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Full Length Research Paper

Larvicidal and insecticidal properties of some marine sponges collected in Palk Bay and Gulf of Mannar waters

J. Venkateswara Rao[1]*, P. K. Usman[1] and J. Bharat Kumar[2]

[1]Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hyderabad-500 007, India
[2]Bharat Institute of Engineering and Technology, Mangalpally, Ibrahimpatnam, Hyderabad- 501 510, India.

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Among marine invertebrates, sponges are one of the most productive marine ecosystems, with regard to presence of novel bio-active compounds. Few sponges (n = 18) were collected from Palk Bay and Gulf of Mannar waters of India and their methanol and dichloromethane (1:1) extracts were screened for larvicidal and insecticidal properties. Among them, around 40% of test extracts were active against the fourth-instar larvae of *Aedes aegypti* (Linn) and three to four day old of female houseflies, *Musca domestica* (Linn) at the concentrations of less than 100 ppm and 100 µg/insect respectively. Among the sponges *Psammaplysilla purpurea* and *Haliclona cribricutis* were found to be more active with both larvicidal and insecticidal properties. Considering both these activities, the following sponges *Psammaplysilla purpurea, Haliclona cribricutis, Dendrilla nigra, Haliclona pigmentifera* and *Petrosia testudinaria* could be used to obtain novel pesticidal molecules.

Key words: Marine Sponges, Biological screening, *Aedes aegypti, Musca domestica,* India.

INTRODUCTION

The marine environment is an exceptional reservoir of bioactive natural products, which produced several novel structures with unique biological properties, which may not found in terrestrial natural products. The ocean environment is massively complex, consisting of extreme variations in pressure, salinity, temperature, and biological habitats. Among the groups of marine organisms, sponges are the most diverse and abundant, due to their soft bodies and sedentary life styles. These marine invertebrates have evolved chemical defense mechanicsms against other invading organisms, which involve the production of secondary metabolites (Li Kam Wah et al., 2006). Recently, studies have also suggested that some bioactive compounds isolated from marine organisms have been shown to exhibit anti-cancer, anti-microbial, anti-fungal or anti-inflammatory and other pharmacological activities (Venkateswara Rao et al., 1998; Venkateswarlu et al., 2001; Proksch et al., 2002; Donia and Hamann, 2003; Haefner, 2003; Jha and Xu, 2004; Gul and Hamann, 2005; Mayer and Hamann, 2005). The current thrust of the investigations involves identifying newer drugs and other pharmaceuticals from marine origin, where as comparatively little attention has been paid with respect to the discovery of pesticide molecules. The Secondary metabolites isolated from marine sponges may be an alternative source for vector control agents to replace existing and highly toxic synthetic insecticides and will play an important role in future insecticide development programme.

The Gulf of Mannar Marine Biosphere Reserve (GoMBR; 8° 49' to 9° 15' N latitude and 78° 11' to 79° 30' E longitude) is located on the southeastern tip of India in

*Corresponding author. E-mail: jv@iict.res.in or jv@india.com. Tel.: Office: +91 (40) 2719 3191; Resident: +91 (40) 2720 5440; Fax: +91 (40) 2719 3227.

