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# Antibacterial activity of the sea cucumber *Holothuria leucospilota*

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ABSTRACT

Aquatics are a source of bioactive compounds that these compounds have different properties such as antimicrobial activity. In this study, antibacterial activity of methanol, chloroform and hexane extracts from body wall, gonad and intestine of sea cucumber *Holothuria leucospilota* was evaluated against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Bioactive compounds of body wall, gonad and intestine of the sea cucumber *Holothuria leucospilota* collected from the north coast of the Persian Gulf were extracted using methanol, chloroform and hexane. The antibacterial activity was determined using the serial dilution method. The minimum inhibitory concentration and minimum bactericidal concentration was evaluated by broth micro dilution method. Results demonstrated that the *P.aeruginosa* was shown to be the most sensitive microorganism. All concentrations of methanol extracts from body wall, gonad and intestine did not show antibacterial activity against *B.subtilis* and *S.aureus*. Methanol extracts of gonad and intestine and chloroform extract from body wall showed no antibacterial activity against *P. aeruginosa*. Hexane extract from gonad had no inhibitory effect on the growth of *B. subtilis* in any of the concentrations. Other extracts had antibacterial effect in certain concentrations studied. None of the extracts showed any bactericidal effect against *B. subtilis*. Based on findings of this study, sea cucumber extracts can be considered as a natural antibiotic in the future research.

## 1. Introduction

Sea cucumber is a marine invertebrate from the class Holothuroidea which is found on the sea floor (Althunibat et al., 2009). These organisms are living in a wide variety of habitats (Graham and Ballaglene, 2004). Sea cucumbers are one of the important marine animals that are used as human food sources, especially in some parts of Asia (Taiyeb- Ali et al., 2003). Due to the growing global demand for food and pharmaceutical applications, many species of sea cucumbers have been exploited (Bruckner et al, 2003; Lawrence et al., 2009; Mehmet et al., 2011). These organisms have a high percentage of protein with no cholesterol and are considered as food stuffs (Carballo et al., 2002). Many bioactive compounds have been reported in different species of sea cucumber. Some of these compounds have biological activity (Bryan et al., 1992; Villasin and Pomory, 2000). In researches that has been conducted recently on the extracts and compounds derived from sea cucumber, it has been proven that they have cytotoxicity (Hawa et al., 1999; Sugawara et al., 2006), anti-oxidant (Ding et al., 2003), antibacterial, anti-inflammatory, antiviral,

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antitumor and anticancer (Chen, 2003; Farouk et al., 2007) properties.

Sea cucumber extract has been demonstrated in various studies as potential antimicrobial agents. Mokhlesi et al (2011) studied the cytotoxic activity, antibacterial and antifungal of sea cucumber Bohadschia marmorata (Mokhlesi et al., 2011). Their results showed that the extract of this species has antifungal effect while no antibacterial activity. Jamali et al (2011) investigated the antibacterial effect of sea cucumber *Holoturia* spp. on three strains of *Escherichia coli*. Their research results showed that the methanol and chloroform extract at a concentration of 100 mg/ml caused death to bacterial strains K12 and TG1, respectively (Jamali et al., 2011).

*Holothuria leucospilota* usually lives in quiet and deep areas on the sandy bottom or on coral rubbles. It is supposed that *H. leucospilota* is the dominant species in the Persian Gulf (Afkhami et al., 2012). Persian Gulf is a unique environment with rich biodiversity; this environment is a very suitable host for the study of marine biological activity. 17 species of holothurians is found in the waters around Iran (Heding, 1940). However, few studies have been conducted about the effectiveness of some sea cucumber species as sources of antimicrobial, antifungal and cytotoxic, worldwide. Therefore, there is little information regarding the biological activity of sea cucumber species in the Persian Gulf (Mokhlesi et al., 2011).

