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Mosquito Larvicidal Activity of Marine Sponge Metabolites

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Abstract: The secondary metabolites of five species of marine sponge viz., *Acanthella elongata, Echinodictyum gorgonoides, Axinella donnani, Callyspongia subarmigera* and *Callyspongia diffusa* collected off Kanyakumari were explored for vector control activity. The extracts were assayed against larvae of *Culex sp*, the mosquito vector of filariasis as per the World Health Organization protocols. The test doses varied from 0.01% to 10.0% for 24h from which LC_{50} were determined. Maximum mosquito larvicidal activity was noted in the methanol extract of *Acanthella elongata* with the LC_{50} of 0.066 mg/ml. *Axinella donnani* was the least active among the tested sponges. The results of present investigation suggest that *A. elongata* extract has the potential for developing novel bioactive larvicidal agent.

Key words: Sponge metabolites · Mosquito larvicidal activity · *Culex* sp

INTRODUCTION

The metabolites and new bioactive compounds extracted from marine sponges are gaining importance as tools not only to human medicine or pharmaceuticals but also effective vector control agents [1]. Vector-borne diseases such as malaria, dengue, yellow fever, encephalitis, chikungunya and lymphatic filariasis, spread by mosquitoes continue to pose public health problems [2]. Considering this, much attention is focused to control the vectors as a preventive measure than investing in treatment protocols. The common insecticides are not target-oriented and hence cause severe ecological imbalances. Eventually, biocontrol of insect pests and vectors is becoming one of the most promising alternatives to chemical pesticides. In this respect, several biological control agents have been tested in India and many other parts of the world to evaluate their potential to control the mosquito vectors [3]. Specific, effective and eco-friendly vector control agents are under investigations [4]. Earlier studies in the laboratory indicated larvicidal activity of methanol extracts of marine sponges such as *Dendrilla nigra, Clathria gorgonoides* and *Axinella donnani* towards *Culex* larvae [5]. In view of the importance of vector control and the scope of using sponge secondary metabolites, studies were carried out with five different methanolic extracts of marine sponges. Different concentrations of the sponge extracts were tested to determine the lethal dose of *Culex* larvae and the results are presented in this research article.

MATERIALS AND METHODS

Sponges entangled in fishing nets off Kanyakumari (8°04'N, 77°36' E) at depths ranging from10 to 15 meters were carefully removed from the nets. Five species of sponge viz., *Acanthella elongata, Echinodictyum gorgonoides, Axinella donnani, Callyspongia subarmigera* and *Callyspongia diffusa* were identified and used for conducting bioassay. The secondary metabolites were extracted using methanol. The extracts were filtered through Whatman No.1 filter paper and concentrated in a rotary evaporator (Buchi-type) under vacuum at low temperature to get crude methanolic extracts.

The larvicidal activities of crude methanol extracts of sponges were evaluated by adopting standard bioassay protocol [6]. Early third instar larvae of mosquito (*Culex sp.*) collected from stagnant water were used. Batches of the early third instar larvae of 10 numbers were introduced in 100 ml of water with varying dilutions ranging from 0.01% to 10.0% along with suitable control sets (without the extract). No activity or lack of reaction of larvae to gentle prodding with a glass pipette was counted for mortality [7]. Tests were conducted as triplicates for statistically significant results. Mortality was recorded after 24h of exposure, during this period, no food was provided to the larvae. The percentage mortality was calculated along with corrections for mortality by using Abbot's formula [8].

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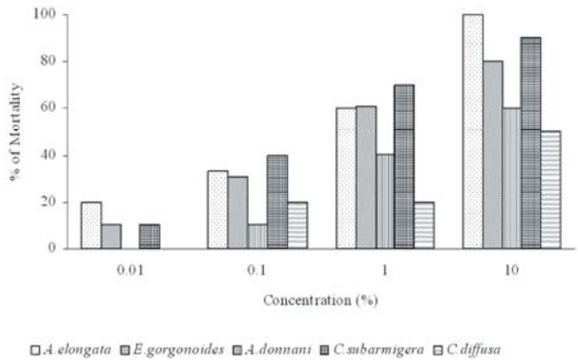