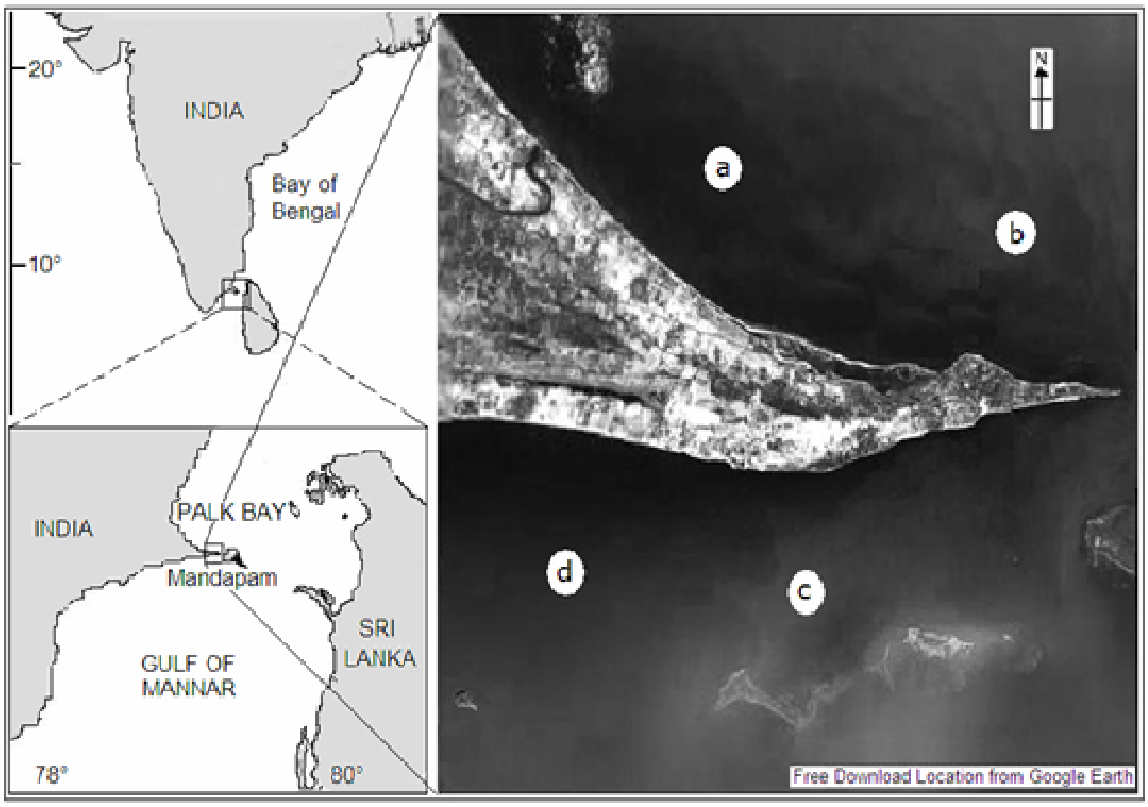

Figure 1. Map showing the collection sites at Palk Bay and Gulf of Mannar, India (9°10' to 9° 50' N latitude and 78°10' to 79°07' E longitude). Samples of sponges (n = 18) were collected from North and South coasts of Mandapam at depths varying from 15 - 25 feet by snorkeling and skin-diving. Sponge pieces were squeezed gently to drain of sea water, and extracted with 1:1 mixture of methanol and dichloromethane. The organic solvent portions were evaporated at reduced pressure for obtaining solvent free crude extracts. The test solutions with desired concentrations were prepared by mixing the known amount of solvent free crude extract in a carrier solvent acetone (w/v) and were then subjected to larvicidal and insecticidal screening. Mortality in each concentration and control was recorded after 24 h and the test mortalities were corrected with the use of the Abbots formula (Abbot, 1925). The median lethal concentration or dose (LC or LD_{50}) was calculated using 'Probit' analysis (Finney, 1971).

the state of Tamil Nadu, in the Indo-Pacific region. It lies between India and Sri Lanka, covering an area of about 10,500 km^2 and runs along (mainland) India coast to about 170 nautical miles. The Gulf of Mannar and its 3,600 Species of flora and fauna is one of the biologically richest coastal regions in all of mainland of India. The general objectives of this work were to evaluate the biodiversity of the marine environment around the seas of Palk Bay and the Gulf of Mannar, to isolate and characterize secondary metabolites from sponges and screen them for potential larvicidal and insecticidal properties. This paper reports the taxonomic identification of some potential sponges as a source for further exploration to obtain new leads of pesticidal molecules. These bioactive principles either may be produced from associated microbes or sponge itself. Hence, it is especially important for greater cooperation and well-coordinated efforts between bacteriologists, mycologists, natural product chemists, and entomologists for exploring the possibilities of developing newer pesticidal molecules from marine sponges. There is a lot of scope to obtain new leads of pesticidal molecules especially new toxophoric groups, which can be appropriately incorporated in molecules to obtain potent synthetic products with targeted features.

