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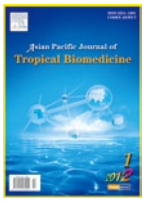

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In vitro repellent and larvicidal efficacy of *Swietenia mahagoni* against the larval forms of *Culex quinquefasciatus* Say

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ABSTRACT

Objective: To analyze the mosquito repellency and larvicidal activity of mature leaves of *Swietenia mahagoni* (*S. mahagoni*) against *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say in laboratory bioassay.**Methods:** Various concentrations of crude and petroleum ether extract of *S. mahagoni* mature leaves were exposed against all instars of *Cx. quinquefasciatus* larvae and the respective LC_{50}, LC_{90}, Regression equations and *R* values were calculated. Mosquito repellency was tested with chloroform: methanol (1:1, v/v) extract of mature leaves. **Results:** Cent percent mortality of 2nd instar mosquito larva was observed at 50 ppm concentration of petroleum ether extract after 72 h of exposure. 1st, 3rd and 4th instar larvae showed 96.66%, 90.00% and 60.00% mortality at 50 ppm concentration of petroleum ether respectively after 72 h of exposure. Preliminary qualitative phytochemical assay revealed the presence of saponins, alkaloids, tannin, flavonoids, and free glycoside bound anthroquinones. Chloroform: methanol extract showed repellency up to 2 h 15 min after application. No mortality was found in non target organisms, such as *Gambusia affinis*, Tadpole of *Bufo* and *Chironomus* larvae. Chloroform: methanol of mature leaves extract of *S. mahagoni* exhibits 100% repellency upto 2 h 15 min as no mosquito bites up to that time periods in the treated hands. **Conclusions:** Different solvent extracts of mature leaves of *S. mahagoni* can be effectively used as a potent ecofriendly biocontrol agent against larval form of *Cx. quinquefasciatus*.

1. Introduction

Mosquitoes are well known vectors of different communicable diseases such as malaria, filariasis, yellow fever, dengue, Japanese encephalitis etc, which produce devastating impacts on human health[1]. These fatal diseases kill more than a million victims/ year around the world[2]. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) transmits human lymphatic filariasis which is caused by *Wuchereria bancrofti*, and is found to be more endemic in the Indian subcontinent[3]. The lymphatic filariasis is a widely distributed tropical disease with around 120 million people infected worldwide and 44 million people have chronic manifestations[4].

Larval stages of mosquitoes are attractive target to control due to their low mobility in breeding habitats and it is easy to control them in these habitats[5].Various strategies have been developed to reduce the prevalence of mosquito borne diseases in different parts of the world. Different synthetic pesticides, organophosphates and insect growth regulators are most commonly used to control mosquitoes[6]. But these methods are not cost effective and the concentration of these hazardous chemicals gradually increases in higher trophic levels through biomagnifications and develop insecticide resistance[7]. Now a day's many new strategies have been developed for selective mosquito larval control, such as, utilization of bioactive herbal products. These products are cost effective, easily applicable in the field usually safe to the non target organisms and do not produce any ill effect on ecosystem. Many secondary metabolites of plant origin, such as saponin[8], steroid[6], isoflavonoids[9], essential oil[10], alkaloids and tannin[11] are effective as mosquito larvicides. Plant derived essential oil showed mosquito repellency and toxicity which was established by Pandey *et al* (2009)[12].

Swietenia mahagoni (*S. mahagoni*) (family: Meliaceae) is a tree which has great medical uses. The bark extracts are used as astringent for wounds. It is used to cure malaria,

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fever, dysentery and depurative. Its wood is also used in furniture making, boat making etc. The present study is the first time attempt to control the *Cx. quinquefasciatus* larvae with crude and petroleum ether extracts of the mature leaves of *S. mahagoni*. We have also studied the phytochemical profile, repellency against adult mosquitoes and the toxic effect on non target organisms found in the same aquatic habitats of mosquito larvae.

2. Materials and methods

2.1. Collection of mosquito larvae

The present study was conducted at Burdwan (23 ° 16′ N, 87 ° 54′ E), West Bengal, India. *Cx. quinquefasciatus* larvae were collected from cemented drains surrounding the University campus. They were kept in large plastic tray (15 L) with artificial foods (powdered mixture of dog biscuits and dried yeast powder in the ratio of 3:1).

