

## **Evaluation bioactivity of a Sea cucumber, *Stichopus hermanni* from Persian Gulf**

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### **ABSTRACT**

*Within the frame of a biodiversity and bioactivity study of marine macro organisms from the Persian Gulf, *Stichopus hermanni* a sea cucumber species extracts were investigated for cytotoxic, antibacterial and antifungal activities against five human pathogenic microorganisms. All concentrations of extracts showed no antibacterial activity towards the tested strains. In antifungal assays the sea cucumber extracts showed significant activity solely against *Aspergillus niger* with MICs ranging from 3 to 15 µg/ml. The highest antifungal activity was found in body wall (MeOH) extract with an inhibition zone of 38 mm against *A. niger* at 18 µg/ml extract concentration. Body wall and Cuvierian organ extracts of this species exhibited cytotoxic activities. It is important for future research to concentrate on finding the mechanisms employed by sea cucumbers to defend themselves against invasion, the mechanism of infections and the type of chemical compounds in *S. hermanni* extracts that inhibit antifungal growth or proliferation in less exploited areas such as the Persian Gulf.*

**Key words:** Antibacterial, Antifungal, cytotoxic, *Stichopus hermanni*, Persian Gulf.

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### **INTRODUCTION**

More than 15,000 natural products, used for different purposes, have been mainly isolated from marine sponges, jellyfish, sea anemones, corals, bryozoans, molluscs, echinoderms, tunicates and crustaceans [1]. Many of these secondary metabolites possess interesting biological activities [2]. Drugs from marine sources are an area, which offers an unprecedented opportunity for their pharmacological exploration and hence have received great attention during recent years for natural product chemistry, a promising area of study. Some of these components were commercialized and reached the market, i.e. the antiviral agent vidarabin (Ara-A), or the antitumor agents cytarabin (Ara-C), ecteinascidin 743 (Yondelis<sup>®</sup>) and eribulin (Halaven<sup>®</sup>).

There is a continuous and urgent need to discover new antimicrobial and antifungal compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and reemerging infectious diseases and development of resistance to the antibiotics in current clinical use.

Sea cucumber is a marine invertebrate of the phylum Echinoderm and the class Holothuroidea, which is found on the sea floor worldwide. There are some identified sea cucumber species in Iranian water (Persian Gulf) and it seems *Stichopus hermanni* is dominant species in coral reef Islands in Persian Gulf [3].

Sea cucumbers have been well recognized as a tonic and traditional remedy in Chinese and Malaysian literature for their effectiveness against hypertension, asthma, rheumatism, cuts and burns, impotence and constipation [4]. These

medicinal benefits and health functions of sea cucumbers can be attributed to the presence of appreciable amounts of bioactive compounds, especially the triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan, sulfated polysaccharides, sterols (glycosides and sulfates), phenolics, peptides, cerberosides and lectins[5].

Several studies have shown multiple biological activities of sea cucumber species. These include wound healing promoters and exhibiting antinociceptive, antibacterial, antifungal, antioxidant and cytotoxic properties [6-7-8-9-10-11]. They are also rich in saponin glycosides that exhibit anticancer activity [12].

Despite of several worldwide studies that have revealed the efficacy of some sea cucumber species as potential sources of antifungal, antibacterial and cytotoxic compounds, there is lack in information about levels of these activities in most sea cucumber species in Persian Gulf. Therefore, in our ongoing program to monitor and investigate the biodiversity and bioactivity of marine macro organisms from the Persian Gulf we studied a sea cucumber *S. hermanni* from Qeshm Island as part of a research hopes to find new potential of these compounds in Iranian sea cucumbers.

## MATERIALS AND METHODS

### *Sampling and identification*

Sea cucumber Samples(n=30) were caught around Qeshm Island in the North Coast of Persian Gulf in June 2012 by scuba diving in the depths of 5-15 m. The samples were dissected to remove internal organs, and packed immediately with ice prior sending to the lab and kept at -20°C until extracted. The taxonomic identity of the samples was confirmed by Professor Chatland Conad from the Laboratoire d'écologie marine, Université de la Réunion, Saint Denis, France.

### *Extraction of the samples and isolation*

Bioactive compounds were extracted as a function of their polarity using water and organic solvents according to method of Mamelona *et al.*, [13]. The samples of Gonad (G), Respiration Tree (RT), Cuvierian Organ (CO), and Body Wall (BW) were defrosted before use. The body wall recuperated was cut into small pieces. The samples were homogenized using a blender and suspended followed by extraction with ethyl acetate, methanol and water-methanol (50%) successively by percolation (72 h for each solvent) at room temperature. After filtration and centrifugation (15 min, 30,000 ×g, 4° C), extracts were evaporated under vacuum at 45° C by a rotary evaporator. The powdered extracts of each sample were obtained by freeze dryer and stored at -20 °C.

