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ORIGINAL ARTICLE

Biomedical Activities of Marine Sponge *Sigmadocia fibulata (Schmidt)collected from* West Coast of Mumbai, India

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ABSTRACT
The sponge Sigmadocia fibulata was collected during low tides from West Coast of Mumbai. Crude extract was obtained by taking 10 gram of sponge samples in10 ml of methanol. In the present investigation we found the crude protein contents in Sigmadocia fibulata as 0.096 mg/ml. The Neuromodulatory Na (+)-K+ ATPase activity and AChE on Sprague dawley rat brain and chicken brain extract may brain may contribute to the pathogenesis of metabolic complications of the central nervous system, and that the undetectable enzyme activity in chicken brain convulsing chicken brain may result from considerable damage or necrosis of brain tissue during seizures. In AChE our study is evident that both the sponge extract showed enzyme inhibitor activity at certain concentrations. In hemolytic activity showed potent toxin which is responsible for hemagglutination. Hemagglutination activity is generated by the presence of protein and the protein found in sponges which usually show hemmaglutination activity that might be because of presence of lectin which showed hemolytic activity. In CAM study showed that methanolic extract has strong antiangiogenic activity. The protein bands showed lectins have variety of effects on cells, such as agglutination, mitogenic stimulation, redistribution of cell surface components, modifying the activity of membrane enzymes, inhibition of bacterial and fungal growth, cell aggregation, toxicity, immunomodulation.
Key words: *Sponge, Neuromodulatory activity, hemolytic activity, lectins, agglutination*

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INTRODUCTION

Humans have been attempting to understand and use oceanic resources since ancient times. Ancient maritime people, notably the Chinese and Japanese, ate a variety of iodine- rich seaweeds that undoubtedly accounted for their low incidence of goiter. Chinese Pharmacopoeia recommends recipes for a number of disorders such as pain, menstrual difficulties, abscesses and cancer [1-14]. Coral Reef products have also been traditionally used for treating various ailments in Taiwan, Japan, China and India. Historical records show that human being has become aware of the venomous nature of some sea creatures for at least 4000 years [5, 6, 15]]. It has been known for centuries that sponges contain bioactive compounds that are of potential medical importance.

Looking at the massive potential of marine pharmacology, there are several organizations all over the world which are concentrating on research in this domain of drug development. The various processes involved in the development of marine-derived drugs, right from the discovery of potential novel compounds from marine organisms to extraction and isolation, along with the evaluation of their safety and efficacy are being planned for the future. However, there are many challenges and pitfalls in the development of drugs from marine sources which may be pulling back the pharmaceutical companies from investing in this domain [16]. However, many encouraging findings worldwide, especially related to the field of cancer chemotherapeutics, still gives the domain of marine pharmacology a ray of hope. Certain inhibitors of the signal transduction pathways involved in cancer development have been obtained from marine organisms, and further studies are under process to attain evidence on their

efficacy in cancer therapy [3, 7-9]). There is hope to find drugs of marine origin to targeted cancer therapy that is difficult currently with other available sources [13, 14]. Drug discovery efforts today, include the inhabitants of the world's oceans as a new source of biodiversity and novel drugs. In contrast to the terrestrial environment, little ethnomedicinal information is available to guide current marine research. With the exemption possibly of southern China, few societies have used marine organisms as crude drugs. Thus studies now in progress have relied on ecological observations of chemical defense and survival to identify those organisms that might be expected to contain drug candidates

MATERIAL AND METHODS

Collection of samples:

The sponges *Sigmadocia fibulata*(Schmidt) were collected during low tides from West Coast of Mumbai. Animal was taken alive to the laboratory in seawater washed under sea water and then with distilled water and sun dried.

