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Original Research Article

Biomedical Activities of Marine Sponge *Suberites carnosus (Johnston)* Collected from West Coast of Mumbai, India

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Abstract

The sponge *Suberites carnosus*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 *Suberites carnosus* as 0.096 mg/mL. The Neuromodulatory Na (+)-K+ ATPase activity and AChE on *Sprague dawley* rat brain and chicken brain extract 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 A Ch E 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.

Keywords: Sponge, Neuromodulatory activity, hemolytic activity, lectins, agglutination.

INTRODUCTION

"Poison kills the poison," the famous proverb is the basis for researchers in finding the biomedical metabolites from living organisms. Sea has got plenty of metabolites and other resources in living or dead form [1]. Sponges (37%), coelenterates (21%) and microorganisms (18%) are the major sources of biomedical compounds followed by algae (9%), echinoderms (6%), and tunicates (6%), molluscs (2%) bryozoans (1%) [2]. Pharmacological investigations of oceanic organisms are relatively new and have been based on the establishment of unprecedented "scientific bridges" between the marine and pharmaceutical sciences. In this day and age, roughly one-half of all cancer drug discovery focuses on marine organisms, and forecasts for the future are brilliant, as well [3]. In fact, some of the most important recent discoveries have been from the oceanic milieu. Marine drug discovery began in the late 1970s by early investigators demonstrating, unequivocally, that marine plants and animals were genetically and biochemically unique [4]. Over 18,000 structurally unique and often highly bioactive metabolites have now been isolated from marine plants and animals. After the uniqueness of marine metabolism became accepted, programs began to evolve that linked academic marine scientists with biomedical researchers in the pharmaceutical industries [5]. Programs, which established the foundations of today's efforts, were created in the 1980s in the United States, in Japan, and in Australia [6].Today these programs are expanding on the basis of their continuing discoveries of novel new drug leads. Unlike the majority of terrestrial drug research, marine drug discovery programs have been applied to selected, difficult to treat diseases that have eluded cures for decades [7]. Yet, progress has been observed in many of these difficult areas. Research into the pharmacological properties of marine natural products has led to the discovery of many potently active agents considered worthy of clinical application [8]. The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit

structural/chemical features not found in terrestrial natural products [9].

MATERIALS AND METHODS

a) Collection of samples

The sponge Suberites carnosus were collected during low tides from West Coast of Mumbai. Animals were taken alive to the laboratory in seawater washed under sea water and then with distilled water and sun dried.

b) 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

c) Preparation of Sponge Extracts

Crude extract was obtained following the method of [10] with some modifications. 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 45^0 C. The resultant compound was subjected to Millipore filter system and finally dried in a vacuum desiccator and stored at 4^0 C in a refrigerator till further use.

d) Ethical approval

Ethical approval is received by Maharashtra State Biodiversity Board, Nagpur for collection of sponges for research purpose. The voucher specimens of Suberites carnosus 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.

RESULTS AND DISCUSSION

Table-1: Showing protein content in crude extract of Suberites carnosus

Type of extract	Concentration of extract	protein (mg/ml) at 750nm	Average concentration of protein (mg/ml)
Methanol extract of Sigmadocia fibulata	0.24	0.096	0.096
	0.24	0.096	
	0.24	0.096	

Table-2: In vitro effect of methanolic extract of Suberites carnosus on Sprague dawley rat brain (20±2g) and chicken (30±2g) brain Na^+-K^+ ATP-ase activity

S.No.	Concentration of Toxins (µg)	Na^+-K^+ATP-ase activity(µ Pi/mg protein/hour)	
		Sprague dawley rat brain	chicken brain
1	10µg	0.061	0.098
2	20µg	0.067	0.086
3	30µg	0.061	0.057
4	40µg	0.067	0.055
5	50µg	0.073	0.068
6	60µg	0.078	0.073
7	70µg	0.080	0.078
8	80µg	0.086	0.087
9	90µg	0.086	0.092
10	100µg	0.092	0.098

(All results are average of triplicate sets)

Table-3: In vitro effect of methanolic extract of Suberites carnosus 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 (30±2g) brain
1.	50 µg	8	168
2.	100 µg	12	96
3.	150 µg	16	32
4.	200µg	19	48
5.	500 µg	24	192

(All results are average of triplicate sets)

Table-4: Angiogenesis in 9 day-old chicken egg after treatment with methanolic extract of Suberites carnosus

