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RESEARCH ARTICLE

SECONDARY METABOLITES OF *SPILANTHES CALVA* AND THEIR EFFECT ON MOSQUITO LARVAE

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ABSTRACT

Bioinsecticides have several advantages over chemical insecticides in the control of major vector borne diseases. The secondary metabolites from plants are generally used as bioinsecticides. The aim of the present work is to evolve a biological or ecofriendly control over the mosquitoes using flower extracts from *Spilanthes calva* by evaluating the effect of phytochemicals in the survival of mosquito larvae. Both aqueous and solvent extracts were tested (LC_{50}) against the mosquito larvae. The concentration of the extract used was in the range of 1-120ppm.Column chromatography was used for the isolation of crude extract. Each fraction was analyzed using TLC. From the results it was seen that petroleum ether fraction is more effective in controlling the mosquito larvae (*Culex quinquefasciatus*).

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INTRODUCTION

Mosquitoes are the vectors of the major public health problem. Of all the insects that transmit diseases, mosquitoes represent the greatest menace. The mosquito Culex quinquefasciatus act as a vector for Wuchereria bancrofti responsible for Filariasis (Kamaraj *et al*., 2011). One of the methods available for the control of mosquitoes is the use of larvicides, which is one of the oldest methods of controlling malaria. Among other advantages, use of larvicides controls mosquitoes before they are able to spread and transmit diseases. While other methods like adult spraying may have direct effects like visible protection of populations and may show quick results, larval control has yielded several successes than adult mosquito control (Omena *et al.,* 2007). Due to the disadvantages associated with synthetic pesticides, including development of pesticide resistant strains, ecological imbalances and harm to non-target organisms, there is a renewed effort to develop substances of plant origin which are considered to be more environmentally friendly due to their innate biodegradability and lower toxicity to most organisms (Ghosh *et al.,* 2008). Many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active constituents, which accounted for various uses by man.

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The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins (Nair, 2002). Mosquito control strategies, especially those that are effective, cheap and environmentally non-hazardous are needed. Hence, crude plant extracts have played an important role in this aspect. So the present study was an attempt to find new larvicidal products from the extracts of *Spilanthes calva* to control the filarial vector Culex quinquefasciatus.

MATERIALS AND METHODS

Plant collection and processing

The plant materials (*Spilanthes calva*) were collected from different localities in Mahatma Gandhi University Campus, were taken from healthy plant parts (flower) free from dust, dirt and other impurities and were brought to the laboratory for subsequent processing. *Spilanthes calva* is an indigenous herb belonging to the family compositae.It grows as an annual herb throughout the tropics. It has conical small flowers. The entire plant (root, stem, leaf and flower) is medicinally active and nontoxic to humans. The plant owes its activity to the antiseptic alkaloid spilanthol, as well as immune stimulating alkylamides. The herb is also strong anti bacterial activity. The flowers are chewed to relieve toothache and the crushed plant used in rheumatism. Leaves are used externally in treatment of skin diseases and it also eaten in raw as vegetable by many tribes of India. About 60 species of *Spilanthes calva* have been reported (Chakraborthy *et al.,* 2004).

Preparation of crude aqueous extracts

The collected plants were washed thoroughly with water to remove the sand and dust particles adhering to the plant parts. These washed plant materials (flower) were chopped properly and kept in clean trays. For the preparation of extracts, a known weight of plant (20gm) was taken, cut into small pieces and separately macerated in tap water and ground in a homogenizer. The extract was filtered and the filtrate was made upto 1000 ml with distilled water and retained as stock solution for further experimentation. Serial dilutions of the stock solutions were prepared for assessing treatment efficiencies.

Extraction of Phytochemicals

Soxhlet extraction

The washed plant materials were chopped properly and dried at controlled temperature (< 50^0C) in clean trays. After 48 to 96 hrs, the materials were dried and ready for further processing. The pulverized materials were continuously extracted in petroleum ether, chloroform and methanol using soxhlet apparatus. The extraction was done nearly 8 to 10 hrs at controlled temperature 50^0C. The extract was dried and the stock solution was prepared from the residue.