MATERIALS AND METHODS

Collection of sponges

Samples of sponges (n = 18) were collected in the Palk Bay and Gulf of Mannar (between 9°10' to 9° 50' N Latitude and 78°10' to 79°07' E Longitude, India) waters from north and south coasts of Mandapam at depths varying from 15 - 25 feet by snorkeling and skin-diving (Figure 1). Sponges were gently removed from the substratum and cut into small pieces and soaked in methanol for preparing crude extracts. The intact sample specimens were sent along with necessary details to the Central Marine Fisheries Res-

earch Institute (CMFRI), Trivandram, Kerala, India for identification.

Preparation of crude extracts

The initial aqueous methanol extract was concentrated in the laboratory under reduced pressure and lyophilized. The lyophilized powder was extracted with 1:1 mixture of methanol and dichloromethane. At the same time, the methanol soaked cut pieces (100 g) were further diced and extracted with the same mixture of solvents. The extracts were pooled and the organic portions were evaporated for obtaining solvent free crude extract. The test solutions with desired concentrations were prepared by mixing the known amount of crude extract in a carrier solvent, acetone (w/v) and were then subjected to larvicidal and insecticidal screening.

Biological screening

The cyclic colonies of *Aedes aegypti* (yellow fever mosquito vector) and *Musca domestica* (housefly) were reared in our insectary at 27 ± 1°C and 80 ± 5% RH with a photo period of 14:10 hour light and dark cycles followed by the methods of Morlan (1966) and Keiding et al. (1991) respectively, with little modifications.

Mosquito larvae lethality test (Larvicidal activity): The larvicidal activities of sponge extracts were evaluated against the fourth-instar larvae of *Aedes aegypti* (WHO, 1981). The each crude extract was tested to determine the larval bio-efficacy by making serial dilutions ranging from 10 to 400 ppm. The bioassays were performed at a room temperature of 27 ± 1°C by exposing 25 larvae in a final volume of 250 ml water in 500 ml glass beaker with minimum of four replicates for each concentration. Simultaneously, control groups were also run with zero concentration of the test extract (carrier solvent alone).

Housefly lethality test (insecticidal activity): Healthy female houseflies of three to four day old were treated topically (1 µl per insect) on the dorsal surface of the thorax with carrier solvent (control) and five to six different doses (ranging from 10 to 300 µg/insect) of test extracts with the help of a microapplicator (Microprocessor controlled insect topical applicator, Model- PAX-100-2; Burkard Scientific, UK). After treatment the flies were transferred into observation jars and a minimum of four replicates (25 flies each) were used for every test dose in three separate experiments.

Data analysis

The housefly and larval mortality in each dose/concentration and control was recorded after 24 h of exposure. Percentage mortalities were corrected for the natural mortality observed in the negative controls using Abbots (1925) formula; $P = PI - C / 1 - C$, where PI denotes the observed mortality rate and C means the natural mortality. The median lethal concentration or dose (LC_{50} or LD_{50}) was calculated using 'Probit' analysis (Finney, 1971) that has been recommended by OECD guideline as appropriate statistical method for toxicity data analysis. After linearization of response curve by logarithmic transformation of concentrations, 95% confidence limits and slope function were calculated to provide a consistent presentation of the toxicity data.

RESULTS AND DISCUSSION

The use of marine natural products is an alternative pest control method, which helps to minimize the usage of toxic pesticides and their deleterious effects on non-target insect species, livestock, wildlife and on the environment (Fatope et al., 1993). The sponge extracts of *Psammaplysilla purpurea* and *Haliclona cribricutis* was found to be the most effective against *A. aegypti* larvae showed LC_{50} values at < 50 ppm. However, other extracts of *Dendrilla nigra*, *Petrosia testudinaria*, *Petrosia similes*, *Haliclona pigmentifera*, *Ircinia fusca*, *Sigmadocia fibulata* showed LC_{50} values at <100 ppm (Table 1). The above eighteen crude extracts were also screened for insecticidal properties using housefly lethality test against *M. domestica* and their LD_{50} values are presented in Table 2. Among the extracts, *P. purpurea* and *H. cribricutis* was once again proved to be the most promising extracts with insecticidal properties against female adult *M. domestica* at LD_{50} values at <50 µg/insect. Among the extracts *Cinachyra cavernosa, Fasciospongia chondroides, Heteronema erecta* and *Sigmadocia pumila* did not show either larvicidal or insecticidal activities even at higher concentrations or doses (400 ppm for larvicidal and 300 µg/insect for insecticidal activity).