Incubated up to 72 hours after treatment	Suberites carnosus					
	0.5 min		2 min		5 min	
	40	80	40	80	40	80
Lysis	+	+	+	+	+	+
Hemorrhage	+	-	+	-	+	-
Coagulation	-	+	-	+	-	+

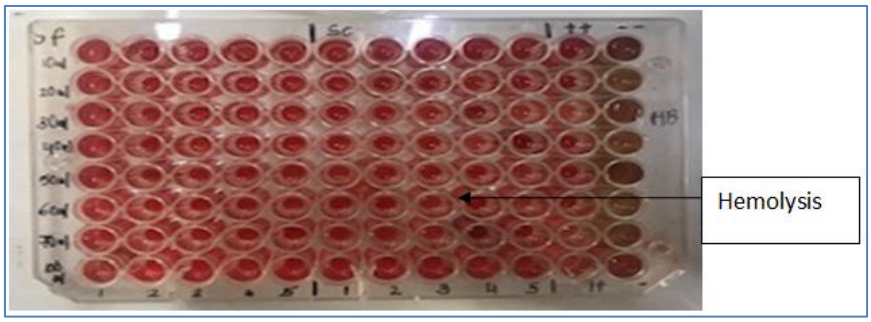


Fig-1: Suberites carnosus Haemolytic Assay on Human erythrocytes

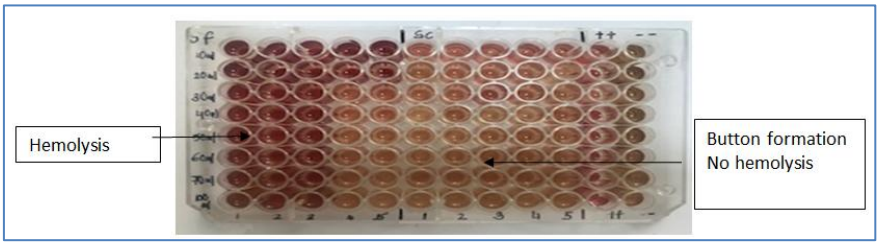
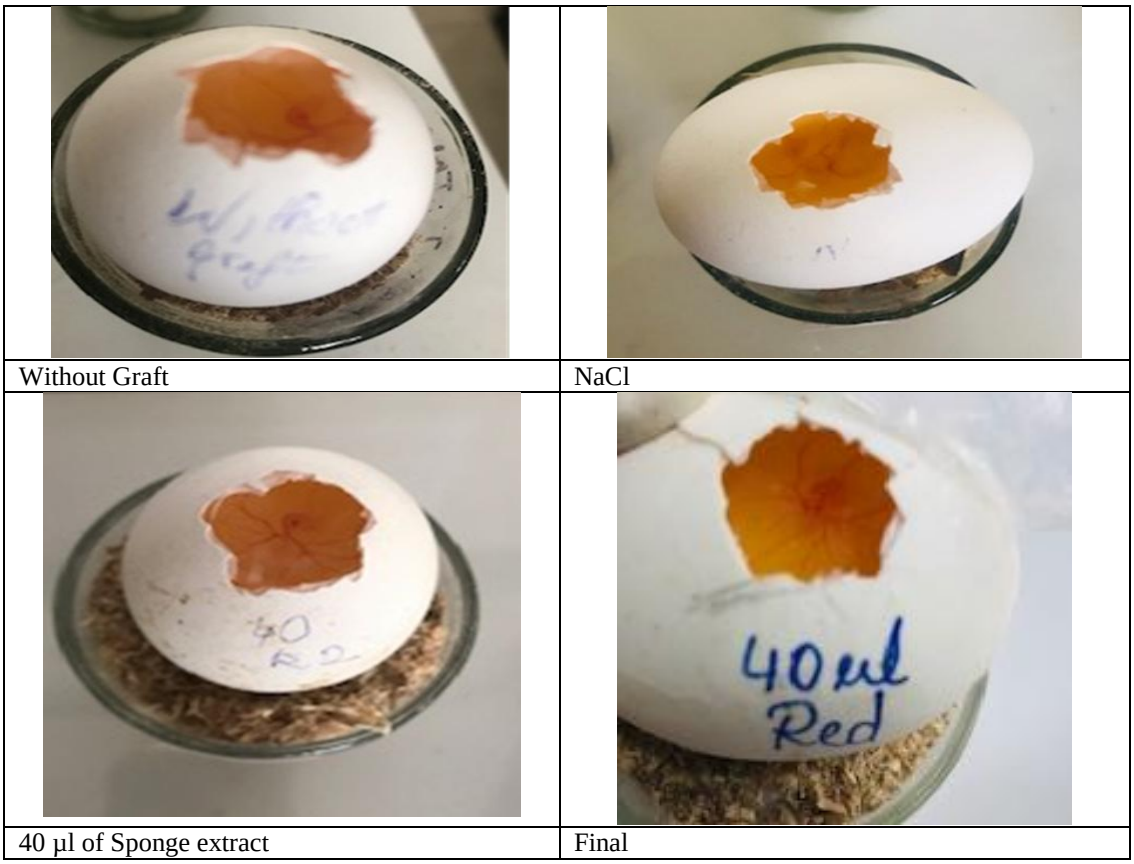
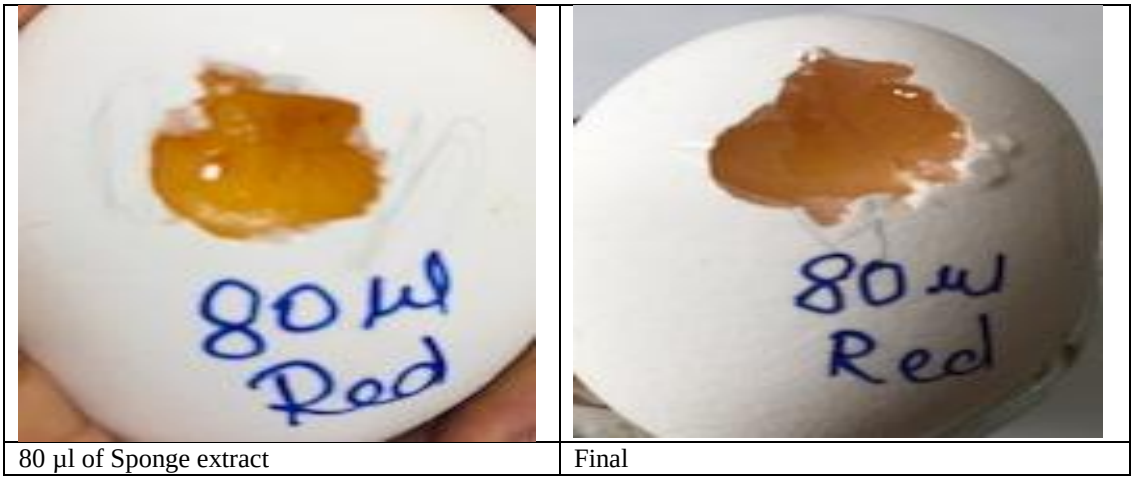


