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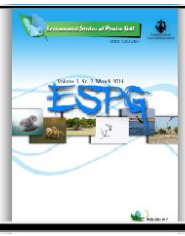

The Investigation of Anti-bacterial Activity of *Holothuria Leucospilota* Sea Cucumber Extracts (Body Wall, Guts and White Strings) at Chabahar Bay in Oman Sea

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ABSTRACT

Echinoderms are independent and quite special branch of world animals. Sea cucumbers are among the strangest members of echinoderm class regarding their structure and physiology. These fauna have been tested with regard to anti-bacterial, anti-fungal, anti-coagulants, anti-virus, cytotoxic, hemolytic and anti-HIV features. In this study, the antibacterial activity of *Holothuria leucospilota* sea cucumber was investigated. Antibacterial test was done on 8 strains of bacteria (i.e. *Staphylococcus epidermidis, Proteus vulgaris, Shigella dysenteriae, Bacillus cereus, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeroginosa*) by two methods of disk diffusion and spot test using aqueous-methanol, methanol, chloroform and n-hexane extracts of body wall, guts and white strings of the sea cucumber collected from Chabahar Bay. Growth inhibition zone was not observed in the disk diffusion method. But a concentration of 2000 micrograms per milliliter of aqueous-methanol extracts of body wall, guts, and white strings had static effect on the growth of some bacteria (Inhibition of bacteria growth).These extracts had no static effect on the growth of bacteria, *Sh. dysenteria*, *P. vulgaris*, *B. cereus*, *S. epidermidis*. The highest influences of the extracts were on *E. coli*, *S. typhimurium*, *S. aureus*, *P. aeroginosa* bacteria, respectively.

1 INTRODUCTION

Echinoderms (Echinodermata) are independent and quite specific branch of the animals that are not comparable with any of the other animals in terms of body construction plan. These animals constitute almost more than 6,500 species of marine animals. Sea cucumbers (*Holothuroidea*) are among the strangest members of echinoderm class regarding the structure and physiology (Flammang & Ribesse, 2002). For a long time, the studies on sea cucumbers have been limited to sea cucumber physiology and ecology. Now, sea cucumbers are tested with regard to anti-bacterial, anti-fungal, anti-coagulants, anti-virus, cytotoxic, hemolytic and anti-HIV features (Michalet al., 2008; Nagaraj et al., 2008). In 2007 and 2008, extensive pharmacological studies were conducted by

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researchers from 26 countries on marine animals and 197 natural chemical makeups of the organisms were isolated, identified and recorded. Anti-fungal, anti-bacterial, anti-thrombotic, anti-malarial, anti-protozoa and anti-virus effects have been reported from some of the isolated compounds (Kumar et al., 2007). During the studies, it has been found that the extracts of sea cucumbers have strong anti-bacterial activities (Ismail et al., 2008). This anti-microbial property is attributed to triterpene glycosides (Ismail et al., 2008), disulfate glycosides (Muniai et al., 2008),steroidal glycosides(Bryan et al., 1993), poly-hydroxy sterols (Jensen et al., 1996), naphthoquinone pigments (Chattapadhyay et al., 1996), lysozymes and various precursors (Findlay & Smith, 1995).

2 MATERIALS AND METHODS

Holothuria Leucospilota sea cucumbers were collected from the sub –tidal zone of Chabahar Bay by a diver. Samples were put in containers of sea water; they were transferred to the laboratory and were frozen at a temperature of- 20° C. Then, they were transferred to the microbiology laboratory of Jondi Shapour University in Ahvaz for performing some microbial tests. After thawing, the samples were washed with distilled water and sea cucumber guts and white strings were brought out and body wall was divided into small pieces by along it udinal incision in the belly of the sea cucumber (Ismail et al., 2008). In order for being dried, each of the three parts was put at the laboratory temperature a way from light and heat. The guts and dried white strings were fully crushed in porcelain mortar and extracted twice by the dried pieces of the body wall. Extraction process was conducted to separate the compounds dissolved in solution or to bring out some compounds out of a solid mixture. There are different methods such as soaking, percolation, digesting and brewing to obtain organic compounds found in plant and animal tissues. Each of these methods is selected according to the type of texture and removable material (Mokhlesi et al., 2012). In this study, soaking was used to extract different tissues of sea cucumber. In order for extraction, the shredded muscles, white strings and guts were put in 300 milliliters of aqueous-methanol, chloroform, methanol and n-hexane solvents in a 72 hours given time period. The obtained extracts were then filtered and concentrated by a rotary device using rotary vacuum. Then, they were dried by a freeze dryer (Mokhlesi et al., 2012; Mariana et al., 2009).