Earlier, we have isolated a molecule, ethylene bis isobutyl xanthate, from marine green alga, *Dictyosphaeria favulosa* (Venkateswarlu et al., 1993) based on the larvicidal activity (LC_{50} value at <50 ppm). The investigation further revealed that this molecule also exhibited insect growth regulator (IGR) properties against *A. aegypti* (Venkateswara Rao et al., 1995). Based on the bio-active properties, several analogues of alkylxanthates were synthesized and evaluated against lepidopteron pest, *Spodoptera litura* and *Helicoverpa armigera*. Three of the analogues i.e., methylene bis (tetrahydrofurfuryl xanthate), m-Fluorobenzyl n-butylxanthate and m-Fluorobenzyl isobutylxanthate have shown antifeedent and IGR activities against mosquitoes and agricultural pests (Venkateswara Rao et al., 2003).

The present preliminary investigations are helped us to short list the bio-active sponge crude extracts, which possess larvicidal and insecticidal activities. These active extracts could be used for obtaining new leads to isolate bioactive pesticidal molecules from marine origin. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Thomas et al., 2004; Dharmagadda et al., 2005; Singh et al., 2005). The percentage of active crude extracts (about 40%) identified in the present experiments is high in comparison to other terrestrial natural products. Previous literature also indicated that the marine organisms possess maximum percentage of bioactive substances with novel biological properties than the molecules originated from terrestrial origin (DOD Progress report, 1991). Use of these invented novel products in mosquito control instead of synthetic insecticides could reduce the environmental pollution. The present results may be useful as

Table 1. LC_{50} values (ppm) for 24 h with their 95% fiducial (lower and upper) limits, regression equation, Chi-square (χ^2) and P-levels of certain marine sponges against 4th instar larvae of *Aedes aegypti*[*].

Species	LC_{50} with fiducial limits	Regression equation $Y = (\bar{Y} - \bar{b}x) + b \log_{10}$ Conc.	χ^2 (df)	P-level	Relative activity
Psammaplysilla purpurea	25.90 (22.06 to 29.78)	Y = 1.40+2.55X	2.05 (4)	0.73	1.00
Haliclona cribricutis	31.46 (21.41 to 44.49)	Y = 0.57+2.96X	6.32(4)	0.18	1.21
Dendrilla nigra	63.99 (55.20 to 72.76)	Y = -0.09+2.82X	2.45(4)	0.65	2.47
Petrosia testudinaria	73.84 (63.85 to 84.62)	Y = 0.11+2.62X	2.32(4)	0.68	2.85
Petrosia similes	76.83 (67.26 to 87.23)	Y =-0.37+2.85X	3.75(4)	0.44	2.97
Haliclona pigmentifera	87.92 (73.28 to 103.78)	Y = 0.76+2.18X	1.88(4)	0.76	3.40
Ircinia fusca	89.68 (79.22 to 102.20)	Y =-0.73+2.93X	1.75(4)	0.78	3.46
Sigmadocia fibulata	97.28 (82.52 to 111.23)	Y = -1.15+3.09X	0.83(3)	0.84	3.76
Clathria reinwardti	110.77 (95.14 to 128.51)	Y = -0.16+2.53X	3.69(4)	0.45	4.28
Spirastrella inconstans	112.03 (94.91 to 132.17)	Y = 0.40+2.24X	2.34(4)	0.67	4.33
Fasciospongia cavernosa	120.59 (112.04 to 129.58)	Y =-6.84+5.68X	0.16(3)	0.98	4.67
Callyspongia diffusa	142.21 (122.64 to 162.86)	Y = -0.64+2.62X	3.28(4)	0.51	5.49
Spongia officinalis var ceylonensis	172.10 (150.49 to 197.83)	Y = -1.01+2.69X	2.66(4)	0.62	6.64
Dysidea herbacea	199.49 (176.86 to 228.70)	Y = -1.95+3.02X	2.13(4)	0.71	7.70
Cinachyra cavernosa	Not active at 400 ppm level				
Fasciospongia chondroides	Not active at 400 ppm level				
Heteronema erecta	Not active at 400 ppm level				
Sigmadocia pumila	Not active at 400 ppm level				

[*]The larvicidal activities of sponge extracts were evaluated against the fourth-instar larvae of *Aedes aegypti* (WHO, 1981). The bioassays were performed at a room temperature of 27±1°C by exposing 25 larvae in each concentration of the extract (n = 6) in a final volume of 250 ml water taken in 500 ml glass beaker. Four replicates for each concentration and the control (without sponge extract), were tested for larval bio-efficacy.