The objective of this study was to determine the antibacterial activity of the methanol, chloroform and hexane extracts from body wall, gonad and intestine of the Holothuria leucospilota on the *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

## 2. Matherials and Methods

### *2.1 Sample collection and extraction of the samples*

Samples of sea cucumber *Holothuria leucospilota* were harvested from the Persian Gulf, around the Lark Island, Iran, at depth of 25-30 meters in January 2013. Samples were transported to laboratory of Fisheries, Department of Natural Resources at University of Tehran and were stored at -20°C until next step. Sea cucumbers were cleaned with fresh water after defreezing. The samples were cut from the anus to the mouth (Mamelona et al., 2007). Then, sections of wall, gonad and intestine were cut into small pieces, separately. Samples were kept at 45°C for 2 days to dry completely. Then the samples dried were crushed by grinding machine (Worldstar) and were powdered thoroughly. Powder prepared was soxhlet with solvents of chloroform, methanol and hexane (Merk, Darmstadt, Germany) for 6 h (Gaidi et al., 2001). The solvents were evaporated under vacuum conditions at each stage (Estrada et al., 2000). Finally, the extracts were dried by freeze dryer for complete removal of solvents; create a solid form and increase of the purity (Mamelona et al., 2007). The extracts obtained from different parts of the body were transported to the microbiology laboratory of Iran University of Medical Sciences for microbial test.

### *2.2 Antibacterial assay*

The antibacterial activity of the *H. leucospilota* extracts were assessed against *Bacillus subtilis* (ATCC 127111), *Pseudomonas aeruginosa* (27853) and *Staphylococcus aureus* (25923) by the Serial dilution method (McDermott et al., 2001). In this study, the OD of extracts was read at zero time and after 24 h by the ELISA reader at 630 nm. If the OD was reduced after 24 h, extracts would have had inhibitory effect on bacteria growth. Minimum inhibitory concentrations (MIC) and Minimal bactericidal concentration (MBC) of the extracts were tested by Broth micro dilution method (Thornsberry and McDougal, 1983).

### *2.3. Statistical analysis*

In this research, Kolmogorov- Smirnov test was performed to determine the normality of the data. One-way analysis of variance (ANOVA) was used to determine significant differences.

## 3. Results

Methanol, chloroform and hexane extracts from body wall, gonad and intestine of *H. leucospilota* were tested against three types of bacteria, *B.Subtilis*, *P.aeruginosa* and *S.aureus*. All concentrations of methanol extracts from body wall, gonad and intestine did not show antibacterial activity against *B. subtilis* and *S. aureus* (Fig 1, 7). Methanol extracts of gonad and intestine and chloroform extracts from body wall showed no antibacterial activity against *P. aeruginosa* (Fig 4, 5). Hexane extract from gonad had no inhibitory effect on the growth of *B. subtilis* in any of the concentrations studied (Fig 3). Apart from the above extracts, the other extracts had antibacterial effect in certain concentrations. The results of the MIC and MBC tests are summarized in Table 1.

