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RESEARCH ARTICLE

MOSQUITO LARVICIDAL ACTIVITY OF INDIGENENOUS PLANT EXTRACTS AGAINST *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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ABSTRACT

Vector control is an essential requirement in control of epidemic diseases such as malaria, filariasis, dengue, etc. that are transmitted by mosquitoes. Excessive use of synthetic pesticides causes emergence of pesticide resistance and harmful effect on non-target organisms. This has necessitated an urgent search for development of new and improved mosquito control methods that are economical and effective as well as safe for non-target organisms and the environment. Herbal insecticides of plant origin become a priority in this search. The present study is to evaluate the larvicidal activity of both aqueous and solvent extracts of twenty two plants were tested against mosquito larvae (*Culex quinquefasciatus).* Mortality percentages and LC_{50} were calculated. Among plants under experimentation, *Spilanthes calva* and *Adenocalymma alliaceum* were potentially effective**.**

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INTRODUCTION

Blood feeding female mosquitoes are responsible for the biting nuisance and also in the transmission of more diseases than any other group of arthropods and play an important role as etiologic agents of vector-borne diseases (Aarthi and Murugan 2011; Prasad *et al*., 2014). Mosquitoes are one of the most significant vectors in medical point of view and they transmit parasites and pathogens which continue to have a devastating impact on human beings (Mousumi Kundu, *et al.,* 2013). The vector-borne diseases caused by mosquitoes are one of the major health problems in many countries. Several species belonging to genera *Culex, Anopheles* and *Aedes* are vectors for the pathogens of various diseases viz., filarial fever, Japanese encephalitis, malaria, dengue and chickungunya (Murugesan Sakthivadivel *et al.,* 2014). The most efficient approach to control the vector is to target the immature stages of the life cycle. A primary element in the current global strategy for the control of vector-borne diseases is vector control; with chemical control remaining a main component of integrated vector management (Anjali Rawani *et al.,* 2012).

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The indiscriminate use of those synthetic chemicals unequivocally produced different adverse consequences on soil, water and air ecosystem, creating undesirable effects like toxicity to non-target organisms, human health and ultimately posing threat to/ for global environment (Govindarajan and Mathivanan, 2011; Govindarajan *et al.,* 2011). In addition, development of resistance in vectors against these widespread chemical insecticides results in rebounding of vectorial capacity thus was reducing the effective efficacy of those chemicals. It is established that repeated use of synthetic larvicides results in the development of resistance in mosquitoes, and hence effective ecofriendly phytochemicals are the need of the day. Plant world comprises a store house of biochemical that could be tapped for use as insecticides and they are the richest source of renewable bioactive phytochemicals (Samuel Tennyson *et al.*, 2015). The use of different parts of locally available plants and their various products in the control of mosquitoes have been well established globally by numerous researchers. In all probability, plants used to control insects contained insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects.

(Anjali Rawani *et al.,* 2012). The present study was taken up to evolve a biological or eco-friendly control over the mosquitoes using plant extracts by evaluating the effects of phytochemicals on the survival of *Culex quinquefasciatus*.

MATERIALS AND METHODS

Plant collection and processing

Plant species were collected from different localities based on their local availability and reported medicinal properties. Selection of plants was based on the strong aromatic character of leaves and stem and their medicinal property. The materials were collected from the plants in field located in Mahatma Gandhi University Campus, were taken from healthy plants free from dust, dirt and other impurities and were brought to the laboratory for subsequent processing.

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 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 Method

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.

Direct Solvent Method

The extracts were prepared with different solvent media such as petroleum ether, chloroform, and Methanol. The plant parts were homogenized with measured quantity of media set up over night. The solvent was evaporated in a water bath and the residue was dried in a dessicator. Afterwards it was redissolved in acetone / tap water.

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 (ie. 40, 30, 20, 10 and 5 ml) were added from the stock solution. To study the synergistic effect equal quantity of two different phytochemical extracts were dissolved in water/ acetone to prepare various doses. 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,

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

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 DISCUSSION

The present study has been carried out to assess the lethal properties of both aqueous and solvent extracts of twenty two species of plants belonging to various families on mosquito larvae. Details of plants used for the present study and larval mortality were noticed Table 1. The effect of various plant extracts on mosquito larvae exposed to 96 hours, for confirming lethality as per WHO (1981) standards is depicted (Table 2, 3, 4 & Fig.1).

Out of twenty two plant species, only seven could induce mortality of mosquito larvae. The rest fifteen plant species did not exhibit any larvicidal property (Table 1). Of the seven test positive plants, two plants (*Spilanthes calva and Adenocalymma alliaceum*) showed more larvicidal activity as compared to others and they were selected for the extraction of phytochemicals. Aqueous extracts of *Allium sativum*, *Spilanthes calva* and *Adenocalymma alliaceum* showed more larvicidal activity as compared to *vitex negundo*, *Aeagle marmelos*, *Carica papaya*, and *Lantana camara* (Table 2). In soxhlet method the methanol (87.2±15.47) and petroleum ether (73.6±28.24) fraction of *Spilanthes calva* were highly lethal to the larvae exhibiting a mortality rate of 100% at a lower concentration. Chloroform fraction did not show any lethal activity.

