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Insecticidal Activity of Aromatic Bromo Compound from *Ipomoea Carnea*

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ABSTRACT

Prevalence of Mosquito borne diseases is one of the world's most notable health hazards. Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever and chickungunya. Management of the disease vector using synthetic chemicals has failed due to vector resurgence and environmental pollution. Considering the recent evidence of these diseases, there is an urgent need to control vector population of mosquitoes. The development of eco-friendly and target specific agents for the control of mosquito level is of prime importance. Ipomoea carnea belonging to convolvulaceae family and fistulosa sub-family can be a potential candidate. The present work reveals some larvicidal activities of ethyl acetate extract of I. carnea and isolated aromatic bromo compound. These were screened against mosquitoes Aedes aegypti and Cules quinquefasciatus. The isolated novel compound as well as extract exhibited significant chronic mosquito-larval toxicities against the two vectors. Experiments were carried out with 4th instar larvae (0-24 h old) of Aedes egypti and Culex quinquefasciatus which were cultured and maintained during the experiment at 80± 5% and relative humidity at 27± 2º. LC50 and LC 90 values for each sample were calculated.

Keywords: *Ipomoea carnea*; *Aedes aegypti*; Cules quinquefasciatus; Aromatic Bromo Compound

INTRODUCTION

Prevalence of Mosquito borne diseases is one of the world's most notable health hazards. Mosquitoes are the most important arthropods in medical entomology. Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever and chickungunya [1]. Nearly 300-500 million people are infected worldwide with mosquito-borne diseases and 1.5 to 2.0 million die per year [2]. The most efficient approach to control the vector is to target the immature stages of their life cycles. The current mosquito control approach is based on synthetic insecticides of organophosphate compounds and insect growth regulators. Continuous use of synthetic insecticides has disrupted natural enemies and led to outbreak of some insect species, resulted in developing resistance, had undesirable effects on non-targeted organisms, pollution and toxic side effect on human being. There is a continuous and urgent need to discover new environmentally safe, biodegradable indigenous method for vector control. Therefore, researchers are increasingly turning their attention to herbal products to use as insecticides for controlling larval mosquitoes. The use of plants for medicinal and insecticidal purposes dates back to antiquity. Plant extracts of roots, leaves and flowers were found to have mosquito larvicidal activity [1]. Many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae. Insecticidal activities of plant – derived compounds have been evaluated and few of these developed commercially [3].

There were reports on synergistic effect of insecticides of I. carnea leaves extract against malarial vector Anopheles stephensi [4]. The steam distilled essential oil from the leaves of I. cairica was found highly toxic against *A. aegypti, A. stephensi and C. quinquefasciatus* [5].
Insecticidal activity of natural halogenated compounds was reported [6]. Several identified and potential natural brominated bio accumulative compounds were studied [7]. Faulkner and Gribble reported different biological activities of natural halogenated products [6].The bio accumulative natural organ halogens investigated so far are active against bacteria, viruses and fungi [6].
In line with the objectives of the current research work, an aromatic bromo compound, propyl 5-bromo-4-(2'-bromo- 3'- methoxypropyl) – 2-

methyl benzoate was isolated from leaves and stem of *I. carnea* for the first time. The mosquito larvicidal activity of the novel compound was investigated for the first time against filarial mosquito vector C. quinquefasciatus and dengue vector *A. aegypti*.

EXPERIMENTAL

Materials and Methods

Collection and identification of plant materials

The plant material was collected from the river sides of Pune, Maharashtra, India. The plant was authenticated at Botanical Survey of India, Pune, India. The authentication number is ELICAI.,BSI/WC/Tech/2009/96.

Preparation of plant extract and isolation

Air shade dried, powdered leaves material (200 g) was extracted using soxhlet extractor with solvent n-hexane followed by ethyl acetate, acetone and ethanol. Entomological activity study was performed for all the crude extracts against two mosquito species *Aedes Egypti*, which is responsible for Dengue fever and *Culex Quinquefasciatus*, which is a vector of Filariasis. Results of the experiment manifested that ethyl acetate, acetone and ethanol extracts were active against both mosquito species. Thus bioassay guided fractionation and isolation was performed by repeated column chromatographic methods and compound was purified using repeated crystallization.

Mosquito Culture

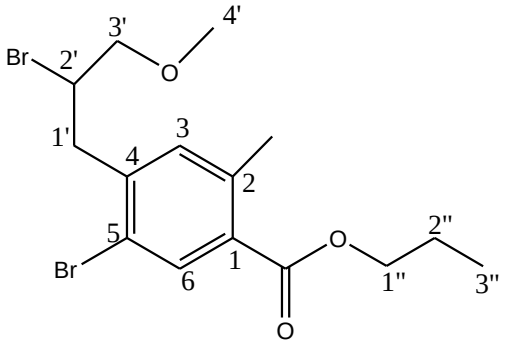
4 th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were drawn from the laboratory culture of mosquitoes maintained at 27 ± 20º C temperature and 80 ± 5% relative humidity.