Disk diffusion (Michel et al., 2008; Mokhlesi, 2012; Mariana et al., 2009) and Spot test methods (two fold dilution) (Khalaj et al., 2004) were used to study anti-bacterial effects of the extracts of *H. leucospilota* sea cucumber. In disk diffusion method, each of the aqueous-methanol, chloroform, methanol and n-hexane extracts of body wall, guts, and white strings with concentrations of 100, 200, 400, 800, 1000and 2000 micrograms per milliliter was prepared in disks and then put in culture medium. Eight-strained was used as bacterial strains based on the research conducted on species of sea cucumbers (*Staphylococcus epidermidis, Proteus vulgaris, Shigella dysenteriae, Bacillus cereus, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeroginosa*). The above mentioned micro-organisms were used to prepare 0.5 suspension McFarland under the hood and then the strains were cultured by swap on Hinton agar medium. The disks with certain concentrations were then placed on culture medium and plates were put for 24 hours in 37°C incubator. After this period of time, the diameter of inhibitory zone of bacteria growth was measured in millimeter by the help of caliper (Haug et al., 2002).

Table 1
Results of different sea cucumber extracts at 2000 micrograms per milliliter concentration on the studied strain

Aqueous-methanol extracts	Body wall	Guts	White strings
Concentrations	2000	2000	2000
Sh.dysenteria	-	-	-
P. vulgaris	-	-	-
B. cereus	-	-	-
S. epidermis	-	-	-

Gentamicin antibiotic was used as a positive controller in this test (Michel et al., 2008). In Spot test method using two-fold dilution method, three parts of sea cucumber body (body wall, guts and white strings) were soaked for 72 hours in aqueous-methanol solvent and concentrated by rotary machine under vacuum conditions. Next, they were dried by freeze dryer after being passed through the filter paper. The extracts with high concentrations were used in this method. Mueller Hinton agar medium (MHA) with different concentrations of extracts were combined separately and bacteria suspensions were placed in culture medium in a drop-form. Anti-bacterial test was performed on the extracts with concentrations of 1000, 1250, 1500, 1750 and 2000 micrograms per milliliter. *Gentamicin* antibiotic was also used as a positive controller (Michel et al., 2008).

3 RESULTS

In disk diffusion method, growth inhibition was not observed in all the studied strains at concentrations of 100 to 2000 micrograms per milliliter of aqueous-methanol, chloroform, methanol and n-hexane extracts (no growth inhibition zone was observed). In the Spot test, concentration of aqueous-methanol extracts of the body wall, guts, and white strings was1000, 1250, 1500, 1750 and 2000 micrograms per milliliter respectively. A concentration of 1,000 to 2,000 micrograms per milliliter did not prevent bacterial growth, but a concentration of 2000 micrograms per milliliter of aqueous-methanol extracts had static influence on the body wall, guts, and white strings of some bacteria (growth inhibition of bacteria). These extracts had no static effect on the growth of *Sh. dysenteria*, *P. vulgaris*, *B. cereus* and *S. epidermidis* bacteria (Table 1). The extracts' maximum influence was on *E. coli*, *S. typhimurium*, *S. aureus* and *P. aeroginosa* bacteria, respectively (Table 2).

As the crude extract (aqueous-methanol) of body wall has better static effect on bacterial strains in comparison with the extracts of guts and white strings, hydrophilic and lipophilic (methanol, n-hexane and chloroform) solvents were used on the body wall of sea cucumber and anti-bacterial test was performed on the obtained extract. However, the results showed that these extracts did not have any inhibitory effect on the bacteria growth too.

Table 2
Results of different sea cucumber extracts at 2000 micrograms per milliliter concentration of the studied strains

Aqueous-methanol extracts	Body wall	Guts	White strings
Concentrations	2000	2000	2000
E. coli	+	+	+
S. typhimurium	+	+	+
S. aureus	+	+	+
P.aeroginosa	+	+	+

4 DISCUSSION

In the recent years, many studies have been done on the anti-microbial effects of various marine organisms such as echinoderms in different countries (Ridzwan et al., 1995). Although researches have shown that echinoderms have the highest anti-bacterial effect in comparison with other marine organisms such as porifera, bryozoa, mollusca, corals and annelida round worms are the most effective anti-bacterial (Shakouri et al., 2009). In the study of *Holothuria Leucospilota* sea cucumber showed anti-bacterial property only in spot test method and at the concentration of 2000 micrograms per milliliter.

