MOSQUITO LARVICIDAL ACTIVITIES OF *Spilanthes paniculata* FLOWER HEAD EXTRACT AGAINST FILARIA VECTOR (*Culex quinquefasciatus* Say)

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ABSTRACT

Vector control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. Although several plants have been reported for mosquitocidal activity, only a few botanicals have moved from the laboratory to field use, because they are poorly characterized, in most cases active principals are not determined and most of the works are restricted to preliminary screening. Spilanthes paniculata is a member of family Asteraceae distributed in many parts of India with medicinal properties, but the active principle of this plant having mosquito larvicidal property has not been reported so far. Aqueous and polar/non-polar solvent extract of fresh, mature, flower heads of S. paniculata was tested against Culex quinquefasciatus, a common vector of pathogen responsible for bancroftian filariasis. A phytochemical analysis of petroleum ether extract was performed to search for the active toxic ingredient. The lethal concentration was determined (log probit analysis) and compared with Malathion. In a 3 hour bioassay experiment with the aqueous extract, the highest mortality was recorded in 0.5% extract. When the mortality of different solvent extracts was compared, the maximum ($p < 0.05$) mortality was recorded at a concentration of 50 ppm of petroleum ether extract. The larvicidal activity was lower when compared with the chemical insecticide, Malathion ($p < 0.05$). Results of regression analysis revealed that the mortality rate (Y) was positively correlated with the period of exposure (X) and the log probit analysis (95% confidence level) recorded lowest value (5.97 ppm) at 3 hours of exposure. In the present study of phytochemical analysis of the petroleum ether extract of S. paniculata containing bioactive phytochemical/s has been reported for the first time.

Key Words: *Spilanthes paniculata*, *Culex quinquefasciatus*, biocontrol, bioassay, LC50

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INTRODUCTION

Culex quinquefasciatus Say is generally known as the vector of the pathogen responsible for bancroftian filariasis in warm and humid areas. Phytochemicals have a major role in mosquito control programs (Hag et al., 1999; Palsson and Janeson, 1999; Markouk et al., 2000; Saktivadivel and Thilagavathy, 2003; Ghosh and Chandra, 2006). Some herbal products such as pyrethrums from *Chrysanthemum cinerarifolium* flowers (Hartzell and Wilcoxon, 1941), and *Anabasis aphylla* (Campbell et al., 1993) have been used as natural insecticides before and after the discovery of synthetic organic insecticides (Jacobson and Crosby, 1971). Since the discovery of DDT, synthetic insecticide based method not only contributes to environmental hazards but also increases resistance to disease vectors (Wattal et al., 1981). In recent years, the search for new insecticides that are easily biodegradable and do not have any ill effect on nontarget organisms remains the top priority (Redwane et al., 2002). The plant product can be obtained from the whole plant or from a specific part by extraction with different types of solvents, such as water, petroleum ether, chloroform, and methanol, depending on the polarity of the phytochemicals. Hartzell and Wilcoxon (1941) evaluated extracts from 150 plant species for their toxicities to mosquito and found several to be very effective. Jantal et al. (2003) evaluated 17 methanol extracts and 9 essential oil preparations of Malaysian plants for their larvicidal activities against Aedes aegypti.

In the light of the above prospects, we have conducted investigations on a traditional medicinal plant *Spilanthes paniculata*, commonly known as Akarkara (Hindi),maratti mogga (Telugu) or piccaraza (Assamese); (Anonymous 1989). A member of the family Asteraceae, the genus is widely distributed in the tropics and sub-tropics. In India, the plants have been found growing in the northern and southern hills and plateaus. There are around five species of *Spilanthes* reported so far (Anonymous 1989) growing in India: *S. acmella* Murr., *S. acmella* L. var *oleraceae* clarke, *S. calva* L., *S. paniculata* L. and *S. mauritiana* L. Of these, *S. calva* L., *S. mauritiana* L. and *S. paniculata* L. are found more commonly, whereas *S. acmella* Murr. and *S. acmella* L. var *oleraceae* Clarke are rare in occurrence. The genus is attributed with immense medicinal (Borges-Del-Castillo et al. 1984), antimicrobial (Rai et al. 2004), larvicidal (Pendse et al. 1945) and insecticidal properties (Ramsewak et al. 1999) because of the presence of several bioactive compounds, which includes Spilanthol and a group of other isobutylamides. The flower heads and root part of the plant have been known to be especially rich in the active principle content (Nayak 2002). Although, some fragmentary studies have been carried out on the larvicidal/insecticidal activity using the flower head extract of S. acmella Murr. Against Anopheles spp.(Pendse et al. 1945), *A. stephensi* Liston and *C. quinquefasciatus* (Saraf and Dixit 2002), *S. mauritiana* against *Aedes* (Jondiko 1986) and *S. oleraceae* against *Helicoverpa zea* (Ramsewak et al.