Fig-2: Suberites carnosus Haemolytic Assay on Chicken erythrocytes



Without Graft | NaCl

40 µl of Sponge extract | Final



80 µl of Sponge extract | Final

Fig-3: Suberites carnosus (Johnston) CAM Assay

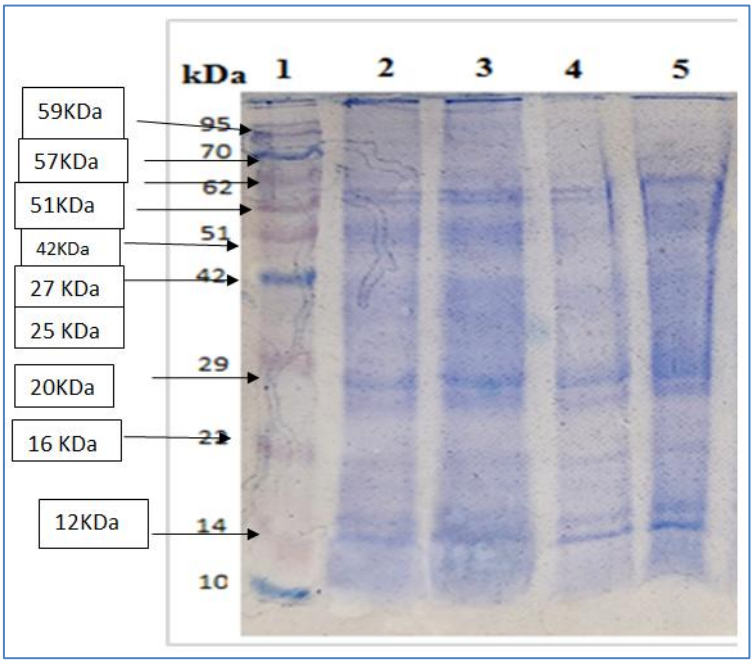


Fig-4: Suberites carnosus(Johnston) SDS PAGE analysis

A). Protein Estimation of Sponge Crude Extracts

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 [11]. Similarly, [12] 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. The crude protein contents were found to be 0.096 and 0.192 mg/mL, respectively, in methanolic and chloroform-methanol extracts, and 0.124 mg/mL in aqueous extract. It ranged from below detectable levels to 0.014 mg/mL in methanolic extract fractions and from below detectable levels to 0.002 mg/mL in aqueous extract [13]. The amount of crude toxin extracted by lyophilization in the current study was higher compared to those reported in earlier findings [14] obtained 1.67 g of crude extract from 200 g of Epipolasis sp. and [15]obtained 5.8 g of crude extract from 490 g of fresh marine sponge Suberea praetensa [16]. The average protein concentration in methanol crude extract of marine sponge was found to be 5.3mg/ml, in aqueous crude extract of marine sponge was found to be 3.4mg/ml and in Methanol: chloroform extract was found to be 2.4mg/ml. Lyophilized toxin-like proteins from bacterialisolates A03 was 2.78 g and A03k was 1.38 gr of sponge Haliclona molitba wasfound toxinlike proteins from A03 and A03k were 0.23 mg/mL and 0.26 mg/mL, respectively [17].

Table No. 1.In the present investigation we found the crude protein content in Suberites carnosus was 0.056 mg/mL as shown in table 1. Very scanty data is available in the literature to study and correlate the crude protein content in sponge with our study. Our data of crude protein content in Suberites carnosus is comparable with the study carried out by[18], 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.

b). Neuromodulatory Activity of Sponge Crude Extracts on ATP-Ase and AChE Enzyme Assay

Although the phylum Porifera are multicellular organism, they are generally considered to be primitive and evolutionally. They lack nervous system but histochemical detection of AChE activity has provided indirect evidence of AChE expression in them a conclusion that is directly confirmed by the present study in three marine sponges belonging to Demospongiae group but different group and family [19]. Three sponges identified as H. glabrata [20-22] S. pachyspira and C. lobata are a new addition to the distribution of these species in the coastal areas of Mumbai, India. These sponges have not been studied for their AChE and antiangiogenic activities to the best of our knowledge. AChE is an enzyme essential to correct transmission of nerve impulses, and inhibition of this enzyme has been used to detect and measure the biological effects of organophosphorus and carbamates pesticides in the marine environment [23]. In an earlier work large polymeric 3-alkylpyridinium salts from the marine sponge-Reniera Sarai were isolated and acted as acetylcholinesterase inhibitors and showed an unusual inhibition pattern [24]. It was tentatively described as quick initial reversible binding, followed by slow binding or irreversible inhibition of the enzyme. Another recent study reports thin-layer chromatography and microplate assays revealing potent AChE inhibitory activities of two ethyl acetate extracts from the sponges Pericharax heteroraphis and Amphimedon navalis [25]. AChE inhibitors from marine sponges have been rarely studied, and this study demonstrates the potential of marine sponges present with AChE enzymes and probable inhibitors as a source of pharmaceutical leads against neurodegenerative diseases and also for cancer therapy, as it was demonstrated for Haliclona sarai and Reniera sarai [26, 27]. The impact of both sponges Halichondria panicea methanolic and aqueous extracts was found to increase activities of Na+ -K+ ATP-ase and Mg++ ATP-ase. In the case of chloroform-methanol extract, higher concentrations increased acetylcholine esterase (AchE) activity[28]. Their results on AChE activity of Halichondria panicea extracts correspond to earlier findings of[29], who reported elevated AChE activity due to higher doses of tetrodotoxin. [30]observed similar neuroinhibitory activity by bile extracts of freshwater carps on the Na+ -K+ ATP-ase enzyme system in mammalian models. The ATP-ase enzyme system is widely accepted as a structure that employs part of the free energy from ATP hydrolysis for active transport of Na+ - K+. [31]found that metabolic stimulation provoked by ionic movement at the membrane level results from a series of reactions that lead to accumulation of ADP and Pi (inorganic phosphate), which play an important role in regulating respiration. Augmented ADP concentration intensifies mitochondrial respiration that, in turn, increases oxygen consumption and accelerates ATP biosynthesis [32]. Studied the tropical marine sponges exhibited various biological activities including anti-acetyl cholinesterase activity.

