



The Investigation of the Anti-fungal Activity of Sea Cucumber (*Holothuria Leucospilota*) Extracts (Body Wall, Guts and White Strings) from Chabahar Bay, Oman Sea

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ABSTRACT

Compounds obtained from marine organisms have been isolated due to their anti-bacterial and antifungal activities. In this study, the antifungal activity of aqueous-methanol, methanol, chloroform and n-hexane extracts of body wall, guts and white strings of sea cucumber (*Holothuria leucospilota*) collected from Chabahar Bay in 100 to 2000 µg/ml concentrations is discussed. The antifungal test on fungal strains (*A. niger*, *A. flavus*, *A. fumigatus*, *A. brasiliensis*) and *C.albicans* yeast was conducted by disk diffusion and broth macro-dilution methods. Growth inhibition zone was not observed in the first method. In the second method, the body wall extract at the concentration of 2000 µg/ml inhibited the growth of all the four fungal strains. Also, white string extracts at concentrations of 1000 to 2000 µg/ml inhibited, to some extent, the growth of *A. niger* and *A. fumigatus* and *brasiliensis*. The best inhibitory effect of this extract in the mentioned concentrations was on *A. flavus*. The guts extract had no effect.

1 INTRODUCTION

As the origin of life, oceans are the source of natural compounds that have been accumulated in various organisms. The natural compounds found in marine organisms can be used as a rich source of compounds with food, pharmaceutical and medical uses (Jha and Xu, 2004). Compounds with biological activity can be separated from different animal groups such as corals, crabs, algae, echinoderms, sponges, fishes and sponges (Yasoda et al., 2006). The scientific attempts to discover effective antifungal and antibacterial drugs have been

mainly focused on chemical synthetic drugs or on the drugs obtained from plants and animals. As a result, in recent decades, seas and oceans have been known as rich sources of metabolites and bioactive compounds with biodiversity and pharmacological activities (James, 2001). Studies have demonstrated that extracts of sea cucumbers have strong anti-microbial activities. This anti-microbial property is attributed to triterpene glycosides (Ismail et al., 2008), disulfate glycosides (Munia et al., 2008), steroidal glycosides (Bryan et al., 1993), poly-hydroxy sterols

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(Jensen et al., 1996), naphthoquinone pigments (Chattapadhyay et al., 1996), lysozymes and various precursors (Findlay and Smith, 1995).

2 MATERIALS AND METHODS

Holothuria Leucospilota sea cucumber was collected from the sub-tidal zone of Chabahar Bay by a diver. This species has been selected due to its wide dispersion in Chabahar Bay region and because it is among the sea cucumbers with low economic value. The samples were put in containers of sea water and were transferred to the microbiological laboratory and were freezed at a temperature of - 20° C. After thawing, the samples were washed with distilled water and by a longitudinal incision in the belly of the sea cucumber, the guts and white strings were brought out and the body wall was divided into small pieces (Ismail et al., 2008). Each of the three parts was dried at laboratory temperature, away from light and heat. The guts and dried white strings were fully crushed in porcelain mortar and were extracted twice along with the dried pieces of body wall (Mokhlesi et al., 2012). In this study, the soaking method was used to extract different tissues of sea cucumber. The small parts of the needed organs (body wall, guts and white strings) were put in a certain amount of aqueous-methanol, chloroform, methanol and n-hexane solvents (300 ml) in a 72-hour time period. The obtained extracts were concentrated and then dried by a freeze dryer (Mokhlesi et al., 2012; Mariana et al., 2009).

Disk diffusion (Michel et al., 2008; Mokhlesi et al., 2012; Mariana et al., 2009) and Broth macro-dilution method (Adibpour et al., 2007) were used to study the antifungal effects of the extracts of *H.leucospilota* sea cucumber. In the disk diffusion method, each of the aqueous-methanol, chloroform, methanol and n-hexane extracts of the body

wall, guts, and white strings with concentrations of 100, 200, 400, 800, 1000 and 2000 µg/ml was prepared in the disks and was put in the culture medium. In this experiment, the four strains of *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus brasiliensis* were used as human pathogens and *Candida albicans* was used as the yeast (Laemmli, 1970; Villasine and Pomory, 2000; Ridzwan et al., 2003). Of the above mentioned micro-organisms, 1 McFarland suspension was prepared under the hood and then the strains were cultured by swap on sabour dextrose medium. The disks with certain concentrations were then placed on culture medium and the plates were put in a 25° C incubator for 24-48 hours. After this time period, the diameter of the inhibitory zone of fungal growth was measured in mm by the help of a caliper (Haug et al., 2002). Ketoconazole antibiotic was used as a positive controller in this experiment (Michel et al., 2008). In Broth macro-dilution method, lyophilized funguses were first put in broth sabour dextrose culture medium (SDB) and then cultured in sabour dextrose agar culture medium (SDA). RPMI culture medium was finally used. Changes in color and opacity of RPMI culture medium indicate the degree of fungus growth (Adibpour et al., 2007). *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus brasiliensis* were tested as fungal strains and *Candida albicans* was used as the yeast (Laemmli, 1970; Villasine and Pomory, 2000; Ridzwan et al., 2003). In this study, anti-fungal test was conducted on aqueous-methanol extracts of the body wall, guts and white strings at concentrations of 1000, 1250, 1500, 1750 and 2000 µg/ml. Culture medium without an extract and culture medium with Ketoconazole

