tested. Each test was repeated for six times. The lethal concentration(LC_{50}) was carried out after 24 h by probit analysis[17] and the level of significance by Duncan's Multiple Range Test[18].

2.5. Repellent activity

The minutes of protection in relation to dose method was used[16]. Three-day-old blood-starved female *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* mosquitoes (100) were kept in a net cage (45 cm× 30 cm× 45 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of volunteer, only 25 cm^2 dorsal side of the skin on each arms was exposed and the remaining area covered by rubber gloves. The crude extract was applied at 1.0, 2.5 and 5.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. The time of the test dependent on whether the target mosquitoes day-or night biters. *Ae. aegypti* was tested during the day time from 7:00 to 17:00, while *Cx. quinquefasciatus* and *An. stephensi* were tested during the night from 19:00 to 5:00. The control and treated arm were introduced simultaneously in to the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arm in to the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood; making out a 5 minutes protection. The percentage of repellency was calculated by the following formula.

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

2.6. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the software using Statistical Package of Social Sciences (SPSS) 13.0 for windows, significance level was set at $P<0.05$.

3. Results

Data of the larvicidal activity of the of crude methanolic leaf extract of *S. acuta* against three species of mosquitoes

Table 1
Larvicidal activity of crude methanol extract of *S. acuta* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

Mosquito	Concentration (mg/L)	24 h mortality (%)	LC_{50} (mg/L)	95%Confidence limits(mg/lL)		LC_{90}(mg/L)	x^2(df)
				Lower	Upper		
Cx. quinquefasciatus	15	19.2±1.8	47.91	40.16	55.91	85.63	15.661*(5)
	30	32.4±1.4					
	45	44.3±2.2					
	60	61.0±1.4					
	75	76.4±1.8					
	90	97.5±1.8					
	Control	0.8±1.4					
Ae. aegypt	15	22.8±2.2	42.08	34.72	49.20	78.87	13.859*(5)
	30	39.2±1.8					
	45	56.4±1.8					
	60	69.2±2.2					
	75	82.4±1.4					
	90	98.0±1.8					
	Control	1.2±1.4					
An. stephensi	15	29.4±1.8	38.64	29.47	47.14	74.78	20.070*(5)
	30	42.8±1.8					
	45	59.2±1.4					
	60	72.4±1.4					
	75	85.8±1.8					
	90	99.8±1.2					
	Control	1.2±1.4					

*Significant at $P<0.05$ level.

Table 2
Repellent activity of crude leaf extract of *S. acuta* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

Mosquitoes	Concentration (mg/cm^2)	% of repellency						
		30 min	60 min	90 min	120 min	150 min	180 min	210 min
Cx. quinquefasciatus	1.0	100.0±0.0	100.0±0.0	86.0±0.9	72.0±1.3	54.0±1.8	32.0±0.8	16.0±1.5
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	89.0±1.5	85.0±1.2	71.0±1.1	41.0±1.2
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.0±1.6	81.0±1.3	68.0±1.2
Ae. aegypti	1.0	100.0±0.0	100.0±0.0	81.0±1.3	69.0±1.6	42.0±1.2	28.0±1.4	17.0±1.2
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	78.0±1.4	65.0±1.8	31.0±1.4	25.0±1.8
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	69.0±1.2	41.0±1.4
An. stephensi	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.0±1.4	64.0±1.8	33.0±1.4
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	78.0±1.2	48.0±1.2
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±00	74.0±1.2

are presented in Table 1. In terms of lethal concentrations for 50% mortality (LC_{50}) crude extract of *S. acuta* appeared to be most effective against *An. stephensi* (LC_{50}=38.64 mg/L) followed by *Ae.aegypti* (LC_{50}=42.08 mg/L) *Cx.quinquefasciatus* (LC_{50}=47.91 mg/L). The repellent activity of the crude extract of *S. acuta* showed significant repellent against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* (Table 2). It showed that repellency depends on the strength of the crude extract concentration. A higher concentration of 5.0 mg/cm^2 provided 100% protection up to 120, 150 and180 min against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively.

