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Research Article

Larvicidal Activity of Methanol and Chloroform Extract of *Swertia celiata* against Three Mosquito Vectors

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Abstract

Background: Mosquitoes are an important public health concern as they spread life-threatening diseases such as malaria, filaria, Japanese encephalitis, dengue fever, chikungunya, and yellow fever. In the last decades, synthetic insecticides were extensively used for the control of these vector-borne diseases but it also reported the detrimental side-effects in human beings and pet animals. To overcome the side effects, plants-derived secondary metabolites were screened and tested for insecticidal properties. The present study deals with the insecticidal activity of chloroform and methanol extracts of *Swertia celiata* leaves against *Culex quenquifasciatus*, *Aedes aegypti,* and *Anopheles stephensi* larvae.

Method: The *S. celiata* leaves were subjected to chloroform and methanol with 1:3 (Weight/ Volume) ratio and the extracted solvent was dried using rotary vacuum evaporator. The larvicidal activity of the extract was tested using WHO method and LC_{50} and LC_{90} were evaluated by probit analysis.

Results: The LC_{50} value of chloroform extract of *S. celiata* was found to be 65.288, 67.406 and 71.608 ppm whereas LC_{90} was 184.721, 186.582 and 192.497 ppm against *C. quinquefasciatus, Ae. aegypti and A. stephensi,* respectively. The methanolic extract was also found potent; LC_{50} was 91.503, 101.574 and 99.104 ppm whereas LC_{90} was 230.823, 271.927 and 234.257 ppm against *C. quinquefasciatus, Ae. aegypti* and *A. stephensi,* respectively. Both chloroform and methanol extract were found significantly lethal to the tested mosquito vectors.

Conclusion: Taken results together, chloroform extract showed higher toxicity as compared to methanolic extract against all the tested species. The study clearly revealed that *S. ciliata* extract or bioactive compounds can be used as an alternative to synthetic insecticides.

Keywords: Larvicidal activity, *Swertia Celiata,* Chloroform extract, methanol extract, Mosquito vectors

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Introduction

Mosquitoes are blood-feeding insects and deadly vectors for spreading human diseases such as malaria, filaria, Japanese encephalitis, dengue fever, chikungunya[1] and yellow fever.[2] These vector-borne diseases affect the health and quality of life of millions of people throughout the world.[3] In addition, mosquito bites can cause severe skin irritation through an allergic response to the mosquito's saliva in humans that include local skin and systemic reactions such as angioederma.[4] Vector-borne diseases represent one of the biggest challenges to the current and future human wellbeing. Vector-borne diseases are also becoming a serious health concern for more developed countries[5-8] due to expansion of vectors throughout the world in response to climatic changes.[9-13] The international migration and commercial exchanges are also a prominent region for accidental introduction of vectors or pathogens.[14-17] Mosquitos worldwide threaten the lives of people every year. In 2010, WHO reported 216 million cases of malaria in the world with an estimated 655,000 malaria deaths.[18] An estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis,[19] more specifically in India, around 23 million circumstances of symptomatic filariasis, 31 million microfilaraemics, and about 473 million persons are potentially at risk of infection.[20] Three billion people in the endemic areas are at risk of infection with Japanese encephalitis (JE) and incidence of the disease is 30,000–50,000 cases annually,[21] whereas approximately 1.9 billion people currently live in rural JE-prone areas of the world, the majority of them in China (766 million) and India (646 million).[22] In India, JE is endemic in a few states and highly endemic in a few districts of Tamil Nadu (Southern India).[23] Over 40% of the world's population (approximately 2.5 billion) is at risk from dengue; WHO has estimated 50–100 million dengue infections worldwide, annually.[24] Dengue transmission now occurs in over 120 countries, mostly in the tropical and sub-tropical regions of the world.[25] Moreover, there are estimated 200,000 annual incidences of yellow fever with 30,000 deaths worldwide.[26] The yellow fever is predominantly epidemic in Africa; current estimates of disease burden are 51,000–380,000 per year.[27] Chikungunya also caused more than 2.5 million infections over the past decade and has more recently been spreading in the Americas and emerging in Europe.[28-30]