Fig. 1: Larvicidal activity of methanol extracts of marine sponge

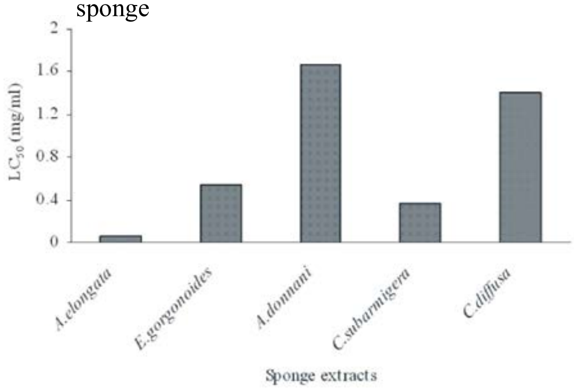

Fig. 2: Comparative LC_{50} values of different sponge extracts towards early 3rd instar larvae of *Culex sp.*

$$\text{Corrected mortality (\%)} = \frac{\text{(\% test mortality -\% control mortality)}}{\text{(100 -\% control mortality)}} \times 100$$

The corrected mortality was subjected to log-probit regression analysis and median lethal concentration (LC_{50}) was calculated [9].

RESULTS AND DISCUSSION

All the tested methanol extracts of marine sponges exhibited larvicidal activity (Fig. 1). Among these, the extracts of *Acanthella elongata* had high larvicidal activity and low LC_{50} value (0.066 mg/ml). Based on the LC_{50} values, the potency of crude sponge extracts could be categorized as: *A. elongata* > *C. subarmigera* > *E. gorgonoides* > *C. diffusa* > *Axinella donnani* in the decreasing order. A dose-dependent lethality was observed in all extracts. No mortality was observed at 0.01% level of *C. diffusa and A. donnani* extracts although 20.0% and 10.0% mortality respectively was recorded at higher concentration of 0.1%.

The extract of *Axinella donnani* was least toxic (Fig. 2). Probit analysis indicated 50% mortality at the higher concentration of 1.70 mg/ml. Selvin and Lipton 2004 [5] reported that, 10% of methanol extract of *A. donnani* killed all larvae while in the present investigation 60% mortality was noted at 10% concentration. This difference could be due to the seasonal variations, which again requires further investigations.

Mosquitoes such as *Aedes aegypti* act as vectors of dengue, yellow fever and chikungunya while *Anopheles stephensi* and *Culex sp* act as vectors of malaria and filariasis respectively. Most of the mosquito control programmes target the larval stage in their breeding sites with larvicides, as adulticides temporarily reduce the adult population only [10]. Due to increasing resistance of mosquitoes to current commercial insecticides such as organochlorides, organophosphates, carbamates and also to biological insecticides [11], researchers are now looking for natural products as larvicides with least hazard effects on non-target population along with easy degradability. Bio larvicides from bacteria viz., *Bacillus thuringiensis* and terrestrial plant extracts were tested for their efficacy [12, 13]. Information on larvicidal activity of mangrove plants, cyanobacteria, seaweeds, shrimps and sea cucumber is also reported [14-16].

Reports on larvicidal activity of sponge extracts are scanty. In India, marine sponges, *Dendrilla nigra, Clathria gorgonoides* and *Axinella donnani* exhibited larvicidal potential against second instar larvae of *Culex sp.* [5]. In the present study, the extract of *A. elongata* had the highest larvicidal activity against mosquito larvae. Detecting the mosquito larvicidal activity of sponge extracts has evident advantages such as the possibilities of developing new antifilarial and antimalarial compounds. Manzamines are the most promising antimalarial compound that has been discovered in many marine sponges [17]. Angerhofer *et al*. [18] isolated an antimalarial compound, sesquiterpene from *Acanthella klethra*.

CONCLUSION

Based on the earlier reports and the result of the present investigation, separation of active principles from *A. elongata* followed by bioassay guided purification and studying their mode of action warrants further research for developing potent antimalarial compounds.

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