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Bioactivities of Protein Isolated from Marine Sponge, *Sigmadocia fibulatus*

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Abstract: The marine sponge *Sigmadocia fibulatus,* collected from Tuticorin coast, was studied for antibacterial and antifungal activity of proteins. Sponge species identified based on spicules morphology. Chloroform and aqueous extracts yielded a total amount of 6.8g and 5.5g from 500g of sponge respectively. Crude protein from the sponge was extracted the concentration of 2.82 g/mL in aqueous and 1.93 mg/mL in chloroform extract. The antimicrobial activity of crude extract against bacterial and fungal pathogens showed the clear inhibition zone against *Vibrio cholerae* and *Aspergillus niger* in chloroform extract and aqueous extracts showed clear inhibition zone for *Pseudomonas* sp. and *Candida albicans*. Both the extracts exhibited hemolytic activity which was estimated as 11.09ht/mg for chloroform extract and 9.8ht/mg for aqueous extract. The partial purification of protein is done by using DEAE cellulose. On SDS-PAGE the crude protein yielded three well defined bands at 109.9, 28.2, 12.4 KDa in both the extracts. The Fatty acid profile showed the dominance of myristic acid (14.67%) in the case of aqueous and chloroform extracts.

Key words: *Sigmadocia fibulatus, Vibrio cholerae,* chloroform exract, myristic acid and *Aspergillus niger*

INTRODUCTION

Sponges are the simplest of the multicellular invertebrate animals. They are exclusively aquatic, most of the sponges found in the deepest oceans to the edge of the sea. Sponges play important roles in so many marine habitats. They can survive in a variety of circumstances, like in places where there is no or abundant light and in cold or warm water. They are found in the shallow tropical reefs (Thompson, 1985). There are approximately 5,000 different species of sponges in the world, of which 150 occur in freshwater, but only about 17 are of commercial value. A total of 486 species of sponges have been identified in India (Thomas, 1998). In the Gulf of Mannar and Palk Bay a maximum of 275 species of sponges have been recorded. The distribution of sponges in other area as reported by Thomas (1998) is Gulf of Kutch - 25 species; and Orissa coast - 54 species. The rapid development of the pharmaceutical market has brought about a bloom of information regarding various toxins native to the sponges. Recently various technology developed to produce novel products from marine sponges; these could contribute to human healthcare (e.g. bioactive compounds that can be used for new medicines), A variety of natural products from the marine sponges have been found to exhibit remarkable antitumour and anti-inflammatory activities Thomas (1998).In the present study an attempt has been made to find out the symbiotic bacteria and bioactive proteins from the marine sponge, *Sigmadocia fibulatus*.

MATERIALS AND METHODS

The study area was located at Tuticorin coast, Gulf of Mannar region. Specimens of *S. fibulatus* were collected at extreme low tide by hand picking. They were brought to the laboratory with seawater. Subsequently they were kept in low temperature and preserved in 10% neutralized formalin for further study. The experiments were conducted from January to June, 2008

Extraction of spicules: The sponge tissue was boiled with concentrated HNO_3 to extrude the spicules that were then identified based on the identification characters given by Thomas (1998).

Extraction of crude toxin:

Aqueous extraction: The aqueous extract of sponge was prepared by squeezing the sand – free specimens in triple distilled water. The resultant solution was filtered and dialyzed by using Sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter -14.3 mm and capacity approx-1.61ml/cm) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4 ºC in a refrigerator for further use as crude aqueous extract.

Chloroform extraction: Crude toxin was extracted following the method of Bakus *et al.,* (1981) with certain modifications. The sponge was dried in air for 2 days and after that 10 g sponge tissue was shocked with 200 ml of chloroform, covered and kept standing for 5 hours. The solvent was then removed after squeezing the sponge and filtered through Whatman No 1 filter paper. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 45 ºC in refrigerator for further use as crude chloroform extracts.

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Antimicrobial activity: Petri dishes with nutrient agar and PDA agar were inoculated with six different species of bacteria and fungus. Sponge extracts were sterilized by passing each through a 0.22 µm Millipore GV filter (Millipore, U.S.A). Round paper discs with a radius of 0.8 cm were dipped into each sponge extract and placed in the center on inoculated petri dishes. The bacterial and fungal colonies were allowed to grow overnight at 37 and 20 ºC respectively, and then the inhibition zone around the disc was measured.

Protein estimation: Protein estimation was done as described by Lowry and Lopaz (1946), using Bovine serum Albumin at the rate of 1mg/ml as the standard. Different concentrations of the standard ranging from 0.1 to 1mg/ml were taken and made up to 1 mg/ml. Then 5ml of alkaline copper reagent was added, mixed well and allowed to stand for 10 minutes at room temperature. Then 0.5ml of diluted Folins phenol reagent was added and mixed well. The mixture was incubated for 30 minutes at room temperature. The absorbance at 720nm was read spectrophotometrically. The protein concentrations of *S. fibulatus* extracts were estimated

Partial purification of crude protein: Partial purification of the crude extract was carried out using DEAE Cellulose Anion Exchange chromatography according to the procedure of Stempion *et al.*, (1970).