In our present investigations we found the neuromodulatory activity on Sprague dawley rat brain and chicken brain are shown in Table No. 2. Showing in vitro effect of methanolic extract of Suberites carnosus on Sprague dawley rat brain (20±2g) Na^+-K^+ ATP-ase activity. The neuromodulatory Activity Na^+ - K^+ ATP-ase activity in methanolic extract of Suberites carnosus was (0.061 to 0.092 %) in Sprague dawley rat brain extract. Whereas, in chicken brain the Na^+ -K^+ ATP-ase activity was found in chicken brain in Suberites carnosus was (0.098to 0.098%). The activity was found enhanced at doses from 10 µg to 100 µg. The methanolic extract of Suberites carnosus, the height activity was noted at 100µg/mL was 0.092 %) in Sprague dawley rat brain extract. The trends in Na^+ -K^+ ATP-ase activity in methanolic extract of Suberites carnosus, the trends in Na^+ -K^+ ATP-ase activity was found steadily increasing trends from lower to higher concentrations in Sprague dawley rat brain extract. Table No. 2, showing in vitro effect of methanolic extract of Suberites carnosus on chicken (30±2g) brain Na^+-K^+ ATP-ase activity. The methanolic extract of Suberites carnosus, the height activity was noted at initial 10µg/mL and 100 µg/mL final concentrations (0.098%) in chicken brain extract. Interestingly in Suberites carnosus the concentration of in Na^+ -K^+ ATP-ase initially it showed increase and then steady declined and then sudden augmentation was observed. The nature of graph showed hyperbola shape from lower to higher concentrations in chicken brain extract.

Table No. 3, showing in vitro effect of methanolic extract of Suberites carnosus on the Sprague dawley rat brain AChE activity. Neuromodulatory activity in Sprague dawley rat brain extract AChE activity in methanolic extract of Suberites carnosus (8% to 96 %) from initial 50 µg/mL to final 500µg/mL concentrations in Sprague dawley rat brain extract. Whereas, in chicken brain AChE activity was found in Suberites carnosus was (168 % to 192 %). The trends in AChE activity in Suberites carnosu, the nature of graph showed hyperbola shape. It was evident that in sponge methanol extract Suberites carnosus (32 %) showed lowest AChE activity at 150 µg/mL. concentrations in Sprague dawley rat brain and chicken brain extract as shown in Table No. 3, in vitro effect of methanolic extract of Suberites carnosus on the Sprague dawley rat brain and chicken brain AChE activity.

c). Hemolytic Activity of Sponge Crude Extracts On Human And Chicken Erythrocyte.