2.2. Adult culture

Cx. quinquefasciatus eggs were collected from same habitats of University campus and were cultured in laboratory condition. After hatching and passing through larval instars, pupae were kept in a 500 mL beaker and were placed into a cage for adult emergence. No food was provided except immobilized pigeon for periodical blood feeding.

2.3. Preparation of crude plant extract

Fresh mature leaves of *S. mahagoni* were randomly collected during April and May, 2011 from the plants growing within the University campus. After proper identification of the plant, the voucher specimen is deposited in the Department of zoology (voucher no.-171), the University of Burdwan. The collected fresh, green leaves were rinsed with distilled water and dried in paper towel. Then the leaves were cut into small pieces by sharp knife and crushed by an electric blender. The juice was filtered by passing through the Whatman no.1 filter paper. The purified filtrate was used as stock solution and required concentration (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were prepared by addition of suitable volume of distilled water.

2.4. Preparation of solvent extracts

The shade dried, crushed leaves were put in Soxhlet apparatus and the plant extracts were prepared using petroleum ether as solvent. Extraction period of the solvent was 72 h. The final extract was concentrated by evaporation in rotary evaporator. Graded concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm) were prepared from collected solid residue of each extract and used in bioassay experiments. After collecting petroleum ether extract, chloroform: methanol (1:1 v/v) was added on the soxhlet apparatus with the same leaves and solvent extract was prepared with same methodologies as described above.

2.5. Dose response larvicidal bioassay

The larvicidal bioassay were conducted according to World Health Organization procedure (1981)[13] with required modifications. Five concentrations of crude extract (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) and five concentrations of petroleum ether extract (10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm) were used during bioassay experiment. The prepared concentrations of sample were transferred into the sterile glass Petri dishes (9 cm. diameter/150 mL.capacity) containing 100 mL tap water. Twenty 1st, 2nd, 3rd and 4th instars larval forms of *Cx. quinquefasciatus* were separately put into each Petri dish containing different concentrations of extract. All experiments including control sets were conducted in triplicate. Larval mortality was calculated at 24 h, 48 h and 72 h exposure. Similar type of experiment was conducted with petroleum ether extract (concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm) of the leaves against 1st, 2nd, 3rd and 4th instar larval forms.

2.6. Phytochemical analysis of the plant extracts

Qualitative phytochemical analysis of crude extracts of the mature leaves of *S. mahagoni* was carried out by appropriate methodologies[14,15]. The phytochmicals included under study were Saponins, cardiac glycosides, terpenoids, alkaloids, tannin, flavonoids, and free glycoside bound anthroquinones

2.7. Repellency activity

The repellent activity of the solvent extract was tested with laboratory reared adult mosquitoes. The adult mosquitoes were kept into a wooden cage (30 cm×30 cm×30 cm) with a clothed envelope and glass top. Both hands of a human volunteer were entered into the cages simultaneously through two passages made in clothed envelope of the cage. One hand was treated with the chloroform: methanol solvent plant extract (treated hand) and on the other hand only chloroform: methanol was applied (control hand). Mosquito biting activity on the control hand was recorded until the first mosquito bites on the treated hand.

2.8. Effect on non target organisms

The effect of crude and petroleum ether extracts of *S. mahagoni* were tested against *Gambusia affinis*, Tadpole of *Bufo* and *Chironomus* larvae that were used as non target organisms due to their habitat similarity with mosquito larvae. The organisms were exposed to appropriate lethal concentration of crude and petroleum ether extract at 24 h to observe mortality and any types of other abnormalities such as sluggishness etc upto 72 h exposure.

2.9. Statistical analysis

Experimental data was performed by using the computer software "STAT PLUS 2007" (Trial version) and MS EXCEL 2002 to calculate the LC_{50}, LC_{90}, regression coefficient values, regression equations (Y=mortality, X=concentration), Mean mortality, Standard error etc. The percentage of corrected mortality was analyzed by Abbott's formula[16].

3. Result

Results of larvicidal bioassay with crude extract of mature leaves are presented in Table 1. From the table it is found that the mortality rate of all larval instars of *Cx. quinquefasciatus* at 0.5% concentration was remarkably higher (P<0.05) than the mortality rates at 0.1%, 0.2%, 0.3% and 0.4% concentrations at 24 h, 48 h and 72 h of exposure. The mortality rate was highest in 72 h larval bioassay.