### *Assay of cytotoxicity effect*

Cytotoxic activity of extracts was determined by Brine-Shrimp Lethality assay (BSA) as described by Meyer *et al.*, [14]. Simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The cysts of the brine shrimp hatched in artificial seawater (3.8% NaCl solution) for 48 hours to mature shrimp called nauplii. Different concentrations of each extract dissolved in normal saline were obtained by serial dilution. Four concentrations of each extract were prepared with 10, 100, 500 and 1000 µg/ml. Twenty nauplii were added to each concentration of the extracts in 24 well chamber slides. Number of nauplii alive noted after 24 h. The mortality end point of the bioassay was determined as the absence of controlled forward motion during 30 seconds of observation. Seawater and berberine hydrochloride (LC50 =26 µg/ml) were used as controls. Lethality percentage was determined and LC50 calculated based on Probit Analysis with 95% of confidence interval using computer software "BioStat-2007".

### *Antibacterial and antifungal assay*

The antibacterial and antifungal activities of the *H. scabra* extracts were assessed against *Escherichia coli* (ATCC 1763), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 25853) *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404) by the Disc Diffusion Susceptibility method triplicates. Minimum inhibitory concentrations (MIC) of the extracts were tested in the lowest concentration at which no growth was observed. Gentamycin, Fluconazole and Ciprofloxacin were used as positive controls [15].

## RESULTS

Curry sea cucumber (*Stichopus hermanni*) has long shaped with a rectangular cross-section and upper side wrinkled or deeply ridged with small black bumps and underside smoother. Entire body has different shades of beige to brown with irregular brown patches and fleshy tubercles projecting along the sides without cuts or small cuts across the mouth. Color of live specimens variable, it is usually brownish yellow and often mottled and with darker papillae giving a spotted appearance. Normally lives between 0 and 22 meter deep but also it likes shallow sandy areas.

**Table 1. Antimicrobial activity of Body Wall (BW), Cuvieran Organ (CO) and Respiration tree (RT) of *Stichopus hermanni* extracts from the Persian Gulf**

Organism	Extract	Zone of Inhibition (mm)						
		MIC $\mu\text{g/ml}$	4 $\mu\text{g/ml}$	8 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	14 $\mu\text{g/ml}$	16 $\mu\text{g/ml}$	18 $\mu\text{g/ml}$
<i>A.niger</i>	BW(methanol)	3	18	20	22	24	30	38
<i>A.niger</i>	BW(methanol-water)	7	0	10	11	13	15	15
<i>A.niger</i>	CO(methanol)	7	0	14	14	20	22	30
<i>A.niger</i>	RT(ethyl acetate)	15	0	0	0	0	20	20

**Table 2. Cytotoxic activity of Body Wall (BW), Cuvieran Organ (CO) of *Stichopus hermanni* extracts from the Persian Gulf**

species	extract	Dose ( $\mu\text{g/ml}$ )	Log dose	total	Alive	Death	Lethality (%)	LC50	chi-square	95% Confidence Limits	
										Lower	Upper
<i>Stichopus hermanni</i>	BW (methanol)	10	1	20	17	3	15	144.827	0.078	56.654	338.21
		100	2	20	11	9	45				
		500	2.7	20	6	14	70				
		1000	3	20	5	15	75				
	BW (methanol- water)	10	1	20	18	2	10	109.76	0.297	52.21	202.731
		100	2	20	10	10	50				
		500	2.7	20	5	15	75				
		1000	3	20	2	18	90				
	CO (methanol- water)	10	1	20	18	2	10	328.562	0.489	142.384	1043.868
		100	2	20	13	7	35				
		500	2.7	20	10	10	50				
		1000	3	20	6	14	70				
	CO (ethyl acetate)	10	1	20	16	4	20	196.291	0.731	65.383	665.654
		100	2	20	12	8	40				
		500	2.7	20	9	11	55				
		1000	3	20	5	15	75				

Table 1 summarizes the biological activity of methanol, water-methanol and ethyl acetate extracts. All concentrations of extracts showed no antibacterial activity towards the tested strains. In antifungal assays the sea cucumber extracts showed significant activity solely against *Aspergillus niger* with MICs ranging from 3 to 15  $\mu\text{g/ml}$ . The highest antifungal activity was found in body wall (MeOH) extract with an inhibition zone of 38 mm against *A. niger* at 18  $\mu\text{g/ml}$  extract concentration. Body wall and Cuvieran organ extracts of this species exhibited cytotoxic activities (Table, 2). Here we must state that at the relevant concentration 10  $\mu\text{g/ml}$  nearly no toxicity is given (only 10-20 % Lethality).

## DISCUSSION

During the past three to four decades many efforts have been devoted to isolating numerous biologically active novel compounds from marine sources. Sea cucumbers are one of the potential marine animal's medicinal values. The medicinal properties of these animals are ascribed to the presence of functional components with promising multiple biological activities [5].

Although some preliminary data on bioactive of sea cucumber is available, this marine animal, due to its species diversity, availability and medicinal utility, remains a highly potential candidate for the search of novel marine compounds for drug discovery especially in Persian Gulf. So far, numerous studies have been conducted on sea cucumber, but still potential exists to isolate and identify new compounds from different parts of various species of this harvestable marine invertebrate.