Ina study by the anti-bacterial effect of natural extracts of Persian Gulf sea cucumbers on three strains of *E.coli* bacteria was investigated. Although, sea cucumbers are studied as *Holothuria* in their research, the type of species is not known in that study. The results obtained on *H. leucospilota* in the present study also confirm those of Jamali et al. (2010). Nonetheless, further studies on other species and also a comparison between their effective features and factors among the species are necessary.

In another study, the methanol-stony extract obtained from the body wall of *Parastichopus Parvimensis* sea cucumber from Santa Catalina island, California was given effect on *Escheriachia coli* and *Bacillis subtilis* bacteria using disk diffusion method (Villasine, 2000).In this study, two antibiotics, *ampicillin* and *tetracyclin*, were used as positive controllers and the anti-bacterial effect of this extract on the mentioned bacteria was confirmed. However, the effect of these extracts was much lower than antibiotics compared to the diameter of inhibitory growth zone in the tested antibiotics. But, disk diffusion method done on *H. leucospilota* species had no inhibitory effect on the growth of none of the bacterial strains. In Spot test method, aqueous-methanol extract of the body wall had static effect on four bacterial strains, *E.coli, S. aureus, P. aeroginosa and S. typhimorium* at the concentration of 2000 micrograms per milliliter. This test shows that the two mentioned sea cucumber species had inhibitory effects on *E.coli* bacteria. So, it can be noted that various body wall extracts in different species of sea cucumber can have the same effects using different tests.

Three lipid, methanol, and phosphate-buffered Saline (PBS) extracts were extracted from the body wall of sea cucumbers in another study (Ridzwan et al., 1995) done on *Holothuria atra, H. scabra* and *Bohadschia argus* sea cucumbers collected from Sabah beach in Malaysia. In this study, the effect of these extracts was tested on *Streptococcus faecalis, S. viridens, S. pneumonia, Staphylococcus aureus, Escheriachia coli, Shigella sonnei* and *Proteus mirabilis* bacteria by disk diffusion method. The results showed that lipid and methanol extracts did not inhibit the bacteria growth and PBS extract had inhibitory effect on the bacteria growth on the other hand. Their results also showed low and weak anti-bacterial property of *H. atra* external guts. PBS extract of *H. atra* also had anti-bacterial effect that depended on the concentration of the extract. The researchers stated that the bacteria growth in the presence of the extracted extracts is evident 48 hours after the test. Comparing this test with the Spot test, it can be said that the effects of phosphate-buffered saline extract of body wall of *H. atra, H. scabra and B. argus* sea cucumbers and aqueous-methanol extract of *H. leucospilota* sea cucumber body wall are similar because of their inhibitory effect on the growth of *S. aureus* and *E.coli* bacteria. So, it can be mentioned that various sea cucumber species with different extracts can have similar anti-bacterial effects.

Since the crude (aqueous-methanol) extract of body wall had better static effect than the other extracts, hydrophilic and lipophilic (chloroform, methanol and n-hexane) solvents were used to investigate the static effect of the extracted material by each solvent. But these extracts had no inhibitory effect on the growth of bacterial strains. It seems that because each solvent separates a special composition from the body wall extract, the mentioned composition cannot have any anti-bacterial effect alone. However, the combined effect of various compounds present in crude extract has such a property. By comparing the tests, it can be stated that sea cucumber extracts have anti-bacterial effects against human pathogenic bacteria like *E.coli, S. aureus* and *P. aeroginosa* due for having materials such as trite pen glycosides (Ismail et al., 2008), disulfate glycosides (Muniai et al., 2008), steroidal glycosides (Bryan et al., 1993), poly-hydroxy sterols (Jensen et al., 1996), Naphthoqui none pigments (Chattapadhyay et al., 1996), Lysozymes and various precursors (Findlay & Smith, 1995). As a result, sea cucumber can be introduced as a source of the compounds with anti-bacterial effect that can act as an appropriate can did ate for making pharmaceutical, medical, and antibiotics compounds.

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