Identification of sponges:

Preliminary identification was done by studying the shape and size of the spicules and by refereeing the relevant literature. The confirmation of identification was done by Dr. P.A. Thomas, Principal Scientist, Central Marine Fisheries Research Institute (CMFRI), Thiruvananthapuram, Kerala

Preparation of Sponge Extracts:

To 10 gram of sponge samples, 10 ml of methanol was added and kept standing for 24 hrs. Solvent were then removed, by squeezing sponge samples, and filtered through Whatman filter paper No.1.The remaining solvent was evaporated at low pressure using Rotary Vacuum Evaporator at 45°C. The resultant compound was subjected to Millipore filter system and finally dried in a vacuum desiccators and stored at 4°C in a refrigerator till further use.

Ethical approval:

Ethical approval is received by *Maharashtra State Biodiversity Board, Nagpur* for collection of sponges for research purpose. The voucher specimens of *Sigmadocia fibulata* was deposited at the repository centre at NIO Goa, India, as per the directions by Maharashtra State Biodiversity Board. The Voucher numbers of the said specimen is 1-NIO1006/18 *(Sigmadocia fibulata)*.

Experimental Design

Protein estimation was done by Kit Method. Haemolytic assay on Chicken and human blood was done by Pani Prasad and Venkateshwaran [11] method. For Na+-K+ATPase and AchE activity the method by Pani Prasad and Venkateshwaran [11] was followed. For Chorio-Allantoic membrane (CAM) assay was performed by the method [11]..

RESULTS AND DISCUSSION

Table No.1. Showing protein content in crude extract of *Sigmadocia fibulata*

Type of extract	Concentration of extract	protein (mg/ml) at 750nm	Average concentration of protein (mg/ml)
Methanol extract of *Sigmadocia fibulata*	0.14	0.056	0.056
	0.14	0.056	
	0.14	0.056	

Table No. 2.In vitro effect of methanolic extract of *Sigmadocia fibulata*on *Sprague dawley* rat brain (20±2g) and chicken (30±2g) brain Na^+-K^+ ATPase activity

S. No.	Concentration of Toxins (µg)	Na^+-K^+ATP-ase activity(µPi/mg protein/hour)	
		Sprague dawley rat brain	Chicken brain
1	10µg	0.087	0.11
2	20µg	0.071	0.11
3	30µg	0.079	0.10
4	40µg	0.063	0.10
5	50µg	0.071	0.096
6	60µg	0.079	0.095
7	70µg	0.079	1.110
8	80µg	0.071	1.122
9	90µg	0.087	1.131
10	100µg	0.046	1.135

(All results are average of triplicate sets)

Table No. 3. *In vitro* effect of methanolic extract of *Sigmadocia fibulata*on Sprague dawley rat brain (20±2g) and chicken (30±2g) brain AChE activity

S. No.	Concentration of Toxins (µg)	Level of Modulation (%)	
		Sprague dawley rat brain	Chicken brain
1.	50 µg	8	8
2.	100 µg	12	9
3.	150 µg	16	12
4.	200µg	19	24
5.	500 µg	24	96

(All results are average of triplicate sets)

Table No. 4. Angiogenesis in 9 day-old chicken egg after treatment with methanolic extract of *Sigmadocia fibulata*

Incubated up to 72 hours after treatment	*Sigmadocia fibulata*					
	0.5 min		2 min		5 min	
	40	80	40	80	40	80
Lysis	+	+	+	+	+	+
Hemorrhage	+	-	+	-	+	-
Coagulation	+	+	+	+	+	+