The most abundant Indian mosquito vector *C. quinquefasciatus,* say, 1823 is a carrier of various deadly diseases, such as West Nile fever, Japanese encephalitis, filariasis, avian malaria, St. Louis encephalitis, and bancroftian filariasis (*Wuchereria bancrofti*).[31] The mosquito *Ae. aegypti* (*Stegomyia aegypti*) is a vector of several globally important arboviruses,[32] including dengue virus (DENV),[33] yellow fever virus,[34] and chikungunya virus (CHIKV).[35] *Ae. aegypti* is predominantly found in artificial containers located in urban regions and exclusively feeds on humans.[36] It is also causing approximately 100 million annual infections throughout the world with half of the population at risk.[37] The *Anopheles stephensi* Liston is a predominant vector of malaria in India,[38] Pakistan and Afghanistan,[39] and south Iran.[40] It is also distributed in Iraq, Saudi Arabia, Oman, South China, Thailand, east of Bangladesh, and Myanmar.[41] To overcome the vector-borne disease burden, various control programs were implemented throughout the world in different time periods. Among these, synthetic insecticides have been used extensively over the past 50 years globally. Due to extensive use of synthetic insecticides in past decades, detrimental side-effects such as neurological effects, respiratory problem, reproductive problem, and cancer in human beings and pet animals was reported.[42-44] Moreover, due to various events such as expansion of genetic resistance,[45, 46] toxicity,[42] high cost, environmental pollutants,[47] and handling hazards[43] have generated worldwide interest in the development of alternative strategies. These include the use of new types of insecticides derived from traditional botanical pest control agents, which are less expensive[48] and comparatively safer to mammals and higher animals.[49] Plants are natural producers of a range of secondary metabolites, some of which have medicinal and insecticidal properties. The chemicals derived from plants have been projected as weapons in mosquito-control programs as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents, and oviposition deterrent.[50] Plant-derived agents belonging to many families have been reported to possess larvicidal properties against *Aedes*, *Anopheles* and *Culex* mosquitoes (Diptera: Culicidae).[51] The present study deals with the larvicidal activity of chloroform and methanol leaf extracts of *S. celiata* against *C. quenquifasciatus*, *Ae. aegypti* and *A. stephensi* larvae and found to be significantly potent.

Materials and Methods

Preparation of Plant Extract

Plant material of *S. celiata* was collected from Garhwal region of the north west Himalaya, India. It was authenticated by Botanical Survey of India, Dehradun. A voucher specimen of the plant was stored in the Institute's herbarium for future reference. Plant material was dried under shade and powdered the leaves. The powdered leaves (1 kg) were subjected to 3 L chloroform and methanol individually for a period of 48 h and extract was filtered through wattman filter paper. The solvent was removed and the extract was concentrated by rotary vacuum evaporator at temperatures of 60°C and 45°C, respectively and the extract was stored at 4°C until used.

Rearing and Maintenance of Test Organisms

The test organism *A. stephensi, C. quenquifasciatus*, and *Ae. aegypti* were reared and maintained in the Entomology

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Laboratory of the National Institute of Malaria Research, Field Unit, Hardwar, India. The culture was free from exposure to pathogens and insecticides, maintained at 26±2°C and 60–80% relative humidity. The hatched larvae were fed with yeast powder and dog biscuits (at the 2:3 ratio) until molting to become pupae. The fourth instar larvae were collected, transferred to plastic bowls and kept inside the mosquito cage for adult emergence.

Larvicidal Bioassay

Larvicidal activities of crude methanol and chloroform leaf extracts of *S. Celiata* were determined in terms of LC_{50} and LC_{90} by using the standard procedure of WHO[52] with slight modification. The early fourth instar larvae (twenty) of *C. quinquefasciatus*, *Ae. aegypti,* and *A. stephensi* were transferred to 500 mL bowls containing 249 mL of de-chlorinated tap water. The extract was dissolved in 1 mL acetone to prepare a serial dilution of test dosage and mixed in 249 mL tap water containing larvae. Three replicates were run simultaneously with different dosages 25–250 µg/mL (ppm) of extract along with control (1 mL of acetone alone to 249 mL of tap water). The bioassay was conducted at room temperature 26±2°C with 60–80% relative humidity, during which time no food was offered to the larvae. Mortality of larvae was recorded 24 h post treatments and evaluated LC_{50} and LC_{90} by probit analysis[53] using StatusPlus 2009 software.

Data Management and Statistical Analysis

Data were arranged in an Excel sheet; statistical analysis of the experimental data was performed using the computer software StatPlus 2009 (AnalystSoft, Canada) to find the lethal concentration against larvae (LC_{50} and LC_{90}) out in 24 h by probit analysis[53] with a reliability interval of 95%. To determine whether there was a statistically significant difference among different doses of methanol and chloroform leaf extracts of *S. celiata* against mosquito larvae, student's t-test was used to analyze the difference of the percentage of mortality. Results with P<0.05 were considered to be statistically significant.