Table 1

The effect of aqueous-methanol extract of *H.leucospilota* sea cucumber's body wall at concentrations of 1000 to 2000 µg/ml on the studied strains.

Aqueous-methanol extract	Body wall				
	1000	1250	1500	1750	2000
Concentrations					
<i>A. niger</i>	-	-	-	-	+
<i>A. fumigatus</i>	-,+	-,+	-,+	-,+	+
<i>A. flavus</i>	-,+	-,+	-,+	-,+	+
<i>A. brasiliensis</i>	-,+	-,+	-,+	-,+	+
<i>C. albicans</i>	-	-	-	-	-

antibiotic as control were also used (Jayaseli et al., 2001; Adibpour et al., 2007).

3 RESULTS

In disk diffusion method, growth inhibition was not observed in all the studied strains of aqueous-methanol, chloroform, methanol, and n-hexane extracts at concentrations of 100 to 2000 µg/ml (the growth inhibition zone was not observed). The aqueous-methanol extract of the body wall at 2000 µg/ml inhibited the growth of 4 fungal strains (*A. niger*, *A. fumigatus*, *A. flavus*, *A. brasiliensis*). *A. fumigatus*, *A. flavus*, *A. brasiliensis* fungal strains at concentrations of 1000 to 2000 µg/ml also showed some increase in growth (average growth) in relation to control culture medium. But these concentrations had no inhibitory effect on the

growth of *A. niger* fungus. None of the above mentioned concentrations had an inhibitory effect on *C. albicans* yeast (Table 1).

The aqueous-methanol extract of white strings at the mentioned concentrations inhibited the growth of *A. flavus* fungus. This extract inhibited, to some extent, the growth of *A. niger* and *A. fumigatus* fungi at concentrations of 1000 to 2000 µg/ml compared to the control culture medium (*A. niger* and *A. fumigatus* fungi had an average growth). The 2000 µg/ml concentration of this extract did not also have any significant influence on the growth of *A. brasiliensis* fungus.

However, *A. brasiliensis* fungus had grown at concentrations of 1000, 1250, 1500 and 1750 µg/ml. This extract also did not inhibit the growth of *C. albicans* yeast at the above mentioned concentrations (Table 2).

Table 2

The effect of white strings extract of *H. leucospilota* sea cucumber on the studied strains at concentrations of 1000 to 2000 µg/ml

Aqueous-methanol extract	White strings				
	1000	1250	1500	1750	2000
Concentrations					
<i>A. niger</i>	-,+	-,+	-,+	-,+	-,+
<i>A. fumigatus</i>	-,+	-,+	-,+	-,+	-,+
<i>A. flavus</i>	+	+	+	+	+
<i>A. brasiliensis</i>	-	-	-	-	-,+
<i>C. albicans</i>	-	-	-	-	-

The aqueous-methanol extract of guts did not inhibit the growth of fungal and yeast strains at any of the tested concentrations.

Because the inhibitory effect of body wall crude extracts (aqueous-methanol) on the growth of fungal strains was better than those of the other two extracts, hydrophilic and lipophilic (chloroform, methanol and n-hexane) solvents at concentrations of 1000 to 2000 µg/ml were used on sea cucumber body wall and anti-fungal test was carried out on the obtained extracts. The results showed that these extracts did not also have any inhibitory effect on the growth of the used fungal and yeast strains.