Fig No.01- Haemolytic Assay on Human erythrocytes

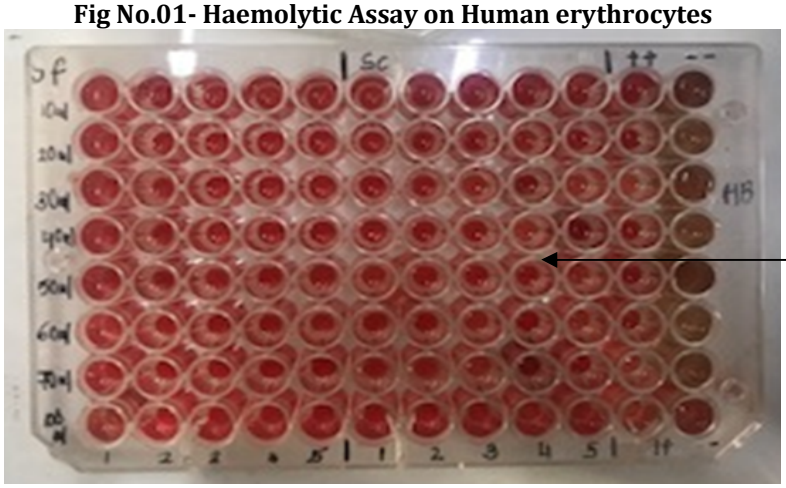


Fig No. 2-Haemolytic Assay on Chicken erythrocytes

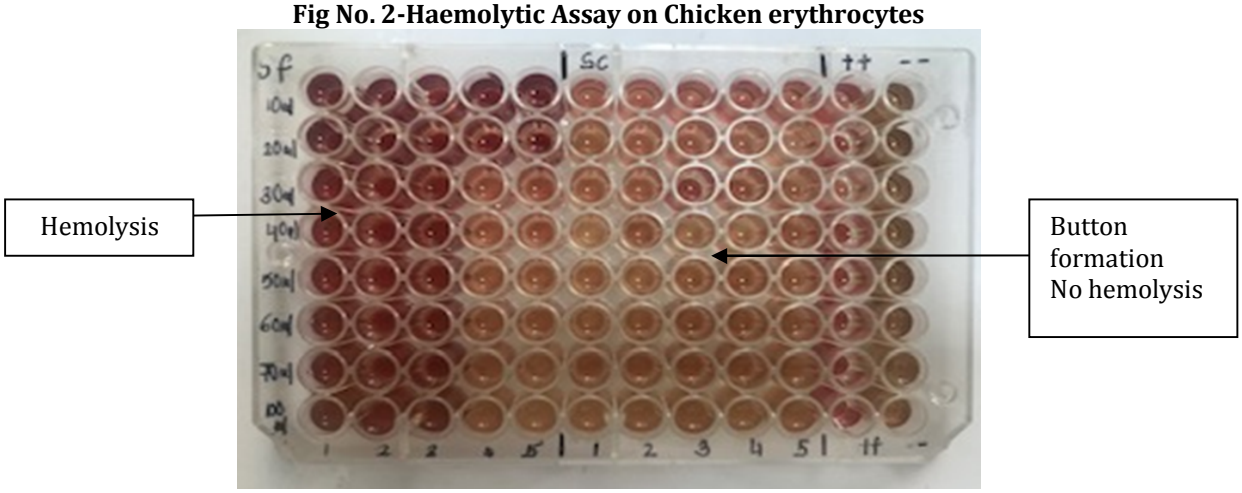
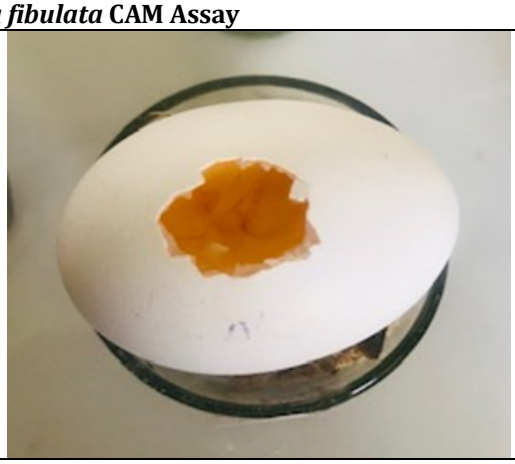


