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Evaluation of mosquito larvicidal activity of *Jasminum* species (Oleaceae) crude extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract

India is endemic to mosquito-borne diseases which are major health problems in tropical regions. Chemical pesticides have been used for several decades in controlling pests and vectors of various human diseases but resulted in several problems such as resistance and resurgence of pests, affecting human health and ecosystem being disrupted leading to the threat that their continued use may further harm the environment. This necessitated the need for environmentally safe and biodegradable indigenous method of vector control. Plant products are considered to be a potential alternative approach as they are environmentally safe, target specific and biodegradable. In the present study, the crude hexane and chloroform flower extracts of *Jasminum auriculatum*, *Jasminum grandiflorum* and *Jasminum officinale* were tested for the larvicidal efficacy against the third instar larvae of *Culex quinquefasciatus* at concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L. Mortality was recorded after 24 and 48 h. Amongst the crude flower extracts of *Jasminum* species tested, the crude chloroform extract of *Jasminum grandiflorum* was found to be effective showing 100% mortality at 500 mg/L and LC₅₀ value of 212.10 mg/L after 48 h. Further investigations are needed to elucidate the larvicidal activity of *Jasminum grandiflorum* crude chloroform flower extract against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

Keywords: Larvicidal efficacy, *Culex quinquefasciatus*, *Jasminum auriculatum*, *Jasminum grandiflorum*, *Jasminum officinale*, crude flower extracts

1. Introduction

India is endemic to mosquito-borne diseases which are major health problems in tropical regions due to favorable ecological conditions. Mosquitoes form a monophyletic family of insects, Culicidae, that inhabit practically every region of every continent in the world except Antarctica and are of significant importance in human and veterinary medicine [1]. Mosquito have the ability of carrying and transmitting human and animal diseases across the countries causing hundreds of millions of clinical cases and millions of death annually [2,3]. WHO has declared the mosquito “public enemy number one” since they are responsible for the transmission of various dreadful diseases [4]. Among several species of mosquitoes, *Culex quinquefasciatus* (Diptera: Culicidae) is a main periodic vector of filarial parasite, *Wuchereria bancrofti*, accredited for human lymphatic filariasis transmission [5]. According to WHO [6], about 90 million people worldwide are infected with *Wuchereria bancrofti*, the lymphatic dwelling parasite, and ten times more people are at the risk of being infected. *Culex quinquefasciatus* is a vector of West Nile virus, filariasis, Japanese encephalitis, avian malaria and bancroftian filariasis [7]. *Culex quinquefasciatus* is a cosmopolitan mosquito with worldwide distribution, especially in the tropical and subtropical areas and is associated with human dwellings. *Culex quinquefasciatus* is responsible for major public health problems in India with around 31 million microfilaraemics, 23 million cases of symptomatic filariasis, and about 473 million individuals potentially at risk of infection [8]. *Culex quinquefasciatus* is a predominant house-resting mosquito in many tropical countries [9] and breeds locally in storm sewer catch basins, clean and polluted ground pools, ditches, animal waste lagoons, effluent from sewage treatment plants and other sites with organic wastes [10]. In India alone, 25 million people harbor microfilaria and 19 million people suffer from filarial disease manifestations [6, 11]. The only way to prevent transmission of mosquito/vector-borne disease is to combat the disease-carrying mosquitoes. Chemical pesticides have been used for several decades in

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controlling pests and vectors of various human diseases as they have a quick knock down effect. However, their indiscriminate use resulted in several problems such as resistance [12] and resurgence of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affect human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environment [13, 14]. This has necessitated the need for a research and development of environmentally safe, biodegradable indigenous method for vector control. Biologically active plant materials have attracted considerable interest in mosquito control programs in the recent times. Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages [15]. Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides [16]. Natural products of plant origin with insecticidal properties have been tried in the recent past in order to control a variety of insect pests and vectors. Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito-borne disease is by killing mosquito at larval stage. The current mosquito control approach is based on synthetic insecticides. Plants may be a source of alternative agents for control of vectors as they are rich in bioactive chemicals, active against a limited number of species including specific target insects, and biodegradable. Phytochemical insecticides have received much attention, in this regard, as they are considered to be more environmentally biodegradable and considered safer than synthetic insecticides [17]. Many researchers have reported on the effectiveness of plant extract against mosquito larvae and during the last decade, various studies on natural plant products against vector mosquito indicate them as possible alternatives to chemical synthetic insecticides for mosquito control [18-26]. Therefore, in the present study, the crude flower extracts of *Jasminum officinale* L. *Jasminum auriculatum* Vahl. and *Jasminum grandiflorum* L. belonging to the family Oleaceae were tested for the larvicidal efficacy against the third instar larvae of *Culex quinquefasciatus*.