1999), but to date, none of the aforesaid work reports the extensive analysis of bioefficacy and comparative analysis of these species (for selection of elite species) against mosquito vectors to achieve 100% mortality with minimum doses.

MATERIALS AND METHODS

Collection of plant material:-

Fresh mature flower heads of S. paniculata were harvested randomly from rural areas of Kulti (latitude 23°43'60N and longitude 86°50'60E), West Bengal, India, from mid December to mid January.

Preparation of extract:-

Fresh mature flower heads harvested, rinsed with distilled water and dried in the shade at room temperature (20 °C), and milled into a fine powder with a Jankel and Kunkel model A10 mill. To determine the efficacy of different extractants, 25 g of finely ground flower heads were plunged in different 250 ml solvents of analytical grades (Merck) of varying polarity: petroleum ether, benzene, chloroform: methanol (1:1 v/v), acetone, and absolute alcohol with vigorous shaking (Kotze and Eloff, 2002). The extracted liquid was subjected to rotary evaporation in order to remove the chemicals. The semisolid extract produced was kept in a deep freeze at –80 °C (REVCO model No. ULT 790-3-V32) overnight and then subjected to freeze drying for 24 h at –60 °C. Then the extract was stored in an air-tight container at 4°C in a refrigerator for further use. The dried residues were weighed and dissolved in suitable volumes of distilled water to make different concentrations. The total yield of each extract from 25 g of flower was as follows: petroleum ether extract, 1.26 g; benzene extract, 2.38 g; chloroform: methanol (1:1, v/v) extract, 4.33 g; acetone extract, 3.00 g; and absolute alcohol extract 2.36 g.

Larvicidal bioassay:-

The larvicidal bioassay followed the World Health Organization (WHO) standard protocols [12] with slight modifications. Each of the concentrations of flower head extract (0.1–0.5%) was transferred into sterile glass Petri dishes (9 cm diameter/150 ml capacity). Ten third instar larval form of *Culex quinquefasciatus* were separately introduced into different Petri dishes containing graded concentrations and the mortality were recorded after 1, 2 and 3 hours of the exposure period. The data of mortality in 2 and 3 hours were expressed by the addition of the mortality at 1 and 2 hours, respectively. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated three times and conducted under laboratory conditions at 25–30°C and 80–90% relative humidity. Similar types of bioassay were conducted with different polar and non-polar solvent extracts (concentrations of 50, 25 and 15 ppm) of the flower

heads and with a chemical insecticide, Malathion, on third instar larval forms, and petroleum ether extract on first and fourth instar larval forms. A Student's *t* test was performed to find the significance between the concentration of plant extract and mortality at different periods with different instars. Statistical analysis of the experimental data was performed using the computer software Statplus 2006 and MS EXCEL 2002 to find the LC50, regression equations (*Y* = mortality; *X* = concentrations) and regression coefficient values.

RESULTS

The results of the present study indicate that the mortality rates at a 0.5% concentration were highest amongst all concentrations of the aqueous extracts tested for larval mortality and it was significantly higher ($p < 0.05$) than the mortality rates at 0.1%, 0.2%, 0.3% and 0.4% concentration of aqueous plant extract at 1, 2 and 3 hours of exposure (Table 1). The mortality of the same instar larval form with petroleum ether extract amongst different polar and non-polar solvent extracts is presented in Table 2. The larvicidal potentiality of petroleum ether extract was further tested for first and fourth instar larvae: the highest mortality was recorded at 15 ppm for first instar larvae and it is statistically more significant ($p < 0.05$) than both the 5 and 10 ppm concentrations and at both 2 and 3hours of exposure (Table 3). However, no significant difference was recorded for fourth instar larvae between 15 and 10 ppm concentrations and 15 and 5 ppm concentrations. An absolute mortality (100%) was observed within 1 hours during the exposure to the chemical insecticide, Malathion (5 ppm concentration). The results of regression analysis revealed that the mortality rate (Y) is positively correlated with the period of exposure (X) having a regression coefficient close to one in each case (Table 4). The results of log probit analysis (95% confidence level) revealed that LC50 values gradually decreased with the exposure periods having the lowest value at 3 hours of exposure to third instar larvae, followed by first and fourth instar larvae.