Table 1. List of plant species used for the preparation of aqueous extracts and their impact on Mosquito larvae (*Culex quinquefasciatus*)

S. No	Name of plant	Family	Part used	Larval mortality
1	*Acalypha indica*	*Euphorbiaceae*	Leaf	No Mortality
2	*Adenocalymma alliaceum*	*Bignoniaceae*	Leaf	Mortality in 6 hrs
3	*Aeagle marmelos*	*Rutaceae*	Leaf	Mortality in 48 hrs
4	*Aerva lanata*	*Amaranthaceae*	Leaf	No Mortality
5	*Alistonia scolaris*	*Apocynaceae*	Leaf	No Mortality
6	*Allium sativum*	*Amaryllidaceae*	Pulp	Mortality in 12hrs
7	*Allium cepa*	*Amaryllidaceae*	Pulp	No Mortality
8	*Bauhinia purpurea*	*Fabaceae*	Aerial part	No Mortality
9	*Capscium annuum*	*Solanaceae*	Leaf	No Mortality
10	*Carica papaya*	*Caricaceae*	Leaf	Mortality in 48 hrs
11	*Cassia alata*	*Fabaceae*	Leaf	No Mortality
12	*Cassia fistula*	*Fabaceae*	Leaf	No Mortality
13	Chrysanthemum *indicum*	*Asteraceae*	Leaf, flower	No Mortality
14	*Citrus limonum*	*Rutaceae*	Tender leaf	No Mortality
15	*Cucurbita pepo*	Cucurbitaceae	Aerial part	No Mortality
16	*Cymbopogon citratus*	*poaceae*	Leaf	No Mortality
17	*Eichhornia crassipes*	*Pontederiaceae*	Bulb	No Mortality
18	*Lantana camara*	*Verbenaceae*	Aerial part	Mortality in 48hrs
19	*Leucas aspera*	Lamiaceae	Aerial part	No Mortality
20	*Macaranga peltata*	*Euphorbiaceae*	Leaf	No Mortality
21	*Spilanthes calva*	*Compositae*	Leaf, flower	Mortality in 6hrs
22	*Vitex negundo*	*Verbenaceae*	Leaf	Mortality in 6hrs

Table 2. Mortality percentages of mosquito larvae exposed to aqueous crude plant extracts after 96 hrs treatment

S. No.	Name of plants	Concentration of crude plant extracts (mg/ ml)					Mean±SD
		Control	500	250	100	50	
1	*Lantana camara*	Nil	48	0	0	0	12±20.78
2	*Allium sativum*	Nil	100	100	60	24	71±31.67
3	*Vitex negundo*	Nil	52	0	0	0	13±22.52
4	*Spilanthes calva*	Nil	100	100	100	100	100±0
5	*Carica papaya*	Nil	8	0	0	0	2±3.46
6	*Aeagle marmelos*	Nil	8	0	0	0	2±3.46
7	*Adenocalymma alliaceum*	Nil	100	100	100	100	100±0

Table 3. Mortality percentages of mosquito larvae exposed to phytochemical extracts of *Spilanthes calva* and *Adenocalymma alliaceum* after 96 hrs treatment. (Soxhlet Method of Extraction)

Plant	*Solvent*	Concentration of plant extracts (mg / ml)						Mean±SD	LC_{50}
		Control	50	40	30	20	10		
	Petroleum Ether	Nil	100	100	80	64	24	73.6±28.24	1.75
Spilanthes calva	Chloroform	Nil	0	0	0	0	0	0±0	0
	Methanol	Nil	100	100	96	80	60	87.2±15.47	1.63
	Petroleum Ether	Nil	100	88	60	52	0	60±34.78	1.84
Adenocalymma	Chloroform	Nil	0	0	0	0	0	0±0	0
alliaceum	Methanol	Nil	0	0	0	0	0	0±0	0

Table 4. Mortality percentages of mosquito larvae exposed to phytochemical extracts of *Spilanthes calva* and *Adenocalymma alliaceum* after 96 hrs. (Direct Solvent Method)

Plant	*Solvent*	Concentration of plant extracts (mg / ml)					Mean±SD	LC_{50}
		Control	40	30	20	10		
	Petroleum Ether	Nil	100	80	64	0	61±37.46	1.77
	Chloroform	Nil	0	0	0	0	0±0	0
Spilanthes calva	Methanol	Nil	100	80	56	0	59±37.46	1.79
	Petroleum Ether	Nil	88	60	52	0	50±31.81	1.84
	Chloroform	Nil	80	68	0	0	37±37.24	1.87
Adenocalymma alliaceum	Methanol	Nil	0	0	0	0	0±0	0

The petroleum ether fraction of *Adenocalymma alliaceum* was highly lethal to the larvae exhibiting a mortality rate of 100% at a lower concentration (60±34.78), but Chloroform and methanol fractions did not show any lethal activity (Table 3). In direct method of extraction, both alcohol and petroleum ether extracts of *Spilanthes calva* showed 100% larvicidal activity at 40 mg/ml.