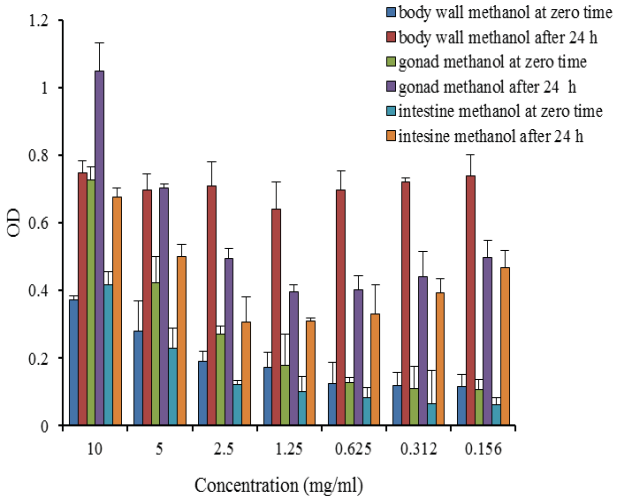


**Fig 1.** Effect of methanol extracts on *Bacillus subtilis*

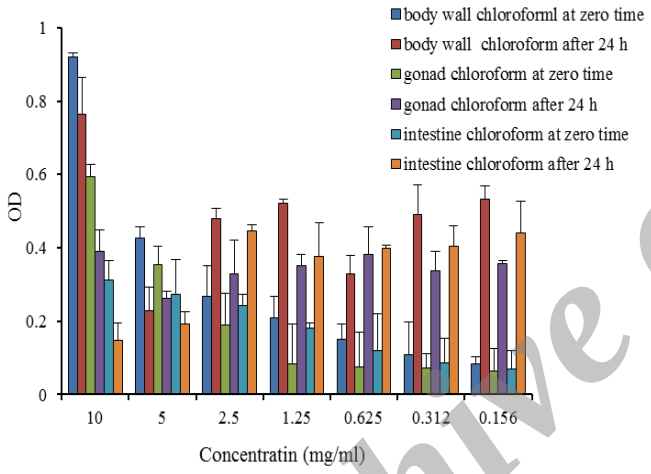


**Fig 2**. Effect of chloroform extracts on *Bacillus subtilis*

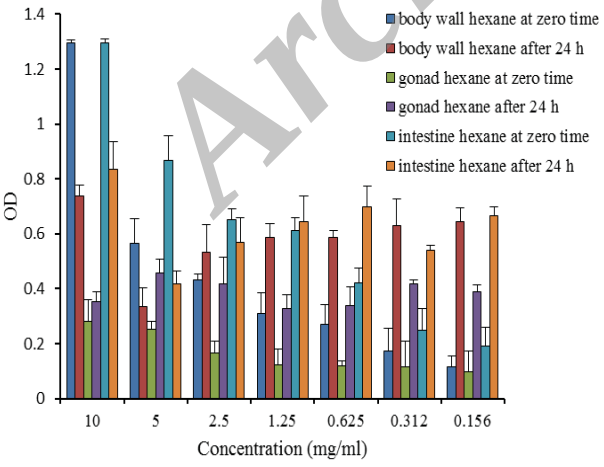


**Fig 3**. Effect of hexane extracts on *Bacillus subtilis*

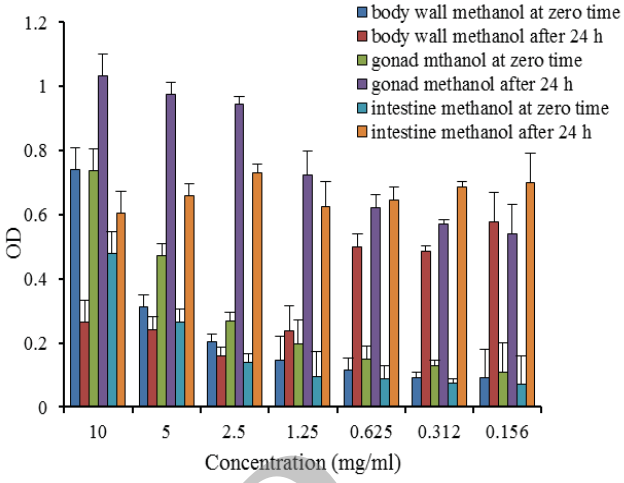


**Fig 4**. Effect of methanol extracts on *Pseudomonas aeruginosa*

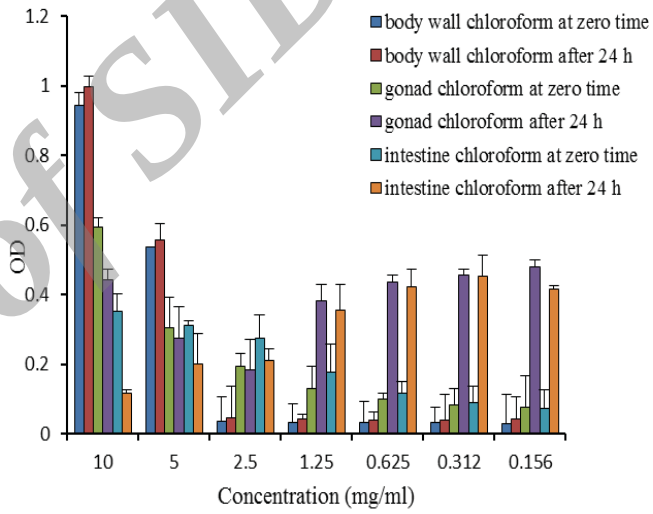


**Fig 5**. Effect of chloroform extracts on *Pseudomonas aeruginosa*

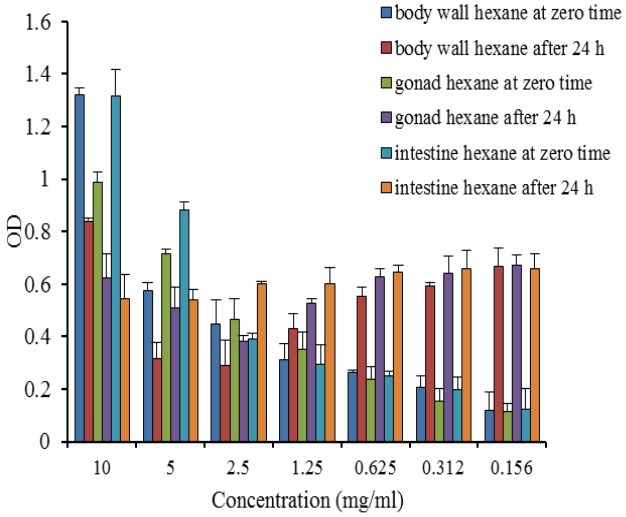


**Fig 6**. Effect of hexane extracts on *Pseudomonas aeruginosa*

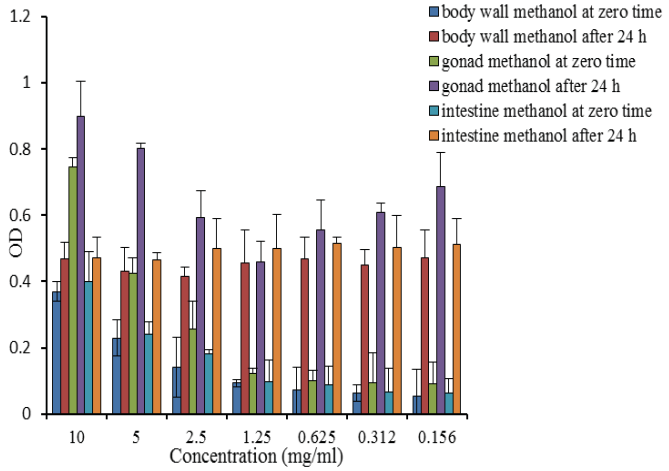


**Fig7**. Effect of methanol extracts on *staphylococcus aureus*

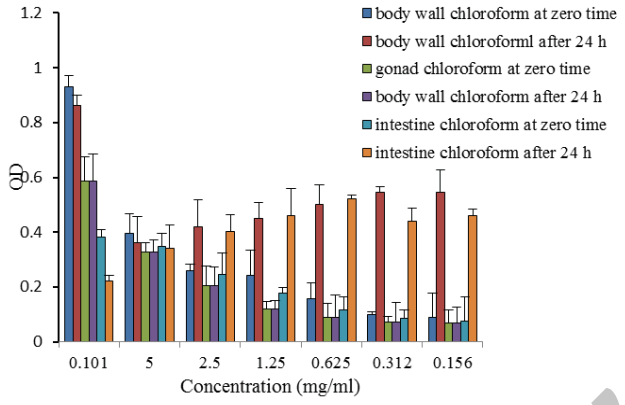


**Fig8**. Effect of chloroform extracts on *staphylococcus aureus*

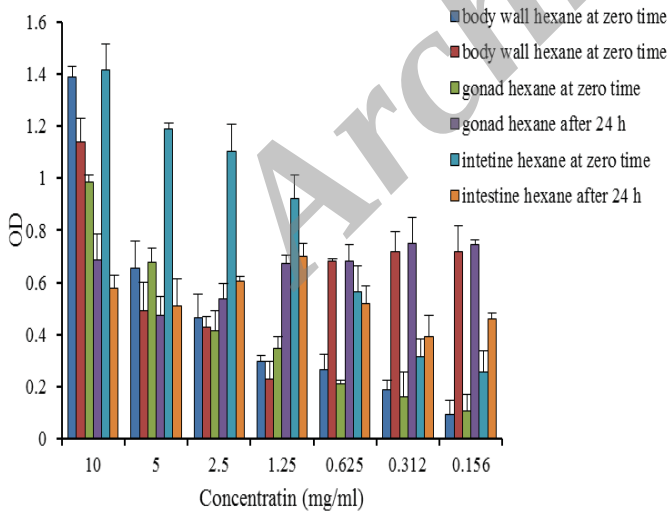


**Fig 9**. Effect of hexane extracts on *staphylococcus aureus*

## 4. Discussion

Our results showed that chloroform and hexane extracts had antibacterial activity more than methanol extract. This could be due to the suitability of chloroform and hexane solvents for dissolving the bioactive compounds present in the bodies of marine creature. Also, some of the microbes become resistant to antibiotics and drugs. At this time, microorganisms use mechanisms that include limiting the antimicrobial agent concentrations within cells by reducing penetration or increasing the exit and neutralizing of the antimicrobial agent that can inactive the drug by enzymes either reversibly or non-reversibly.