Many authors have observed the haemolytic activity of sponge extracts on erythrocytes of human and other animals. The study carried out by [33, 34] indicated significant levels of haemolytic activity of the sponge Renierasarai extracts and moderate haemolytic activity of extracts from Saracotragusm uscarum and Aplysina aerophoba [35]. The sponge Sigmadocia pumila extracts showed high activity on the human erythrocytes [36]. The compounds from sponge Topsentia sp showed strong hemolytic activity on fresh bovine erythrocytes [37]. The Spongia sp and Spirastrella sp released the spongistatins compound, which exhibited antiprolific activity [38]. The results of haemolytic activity of sponge Halichondria panicea on human blood, chick blood and goat blood showed least activity with human erythrocytes compared to other animals [39]. Tropical marine sponges exhibited various biological activities including hemolytic, hemagglutination. The hemolytic activity exhibited from the sponge Pandaros acanthifolium showed effective amount of proteins. Then the sponge Myrem ekiodermastyx showed the hemagglutinating activity against human RBC [40]. Both the methanolic and aqueous extracts of C. diffusa, induced pronounced hemolysis on chicken erythrocytes [41, 42] reported that halitoxin showed better hemolytic activity isolated from genus Haliclona [43] reported hemolytic activity in the chloroform extract from Tethya sp. Moreover, [44, 45] reported that the sterol derivatives viz., halistanol sulphate and sokotrasterol sulphate, obtained from Halichondriidae sponges possessed hemolytic activity [46]. Reported the haemagglutination and hemolytic activity of the aqueous extract obtained from 48 tropical sponge species [47]. In their analysis they found that the Spongosorites halichondriodes showed hemolytic activity in both the extracts exhibited which methanol and aqueous extract. Haemolytic activity of halitoxin from sponges of the genus Haliclona of aqueous extracts from 48 tropical sponge species have been evaluated by [48]. In their study they recorded the haemolytic activity in crude extract of sponge. [49] reported that sterol derivatives from halichondriid sponges, namely halistanol sulfates and sokotrasterol sulfates, possessed hemolytic activity. It also exhibited hemolytic activity on chicken and human erythrocytes [50]. A bioassay-guided pharmacological screening study with sponge-derived extracts from specimens collected from the coast of Sao Paulo State, on the south-eastern region of Brazil, recorded that the organic extracts of Geodia corticostylifera was hemolytic on mice erythrocytes [51]. The study carried out by [52] on haemolytic activity of human erythrocytes with the extracts of A. carteri, they found that the extract in crude methanol and crude chloroform methanol showed high level of haemolysis which exhibited concentration dependent haemolytic activity. Their study on the effect of different extracts of sponge A. carteri on human blood plasma showed that there was no coagulation with any of the extracts indicating that A. carteri extracts have no effect on blood clotting. DEAE methanol and DEAE chloroform showed some activity but it was comparatively very low. The extracts in hexane and acetone did not have any effect on human erythrocytes [53]. Their study is in agreement with the results reported for haemolytic activity using the venom of Palythoacaribaeorum, a Cniderian [54], and [55] they observed that there was pronounced haemolysis in crude methanolic and chloroform- methanol extracts of Halichondria panicea with chicken blood and human blood while aqueous extract failed to elicit haemolysis in chicken and human blood [56]. In their investigation, they have screened 22 species from 12 families of tropical sponges for the presence of lectins. Amongst those nine saline extracts were found strong hemagglutinating activity against pronase-treated hamster red blood cells; five of these reacted against rabbit red blood cells, four with trypsin treated bovine red blood cells, and five with human red blood cells regardless of the blood group type. Extracts from the three species studied from genus Aplysina (archeri, lawnosa, and cauliformis) were highly reactive and panagglutinating against the panel of red blood cells tested. Haemagglutination in twelve species of sponges was observed and found that it was different for different blood types suggesting the presence of lectin like molecules in the sponges [57]. Since the sponge A.carteri extracts failed to agglutinate human blood, it may be lacking lectins. In at least twelve tested species, hemagglutinating activity was different for different blood types, suggesting the presence of lectin-like molecules in these species; namely, hemagglutinating activity was observed in 60% of the tested species [58]. This value is similar to those found in other screenings of hemagglutinating / hemolytic activity of marine invertebrates [59, 60].

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 [61].

The hemolytic activities of marine sponge Suberites carnosus crude toxin at 5 mg/mL in human and chicken erythrocytes are represented in Table No.4, Fig No 1 and 2, in human blood, crude methanolic extracts induced pronounced hemolysis. The hemolytic titer in methanolic extract of Suberites carnosus, the hemolytic titer in methanolic crude extract was also 40 and but the specific hemolytic activity was found to be 714.28 (HT/mg). In chicken erythrocytes, the hemolytic titer in methanolic crude extract was also 40 and but the specific hemolytic activity was 714.28 (HT/mg). It was found that the methanolic extract elicit in both the blood

(human and chicken) samples, induced hemolysis of erythrocytes observed in Suberites carnosus. 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 and Suberites carnosus 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.

d). 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 [62]. 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 [63].

The CAM was first used to study tumor angiogenesis by grafting tumor samples onto its surface on day 8 of incubation. Since then, the CAM assay has been used to identify almost all of the known angiogenic factors and to assess the angiostatic activity of a variety of natural and synthetic compounds[64].Several CAM angiogenic assays have been introduced since almost a century ago when rat Jensen sarcoma cells, implanted into the CAM on the day 6 of incubation[65]. All modifications of the original angiogenic assay in the chick embryo involve grafting of test material onto developing CAM. The grafting is often performed through a window cut in the egg shell over the CAM. The angiogenic material is usually introduced in the form of small disks soaked in angiogenic factors or small pieces of polymerized materials such as gelatin sponges or biologically inert synthetic polymers, containing either purified angiogenic factors or impregnated with tumor cells. Another less traumatic way of introducing angiogenic material onto the CAM involves the use of shell-less embryos grown exovo, which makes the CAM more accessible for repetitive manipulations, for quantitation of angiogenesis, and for direct visualization of the angiogenic process under a stereoscope [66].