Results of Log probit analysis and Regression analysis of larvicidal activities of crude extract (Table 2) and petroleum ether extract (Table 3) of mature leaves against different instars larval forms of *Cx. quinquefasciatus* expressed that the mortality rate (Y) positively correlated with the concentration of exposure (X) and have a regression coefficient (R) close to 1 in each case. The result of Log probit analysis (at 95% confidence level) revealed that LC_{50} values gradually decreased with exposure times and has the lowest value at 72 h of exposure (Table 2 and Table 3).

Results of larvicidal activity with petroleum ether extract are presented in Table 4. Cent percent mortality was found at 40 ppm concentration for 1st instar larva and at 50 ppm concentration for 2nd instar larvae. The mortality was highest at 50 ppm concentration for 3rd and 4th instar larvae. Qualitative phytochemical analysis of crude extracts of the

Table 1.
Efficacy of *S. mahagoni* leaf crude extract at different concentrations on different larval instars of *Cx.quinquefasciatus* (Mean±Standard errors).

Instar	Concentration	Mortality		
		24 h	48 h	72 h
1st	0.1	40.00 ± 0.00	53.33±3.33	63.33±3.33
	0.2	46.67±3.33	46.66±3.33	56.66±3.33
	0.3	60.00±0.00	60.00±0.00	66.66±3.33
	0.4	70.00±0.00	80.00±0.00	83.33±3.33
	0.5	90.00±0.00	93.33±6.66	93.33±6.66
2nd	0.1	46.66±3.33	46.66±3.33	60.00±5.77
	0.2	46.66±3.33	53.33±3.33	53.33±3.33
	0.3	60.00±5.77	63.33±3.33	66.66±3.33
	0.4	66.66±3.33	70.00±5.77	70.00±5.77
	0.5	80.00±0.00	90.00±5.77	90.00±5.77
3rd	0.1	33.33±3.33	43.33±3.33	43.33±3.33
	0.2	53.33±3.33	66.66±3.33	70.00±0.00
	0.3	50.00±0.00	60.00±5.77	6.66±8.81
	0.4	50.00±0.00	60.00±0.00	63.33±3.33
	0.5	76.67±6.66	83.33±6.66	86.66±3.33
4th	0.1	30.00±0.00	33.33±3.33	36.66±3.33
	0.2	33.33±3.33	40.00±0.00	40.00±0.00
	0.3	46.66±3.33	56.66±3.33	60.00±0.00
	0.4	43.33±6.66	50.00±10.00	53.33±6.66
	0.5	63.33±6.66	70.00±5.77	70.00±5.77

Table 2.
Log probit analysis and regression analysis of larvicidal efficacy of *S. mahagoni* leaf crude extract against different larval instars of *Cx. Quinquefasciatus*.

Instar	hours	LC_{50}	LC_{90}	Regression equation	R value
1st	24 h	0.17	0.90	Y=123.33A+24.33	0.92
	48 h	0.13	0.83	Y=113.33A+32.66	0.88
	72 h	0.09	0.80	Y=86.66A+46.66	0.82
2nd	24 h	0.16	1.83	Y=86.66A+34.00	0.89
	48 h	0.14	0.99	Y=103.33+33.66	0.89
	72 h	0.09	1.45	Y=76.66A+45.00	0.76
3rd	24 h	0.23	2.71	Y=83.33A+27.66	0.79
	48 h	0.13	1.85	Y=73.33A+40.66	0.72
	72 h	0.12	1.19	Y=80.00A+42.00	0.73
4th	24 h	0.37	5.77	Y=76.66A+20.33	0.80
	48 h	0.26	3.07	Y=83.33A+25.00	0.78
	72 h	0.23	3.10	Y=80.00A+28.00	0.82

Table 3.
Log probit analysis and regression analysis of larvicidal efficacy of *S. mahagoni* leaf petroleum ether extract against different larval instars of *Cx. quinquefasciatus*.

Instar	hours	LC_{50}(ppm)	LC_{90}(ppm)	Regression equation	*R* value
1st	24 h	26.81	41.29	Y=2.63A+25.66	0.95
	48 h	24.19	41.48	Y=2.56A+20.33	0.95
	72 h	22.56	41.33	Y=2.43A+13.66	0.96
2nd	24 h	33.45	52.91	Y=2.53A−36.66	0.94
	48 h	29.37	63.41	Y=2.26A−22.66	0.94
	72 h	26.25	69.69	Y=2.03A−11.00	0.91
3rd	24 h	13.97	94.36	Y=0.93A+37.33	0.79
	48 h	10.74	61.85	Y=0.90A+45.66	0.81
	72 h	8.90	54.56	Y=0.83A+51.00	0.78
4th	24 h	62.41	204.70	Y=1.03A−9.66	0.83
	48 h	59.69	334.54	Y=0.93A+0.66	0.79
	72 h	45.39	251.98	Y=1.03A+4.33	0.85

Table 4.
Mortality effect of *Cx. quinquefasciatus* exposed to different concentrations of petroleum ether extracts of *S. mahagoni* .

