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Research Article

Larvicidal and repellent activities of ethanolic extract of datura stramonium leaves against mosquitoes

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ABSTRACT
Mosquitoes are responsible for spread of many diseases than any other group of arthropods. Diseases such as malaria, filariasis, dengue haemorrhagic fever (DHF) and chikunguinya are real threat to mankind. In the present study ethanolic extracts of leaves of *Datura stramonium* were evaluated for larvicidal and mosquito repellent activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The LD50 values for larvicidal activity were found to be 86.25, 16.07 and 6.25 ppm against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* respectively. The ethanolic leaves extract of *Datura stramonium* provided complete protection time (Mosquito repellency) of 2.73, 71.66, 117.7 mins against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at higher concentration (1%).

Keywords: *Datura stramonium*, Larvicidal, Mosquito repellent, Mosquitoes.

INTRODUCTION

Mosquitoes are well known vectors of several disease causing pathogens[1]. Mosquito-borne diseases, such as malaria, filariasis, dengue haemorrhagic fever (DHF) and chikunguiniya are still imposing to be major public health problems in the Southeast Asian countries because of the tropical or subtropical climate[2]. *Aedes aegypti* is known to transmit dengue and yellow fever; malaria is transmitted by *Anopheles species*; and filarial disease by *Culex quinquefasciatus*[1]. Urbanization and changed lifestyles mainly contributed to the proliferation of larval habitats resulting in the disease epidemics[3].

WHO has declared the mosquito "Public enemy number one"[4]. One of the method available for the control of mosquitoes is the use of insecticides. Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air[5]. The higher number of breeding places in urban agglomeration, increasing resistance of mosquitoes to current commercial insecticides such as organochlorides, organophosphates and carbamates resulted in the recrudescence of diseases[4]. The ideal control method would be the systematic treatment of their breeding places through larvicides. Botanical pesticides are promising in that they are effective, environment – friendly, easily biodegradable and also inexpensive[6].

Some repellents of synthetic origin have limitations such as skin irritation, unpleasant smell, oily feeling to some users and also potentially toxic. Majority of commercial repellents are prepared by using chemicals like allethrin, N-N-diethyl-m-toluamide (DEET), dimethyl phthalate (DMP) and N, N-diethyl mendelic acid amide (DEM). These steadily growing problems demand an intensive search for new products that are environmentally safe, target specific and degradable[7].

In Traditional Siddha medicine, *Datura stramonium* was used as mosquito repellent.The present study was an attempt to explore the larvicidal and mosquito repellent activities of ethanolic extract of *Datura stramonium* leaves against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus.*

MATERIALS AND METHODS

Collection of Plants and extraction: Fully developed leaves of *Datura stramonium* were collected and authenticated by Dr. Rajanna (Botanist), Department of Botany, G. K. V. K, Bangalore, India. The leaves were washed with tap water, shade dried and powdered. The powdered plant material was loaded in soxhlet apparatus and was extracted with ethanol. The extract was subjected to vacuum evaporator to collect the crude extract. Standard stock solutions were prepared by dissolving the residues in the ethanol. These solutions were used for larvicidal and mosquito repellent bioassays. Larvicidal Bioassay: Larvicidal efficacy of ethanolic extract of *Datura stramonium* leaves were tested against late 3rd or early 4th instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* employing standard WHO procedure[8]. Batches of 25 larvae were exposed to known concentration of test solution (249ml dechlorinated tap water and 1 ml of DMSO dissolved test extract)in glass beakers of 500 ml capacity. Three replicate sets were tested with a final tally of 75 larvae for each concentration. Solutions containing 249 ml tap water and 1 ml of DMSO without plant sample served as controls. No food was provided to the larvae during the

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Table 1: Larvicidal efficacy of Datura stramonium leaves against Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus

S. No	Mosquito species	LD_{50}(50% lethal dose)	LD_{90}(90% lethal dose)	95% confidence limit	Chi-square value (x^2)
1.	*Aedes aegypti*	86.2518	196.389	0.0169±0.0091	2.8521
2.	*Anopheles stephensi*	16.0783	41.9599	0.0902±0.0488	9.6259
3.	*Culex quinquefasciatus*	6.25	11.25	0.1204±0.0947	11.3257

ppm- parts per million, Statistical significance:- With increase in concentration the mortality is decreasing

Table 2: Mosquito repellent activity of *Datura stramonium* leaves against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus.*