The concept of using marine bioactive compounds in the field of antiangiogenesis has gained momentum in the last three decades after the landmark study by [67]. Marine organisms including sponges, sponge-associated bacteria, gorgonia, molluscs, soft coral and actinomycetes, have been extensively explored for potential antiangiogenic agents. According to a recent review, currently more than 43 marine derived compounds are known to possess anti-angiogenic properties, out of which 10 have entered the different phases of clinical trials for cancer therapy [68]. These compounds show great structural and chemical diversity which includes saccharides, macrocycles, terpenes, peptides, saponins, pyrones, alkaloids and xanthones. Due to their unique structures and diversity, these compounds show specific mechanism of their anti-angiogenic activity by altering distinct targets. Therefore, it has been suggested that microtubule-targeted drugs can be very beneficial as anti-angiogenic agents [69]. Until today, nature has proven to be the best source of new microtubule-stabilizing compounds by far, with most of the microtubule targeting agents (MTAs) originating from marine organisms. Although several MTAs have been isolated or derived from marine biota, marine sponges remain as the chief source of MTAs [70].

Bastadins are brominated tyrosine derivative initially isolated from the marine sponge Ianthella basta. Around 30 different Bastadins have been reported till date, out of which Bastadin-6, 9 and 16 are some of the more potent forms [71]. Bastadins generally exhibit anti-angiogenic as well as cytotoxic activity [72]. It is suggest that the anti-angiogenic effect of bastadin 6 is closely related to selective induction activity of apoptosis against endothelial cells [73]. The four novel steroidal alkaloids isolated from marine sponge Corticium simplex named cortistatins A (1), B (2), C (3), and D (4), which exhibited highly selective anti-proliferative activity against HUVECs cells [74]. Analogs of cortistatins were synthesized from estrone, the estrone-isoquinoline hybrid (EI-hybrid A) as a candidate for a new anti angiogenic agent due to its inhibitory activities against the proliferation and migration of HUVEC cells [75]. PM050489 and PM060184 are a new class of marine polyketides, initially isolated from the sponge Lithoplocamia lithistoides and first synthesized by [76]. These polyketides have shown in vitro anti-mitotic activity against multiple types of human tumours, at sub -nanomolar concentrations. They potently disrupt cellular microtubules and mitosis by binding to αβ-tubulin dimers and distinctly modulating tubulin association reactions [77]. Peloruside A is a polyketide isolated from New Zealand marine sponge Mycale hentscheli showed ample evidence of in vitro anti-mitotic effect of Peloruside A, [78]. Aeroplysinin-1 is a brominated metabolite isolated from the sponge Verongia aerophoba, possessing antibacterial, anti-

parasitic, anticancer and anti-angiogenic activities [79]. Aeroplysinin-1 is a naturally occurring alkaloids of brominated tyrosine metabolite extracted from the marine sponge Aplisina aerophoba screening for new potential inhibitors of angiogenesis in vitro and in vivo[80]. Bioassay guided-fractionation of marine sponge Xestospongia exigua extracts led to the identification of a family of anti-invasive and anti-angiogenic alkaloids, particularly the motuporamines A, B, and C [81].Smenospongine, a sesquiterpene aminoquinone isolated from the marine sponge Dactylospongia elegans inhibited proliferation, migration and tube formation of human endothelial cells, suggesting favorable antiangiogenic activity [82]. It is also suggested that smenospongine exhibits potential antitumor efficacy on solid tumors as a promising anticancer drug candidate. A Petrosaspongia mycofijiensis marine sponge extract yielded mycothiazole, a solid tumor selective compound with no known mechanism for its cell line-dependent cytotoxic activity and inhibited hypoxic HIF-1 signaling in tumor cells that correlated with the suppression of hypoxia-stimulated tumor angiogenesis in vitro [83, 84] studied the anti-angiogenic potential of mycothiazole on human umbilical vein endothelial cell (HUVEC) based tube formation assay was employed as an in vitro model. [85] in their studies revealed that extract obtained from the bacterium (PB2) isolated from sponge primmorphs is a potent angiogenesis inhibitor. [86] studied the antiangiogenic activity of the crude extract of the sponge S. pachyspira by performing CAM assay. The extracts were highly toxic to the eggs by causing hemorrhage by reducing the blood supply. The extract disrupted mostly newly forming blood vessels without affecting the preexisting vasculature. The extract obtained from S. pachyspira was a potent angiogenesis inhibitor [87]. The effect of methanol and aqueous extracts of marine sponge Halichondria panacea found an immunostimulating effect and all extracts revealed angiogenic activity. Hemiasterlins are small cytotoxic tri-peptides found as secondary metabolites in several sponges and were first isolated from the sponge Hemiasterella minor. The potent cytotoxicity of hemiasterlins is because of their interference with mitotic spindle formation at low concentrations and tubulin de-polymerization at higher concentrations [88]. In addition, hemiasterlin was found to be more potent in vitro cytotoxic and antimitotic agent than both taxol as well as vincristine, which are anticancer drugs. Eribulin mesylate is an analogue of Halichondrin B, which is isolated from the marine sponge Halichondria okadai. Eribulin exerts antitumour effects by inducing apoptosis and acting as anti-microtubule agent [89]. Fascaplysin is a pigment, first isolated from marine sponge Fascaplysinopsis Bergquist sp in 1988 and identified as an anti-microbial agent [90]. It was later found to exert anti-proliferative activity against HeLa (ovarian cancer) cell line though apoptosis. Fascaplysin showed in vitro anti-angiogenic activity via vascular endothelial growth factor (VEGF) blockage, cell cycle arrest and apoptosis on human umbilical vein endothelial cells (HUVEC) [91]. Ageladine A is a fluorescent alkaloid present in the marine sponge Agelas nakamurai Ageladine A exerts anti-angiogenic activity in vivo as well as in vitro[92].

