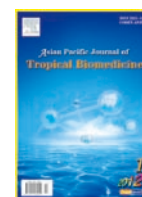




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)

## Document heading

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

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## ARTICLE INFO

## Article history:

Received 12 January 2012

Received in revised form 23 February 2012

Accepted 15 April 2012

Available online 28 April 2012

## Keywords:

*Culex quinquefasciatus*

Larvicide

Repellency

Phytochemical

Petroleum ether extract

Chloroform: methanol

Non target organisms

## 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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Foundation project : It is supported by NET CSIR JRF scholar bearing NO: 10-2(5)/2007(ii)-E.U.II activated from 1.7.2008.

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 phytochemicals 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.

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