Isolation of Phytochemicals

Column chromatography was used for the isolation of crude extract. Each fraction was analyzed using TLC (Pavia etal 1976).

Larval Screening and Rearing

Mosquito larvae, collected from controlled breeding sites maintained with coconut shells, broken earthen pots, plastic containers etc kept at varying distances round households were used in the present study. The collected larvae were pooled in the laboratory and subjected to species level identification using standard manual (Christophers, 1933). The larvae were reared in tap water and fed with dog biscuits and yeast at the ratio of 3:1.

Larvicidal Bioassay

Bioassay for the larvicidal activity was carried out using WHO (1981) procedure with slight modifications. To the treatment set, varying concentrations of the crude plant extracts and separated phytochemicals (1, 5, 10, 20,40,60,80,100 and 120 ppm) were added from the stock solution. A control was also maintained for the treatment set. Twenty five early third instar larvae were introduced into treatment trays containing 250 ml of their natural growth media (Tap water). Mortality counts of larvae were monitored at regular intervals i.e. 6, 12,24,48,72 and 96hours after treatment and the control mortality was corrected using Abbott's (1925) formula when the control mortality ranged between 5-20 per cent,

$$\text{Per cent mortality} = \frac{\%\ \text{Mortality in treated} - \%\ \text{Mortality in control}}{100 - \%\ \text{Mortality in control}} \times 100$$

Statistical analysis

The mortality observed (mg/ml) was corrected using Abbott's formula during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed with MS Excel 2007 to find the Standard deviation and LC_{50} using Regression method (Probit analysis, Finney, 1971).

RESULTS AND DISCUSSIONS

The efficacy of aqueous flower extract of *Spilanthes calva* against Culex quinquefasciatus at 96 hrs generally found that highest larval mortality was observed in highest concentrations (500 and 250ppm) i.e. 16.667±40.825% at 6 h of exposure time, whereas it was lowest in the lower concentrations (120and100ppm) i.e. 47.333±39.631, 58.667±25.633 at 72 and96 hrs of time exposure. The control did not show any mortality (Table 1 & Fig.1).

Table 1. Efficacy of aqueous crude flower extracts of *Spilanthes calva* against *Culex quinquefasciatus*

Conc.(ppm)	Time(Hour)						*n=25	
	6	12	24	48	72	96	Mortality%	Mean±S.D
Control	0	0	0	0	0	0	0	0±0
500	100	0	0	0	0	0	100	16.667±40.825
250	100	0	0	0	0	0	100	16.667±40.825
120	64	60	60	100	0	0	71	47.333±39.631
100	24	44	52	64	68	100	56.667	58.667±25.633

*n = number of larvae used for bioassay test

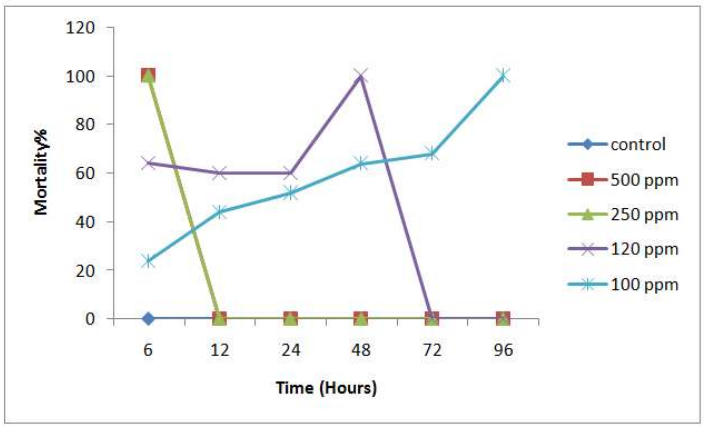

Figure 1. Mortality percentages of mosquito larvae exposed to aqueous crude plant extracts after 96 hrs treatment

According to the data obtained, petroleum fraction of *Spilanthes calva* (80±33.41855772) show more activity and highly lethal to the larvae exhibiting the LC_{50} value was 1.64ppm (Table 2). In hexane the LC_{50} value was 2.18ppm, following chloroform (3.92) and methanol (5.64).