Instar	Concentration(p.p.m)	Mortality		
		24 h	48 h	72 h
1st	10	0.00±0.00	6.66±3.33	10.00±0.00
	20	23.33±3.33	23.33±3.33	30.00±0.00
	30	50.00±0.00	56.66±3.33	60.00±0.00
	40	100.00±0.00	100.00±0.00	100.00±0.00
	50	93.33±3.33	96.66±3.33	96.66±3.33
2nd	10	3.33±3.33	13.33±3.33	23.33±6.66
	20	3.33±3.33	13.33±3.33	20.00±5.77
	30	26.66±3.33	33.33±3.33	36.66±3.33
	40	63.33±8.81	66.66±6.66	70.00±5.77
	50	100.00±0.00	100.00±0.00	100.00±0.00
3rd	10	40.00±5.77	43.33±3.33	50.00±5.77
	20	60.00±10.00	76.66±3.33	80.00±0.00
	30	73.33±3.33	76.66±3.33	76.66±3.33
	40	73.33±3.33	80.00±0.00	83.33±3.33
	50	80.00±5.77	86.66±3.33	90.00±5.77
4th	10	3.33±3.33	10.00±0.00	13.33±3.33
	20	13.33±6.66	23.33±3.33	30.00±0.00
	30	13.33±3.33	23.33±8.81	33.33±3.33
	40	30.00±10.00	36.66±8.81	40.00±10.00
	50	46.66±3.33	50.00±0.00	60.00±5.77

mature leaves of *S. mahagoni* revealed the presence of Saponins, alkaloids, tannin, flavonoids, and free glycoside bound anthroquinones as effective biocontrol agent (s).

Chloroform: methanol extract exhibited cent percent repellency or biting deterrence up to 2 h 15 min as no mosquito bit up to that time periods on the treated hand. However, upto that time 11 mosquito bites were recorded in the control hand.

Crude and petroleum ether extracts of *S. mahagoni* did not produce any mortality and any types of other abnormalities when tested against *Gambusia affinis*, Tadpole of *Bufo* and *Chironomus* larvae that were used as non target organisms.

4. Discussion

Mosquito biocontrol by plant extract is ecofriendly, safe, non hazardous to environment and suitable alternative for chemical mosquito control[17]. Plant extract could be a potential source of biodegradable, bioactive materials to control mosquitoes. Over last few decades' botanical derivatives based insecticides have been used to control mosquitoes. Many plant species have been mentioned and established to have mosquito larvicidal, pupicidal and adulticidal activity[18-20]. Present study showed that the mortality of *Cx. quinquefasciatus* larvae exposed to the phyto-derivatives of *S. mahagoni* gradually increased with time of exposure and concentration of plant extracts which is similar to the studies of Rawani *et al*[21].

Several authors have reported that petroleum ether extract of leaves show mosquito larvicidal activity[22,23]. Larval mortality gradually increased probably due to accumulation of active ingredients of plant extract in larval tissue. 0.5% concentration of crude extract exhibited 93.33% larval mortality at 72 h of exposure for 1st instar larva *i.e.* highest for all instar and at 40 ppm concentration of petroleum ether

extract showed 100% mortality within 24 h for 1st instar larva *i.e.* highest for all instar and all cases.

The chloroform: methanol extracts exhibited repellency during the present investigation, and thus it can be used for personal protection. The most common active materials in mosquito repellent are pyrethroids, bischloromethyl ether, octa chlorodipropyl ether; a genotoxic agent[24]. The pure plant material is safe for a person who uses it as mosquito repellent[24].

Our study is interesting because this plant is generally used as furniture but we have proved that *S. mahagoni* exhibits larvicidal and repellent activities against *Cx. quinquefasciatus*.

The results reported here open a new horizon of researches of biocontrol efficacy of plants. The purification and determination of the structure of active ingredients of the plant is necessary for its wide use in mosquito control programme.

Conflict of interest statement

We declare that we have no conflict of interest.

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