Gas chromatography: Gas chromatography of the crude extract was done as described. Identification of fatty acid was carried out on the basis of retention times of the standard mixtures of fatty acids.

Hemolytic assay: The micro hemolytic test was performed as described by Venkateshwaran (2001) in 96 well 'V' bottom micro titer plates. Different rows were selected for chick blood. Serial two fold dilutions of the crude toxin were made in 100ml of normal saline. This process was repeated upto the last well. Then 100 µl of RBC was added to all the wells. Appropriate controls were included in the test. To the 1% RBC suspension 100µl was added normal saline, which served as negative control. The plate was gently shaken and then allowed to stand for two hours at room temperature and the results were recorded. Uniform red colour suspension in the wells was considered as positive hemolysis and a button formation in the bottom of these wells was considered as lack of hemolysis. Reciprocal of the highest dilution of the crude toxin showing pattern was taken as 1 Hemolytic Unit (HU).

SDS – PAGE: One dimension sodium dodecyl sulphate (SDS) Polyacrylamide gel electrophoresis (PAGE) was carried out following the modified method of Laemmli. (1970). SDS – PAGE was run on vertical slab gel system. Proteins were electrphorised on 12% separating gel (0.75 mm thickness) overlaid with 5% stacking gel. A 10 % (w/v) stock solution was prepared in deionized water and stored in room temperature.

RESULTS AND DISCUSSION

The sponge identification was confirmed based on spicules morphology. Chloroform extract of marine sponge *Sigmadocia fibulatus* yielded a total amount of 6.8g of crude extract from 500g of sponge. Similarly aqueous extract yielded a total amount of 5.25g of crude extract (Table1).

The crude of aqueous and chloroform extracts at different concentration of 5mg/ml, 10mg/ml and 15mg/ml were tested against 6 species of bacteria *Pseudomonas* sp., *Streptococcus aureus, Vibrio parahaemolyticus, V. cholerae, E. coli* and *V. parahaemolyticus* and 3 species of fungus, *A. flavus, A. niger and Candida albicans.* The results showed that the crude aqueous extract inhibit the growth of *V. cholerae* whereas in the chloroform extract a clear inhibition zone were observed only against *Pseudomonas* sp., The inhibition zone was measured and it was found to be 2.7 cm for *Pseudomonas* sp, and 1.8 cm for *V. cholerae* and the crude aqueous extracts inhibit the growth of *A. niger* whereas in the chloroform extract a clear inhibition zones were observed only against *C.albicans*. The inhibition zone was measured and it was found to be 3.2 cm for *A. niger* and 29 cm for *Candida albicans.* Burholder (1973) isolated two bromo compounds from *Verongi fistularies* and *V. vauliformis* that inhibited the growth gram positive and gram negative bacteria.

Bergquist and Bedford (1978) have suggested that the antibacterial agents produced by sponges may have a role in enhancing the efficiency with which sponge retain bacterial food and also reported that the activity was higher in temperate species than tropical species (87% as opposed to 58%) and the sponge extract more frequently inhibited the growth of marine bacteria.

The protein content in crude extract of *S. fibulatus* was found to be 1.6mg/ml in the case of chloroform extract and 1.4mg/ml in the case of aqueous extract (Table 2). Similarly, Boobathy *et al.* (2009) obtained the crude protein content of 1.62 mg/mL in methanolic crude extract and 1.43 mg/mL in the aqueous extract of marine sponge *Callyspongia diffusa*.

Gas chromatography study reveled that palmitic acid is the most abundant fatty acid in aqueous extract of *S. fibulatus* followed by stearic acid, myristic acid and lauric acid. In chloroform extract, myristic acid was found to be dominant and followed by palmitic acid, strearic acid, oleic acid and lauric acid (Tables, 3-4)..