Fig No.3- *Sigmadocia fibulata* CAM Assay

Without Graft

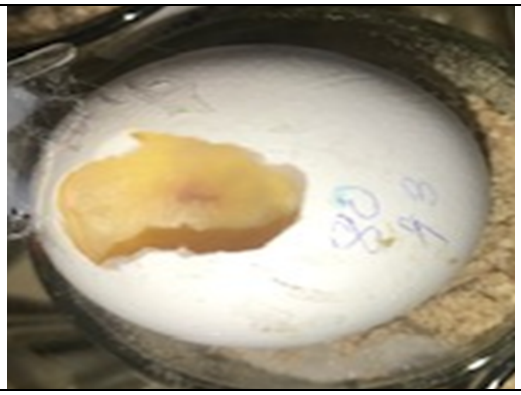


NaCl

Adding 40 µl of Sponge extract

Final

Adding 80 µl of Sponge extract

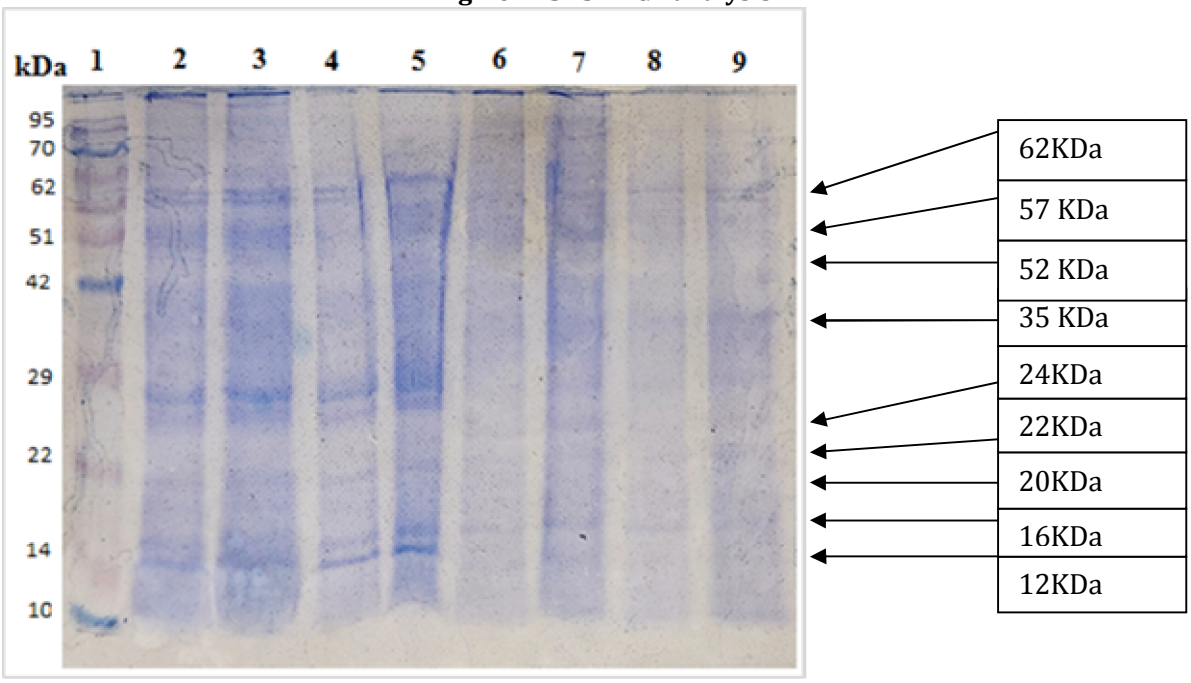


Final

PROTEIN ESTIMATION OF SPONGE CRUDE EXTRACTS

In the present investigation we found the crude protein contents in *Sigmadocia fibulata*0.056mg/mL. Very scanty data is available in the literature to study and correlate the crude protein content in sponges with our study. Our data of crude protein content in *Sigmadocia fibulata* is comparable with the study carried out by Purushottama [11], whereas the other various studied extracts could not be compared with previous studies because similar data on protein contents of sponge toxins are not comparable which is available in the above cited literature [10].