2. Materials and Methods

2.1 Plant collection

Mature fresh flowers of *Jasminum auriculatum*, *Jasminum grandiflorum* and *Jasminum officinale* collected in and around Chennai, Tamil Nadu, India were brought to the laboratory, and shade dried at room temperature. Taxonomical identity of the flowers was confirmed at the Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, Tamil Nadu, India.

2.2 Plant extraction

Dried flowers of *Jasminum auriculatum*, *Jasminum grandiflorum* and *Jasminum officinale* were powdered and the powdered flowers (1 Kg) each was extracted with 3 L of hexane and chloroform each separately using a Soxhlet apparatus at a temperature of 45 °C and 57 °C respectively [27]. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary evaporator. The crude hexane and chloroform flower extracts of *J. auriculatum*, *J. grandiflorum* and *J. officinale* thus obtained were lyophilized and one per cent stock solution prepared by adding adequate volume of acetone was refrigerated at 4 °C until testing for bioassay.

2.3 Test mosquitoes

Culex immatures collected from various places in Chennai, Tamil Nadu, India were transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified upto species level and confirmed before rearing. Cyclic generations of *Culex quinquefasciatus* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of 27 ±2 °C and a relative humidity of 70-80% were maintained in the insectary. The adult mosquitoes were fed on ten per cent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with laboratory reared albino mice. Ovitrap were placed inside the cages for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

2.4 Larvicidal bioassay

Standard WHO [28] protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. *Cx. quinquefasciatus* immature particularly early third instar larvae were obtained from laboratory colonized mosquitoes of F₁ generation. Larvicidal activity at test concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L of the crude extract was assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of the crude extract. Twenty healthy larvae were released into each 250 ml glass beaker containing water and test concentration. Mortality was observed for 24 and 48 h after treatment. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of Tween 80 to distilled water. Distilled water served as untreated control. The larval per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula [29].

2.5 Statistical analysis

Data from all replicates were pooled for analysis. LC₅₀, LC₉₀ and regression coefficient values were calculated using SPSS software by probit analysis [30]. One way ANOVA followed by Tukey's test was performed to determine the difference in larval mortality between concentrations. Results with P<0.05 level were considered to be statistically significant.

3. Results and Discussion

The results of the present study indicate the crude chloroform extract of *J. grandiflorum* flowers to possess larvicidal activity against *Cx. quinquefasciatus*. Results of the larvicidal effects of crude flower extracts of *Jasminum* species against *Cx. quinquefasciatus* are presented in Table 1 and 2. No larval mortality was observed in treated and untreated control. Among the plant species and extracts tested, the crude chloroform flower extract of *J. grandiflorum* was found to be effective with one hundred per cent mortality at 500 mg/L at 48 hours (Table 2) and LC₅₀ value of 212.10 mg/L after 48 hours (Table 3). The crude hexane, diethyl ether, dichloromethane and ethyl acetate extract of leaves of *Cleistanthus collinus*, *Hydrocotyle javanica*, *Murraya koenigii*, whole plant of *Leucas aspera* and *Sphaeranthus*

indicus and *Zanthoxylum limonella* bark exhibited 100% larval mortality at 1000ppm at 48 hours against the larvae of *Cx. quinquefasciatus* [26]. The results of the present study corroborate with earlier reports of *J. grandiflorum* crude chloroform flower extracts tested against the larvae of *Aedes aegypti* exhibiting LC₅₀ values of 344.01 and 300.47 mg/L after 24 and 48 hours respectively [31]. Crude extracts of plants that showed larvicidal activity and the LC₅₀ values ranging above 100 ppm were found in petroleum ether leaf extracts of *Ageratum conyzoides* [32] and ethyl acetate leaf extracts of *Ageratum houstonianum* [33]. The ethyl acetate leaf extract of *Strychnos nuxvomica* indicated larvicidal activity against *Cx. quinquefasciatus* and LC₅₀ value was 222.28 and 146.99 ppm after 24 and 48 hours respectively [34]. The hexane aerial

extract of *Hyptis suaveolens* (LC₅₀ 203.37 ppm), diethyl ether whole plant extract of *Sphaeranthus indicus* (LC₅₀ 211.42 ppm), dichloromethane whole plant extract of *Citrullus colocynthis* (LC₅₀ 240.36 ppm), diethyl ether leaf extract of *Abutilon indicum* (LC₅₀ 395.50 ppm), diethyl ether leaf extract of *Murraya koenigii* (LC₅₀ 399.64 ppm), hexane leaf extract of *Leucas aspera* (LC₅₀ 652.52 ppm) and ethyl acetate leaf extract of *Cleistanthus collinus* (LC₅₀ 755.75ppm) exhibited larvicidal activity against *Cx. quinquefasciatus* [18]. Methanolic leaf extract of *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* when tested against *Cx. quinquefasciatus* larvae showed LC₅₀ values of 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm respectively [35].