Table 1: The larvicidal activity (mean mortality ± standard error) of different concentrations of aqueous extract of the flower heads of *S. paniculata* on third instar larvae of *C. quinquefasciatus*. Student's *t*-test *t* = 29.42*, 5.5*, 17.0* (between 0.5% and 0.1%) 12.43*, 3.32*, 14.0* (between 0.5% and 0.2%) and 1.73*, 4.33*, 4.0* (between mortality in 0.5% and 0.3% plant extract at 1, 2 and 3 hours bioassay); * denotes significant ($p < 0.05$); table value = 2.92 at five degrees of freedom. M, mortality (%); SE, standard error.

	Period of exposure (hours)		
Concentration (%)	1	2	3
0.1	20 ± 5.77	26.67 ± 8.67	30 ± 8.81
0.2	30 ± 7.69	36.67 ± 5.77	40 ± 7.69
0.3	60 ± 5.57	70 ± 1.92	73.33 ± 3.84
0.4	66.66 ± 1.92	70 ± 1.92	76.66 ± 5.57
0.5	76.66 ± 1.92	86.66 ± 5.77	90 ± 1.92

Table 2: Efficacy of different concentrations of petroleum ether solvent extracts of the flower heads of *S. paniculata* on third instar larvae of *Culex quinquefasciatus.* M, mortality (%); S, survivality (%).

Type of solvents	Concentrations (ppm)	Period of exposure (hours)					
		1		2		3	
		M	S	M	S	M	S
Petroleum ether	50	70	30	73.33	26.66	76.66	23.33
	25	53.33	46.66	56.66	43.33	56.66	43.33
	15	40	60	43.33	56.66	43.33	56.66

Table 3: The larvicidal potentiality (mean mortality ± standard error) of different concentrations of petroleum ether extract of the flower heads of *S. paniculata* and a synthetic insecticide, Malathion, on first and fourth instars larvae of *C. quinquefasciatus*. For first instar larvae: t = 2.07NS, 3.14*, 7.56* (between 15 and 10 ppm at 1,2 and 3 hours); t = 5.2*, 26.62*, 13.99* (between 15 and 5 ppm at 1,2 and 3 hours). For fourth instar larvae: t = 2NS, 1.99NS, 0.91NS (between 15 and 10 ppm at 1,2 and 3 hours); t = 1.73NS, 1.89NS, 1.82NS (between 15 and 5 ppm at 1,2 and 3 hours). * denotes significant ($p < 0.05$); NS, not significant ($p > 0.05$). Table value = 2.92 at five degrees of freedom. M, mortality (%); SE, standard error.

Instars of mosquito larvae	Concentrations (ppm)	Period of exposure (hours)		
		1	2	3
First	15	60 ± 1.92	66.67 ± 5.67	70 ± 2.93
	10	40 ± 5.57	43.33 ± 3.84	46.66 ± 3.84
	5	40 ± 5.57	40 ± 5.67	53.33 ± 1.92
	Malathion (5 ppm)	100 ± 0.00	100 ± 0.00	100 ± 0.00
Fourth	15	15 40 ± 1.92	43.33 ± 3.84	46.66 ± 5.57
	10	33.33 ± 3.84	40 ± 1.92	43.33 ± 5.56
	5	30 ± 5.57	33.33 ± 2.93	36.67 ± 2.84
	Malathion (5 ppm)	100 ± 0.00	100 ± 0.00	100 ± 0.00

Table 4: Log probit analysis of the larvicidal activity of petroleum ether extract of the flower heads of *S. paniculata* on different instar larvae of *C. quinquefasciatus*. LC, lethal concentration; *R*, coefficient of regression equations.