4 DISCUSSION

Most of the experiments have been performed to investigate the anti-fungal activity of sea cucumbers on methanol, aqueous-methanol, ethanol, chloroform and triterpene glycosidic compounds extracts (Se-kwon et al., 2012). The study on the anti-microbial activity of the Mediterranean *H. polii* sea cucumber by disk diffusion method showed that the ethanol extract of body wall at the concentration of 2.5 mg/ml (2500 µg/ml) had a potent anti-fungal activity on *A. flavus* fungi, *A. niger* and *C. albicans* yeast (Omran and Allam, 2012). They had a strong inhibitory effect on the growth of *A. fumigatus* and a less strong inhibitory effect on *Trichophyton rubrum* in the study of the anti-fungal activity of the body wall crude extract (aqueous-methanol) of *H. polii* sea cucumber by disk diffusion method and at the concentration of 150 to 300 µg/ml. They had no significant effect on *C. albicans* yeast (Ismail et al., 2008). In the comparison of the anti-fungal test done by culturing fungi in broth macrodilution method with the mentioned tests (disk diffusion method), it can be observed that in *H. leucospilota* species, the best inhibitory effect

of the crude extract of body wall (aqueous-methanol) has been at a concentration of 2000 µg/ml so that like these tests, the activities of *A. niger* and *A. fumigatus* fungus and also *A. flavus* and *A. brasiliensis* two fungal strains were inhibited, but this extract had no inhibitory effect on *C. albicans* yeast at the concentration of 2000 µg/ml. It should be noted that the ethanol extract of *H. polii* sea cucumber body wall at the concentration of 2.5 mg/ml (2500 µg/ml) had a strong inhibitory effect on the growth of *C. albicans* yeast but the crude extract (aqueous-methanol) of *H. polii* sea cucumber at the concentration of 150-300 µg/ml had no strong inhibitory effect on *C. albicans* yeast. It can also be noted that various extracts at different concentrations in *H. polii* sea cucumber can have different inhibitory effects on fungal and yeast strains. Meanwhile, comparing the results of the tests done on *H. polii* species from Mediterranean Sea with *H. leucospilota* species from Chabahar Bay, it can be concluded that the aqueous-methanol extract of body wall at different concentrations and in various methods had different anti-fungal effects.

By comparing the conducted tests with various concentrations, it can be stated that sea cucumbers have different pharmaceutical, medical and food activities because of having different combinations in their body organs (Batrakov et al., 1980).

The investigation of the anti-fungal activity of methanol, aqueous-methanol and ethyl acetate extracts on white strings of *H. leucospilota* and *Bohadschia marmorata* sea cucumbers was done by disk diffusion method in the Persian Gulf (Mokhlesi et al., 2012). In *H. leucospilota* species, the methanol extract of white strings at the concentration of 8 mg per mL had the highest inhibitory effect on *A. niger* fungus with 20 mm growth inhibition zone and *C. albicans* yeast with 13 mm growth

inhibition zone. In *B. marmorata* species, the aqueous-methanol extract of white strings had inhibitory effects on the growth of *A. niger* fungus with 13 mm growth inhibition zone and *C. albicans* yeast with 8 mm growth inhibition zone, but in the performed test by culturing the fungi in broth macrodilution method, the aqueous-methanol extract of white strings had the ability to inhibit the growth and reproduction of *A. flavus* fungus at concentrations of 1000 to 2000 µg/ml by broth macro-dilution method. However, it did not have a strong inhibitory effect on the growth of *A. niger*, *A. fumigatus* and *A. brasiliensis* funguses. Meanwhile, the aqueous-methanol extract of white strings at concentrations of 1000 to 2000 µg/ml had no inhibitory effect on the growth of *C. albicans* yeast. The aqueous-methanol extract of the guts at concentrations of 1000 to 2000 Mg/ml had the weakest growth inhibition effect, indicating that the guts extract had no inhibitory effect on the growth of 4 fungal and yeast strains. By comparing the results of the test on the methanol extract of *H. leucospilota* sea cucumber white strings from the Persian Gulf with the same species from Chabahar Bay, one can say that the identical species can yield different results. This can be attributed to such factors as different geographical locations, different extracts, different concentrations, and different test methods. Since in this experiment the crude extract (aqueous-methanol) of body wall had a better effect compared with other extracts used, hydrophilic and lipophilic (chloroform, methanol and n-hexane) solvents were used in order to investigate the effect of the material extracted by each solvent in this study. However, these extracts had no inhibitory effect on the growth of fungal strains. It seems that because each solvent separates a certain compound from the body wall extract, the mentioned compound cannot

have any anti-fungal effect by itself. Rather, the combined effect of the various compounds present in crude extracts shows such property. These results suggest that echinoderm species are potential sources of antibiotic substances for pharmaceutical and pharmaceutical uses.

Sea cucumbers have an innate immune system which is regarded as a potential source for the discovery of anti-microbial peptides. The difference between molecular mass and sequences of amino acids of peptides derived from these organisms is the reason for the difference between their effects on various bacterial and fungal organisms (Beauregard et al., 2001). As a result, sea cucumber can be introduced as a source of the compounds with anti-fungal effects making it an appropriate candidate for making pharmaceutical, medical and antibiotics compounds.

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