In this study, the antibacterial activity of extracts obtained from *H. leucospilota* can be linked to the presence of a wide array of bioactives, especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulfated polysaccharides, sterols peptides, glycoprotein, glycosphingolipids and essential fatty acids (Bordbar et al., 2011).

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including natural sources from any terrestrial or marine source (Blunt et al., 2007). The present study was designed with the aim of covering wide range of active substances in various extracts obtained from different parts of the sea cucumber.

Antibacterial activity of extracts obtained from *P. paruimensis* wall was confirmed by Villasin and Pomory (2000) (Villasin and Pomory, 2000). One of the important factors that could cause the antibacterial activity of the sea cucumber extracts is secondary metabolites such as triterpene glycosides (Mulyndin and Kovalev, 2001). In previous studies, asterosaponins were isolated from the sea cucumber, which showed toxic effects on marine invertebrate larvae (Fusetani, 2004). Mokhlesi et al (2011) examined the effect of ethyl acetate, methanol and water-methanol extracts from cuvierian organ and body wall of Gulf Persian sea cucumber Bohadschia marmorata on *P. aeruginosa*, *Escherichia coli* and *S. aureus* (Mokhlesi et al., 2011). Their results showed that none of the extracts had any inhibitory effect on the growth of bacteria. The difference in these results and the results of our