Results and Discussion

Larvicidal agents for mosquito larval control are a major module for controlling vector-borne diseases. Plant extracts as potential larvicides are considered as doable and favored alternatives in the control of the mosquito species. In the present study, larvicidal activity of methanol and chloroform extract of *S. ciliata* was evaluated at different concentrations (range 25–250 ppm) against early fourth instars larvae of *C. quinquefasciatus, Ae. aegypti* and *A. stephensi* after 24 h of exposure.

Larvicidal potential of methanolic extract: The mean percent mortality (±standard error) of the methanol extract of *S. ciliata* at different concentration (25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 ppm) was evaluated and found 5±0.041, 15±0.041, 38.33±0.062, 53.33±0.047, 61.66±0.024, 73.33±0.024, 81.66±0.024, 91.66±0.024, 98.33±0.024 and 100±0.000% of *C. quinquefasciatus,* 5±0.040, 11.66±0.023, 33.33±0.047, 46.66±0.023, 58.33±0.023, 66.66±0.023, 76.66±0.023, 83.33±0.023, 88.33±0.023 and 100±0.000% of *Ae. aegypti* and 3.33±0.024, 11.66±0.024, 30±0.041, 40±0.041, 51.65±0.024, 68.33±0.062, 81.66±0.024, 88.33±0.024, 96.66±0.024 and 100±0.000% of *A. stephensi*, respectively whereas no mortality was recorded in the control experiment (Table 1). On the basis of dose-response, we calculated the LC_{50} and LC_{90} value. The LC_{50} of methanol extract was 91.503, 101.574 and 99.104 ppm with lower control limit (LCL) of 74.468, 82.159 and 82.128 and upper control limit (UCL) of 112.435, 125.577 and 119.590 against *C. quinquefasciatus, Ae. aegypti* and *A. stephensi,* respectively. The LC_{90} of methanolic extract was 230.823, 271.927 and 234.257 ppm with LCL of 187.851, 219.951 and 194.129 and UCL of 283.626, 336.186 and 282.679 against *C. quinquefasciatus, Ae. aegypti* and *A. stephensi,* respectively (Table 3). The data were analyzed using student's t-test and found statistically significant with p values <0.05. Result analysis clearly indicates that methanol extract of *S. ciliata* showed higher potency against *C. quinquefasciatus* and *A. stephensi* as compared to *Ae. aegypti* (Fig. 1). The phytochemicals or crude extracts derived from plant sources also act as a larvicide against mosquito vectors.[54, 55] The methanol extract of *Nelumbo nucifera* has larvicidal activity against *C. quinquefasciatus* with LC_{50} and LC_{90} of 9.51 and 28.13 ppm, respectively.[56]

Larvicidal potential of chloroform extract: The chloroform extract of *S. ciliata* also showed the potential larvicidal property against the tested organism. The mean percent mortality (±standard error) of the chloroform extract at varying concentration 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 ppm and found 15±0.041, 31.66±0.024, 51.67±0.024, 66.67±0.024, 78.33±0.024, 90±0.024, 100±0.000, 100±0.000 and 100±0.000% of *C. quenquifasciatus,* 15±0.041, 30±0.041, 53.33±0.024, 56.66±0.047, 68.33±0.024, 88.33±0.024, 96.33±0.024, 100±0.000 and 100±0.000% of *Ae. aegypti* and 13.33±0.024, 28.33±0.024, 43.33±0.024, 55±0.041, 68.33±0.024, 83.33±0.024, 91.66±0.024, 96.66±0.024 and 100±0.000% of *A. stephensi,* respectively whereas no mortality was recorded in the control (Table 2). The data were analyzed using student's t-test and found statistically significant with p values <0.05. The results clearly indicate that the chloroform extract of *S. ciliata* at very low concentrations was toxic against all the three tested mosquito species (Figs. 1 and 2).