Table 1: Larvicidal activity of crude flower extracts of *Jasminum* species against *Culex quinquefasciatus* at 24 hours

Plant species	Solvents	Larval mortality (Mean ±S.D.)							
		Concentration (mg/L)							
		62.5	125	250	500	1000	2000	4000	8000
<i>Jasminum auriculatum</i>	Hexane	0.00 ±0.00 ^a (0.00)	0.00 ±0.00 ^a (0.00)	0.66 ±1.15 ^a (3.30)	2.00 ±2.00 ^{ab} (10.00)	4.00 ±0.00 ^{abc} (20.00)	4.00 ±3.46 ^{abc} (20.00)	5.33 ±1.15 ^{bc} (26.65)	6.66 ±2.30 ^d (33.30)
	Chloroform	0.00 ±0.00 ^a (0.00)	0.66 ±1.15 ^a (3.30)	2.00 ±2.00 ^{ab} (10.00)	4.00 ±2.00 ^{abc} (20.00)	6.00 ±0.00 ^{bc} (30.00)	6.00 ±2.00 ^{bc} (30.00)	6.66 ±3.05 ^c (33.30)	7.33 ±1.15 ^c (36.65)
<i>Jasminum grandiflorum</i>	Hexane	2.66 ±2.30 ^{ab} (13.30)	5.33 ±1.15 ^{bc} (26.65)	7.33 ±1.15 ^{bcd} (36.65)	10.00 ±2.00 ^{cde} (50.00)	11.33 ±1.15 ^{def} (56.65)	13.33 ±1.15 ^{ef} (66.65)	14.66 ±3.05 ^{ef} (73.30)	15.33 ±3.05 ^f (76.65)
	Chloroform	3.33 ±1.15 ^{ab} (16.65)	6.00 ±2.00 ^{bc} (30.00)	10.00 ±0.00 ^{cd} (50.00)	13.33 ±1.15 ^d (66.65)	14.66 ±1.15 ^{ef} (73.30)	17.33 ±3.05 ^{ef} (86.65)	18.00 ±2.00 ^f (90.00)	18.66 ±1.15 ^f (93.30)
<i>Jasminum officinale</i>	Hexane	0.00 ±0.00 ^a (0.00)	2.33 ±1.52 ^{ab} (11.65)	2.66 ±1.15 ^{ab} (13.30)	5.33 ±1.15 ^{ab} (26.65)	7.33 ±1.15 ^{bc} (36.65)	12.00 ±5.29 ^{cd} (60.00)	14.00 ±4.00 ^d (70.00)	16.66 ±1.15 ^d (83.30)
	Chloroform	0.00 ±0.00 ^a (0.00)	0.00 ±0.00 ^a (0.00)	1.33 ±1.15 ^{ab} (6.65)	4.00 ±2.00 ^{bc} (20.00)	6.00 ±2.00 ^{cd} (30.00)	9.33 ±1.15 ^d (46.65)	13.33 ±2.30 ^e (66.65)	16.00 ±0.00 ^e (80.00)

Values are mean of three replicates of three trials ±standard deviation. Different superscript alphabets indicate statistical significant difference in larval mortality between concentrations at P<0.05 level by one way ANOVA followed by Tukey's test. Values in parenthesis denotes per cent larval mortality.

Table 2: Larvicidal activity of crude flower extracts of *Jasminum* species against *Culex quinquefasciatus* at 48 hours