In our study, the results of the chorioallantoic membrane (CAM) assay are presented in Fig. No. 3, 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 of Suberites carnosus showed antiangiogenic response including lysis, hemorrhage, and goagulation of 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 were 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 Suberites carnosus the hemorrhages were observed at 40 µg/ mL concentration whereas no effect was noted at 80 µg/ mL concentration. In Suberites carnosus coagulation was seen only at 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.

e) SDS-PAGE Electrophoresis For The Separation Of Proteins From Sponge Crude Extracts

The presence of protein in crude extract in cultured sponge Haliclona molitba twobacterial isolatesA03k and A03 [93]. The Protein profiles in 15% SDS-PAGE showed twenty bands obtained from A03 and A03k. They found five protein bands in A03 include size around 17, 24, 36, 96, and 120 kDa, and four protein bands are found from A03k with size around 17, 37, 44 and 96 kDa. They concluded that Protein extract from A03k showed hemolytic activity. Based on protein profiles upon SDS-PAGE, A03k has protein size at 24 kDa. [94] studied the tropical sponges collected in Los Roques National Park (Venezuela) and they found the lectins present in sponge exhibited a native molecular mass of 63 kDa and by SDS-polyacrylamide gel electrophoresis under reducing conditions have an apparent molecular mass of 16 kDa, thus suggesting they occur as homotetramers. The purified lectins contain 3–4 mol of divalent cation per molecule, which are essential for their biological activity. [95] found that the sponge Tethya lyncurium showed pore-forming proteins that have hemolytic activity at protein size 21 kDa. This might suggest the possibility protein that responsible in hemolytic activity

is protein with size 24 kDa. [96]calculated the protein from marine sponge Cliona varians and the sea cucumber Holothuria grisea as 17 kDa (data not shown). In addition, on SDS-PAGE, under reducing and non-reducing conditions, HGL showed an apparent molecular mass of 15 kDa. [97] studied the proteins on SDS-PAGE analysis in marine sponge Halichondria panicea in aqueous extract and found that the proteins in crude toxin from the aqueous extract are 19.5, 39.0 and 66.2 kDa.[98]. 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 analysis that the presence of 3 protein bands viz, 19.5, 39.0 and 66.2 kDa already reported in C. diffusa.

Fig No 4. Showing separation of proteins by SDS PAGE electrophoresis in Suberites carnosus (Johnston). In the present investigation in aqueous crude toxin extract of marine sponge, we found around nine bands in both the sponges. The protein bands are found in Suberites carnosus are 12kDa, 16kDa, 20kDa, 25kDa, 27kDa, 42kDa, 51kDa, 57kDa and 59kDa. Our data is found comparable with the data cited above. The presence of protein at 20kDa showed hemolytic activity. It was also confirmed that the protein present in sponge are having corresponding proteins at 25kDa which confirm the presence of lectins present in both 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 Suberites carnosus showed biomedical properties. Our findings are significant for the development of multi-drug therapy for both pharmaceuticals and biomedical applications. Therefore we have also screen the crude extracts of sponges for its structural elucidation to find the new drugs for pharmaceutical industry. The study further suggests that, Suberites carnosus, 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.

Ethics statement

Ethical approval is received by Maharashtra State Biodiversity Board, Nagpur for collection of sponges for research purpose. The voucher specimens of Suberites carnosus 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.

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