4. Discussion

The mosquitocidal activity of the crude leaf extract results are also comparable with earlier reports. Rahuman and Venkatesan[3] reported that the petroleum ether extract of *Citrullus colocynthis (C. colocythis)*, methanol extracts of *Cannabis indica*, *Cannabis sativus*, *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 *Cx. quinquefasciatus* (LC_{50}=88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. Larvicidal activity of acetone extracts of *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida*, and *Trigonella foenum graceum* reported maximum activity ranging 25–900 ppm against *Ae. aegypti*[19]. Mullai and Jebanesan[20] have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *C. colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae. The methanol extract of *Cassia fistula (C. fistula)* exhibited LC_{50} values of 17.97 and 20.57 mg/L, *An. stephensi* and *Cx. quinquefasciatus*, respectively[4]. The petroleum

ether (60-80℃) extracts of the leaves of *Vitex negundo (V. negundo)* were evaluated for larvicidal activity with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/L, against larval stages of *Culex tritaeniorhynchus (Cx. Tritaeniorhynchus)*, respectively[21]; the leaf benzene, petroleum ether, ethyl acetate and methanol of *Citrullus vulgaris* were tested for larvicidal activity with LC_{50} values of 18.56, 48.51, 49.57 and 50.32 ppm, respectively, against *An. stephensi*[22]. Larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis* with three different solvents like methanol, benzene and acetone was tested against the early second, third, fourth instar larvae of *Cx. quinquefasciatus, Ae. aegypti and An. stephensi*. Among the three solvents the maximum efficacy was observed in methanol. The LC_{50} values of *Ficus benghalensis* against early second, third and fourth larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm, respectively[23].

The leaf extract of *C. fistula* with different solvens viz, methanol, benzene and acetone were studied for the larvicidal and repellent activity against *Ae. aegypti*. The 24 h LC_{50} concentration of the extract against *Ae. aegypti* were observed at 10.69, 18.27 and 23.95 mg/L, respectively. The crude extract of *C. fistula* shows significant repellency against *Ae. aegypti*[24]. Komalamisra *et al*[25] have reported that the petroleum ether and methanol (MeOH) extracts of *Rhinacanthus nasutus* and *Derris elliptica* exhibited larvicidal effects against *Ae. aegypti, Cx. quinquefasciatus*, *Anopheles dirus* and *Mansonia uniformis* with LC_{50} values between 3.9 and 11.5 mg/L, whilst the MeOH extract gave LC_{50} values of between 8.1 and 14.7 mg/L. *Derris elliptica* petroleum ether extract showed LC_{50} values of between 11.2 and 18.84 mg/L and the MeOH extract exhibited LC_{50} values between 13.2 and 45.2 mg/L. Earlier authors reported that the n-hexane, ethyl acetate and methanol extracts of *Corynebacterium nigricans* showed 100% larval mortality against *Ochlerotatus triseriatus*[26].

The leaf extract of *Acalypha indica* with different solvents viz, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal activity against *An. 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[5]. Jang *et al*[27] 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*[28]; 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*[8]. The *Ricinus communis* seed extract exhibited larvicidal effects with 100% killing activities at concentrations 32-64 μg/mL, and with LC_{50} values 7.10, 11.64 and 16.84 μg/mL for *Cx. quinquefasciatus, An. stephensi* and *Ae. albopictus* larvae, respectively[29].

Venkatachalam and Jebanesan[30] 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 essential oil of *Zingiber officinalis* showed repellent activity at 4.0 mg/cm^2, which provided 100% protection up to 120 min against *Cx. quinquefasciatus*[31]. The first hand information of the current research clearly shows the potential of *S. acuta* as a possible larvicidal and repellent agent against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*.

Conflict of interest statement

We declare that we have no conflict of interest.

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