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Table 1.Mean Percent Mortality of Methanol Extract of *S. ciliata* against *C. quenquifasciatus*, *Ae. aegypti* and *A. stephensi* at Different Concentration Ranges

Concentrations (PPM)	% Mortality±SD		
	C. quenquifasciatus	*Ae. aegypti*	*A. Stephensi*
0	0±0.000	0±0.000	0±0.000
25	5±0.041	5±0.040	3.33±0.024
50	15±0.041	11.66±0.023	11.66±0.024
75	38.33±0.062	33.33±0.047	30±0.041
100	53.33±0.047	46.66±0.023	40±0.041
125	61.66±0.024	58.33±0.023	51.65±0.024
150	73.33±0.024	66.66±0.023	68.33±0.062
175	81.66±0.024	76.66±0.023	81.66±0.024
200	91.66±0.024	83.33±0.023	88.33±0.024
225	98.33±0.024	88.33±0.023	96.66±0.024
250	100±0.000	100±0.000	100±0.000

Table 2.Percent Mortality of Chloroform Extract of *S. ciliata* against *C. quenquifasciatus*, *Ae. aegypti* and *A. stephensi* at Different Concentration Ranges

Concentrations (PPM)	% Mortality±SD		
	C. quenquifasciatus	*Ae. aegypti*	*A. stephensi*
0	0±0.000	0±0.000	0±0.000
25	15±0.041	15±0.041	13.33±0.024
50	31.66±0.024	30±0.041	28.33±0.024
75	51.67±0.024	53.33±0.024	43.33±0.024
100	66.67±0.024	56.66±0.047	55±0.041
125	78.33±0.024	68.33±0.024	68.33±0.024
150	90±0.024	88.33±0.024	83.33±0.024
175	100±0.000	96.33±0.024	91.66±0.024
200	100±0.000	100±0.000	96.66±0.024
225	100±0.000	100±0.000	100±0.000

Table 3.LC_{50} and LC_{90} of Methanol Extract of *S. ciliata* against *C. quenquifasciatus*, *Ae. aegypti* and *A. stephensi*

Spp.	Methanol Extract					
	LC_{50}	LCL	UCL	LC_{90}	LCL	UCL
C. quenquifasciatus	91.503	74.468	112.435	230.823	187.851	283.626
Ae. aegypti	101.574	82.159	125.577	271.927	219.951	336.186
A. stephensi	99.104	82.128	119.590	234.257	194.129	282.679

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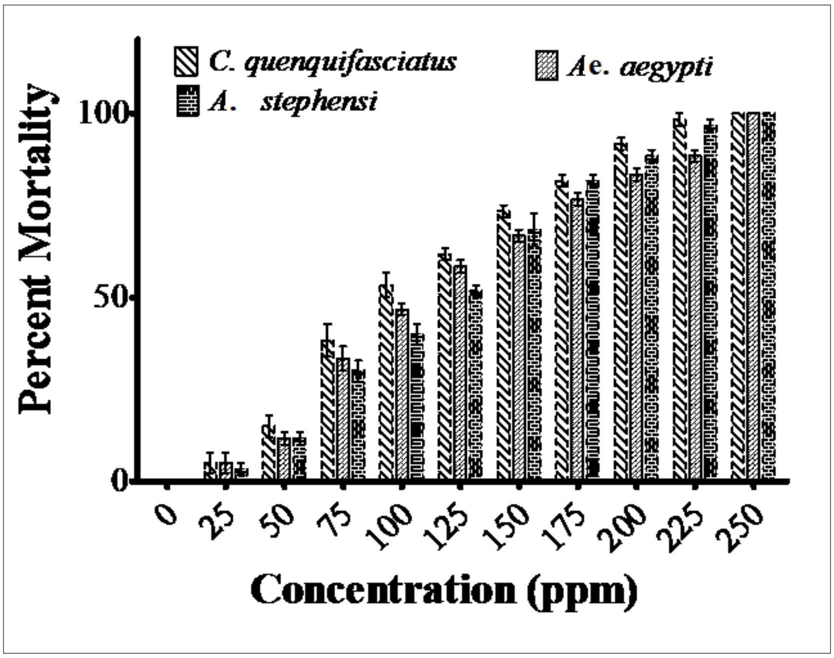

Figure 1.Larvicidal Activity of Methanol Extract of *S. ciliata* against *C. quenquifasciatus*, *Ae. aegypti* and *A. stephensi* at Different Concentration Ranges