Fig No. 4-SDS PAGE analysis



NEUROMODULATORY ACTIVITY OF SPONGE CRUDE EXTRACTS ON ATP-Ase AND AChE ENZYME ASSAY

In our present investigations we found the neuromodulatory activity on *Sprague dawley* rat brain and chicken brain are shown in Table No.2showing in vitro effect of methanolic extract of *Sigmadocia fibulata* on Sprague dawley rat brain (20±2g) Na^+-K^+ ATP-ase activity. Neuromodulatory Activity Na^+ -K^+ ATP-ase activity in methanolic extract of *Sigmadocia fibulata* (0.087 to 0.046 %) in Sprague dawley rat brain extract. Whereas, in chicken brain the Na^+ -K^+ ATP-ase activity was found in chicken brain in *Sigmadocia fibulata* was (0.11 to1.135 %). However, in both the cases, the activity was enhanced at doses from 10 µg to 100 µg. The methanolic extract of *Sigmadocia fibulata* the Na^+ -K^+ ATP-ase activity was found height at 10µg/mL and 90µg/mL was (0.087%) in Sprague dawley rat brain extract. The trends in Na^+ -K^+ ATP-ase activity in methanolic extract of *Sigmadocia fibulata* was found irregularity in increasing and decreasing trends it is not steadily increasing or decreasing. It was found higher at the initial 10µg/mL and 90µg/mL (0.087%) concentrationsin Sprague dawley rat brain extract. Table No. 2 showing in vitro effect of methanolic extract of *Sigmadocia fibulata* on chicken (30±2g) brain Na^+-K^+ ATP-ase activity. The methanolic extract of *Sigmadocia fibulata* the Na+ -K+ ATP-ase activity was found highest at final concentration 100µg/mL (1.135 %) in chicken brain extract. The trends in Na^+ -K^+ ATPase activity in methanolic extract of *Sigmadocia fibulata* was found increasing trends in chicken brain extract. Table No.3 showing in vitro effect of methanolic extract of *Sigmadocia fibulata* on the Sprague dawley rat brain AChE activity. Neuromodulatory activities in Sprague dawley rat brain extract AChE activity in methanolic extract of *Sigmadocia fibulata* (8% to 24 %). Whereas, in chicken brain AChE activity was found in *Sigmadocia fibulata* was (88% to 48 %). The trends in AChE activity in *Sigmadocia fibulata* was registered increasing order at higher concentrations (from 50 µg/mL to 500 µg/mL), It was evident that in sponge methanol extracts *Sigmadocia fibulata* (24%)showed lowest AChE activity at 150 µg/mL concentrations in Sprague dawley rat brain and chicken brain extract as shown in Table No. 2 in vitro effect of methanolic extract of *Sigmadocia fibulata* on the Sprague dawley rat brain and chicken brain AChE activity.

HEMOLYTIC ACTIVITY OF SPONGE CRUDE EXTRACTS ON HUMAN AND CHICKEN ERYTHROCYTE.

In our present study, in the hemolysis assay, human and chicken red blood cells and test materials are co-incubated in buffers at defined pH that mimic extracellular, early endosomal, and late endo-lysosomal environments. The hemolytic activity of crude toxin on human and chicken RBC was tested by micro hemolytic essay method as proposed by [11].

The hemolytic activity of marine sponge *Sigmadocia fibulata*crude toxin at 5 mg/mL in human and chicken erythrocytes is represented in Fig No. 1. In human blood, crude methanolic extracts induced pronounced hemolysis. The hemolytic titer in methanolic extract was 40 and the specific hemolytic activity of *Sigmadocia fibulata* was 416.67(HT/mg). In chicken erythrocytes, the hemolytic titer in

methanolic crude extract was 28 and the specific hemolytic activity of *Sigmadocia fibulata* was 291.66 (HT/mg). Our results are in agreement with the results cited herein above. Thus it is confirmed that the methanolic crude extract of marine sponge *Sigmadocia fibulata* contains potent toxin which is responsible for hemagglutination. Hemagglutination activity is generated by the presence of protein and the protein found in sponges which usually show hemmaglutination activity that might be because of presence of lectin which showed hemolytic activity.

