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Larvicidal activity of *Oestrum nocturnum* on *Aedes aegypti*

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Abstract

Aedes aegypti is a vector parasite of the Dengue. New method to control the population of this insect is necessary. In the present work we evaluated the potential of extract from *Cestrum nocturnum* as larvicide. Methanol extract outstand as highly active larvicide, achieving 100% larval mortality in 24 hours when tested in the concentration of 45 pg/mL (soxhlet) and 25 pg/mL (percolation). Any extract exhibiting significant larvicide activity was further fractioned and the fraction tested according to the WHO protocol. One fraction derived from methanol extract present remarkable LC_{100} at 12 pg/mL. LC_{50} of methanol extract and active fraction were found 14 pg/mL and 6 pg/mL respectively. These fractions will be submitted to further fractions aiming to identify the molecules responsible for the larvicide activity.

Introduction

Dengue fever is considered as a serious public health problem in the world, mainly in tropical countries where the favorable environmental conditions are responsible for the proliferation of vectors Aedes aegypti. Among the arbovirus in India, distribution of all the dengue virus type is continuously expanding. Remarkably the reemergence of Chikungunya virus (CHIK) since 2005 is posing an additional concurrent diseases burden in the country including the Maharashtra. Both these virus are born by the mosquito A. aegypti (L) (Diptera:Culicidae) (Fulmali et al., 2008; Kumar et al., 2008). Periodic treatment with chemical insecticides and synthetic pyrethroids are done in breeding sits. Due to environmental concern on use of existing synthetic insecticides for vector control and further risk of development of widespread insecticides resistance in disease vector; interest on possible use of environment friendly natural products such as extracts of plants or plant parts increased for vector control. Sukumar et al. (1991) listed 346 species for 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, growth inhibition, ovipositional determinacy and repellent. This list includes many species from Solanaceae family. Recently, Ghosh and Chandra (2006) and Ghosh et al. (2008) evaluated phytosteroidal compound of mature leaves of day jasmine *Cestrum diurnum* (Solanaceae:Solanales) against larvae of Culex quinquefasciatus (Diptera:Culicidae) and Anopheles stephensi. This study with plant extracts has been pointed as a promising alternative to combat this vector. In this work we evaluate the potential of extract from C. nocturnum as larvicide.

Materials and Methods

Plant species

C. nocturnum leaves were collected from gardens at Nashik city, Maharashtra State, India, in December 2008. Leaves were shed dried and powdered.

Plant extraction

The leaves were macerated and sequentially extracted with hexane, ethyl acetate and methanol using soxhlet and percolation extraction separately. The solvent were evaporated on rotary evaporator. Any extract exhibiting significant larvicide activities were further fractioned by filtration in silica get eluted with hexane-ethyl

Table I

Lethal concentration of *C. nocturnum extracts*

Extraction method	Extracts	LC100 (pg/mL)	Regression equation
Soxhlet	Hexane	[a]	[a]
	Ethyl acetate	300	Y=2.1180X + 2.3457
	Methanol	65	Y=1.9441X + 3.3592
Percolation	Hexane	[a]	[a]
	Ethyl acetate	210	Y=1.7586X + 2.0573
	Methanol	35	Y=1.4832X + 1.3753

[a]Indicate no larvicidal activity in concentration <350 µg/mL

acetate mixture of increasing polarity, eight fraction were obtained.

Bioassay

Larvae were reared (Pelah et al., 2002) and third instars larvae were selected for bioassay. Larvae were transferred into the test solution with pasture pipette (20 larvae/solution). As a solvent, DMSO is used to soluble the extract in test water. Mortality of each test extract and fractions were determined after 24 hours exposure at 28°C following the protocol of WHO (1981). Mortality was corrected using Abbot formula (Finney, 1971) and the concentration at which 50% of the test population were dies (LC50) was determined by probit program (Finney, 1971).

Results and Discussion

Among the three extracts of *C. nocturnum*, percolation method extracts shows effective larvicidal activity over the soxhlet method (Table I). Methanol extract exhibit significant larvicidal activity causing 100% mortality in a concentration of 100 pg/mL. This extract was further fractioned and the fractions obtained (CNM1-CNM6) were tested for larvicidal activity. The fraction CNM2, 3 and 4 presented the best results (Table II). CNM1, 5, 6 fraction didn't presented good activity in concentration lower than 350 pg/mL (result not shown). The LC_{50} of the methanol extract and its potent fraction (CNM3) is found 14 (± 0.3113) pg/mL and 6 (± 0.1532) pg/mL respectively (LC_{50} values are presented as average of four observations ± SE).

In all larvicidal assays, the methanol extract of *C. nocturnum* leaves extracted with percolation and its fractions presented higher larvicidal activity. Various authors have evaluated larvicidal activity of cestrum species on mosquitoes; where they found a steroidal bioactive compounds responsible for mosquitocidal activity (Ghosh and Chandra, 2006; Ghosh et al., 2008). Eight steroidal glycosides have been isolated from the leaves of *C. nocturnum* (Mimaki et al., 2002). Thus to establish relevance of these steroidal compound with the mosquitocidal activity, fractions will be further fractionated to evaluate their potential to broad use and their possible toxic effect upon the other organism.

Table II

Lethal concentration of fractionated methanol extract of *C. nocturnum*

Extracts	Hexane:Ethyl acetate	LC100 (Pg/mL)	Regression equation
CNM2	1:10	60	Y=2.0045X + 1.0231
CNM3	1:1	12	Y=1.4324X + 2.1042
CNM4	10:1	75	Y=2.2342X + 1.1103

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