Plant species	Solvents	Larval mortality (Mean ±S.D.)							
		Concentration (mg/L)							
		62.5	125	250	500	1000	2000	4000	8000
<i>Jasminum auriculatum</i>	Hexane	0.00 ±0.00 ^a (0.00)	0.00 ±0.00 ^a (0.00)	2.66 ±2.30 ^{ab} (13.30)	3.33 ±1.15 ^{abc} (16.65)	5.33 ±1.15 ^{bcd} (26.65)	6.66 ±3.05 ^{bcd} (33.30)	7.33 ±1.15 ^{cd} (36.65)	8.66 ±2.30 ^d (43.30)
	Chloroform	0.00 ±0.00 ^a (0.00)	2.00 ±2.00 ^{ab} (10.00)	2.66 ±2.30 ^{abc} (13.30)	4.66 ±1.15 ^{abcd} (23.30)	6.00 ±2.00 ^{bcd} (30.00)	6.66 ±3.05 ^{bcd} (33.30)	8.00 ±0.00 ^{cd} (40.00)	10.00 ±3.46 ^d (50.00)
<i>Jasminum grandiflorum</i>	Hexane	4.00 ±2.00 ^b (20.00)	6.00 ±0.00 ^{bc} (30.00)	8.66 ±1.15 ^{cd} (43.30)	10.66 ±1.15 ^d (53.30)	12.66 ±1.15 ^{ef} (53.30)	14.66 ±1.15 ^f (73.30)	16.00 ±2.00 ^{fg} (80.00)	19.33 ±1.15 ^g (96.65)
	Chloroform	4.00 ±0.00 ^b (20.00)	6.00 ±2.00 ^b (30.00)	10.66 ±2.30 ^c (53.30)	20.00 ±0.00 ^d (100.00)	20.00 ±0.00 ^d (100.00)	20.00 ±0.00 ^d (100.00)	20.00 ±0.00 ^d (100.00)	20.00 ±0.00 ^d (100.00)
<i>Jasminum officinale</i>	Hexane	0.00 ±0.00 ^a (0.00)	2.66 ±1.15 ^{ab} (13.30)	2.66 ±1.15 ^{ab} (13.30)	4.33 ±3.51 ^{ab} (21.65)	6.66 ±1.15 ^b (33.30)	13.33 ±2.30 ^c (66.65)	15.33 ±1.15 ^{cd} (76.65)	18.66 ±1.15 ^d (93.30)
	Chloroform	0.00 ±0.00 ^a (0.00)	0.00 ±0.00 ^a (0.00)	2.66 ±1.15 ^{ab} (13.30)	4.66 ±1.15 ^b (23.30)	6.66 ±1.15 ^b (33.30)	11.33 ±1.15 ^c (56.65)	14.00 ±4.00 ^{cd} (70.00)	18.00 ±2.00 ^d (90.00)

Values are mean of three replicates of three trials ±standard deviation. Different superscript alphabets indicate statistical significant difference in larval mortality between concentrations at P<0.05 level by one way ANOVA followed by Tukey's test. Values in parenthesis denotes per cent larval mortality.

Table 3. Probit analysis of crude flower extracts of *Jasminum* species against *Culex quinquefasciatus*

Plant species	Solvents	24 hours			48 hours		
		LC ₅₀ (mg/L)	LC ₉₀ (mg/L)	R ² value	LC ₅₀ (mg/L)	LC ₉₀ (mg/L)	R ² value
<i>Jasminum auriculatum</i>	Hexane	9006.62	16135.79	0.700	7267.26	14211.50	0.809
	Chloroform	8318.52	16514.76	0.783	7059.14	14783.34	0.801
<i>Jasminum grandiflorum</i>	Hexane	2679.77	8104.62	0.915	1525.60	4611.07	0.966
	Chloroform	1182.67	4278.34	0.935	212.10	365.36	0.949
<i>Jasminum officinale</i>	Hexane	3308.76	7084.54	0.839	2662.65	5491.21	0.852
	Chloroform	3909.23	7589.88	0.839	3136.68	6231.08	0.848

LC₅₀: Lethal concentration that kills 50% of the exposed larvae; LC₉₀: Lethal concentration that kills 90% of the exposed larvae; R²: Regression co-efficient.

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting counter measures such as developmental of novel insecticides [36]. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. The preliminary screening of plant extracts against mosquitoes is a good means of evaluating the potential mosquitocidal property present in it [25, 26, 37]. Natural insecticides of plant origin have been given importance due to their ecofriendly nature and biodegradability as a substitute of synthetic insecticides for the control of vectors of public health importance. Plants are the chemical factories and rich source of bioactive chemicals, some of which have medicinal and pesticidal properties [38]. Different types of phytochemicals of plant either from the whole part or from the specific parts come out with solvent during chemical extraction depending on the polarity of the solvent [39-41]. In conclusion, the results reported in the present study open the possibility for further investigations of the efficacy of larvicidal property of the crude chloroform extract of *Jasminum grandiflorum* against *Culex quinquefasciatus* as an agent for combating mosquitoes. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also to identify the active ingredient(s) of the extract responsible for larvicidal activity.

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