Type of instars of mosquito larvae	Period of bioassay (hours)	Regression equations	R^2	LC_{50} values (ppm)	Lower and upper fiducidal limits (ppm)
First	1	$Y = 29.82 + 0.820x$	0.97	22.06	16.05–27.66
	2	$Y = 33.16 + 0.820x$	0.97	19.58	14.36–24.20
	3	$Y = 31.19 + 0.923x$	0.98	19.19	13.97–23.45
Third	1	$Y = 29.83 + 0.820x$	0.97	11.67	8.49–14.84
	2	$Y = 33.16 + 0.820x$	0.97	9.54	6.82–12.25
	3	$Y = 31.19 + 0.923x$	0.98	5.97	2.15–9.79
Fourth	1	$Y = 25.98 + 0.282x$	0.99	49.84	44.53–54.77
	2	$Y = 31.19 + 0.256x$	0.82	21.22	13.30–29.13
	3	$Y = 34.53 + 0.256x$	0.82	21.02	15.97–73.94

DISCUSSION

Nowadays, mosquito control is mostly directed against larvae and only against adults when necessary. This is because the fight against adult is temporary, unsatisfactory and polluting for the environment, while larval treatment is more localized in time and space resulting in less dangerous outcomes. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified (Howard et al.2007). The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities. Most studies report active compounds as steroidal saponins. Saponins are freely soluble in both organic solvents and water, and they work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which is the most probable reason for larval death (Hostettmann et al.). Saponin extracted from the fruit of *Balanites aegyptica* showed 100% mortality against larvae of *S. aegypti* (Wiesman and Chapagain 2005) The larvicidal property of a saponin mixture isolated from *Cestrum diurnum* was also evaluated against *Anopheles stephensi* mosquito (Ghosh and Chandra 2006). The impact of phenolic compounds on the mosquito larvae has also been reported by many authors (Tripathi and Rathore 2001, Marston et al. 1993). Aluminum chloride obtained from alder leaf, known for its phenolic complexing activity, is also reported to have the larvicidal activity against *S. aegypti* (David et al. 2000) Isoflavonoids from tubers of *Neorautanenia mitis* had a larvicidal effect against the malaria and filariasis transmitting mosquitoes, *Anopheles gambiae* and *Cx. quinquefaciatus*, respectively (Joseph et al. 2004). Essential oils extracted from Brazilian plants exhibited larvicidal activity against *S. aegypti*, with LC50 values ranging from 60 to 538 ppm (Cavalcanti et al 2004). Studies with *Lippia sidoides* (Carvalho et al. 2003) and *Cymbopogon citrates* (Sukumar et al.1991) essentials oils suggested that they are a promising biocontrol agent against *S. aegypti*. D-pinitol, from the EtOH extract of *Acacia nilotica*, which showed larvicidal activity (Rohini et al. 2005). Alkaloids derived from *Piper longum* fruit (Lee 2000) and *Triphyophyllum pellatum* (Francois et al. 1996) showed larvicidal activity against *C. pipiens and A. stephensi*, respectively. Tannin compounds extracted from *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrate* that causes mortality in *Cx. quinquefasciatus* larvae (Khanna and Kannabiran 2007). The present study indicates that flower heads of *S. paniculata* had biocontrol activity against *C. quinquefasciatus*. The highest mosquitocidal activity was noted in petroleum ether extract.

CONCLUSION

In conclusion, *S. paniculata* offers promised as a potential bio control agent against *C.quinquefasciatus* particularly in its markedly larvicidal effect. The biocontrol potentiality was lower than chemical insecticides such as Malathion. The extract or isolated bioactive phytochemical from the plant could be used in

stagnant water bodies which are known to be the breeding grounds for mosquitoes. Product used to combat and protect from mosquitoes in a control program. Further field trials are in progress for making this technology viable on a large scale to eradicate this recurrent problem. Besides, the active crude extract has already been separated into different fractions through column chromatography. Their bioassays, further isolation (through thin-layer chromatography [TLC] and preparatory TLC) and characterization of the larvicidal compounds (through nuclear magnetic resonance) is in progress.

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