The crude chloroform extract induced hemolysis on chicken erythrocytes. The hemolytic titer in methanolic extract was 14 and its specific activity was estimated at 8.64 HU/mg of protein (Tables 5&6). The hemolytic titer of aqueous extract was found 10 and its hemolytic activity was 6.99 HU/mg of protein. Stempien (1970) reported that halitoxin showed better hemolytic activity isolated from *genus Haliclona.*

Table 1: Preparation of crude extract

S No	Name of the solvent	Yield (in grams for 500 g of sample)
1	Chloroform	6.8
2	Aqua	5.25

Table 2: Protein estimation from aqueous and chloroform extracts of *Sigmadocia fibulatus*

S No.	Type of extract	Absorbance at 750 nm	Concentration of protein In mg / ml
1	Chloroform	1.6	1.632
2	Aqua	1.4	1.442

Table 3:Gas Chromatography profile of chloroform extract

Peak No.	Reten Time	Area (mV.s)	Amount (ul)	Amount (%)	Peak Type	Component Name
01.	0.963	4.3753	4.3753	1.984	Free	Lauric
02.	1.593	32.3586	32.3586	14.676	Free	Myristic
03.	2.495	32.22083	32.2208	14.614	Free	Palmitic
04.	2.907	19.5677	19.5677	8.875	-	-
05.	3.637	22.5232	22.5232	10.215	Free	Stearic
06.	4.600	17.3247	17.3247	7.858	Free	Oleic
07.	37.355	92.1123	92.1123	41.778		
	Total	220.4827	220.4827	100.000	-	-

Table 4. Gas Chromatography profile of aqueous extract

Peak No.	Reten Time	Area (mV.s)	Amount (ul)	Amount (%)	Peak Type	Component Name
01.	1.063	2.8542	2.8542	4.426	Free	Lauric
02.	1.563	3.4139	3.4139	8.882	Free	Myristic
03.	2.405	17.5114	17.5114	45.561	Free	Palmitic
04.	3.578	14.6554	14.6554	38.131	Free	Stearic
	Total	38.4349	38.4349	100.000	-	-

Table 5: Hemolytic activity from aqueous and chloroform extracts of Sigmadocia fibulatus

S.No	Type of extract	Amount of protein (mg)	Total hemolysis	Hemolytic titre	Specific hemolytic activity (HT/mg)
1	Aqueous	1.632	4	16	9.8
2	Chloroform	1.442	4	16	11.09

Table 6: Hemolytic activity of crude extract of *S. fibulatus* at 1 mg/mL on chicken erythrocytes

Type of extract	Name of the sample	Total activity (100mL)	Total protein (100mL)	Specific activity HT/mg	Yield (%)	Purification fold
Aqueous	Crude	9.15	1.0	6.98	100	1.0
	Partial Purification	12.80	1.40	8.49	120	1.10
Chloroform	Crude	4.55	0.85	5.21	100	1.0
	Partial Purification	9.56	1.21	6.33	155	1.21

The SDS-PAGE on gel, crude protein toxins yielded 6 bands in the chloroform extract and 5 bands in the aqueous extract of *S. fibulatus,* ranging from 14.4 to 116 kDa molecular weight with 5 well-defined bands of 28.5, 35.4, 45.0, 59.5, 72.3 kDa in both the extracts. Boobathy *et al.,* (2009) analysis that the presence of 3 protein bands viz, 19.5, 39.0 and 66.2 kDa already reported in *C. diffusa*.

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REFERENCES

Bakus, G., 1981. Chemical defence mechanisms on Great Barrier Reef, Aus. Sci., N. Y., 211: 497-499.

Begquist, P.R. and J.J.Bedford, 1978. The incidence of antibacterial activity in marine Demospngie, Systemic and Geographic consideration. Mar. Biol., 46: 215-221.

Boobathy, K ., T.T. Ajith kumar and K. Kathiresan, 2009. Isolation of symbiotic bacteria and bioactive proteins from the marine sponge, *Callyspongia diffusa*.

Burkholder, P.R., 1973. The Ecology of Marine Antibiotic and Coral Reefs. In: Biology and Geology of Coral Reefs. O.A. Jones and R. Endean, (Eds.). New York, London, Academcic press., 2: 117-182.

Laemmli U.K, 1970. Cleavage of structural proteins during the assembly of bacteriophage T4, Nature (Lond), 227: 680-688.

Lowery, O.H. and J.A. Lopez, 1946. The determination of inorganic phosphate in the presence of labile phosphate ester. J. Biol. Chem., 162: 421-428.

Stempein, M.F., 1970. Physiological Active Substance from Extracts of Marine Sponges. In: Food Drugs from the Sea, pp: 295-305.

Stempein, M.F., G.D., RuggieriNegrelli and J.T.Cecil, 1970. In: Food Drugs from the Sea, Proceeding 1969. H.W. Youngken, Jr., (Ed.). Marine Technology Society, Washington, D.C., pp: 295.

Thomas, P.A., 1998. Porifera.. In: Faunal Diversity in India. J.R.B Alfred, A.K.Das and A.K. Sanyal, (Eds.). ENVIS center, Zoological survey of India, Culcutta, pp: 28-36.

Thompson, J.E., 1985. Exudation of biologically – active metabolities in the sponge Aplysina fistularies. I. Biological evidence, Mar. Biol., 88: 23-26.

Venkateshvaran, K., 2001. Bioactive substance from marine organisms. Aquat. Animal Toxians Pharmacol. Bioresources., pp: 14-29.