Table 2. LD_{50} values (µg/insect) for 24 h with their 95% fiducial (lower and upper) limits, regression equation, Chi-square (χ^2) and P-levels of certain marine sponges against 3 – 4 day old of female houseflies, *Musca domestica*[**].

Species	LD_{50} with fiducial limits	Regression equation $Y = (\bar{Y} - \bar{b}x) + b \log_{10}$ Dose	χ^2 (df)	P-level	Relative activity
Psammaplysilla purpurea	25.56 (21.29 to 31.21)	Y = 2.27+1.94X	2.31(4)	0.68	1.00
Haliclona cribricutis	36.13 (31.55 to 40.71)	Y = 3.73+0.04X	2.02(4)	0.73	1.41
Dendrilla nigra	57.55 (48.28 to 66.45)	Y = 0.55+2.26X	2.47(4)	0.65	2.25
Haliclona pigmentifera	70.43 (59.49 to 82.13)	Y = 0.79+2.27X	1.12(4)	0.89	2.76
Petrosia testudinaria	81.63 (71.70 to 92.81)	Y = -0.46+2.86X	3.77(4)	0.44	3.19
Spirastrella inconstans	93.46 (55.66 to 142.03)	Y = 0.36+2.36X	5.18(4)	0.27	3.67
Spongia officinalis var ceylonensis	99.72 (83.39 to 120.55)	Y = 0.56+2.22X	0.91(3)	0.82	3.90
Clathria reinwardti	114.43 (96.73 to 135.59)	Y = 0.48+2.19X	1.95(4)	0.75	4.48
Sigmadocia fibulata	134.27 (114.67 to 154.41)	Y = -0.38+2.53X	1.73(4)	0.79	5.25
Ircinia fusca	147.35 (82.59 to 230.54)	Y = -0.95+2.74X	7.03(4)	0.13	5.76
Fasciospongia cavernosa	161.22 (107.32 to 239.06)	Y =-1.02+2.73X	5.03(4)	0.28	6.31
Dysidea herbacea	162.17 (142.56 to 184.13)	Y = -1.38+2.89X	3.52(4)	0.48	6.34
Petrosia similes	183.65 (165.51 to 202.72)	Y = -4.01+3.98X	2.33(3)	0.51	7.19
Callyspongia diffusa	Not active at 300 µg/insect				
Cinachyra cavernosa	Not active at 300 µg/insect				
Fasciospongia chondroides	Not active at 300 µg/insect				
Heteronema erecta	Not active at 300 µg/insect				
Sigmadocia pumila	Not active at 300 µg/insect				

[**]Healthy female houseflies of three to four day old were treated topically on the dorsal surface of the thorax with five to six different doses (ranging from 10 to 300 µg/insect) of test solutions (1 µl per insect) with the help of a microapplicator. A minimum of three replicates (20 flies each) were used for every test concentration. Simultaneously, control groups were also run with zero concentration of the test substance (carrier solvent alone). After treatment the flies were transferred into observation jars and the mortality was recorded after 24 h.

blue prints to isolate the active principles from these active crude extracts. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these sponge extracts for development of eco-friendly chemicals for control of insect vectors.

Conclusion

Among the extracts evaluated, five of the sponge extracts i.e., *P. purpurea, H. cribricutis, D. nigra, H. pigmentifera* and *P. testudinaria* showed significant activity in both larvicidal and insecticidal assays. Based on the results the most promising extracts are from *P. purpurea* and *H. cribricutis* that showed both larvicidal and insecticidal activities with LC and LD_{50} at < 50 ppm and < 50 µg per insect, respectively. The results obtained from this study suggested that the above said sponges could be useful for searching newer pesticidal molecules from marine origin.

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