The indiscriminate use of antimicrobial agents has resulted in the emergence of a number of drug-resistant bacteria, fungi, and viruses. To overcome the increasing resistance of pathogenic microbes, more effective antimicrobial agents with novel modes of action must be developed. Sea cucumbers used in traditional medicines to treat infectious diseases seem to be an abundant source of new bioactive secondary metabolites. Therefore the screening of sea cucumber extracts can be a great interest to scientists in the search for new drugs for greater effective treatment of several diseases.

Although there were significant antifungal and cytotoxic activity for methanol and methanol-water extracts of the isolated organs of *S. hermanni*, no antibacterial activity of these extracts was observed. As shown in Table 1, methanolic and water methanolic extracts have indicated antifungal activity against *A. niger*.

The higher antifungal and cytotoxic activity of the Methanol and water Methanol extracts may be due to the greater solubility of the extracts in these organic solvents. Majority of the positive results have been recorded in the methanol and water methanol fractions it shows that they are a good solvent systems for the solubility of bioactive compounds present in the *S. hermanni*.

The effect of extract against *A. niger* was so higher implying that this sea cucumber can be utilized as anti-mycotics drugs against infection of *A. niger* in patients with pulmonary tuberculosis [16]. The Antifungal property of the *S. hermanni* extracts reveals that they are high enough to bring the effect against fungal pathogens than the bacterial. It may due to the incidence of fungal presence in their habitat induce the sea cucumber to produce the antifungal compounds. *S. hermanni* is considered to be of low value in the export market because of the tendency of the body wall disintegrate easily, while exposed to air after harvesting and during boiling[3]. Therefore, uses of sea cucumbers, as potential source, for isolation of antimicrobial agents can be suggested.

Although holothurians are known to contain triterpene glycosides, which show antifungal activity, fungi can still be isolated from these organisms. *Cladosporium brevicompactum* and *C. sphaerospermum* are common in the holothuroids' coelom. *Cladosporium spp.*, *Aspergillus spp.* and *Penicillium spp.* were found on the body surface of the holothurians [17].

In this study, it was shown that *S. hermanni* extracts were not effective against any of the bacterial species tested even at a concentration of 18 µg/ml (Table, 2). They did not inhibit bacterial growth at the highest tested extract concentration as well. This shows that the amount of extract present in the tissues of this species bore no relationship with its antibacterial activity. It seems there are different bacterial spectrums in the sea which is pathogenic for this sea cucumber or it would possibly harm endosymbiotic bacterial species in this area.

The negative results of *S. hermanni* against *Staphylococcus aureus* and *E. coli* and antifungal potential are highly supported by the investigations of our previous studies in body wall and respiration tree from other Iranian sea cucumber species; i.e., *Holothuria leucospilota* and *Holothuria scabra* from Persian Gulf [10-11]. Rafat *et al.* [18] found the antifungal property of the hard corals extracts reveals that they are strong enough to be less affected by fungal organisms than the bacteria.

Methanol extract of sea cucumber (*Actinopyga lecanora*) showed promising antifungal activity, *in vitro* [19]. Farouk *et al.* [20] also isolated some bacterial strains from various tissues of the sea cucumber species, (*Holothuria atra*). Antifungal activity isolated from (*Bohadschia marmorata*) species of sea cucumber [21].

A wide variation of bioactivities among the sea cucumbers extracts suggests that a variety of substances are capable of antimicrobial functionalities. Therefore, marine echinoderms can be explored as a sustainable natural source for the discovery of novel antibiotic compounds [5].

The highest cytotoxic effect on water-methanol extract of body wall with LC<sub>50</sub> values about 109.76b µg/ml continue with methanol extract from body wall with LC<sub>50</sub> values about 144.82µg/ml and ethyl acetate extract from cuvierian organ with LC<sub>50</sub> values about 196.29 µg/ml. Sea cucumbers are reported to contain several compounds with anticancer and antiproliferative properties [5]. Antiproliferative and anticancer functionality of sea cucumber extracts might be ascribed to the presence of considerable amounts of total phenols and flavonoids which are valued as effective antioxidants to protect from oxidative stress and degenerative diseases including certain cancers [22]. The isolation of sphenoid bases of sea cucumber (*Stichopus variegatus*) cerberosides along with their cytotoxic effects against human colon cancer cell lines were reported [23].

## CONCLUSION

Based upon the antimicrobial potential as revealed by several studies, it would be interesting to explore sea cucumbers as a natural source for isolation of novel antimicrobial agents for drug development against infectious diseases. *A. niger* is still relevant bug in medical area; therefore chemistry investigations from new resources are ongoing. These findings have encouraged us to continue our work to determine the active ingredients. Therefore further isolation and identification of active structures from the active extracts of sea cucumber species from Persian Gulf is in progress. In addition, further investigations are needed to clarify intracellular pathways involved in the mechanism of the growth inhibition activity.

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