Biological assay

The test samples were dissolved in organic solvent according to their solubility. They were tested to determine the larvicidal activity by making serial dilutions ranging from 1000 to 10 ppm in bioassays against larvae of the mosquito species. All experiments were performed with 4th instar larvae (0- 24 h old) of *Aedes aegypti* and *Culex quinquefasciatus* which were cultured and maintained during the experiment at 80± 5 % relative humidity. The bioassays were performed at room temperature of 27 ± 2 ^{0} C by exposing 10 larvae in each concentration of the extract in the final volume of 50 ml in 100 ml beaker. Larva food, ground dog biscuits/ yeast tablets (1: 1), was provided every alternate day. Five replicates of each concentration were tested for larval bio-efficasy and each experiment was repeated three times. The larval mortality in each concentration and control was recorded after 24 hours of continuous exposure, where there was no 100 % kill, the larvae was allowed to stay in water for 48 and till 72 hours. The mortality was recorded after 48 hours. Untreated controls were also taken in each test. The corrected mortality was determined using Abbott's formula whenever required [8]. The dose mortality data was analyzed by log Probit – method of Finney [9] and lethal concentration for 50 % and 90 % mortality were calculated (LC_{50} and LC_{90}).

RESULTS AND DISCUSSION

Aromatic bromo compound is isolated from ethyl acetate extract and characterized. The white square crystals are purified and its structure was determined by modern spectral methods. It exhibits sharp melting nature at 278ºC.



Propyl 5-bromo-4-(2'-bromo- 3'- methoxypropyl) – 2- methylbenzoate

Larvicidal efficacy of ethyl acetate extract and isolated compound

Five different concentrations (1000, 750, 500, 250, 100 ppm) of EA are tested against 4th instar larvae of *A. aegypti* and *C. quinquefasciatus.* 500 ppm to 10 ppm concentrations are used for isolated pure compound. The results of this study are reported (Table 1).

The results exhibit that ethyl acetate extract exhibits 100 % kill for both species after 24 hrs. Exposure at 1000 ppm concentration. At 750 ppm concentration 100 % kill is observed after 48 hrs exposure for *A. aegypti* while it shows 100 % kill after 48 hrs exposure for *Cx. Quinquefasciatus*. At 500 ppm concentration 100 % mortality is illustrated after 48 hrs. Exposure for *A. aegypti* while for *Cx. quinquefasciatus* 80.5 % kill is produced after 48 hrs Exposure. The pure compound exhibits remarkable activity. It shows 100% mortality at 50 ppm after 24 hours exposure against both species. At 40 ppm it shows 100 % and 82% mortality after 48 hours against *A.aegypti* and *C. quinquefasciatus* respectively. At lower concentration of 10 ppm also it is found to be active. The details are reported (Table 1).

Table 1: Larvicidal Activity of Ethyl acetate extract and Bromo Compound.

Test sample	Conc. (ppm)	% Mortality ± S.E after hours			
		Aedes aegypti		*Culex quinquefasciatus*	
		24	48	24	48
Ethyl acetate extract	1000	100	-	100	-
	750	82.5 ± 1.7	100	100	-
	500	55.0 ± 1.80	**100**	61.5 ± 1.83	80.5 ± 1.82
	250	40.5± 1.62	90.5 ± 2.15	20.0 ± 0	52.5 ± 1.32
	100	31.0 ± 1.30	50.0± 0.83	10.5 ± 0.85	30.0 ± 1.04
Bromo comp.	**50**	**100 ± 0**	-	**100 ± 0**	-
	40	80.0 ± 1.38	100 ± 0	60.00 ± 1.95	82.66± 1.18
	30	60.0 ± 1.95	82.0± 1.74	40.66 ± 1.53	64.0 ± 1.31
	25	30.66 ± 1.81	69.33 ± 1.81	20.66 ± 0.66	25.33 ± 1.65
	10	20.0± 1.69	30.66± 1.81	10± 0	12.0± 1.06

Bio efficacy of Ethyl acetate extract and Bromo Compound

Bioefficasy of Ethyl acetate extract and pure compound is tested against 4th instar larvae of *C. quinquefasciatus* and *A. aegypti*. The details of lethal concentrations, 50 % and 90 % kill using Probit Analysis data are reported (Table 2). The LC_{50} and LC_{90} for ethyl acetate and bromo compound are (260.33 and 23.38 ppm) & (1272.79 and 55.73 ppm) against *A. aegypti* and (336.60 and 30.67 ppm) and (802.68 and 64.57 ppm) against *C. quinquefasciatus.*

The outcome of the experiment indicates that the isolated bromo compound is more active molecule than the total extract. The compound exhibits significant activity against both the mosquito species as it shows activity at lower concentrations. As per LC_{50} and LC_{90} values it is more effective against *A. aegypti* than *C. quinquefasciatus.* It is noticed from the results that the percentage mortality is increased with the increase in concentration hence; the effect of the samples is dose dependant (Table 2).

Table2: Lethal Concentration of Ethyl acetate extract and Bromo Compound.

Sample	*Aedes aegypti.*		*Culex quinquefasciatus*	
	Lethal Conc. (24hrs) (ppm)	**Regression equation**	**Lethal Conc. (24hrs) (ppm)**	**Regression equation**
Ethyl acetate	LC_{50} =260.33 LC_{90} =1272.79	Y = 0.507 + 1.859 x SE ± 0.625	LC_{50} =336.60 LC_{90} =802.68	Y = -3.582 + 3.39 x SE ± 0.897
Bromo Comp.	**LC_{50} =23.38** **LC_{90} =55.73**	Y=0.349+3.97x	**LC_{50} =30.67** **LC_{90} =64.57**	Y= - 893+3.964x

CONCLUSIONS

The present study shows that the crude extract and isolates of I. carnea leaves extract exhibit potent larvicidal activity against *C.quinquefasciatus* and *A. aegypti*. Aromatic bromo compound exhibits 100% kill at 50 ppm after 24 hr exposure for both species. This suggests that the extract and isolated compound can be employed for developing cost effective and environment friendly new type of larvicide for mosquito control. The bromo compound isolated from stem also exhibits same activity.

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