CHICK EMBRYO:- CHORIO ALLANTOIC MEMBRANE (CAM) ASSAY OF SPONGE CRUDE EXTRACTS

Angiogenesis is a physiological process of growth of nascent blood vessels from the existing vasculature. It is a complex multistep process that involves the activation, migration, invasion, and proliferation of vascular endothelial cells, followed by formation of sprout, tube-like structures, and finally capillary network formation Feng X (2014). It is a well-known fact that angiogenesis plays a prime role in the development of primary tumour into metastasis or malignancies. Physiological angiogenesis varies significantly from tumour angiogenesis. These differences are mainly in the form of altered endothelial-cell–pericyte interactions, unusual vasculature morphology, higher blood vessel permeability, irregular blood flow and delayed maturation. Such abnormal features of the tumour vasculature lead to tissue hypoxia which in turn upsurges the expression of angiogenic promoters or proangiogenic factors [12].

In our study, the results of the chorioallantoic membrane (CAM) assay are presented in Fig No. 4 After treatment with crude methanolic extract; the eggs were incubated for 72 hours. After 72 hours the CAM was measured at 0.5 min, 2.00 min, and 5.00min intervals. All the extract from sponge *Sigmadocia fibulata* showed antiangiogenic response including lysis, hemorrhage, and coagulationof blood vessels at 40 µg/ mL and 80 µg/ mL doses. In control CAM the blood vessels were distributed in tree branches like patterns in which primary blood vessels gives off secondary blood vessels. In experimental group, It was found that the extracts was highly toxic to the eggs at concentrations of 40 µg/ mL and 80 µg/ mL after 72 hours of time intervals. All the concentrations of extract showed disintegration of blood vessels by rupturing the membrane which leads to lyses by reducing the blood supply. In case of sponge *Sigmadocia fibulata* the hemorrhages were observed at 40 µg/ mL concentration whereas no effect was noted at 80 µg/ mL concentration in the sponge extract. In case of *Sigmadocia fibulata* the coagulation was observed at 40 µg/mL and 80 µg/mL concentration. It was also observed that in some cases embryo had reduced blood supply, whereas the others were found complete lysis. The eggs treated with extract disrupted mostly newly forming blood vessels by affecting the preexisting vasculature.

SDS-PAGE ELECTROPHORESIS FOR THE SEPARATION OF PROTEINS

In the present investigation in aqueous crude toxin extract of marine sponge, we found around nine bands in the sponge. The protein bands are found in *Sigmadocia fibulata* are 12kDa, 16kDa, 20kDa, 22kDa, 24kDa, 35kDa, 52kDa, 57kDa, and 62kDa. Our data is found comparable with the data cited above. The presence of protein at 20kDa, and 22kDa showed hemolytic activity. It was also confirmed that the protein present in sponge are having corresponding proteins at 24kDa which confirm the presence of lectins present in the sponges. Lectins are a special class of proteins. The presence of lectins in invertebrate animals occurs in almost all phyla in the haemolymph and coelomic fluid, which can be detected through haemagglutination assays; interact with different carbohydrates present in cell surfaces. Lectins and their characteristic properties, mainly due to their ability to bind glycoconjugates, stand out as important tools in research covering various areas of science, especially in biochemistry, cellular and molecular biology, immunology, pharmacology, medicine and clinical analysis. Lectins have a variety of effects on cells, such as agglutination, mitogenic stimulation, redistribution of cell surface components, modifying the activity of membrane enzymes, inhibition of bacterial and fungal growth, cell aggregation, toxicity, immunomodulation.

CONCLUSION

From the above results it is concluded that, the compounds extracted from sponge *Sigmadocia fibulata* showed biomedical properties. Our findings are significant for the development of multi-drug therapy for both pharmaceuticals and biomedical applications. Therefore we have screen the crude extracts of sponges for its structural elucidation to find the new drugs for pharmaceutical industry. The study further suggests that, *Sigmadocia fibulata*, further screening is required for molecular level to understand the physiology and mode of action of the compound. The clinical study is also required which may be useful for pharmaceutical industry to manufacture the new drugs for safe performance and safety indexes to be studied to eradicate the diseases from mankind in future.

CONFLICT OF INTEREST

There is no conflict of interest

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