The petroleum ether fraction of *Adenocalymma alliaceum* exhibited more activity than chloroform fraction i.e., 88% of mortality at 40 mg/ ml but in the latter it was 80% at 40 mg/ ml (Table 4). The LC_{50} estimate for the promising plants like *Spilanthes calva* and *Adenocalymma alliaceum* were noted (Table 3 & 4). In synergic effect the larval mortality rate was 100% in 20 mg/ ml (Figure 3).

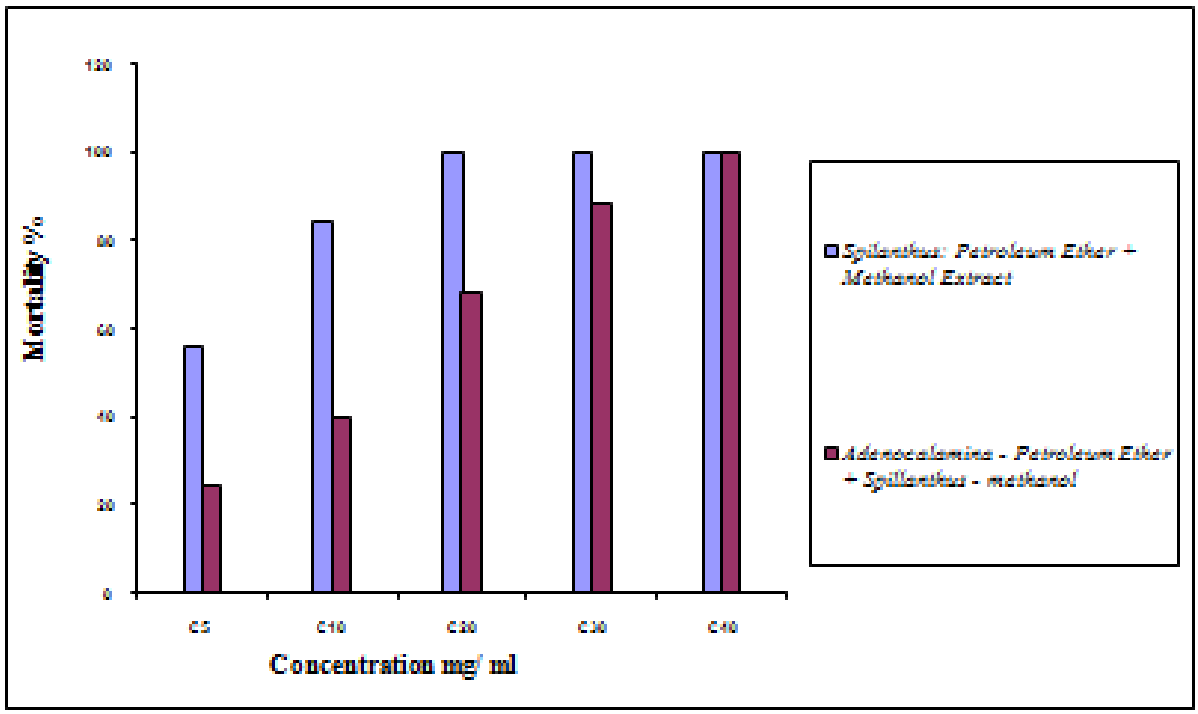

Fig.1. Synergic effect on the Mortality percentages of Mosquito larvae (Soxhlet Method of extraction)

According to the data obtained, the Soxhlet method is more effective than direct solvent method. Both *Spilanthes calva* and *Adenocalymma alliaceum* were highly lethal to the larvae exhibiting mortality rate and the former is found to be more effective. 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 Sukumaran (1997), Murugan and Jayabalan (1999), Mehra and Hiradhar (2002), Kalyanasundaram and Das (1985), all of whom have, but used different plant extracts. Similar studies conducted by Sing *et al.* (2002), Mital *et al*. (2002) and Reena and Ramakrishnan (1997) showed that acetone extracts 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. The two species, *Spilanthes calva,* and *Adenocalymma alliaceum* offer great promise as source of phytochemicals for the control of mosquitoes. Of the two *Spilanthes calva* is more active than *Adenocalymma alliaceum*. Isolation of the active principle of these plants may prove useful in the development of safer bio-insecticides. It was also observed a differential larvicidal activity for the different solvent extracts prepared. Though the plant extracts were found to be effective, their utility in the field was tried.

Conclusion

The present study revealed that plant extracts could be effectively utilised for the control of mosquitoes.

Out of twenty two plants tried only seven could induce mortality of mosquito larvae. Of the seven plant species, only two species (*Spilanthes calva* and *Adenocalymma alliaceum*) are potentially effective against mosquito larvae. According to the observation, extracts obtained by Soxhlet method are more effective than those prepared by direct solvent method. Both *Spilanthes calva* and *Adenocalymma alliaceum* were highly lethal to the larvae exhibiting mortality rate and the former is found to be more effective. So the *Spilanthes calva* offer great promise as a source of phytochemicals for the control of mosquitoes. Isolation of the active principle of plants may prove useful in the development of safer bio-insecticides for the future.

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