S. No	Concentration(%)	Complete Protection Time (Min)		
		Aedes aegypti	*Anopheles stephensi*	*Culex quinquefasciatus*
1.	Control	0.74	3.53	6.98
2.	0.1	1.63	21.91	51.31
3.	0.5	2.1	43.6	71.11
4.	1	2.73	71.66	117.7

Statistical significance:- With increase in concentration, the complete protection time is increasing.

test period. Mortality and survival were monitored after 24h of treatment. The moribund and dead larvae in replicates were combined and expressed as percentage mortality. The larvae were considered as dead or moribund, if they were not responsive to a gentle prodding with a fine needle. Experiments were conducted at 25±2ºc, 12 h light/dark regime. The observed percent mortality was adjusted with the corrected mortality using Abott's formula and expressed as corrected mortality[9]. These were subjected to regression analysis of probit mortality on log dosage using computerised log-probit analysis, which provided the lethal dosage of 50, 90, 95 and 99ppm as well as their 95% confidence limit[10].

Repellency tests: The ethanolic extract of Datura stramonium leaves was evaluated for repellent activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* using the human – bait technique[11]. The plant extract was dilutedin ethanol and 0.1, 0.5 and 1% concentrations were prepared. For each test, 10 disease free, laboratory-reared female mosquitoes were placed into seperate laboratory cages(45x38x38 cm)[12]. The mosquito progenies obtained form the laboratory colny were maintained in a cloth cage under controlled temperature (28±2ºc) and relative humidity range (75-80%)[13]. Three volunteers were considered for performing human- bait technique. Before each test, the volunteers skin was washed with unscented saop and the tested solution was applied from the elbow to the finger tips[12].

The test was repeated at every 30 min interval. The interval between the application of repellent and the first two consecutive bites occuring within 30 min was considered as protection time against the bites afforded by each of the concentrations of the test repellents[13]. In each cage one arm was inserted for one test concentration and the other arm applied with ethanol serves as control The trated and control arms were ineterchanged regularly to eliminate bias. Each test concentration was repeated thre times and in each replicate subject different volunteers to nullify any effect of color of the skin of repellent. Volunteers were asked to follow the testing protocol[12]. The complete protection time(min0 for each concentration was claculated[13].

RESULTS

Results of the larvciidal and repellent activities of ethanolic aextract of Datura stramonium leaves against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* are presented in Table:-1 and 2. The data was recorded, the LD_{50} (50% lethal dose), LD_{90} (90% lethal dose), 95% confidence limit and Chi-square value were calculated.

DISCUSSION

Previous studies on ethanolic extract of *Annona squamosa* leaves has shown LD50 (20.70 ppm), LD90 (76.73 ppm) against *Aedes albopictus* and LD50 (6.96 ppm), LD90 (31.80 ppm) against *Culex quinquefasciatus*[14]. The ethanolic extract of *Capsicum annum* fruits shown LD50 (0.011ppm), LD90 (0.027 ppm) against *Anopheles stephensi*[15]. The ethanolic extract of *Datura stramonium* leaves showed that LD50 (86.2518 ppm), LD90 (196.389 ppm) against *Aedes aegypti*, LD50 (16.0783 ppm), LD90 (41.9599 ppm) against *Anopheles stephensi* and LD50 (6.25 ppm), LD90 (11.25 ppm) against *Culex quinquefasciatus*. Earlier studies done on essential oil from leaves of *Ocimum basilicum* showed effective repellency 82.4±0.7, 75.0±1.2 and 115.3±1.9 mg/mat against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*[16]. Other studies with petroleum ether extract of *Zanthoxylum limonella* fruits provided protection time of 296 min and 223.5 min against *Aedes albopictus* in mustard oil base and coconut oil base

respectively[17]. The ethanolic leaf extract of *Datura stramonium* provided complete protection time of 2.73, 71.66 and117.7 mins against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at higher concentration (1%).

CONCLUSION

Datura stramonium showed an effective larvicidal activity in comparision with Coleus forskohlii and Pongamia pinnata against *Anopheles stephensi* and *Culex quinquefasciatus.* The effective repellency against *Culex quinquefasciatus* was better cmpared to *Anopheles stephensi* and *Aedes aegypti*. Larvicidal and repellent activities of ethanolic leaf extract of *Datura stramonium* in this study against the vectors are encouraging.

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