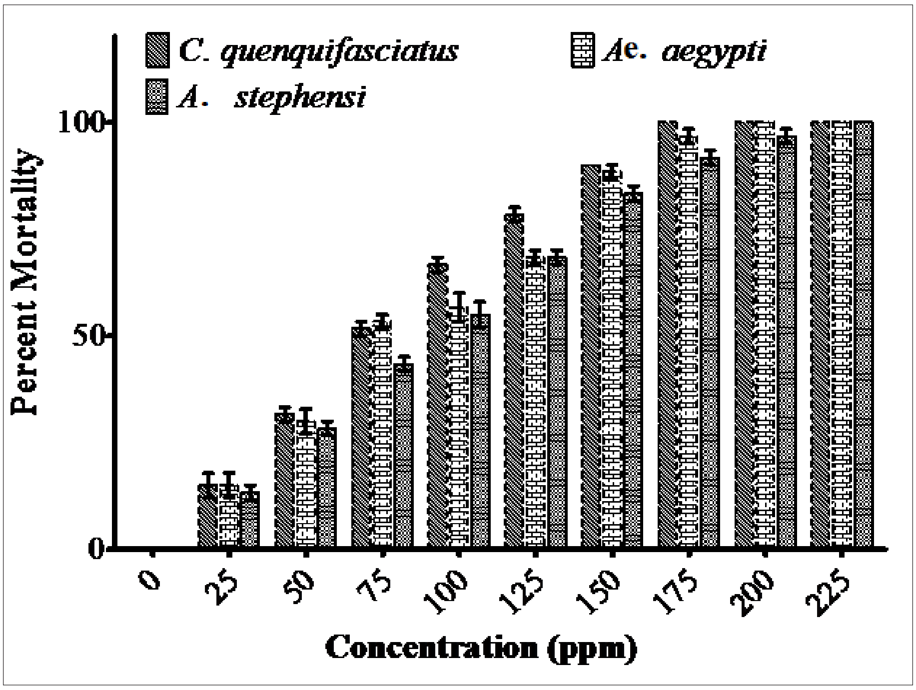

Figure 2.Larvicidal Activity of Chloroform Extract of *S. ciliata* against *C. quenquifasciatus*, *Ae. aegypti* and *A. stephensi* at Different Concentration Ranges

The LC_{50} value of chloroform extract was found to be 65.288, 67.406 and 71.608 ppm with LCL of 51.000, 53.287 and 56.975 and UCL of 83.580, 85.268 and 90.000 against *C. quinquefasciatus, Ae. aegypti and A. stephensi,* respectively. The LC_{90} of chloroform extract was 184.721, 186.582 and 192.497 ppm with LCL of 144.294, 147.498 and 153.160 and UCL of 236.475, 236.021 and 241.935 against *C. quinquefasciatus, Ae. aegypti* and *A. stephensi,* respectively (Table 4).

Table 4.LC_{50} and LC_{90} of Chloroform Extract of *S. ciliata* against *C. quenquifasciatus*, *Ae. aegypti* and *A. stephensi*

Spp.	Chloroform Extract					
	LC_{50}	LCL	UCL	LC_{90}	LCL	UCL
C. quenquifasciatus	65.288	51.000	83.580	184.721	144.294	236.475
Ae. aegypti	67.406	53.287	85.268	186.582	147.498	236.021
A. stephensi	71.608	56.975	90.000	192.497	153.160	241.935

Investigation of the results clearly indicates that chloroform extract of *S. ciliata* showed comperatively higher potency than methanolic extract against *C. quinquefasciatus, Ae. aegypti* and *A. stephensi*. Furthermore, chloroform extract showed higher potency against *C. quinquefasciatus, Ae. aegypti* with respect to *A. Stephensi.* A number of plant extracts have been reported to have mosquito larvicidal activities against mosquito vectors, but few plant products have shown practical utility for mosquito control.[57] The chloroform extract of *Saraca indica* has larvicidal activity against *C. quinquefasciatus* with LC_{50} of 291.5 ppm.[58] Furthermore, chloroform and methanol extracts of *Nyctanthes arbor-tristis* flowers reported larvicidal activity against *A. stephensi* with LC_{50} values of 747.7 and 244.4 ppm.[58]

The *S. ciliate* contains various biologically active phytochemicals such as glucosides (amaroswerin and amarogentin) and C-glucoxanthone mangiferin.[59] Among these, amarogentin have anthelmintic, hypoglycemic and antipyretic properties,[60] whereas mangiferin has anti-tubercular,[61,62] hypoglycemic,[63] anti-inflammatory,[64,65] hepatoprotective,[66] anti-oxidative,[67,68] and antifungal[69] activities. The pharmacological properties of these major compounds revealed that larvicidal properties of *S. ciliate* occur due to these compounds.

Conclusion

The present study clearly revealed that the plant *S. ciliata* chloroform and methanol extract have potential larvicide against mosquito's vector *C. quinquefasciatus, Ae. aegypti* and *A. stephensi*. Crude extract or isolated bioactive compounds from the plant *S. ciliata* could be used in breeding grounds of the mosquitoes and can be used as an alternative against synthetic insecticides.

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Conflict of Interest: None

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