In hexane the LC_{50} value was 2.18ppm, following chloroform 3.92ppm and methanol 5.64ppm.The initial screening of the petroleum ether crude extract by TLC showed the presence of 10 compounds. The results indicated that the active constituents were responsible for exhibited larvicidal activity.

Table 2. Mortality percentage of mosquito larvae exposed to phytochemical extracts of *Spilanthes calva* at different time duration

Conc. (ppm)	120	100	80	60	40	20	10	5	1	Control	Mortality%	LC_{50}
Time	24hr	24hr	48hr	72hr	72hr	78hr	78hr	96hr	96hr	96hr	Mean±S.D	
Hexane	100	100	100	100	100	80	68	32	0	0	68±39.7592756	2.18
Petroleumether	100	100	100	100	100	100	88	80	32	0	80±33.41855772	1.64
Chlorofm	100	100	100	96	80	68	0	0	0	0	54.4±45.43830983	3.92
Methanol	100	100	100	96	80	60	32	4	0	0	57.2±41.87791781	5.64

Table 3.TLC of flower extract of *Spilanthes calva*

Crude extracts	Mobile phase	Number of spots	Rf value
Hexane	Hexane: Ethyl acetate(2:1)	8	0.13,0.20,0.25,0.29,0.37,0.54,0.82,0.92
Petroleum ether	Hexane: Ethanol(2:1)	10	0.15,0.16,0.36,0.38,0.41,0.6,0.68,0.72,0.77,0.8
Chloroform	Chloroform: Methanol(3:1)	5	0.14,0.17,0.19,0.46,0.93
Methanol	Ethyl acetate: Ethanol(2:1)	4	0.18,0.41,0.61,0.94

The present study revealed that plant extracts could be effectively utilized for the control of mosquito menace. The efficacy of the plant extracts was already reported by Kalyanasundaram and Das (1985), Mathai and Devi (1992), Latha and Vijayakumar *et al*. (1998), Kamali (2001), all of whom has, but used different plant extracts. Similar studies conducted by Jaswanth *et al*. (2002) Mehra and Hirdhar (2002) showed that methanolic extract of *Annona squamosa* and acetone extract of *Tridax procumbens* possess effective potential larvicidal activity. Since the plants studied in the present experiment are perennially available in large quantity with ease and little coast, the result of the present study have opened up the prospects for the large scale extraction of the active ingredients for effective mosquito larvicidal control. The fact that non-target organisms in the ecosystem have been little affected by the application of these plant extracts emphasises the significance and utility of the present study. The identification of the effective plant species will be the first step in the development of larvicides in mosquito control. *Spilanthes calva,* offer great promise as source of phytochemicals for the control of mosquitoes. Isolation of the active principle of these plants may prove useful in the development of safer bioinsecticides.

The crude extracts of hexane, petroleum ether, chloroform, and methanol were prepared.TLC was used for the initial screening of the extracts. The number of spots obtained and the corresponding Rf values are depicted in Table.3.To isolate the individual compounds the hexane crude extract was run through column. Fractions were collected and further were run again. The collected fractions were tested for larvicidal activity. The initial screening of the petroleum ether crude extract by TLC showed the presence of 10 compounds. The results indicated that the active constituents (petroleum ether fraction) were responsible for larvicidal activity.

Conclusion

The present study revealed that *Spilanthes calva* could be effectively utilized for the control of mosquitoes. The petroleum ether fraction was highly lethal exhibiting the LC_{50} value 1.64ppm.

The flower of *Spilanthes calva* suggests that this plant deserves further pharmacological and phytochemical investigation to identify the active constituents was responsible for the exhibited larvicidal activity.

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