study can be due to various reasons, such as different methods of extraction, the use of different solvents, different species of sea cucumber and different ecological conditions of studied species. In general, antibacterial activity of extracts derived from different parts of the sea cucumber is attributed to the presence of bioactive compounds in the body. Also, due to the uneven distribution of these compounds in different parts of the sea cucumber, the antibacterial effect of extracts isolated from various sectors is different.

**Table 1**. Minimum inhibitory concentration and minimum bactericidal concentration (mg/ml)

| Bacteria | Extract | MIC | MBC |
|---|---|---|---|
| *Bacillus subtilis* | Body wall methanol | - | - |
| | Gonad methanol | - | - |
| | Intestine methanol | - | - |
| | Body wall chloroform | 5 | - |
| | Gonad chloroform | 5 | - |
| | Intestine chloroform | 5 | - |
| | Body wall hexane | 5 | - |
| | Gonad hexane | - | - |
| | Intestine hexane | 2.5 | - |
| *Pseudomonas aeruginosa* | Body wall methanol | 2.5 | 2.5, 5, 10 |
| | Gonad methanol | - | - |
| | Intestine methanol | - | - |
| | Body wall chloroform | - | - |
| | Gonad chloroform | 2.5 | 5, 10 |
| | Intestine chloroform | 2.5 | - |
| | Body wall hexane | 2.5 | - |
| | Gonad hexane | 2.5 | - |
| | Intestine hexane | 5 | 5, 10 |
| *Staphylococcus aureus* | Body wall methanol | - | - |
| | Gonad methanol | - | - |
| | Intestine methanol | - | - |
| | Body wall chloroform | 5 | - |
| | Gonad chloroform | 10 | - |
| | Intestine chloroform | 5 | - |
| | Body wall hexane | 1.25 | - |
| | Gonad hexane | 5 | 10 |
| | Intestine hexane | 0.625 | 10 |

## Conclusion

Antibacterial activity in crude extracts of the sea cucumber *Holothuria leucospilota* suggests a possible ecological function for their secondary metabolites.

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