Derivation of extracts from Persian Gulf sea cucumber (*Holothuria leucospilota*) and assessment of its antifungal effect

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Abstract

Sea cucumber is presented as potential marine source of antimicrobial compounds. The purpose of this study is to evaluate antifungal effects of sea cucumber, *Holothuria leucospilota*, extracts on *Aspergillus niger* and *Candida albicans*. Methanol and chloroform extracts of the body wall, gonad and intestine of sea cucumber, *H. leucospilota*, collected from Persian Gulf, were evaluated for their antifungal activity against *A.niger* and *C.albicans*. The activity was determined using serial dilution method. Antifungal activity, minimum inhibitory concentration and minimum fungicidal concentration were evaluated by the different concentrations. Results showed that each of the extracts had antifungal effect at specified concentrations on the *A.niger*. All examined concentrations of Gonad methanol and intestine chloroform extracts had no inhibitory effect on *C.albicans*. Chloroform extracts of gonad and intestine had more fungicidal effect against *A.Niger* compared with *C.albicans*. Gonad chloroform extracts showed more fungicidal effect on the *C.albicans* at concentrations of 2.5, 5 and 10 mg/ml. Sea cucumber extract can be considered as an antifungal agent in various industries such as medicine and pharmaceutical industry.

Keywords: Sea cucumber extract, Persian Gulf, Antifungal activity, Inhibitory effect

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Introduction

In recent years, many bioactive compounds are extracted from different marine organisms. Sea cucumber is a marine invertebrate of the phylum Echinodermata and the class Holothuroidea, which is found on the sea floor worldwide (McDermott *et al*., 2001). Sea cucumbers are numerous and are various groups of echinoderms that have a soft, fleshy and elongated body (Castro and Huber, 2000). Among the sea creatures, sea cucumbers are a large and diverse group that many of secondary metabolites are extracted from them. Some of these compounds have biological activities such as cytotoxic activity, anti-bacterial, anti-fungal, anti-viral, anti-cancer and other proprietary activities (Bryan *et al*., 1992; Villasin and Pomory, 2000).

For a long time, sea cucumbers' information was confined to their physiological and ecological aspects (Bakus, 1973). However, sea cucumbers are recognized as a potential source of food by native Asian countries (Trinidad-Roa, 1987). Also scientific researchers investigated medicinal properties of these organisms (Canicatti and Roch, 1989; Hawa *et al*., 1999; Mamelona *et al*., 2007) and showed drug's potential for most triterpene glycosides isolated from various species of sea cucumber such as *Cucumaria japonica* (Batrakov *et al*., 1980), *Hemioedema spectabilis* (Chludil *et al*., 2002), and *H.pervicax* (Yamada, 2002). These glycoside compounds have anti-fungal (Anisimov *et al*., 1972; Bakus, 1973; Murray *et al*., 2001; Chludil *et al*., 2002; Kumar *et al*., 2007), anti-inflammatory (Herencia *et al*., 1998; Aminin *et al*., 2001) and cytotoxic activities (Chludil *et al*., 2002; Zou *et al*., 2003).

Antimicrobial activity of extracts derived from different body parts of sea cucumbers (*C.frondosa*), and common starfish (*Asterias rubens*) has been studied (Haug *et al*., 2002). Wide variety of bioactive compounds in the extracts, showed the presence of various substances that have anti-microbial performance capabilities. So marine echinoderms can be surveyed as sustainable resources for the discovery of new antibiotic compounds. Antifungal activity is one of the most useful biological functions that can be obtained from sea cucumbers (Kitagawa *et al*., 1989; Murray *et al*., 2001; Chludil *et al*., 2002; Kumar *et al*., 2007). Several studies have been conducted on the antimicrobial properties of sea cucumbers. Ismail *et al* (2008) studied the antifungal activity of aqueous and methanol extract of the sea cucumber *H. poli.* Some studies have discussed the biological activity of bioactive compounds extracted from sea cucumber. Wang *et al* (2012) tested antifungal properties of triterpene and non triterpene glycosides derived from the sea cucumber *Apostichopus japonicus*. Most of these studies have demonstrated antimicrobial activity of sea cucumber extract.

Sea cucumbers are commercially important aquatics in the Persian Gulf and Oman Sea. These aquatics have important composition and properties, but there is no information about the biological activity of

these species (Mokhlesi *et al*., 2011). In the present study, antifungal activity of the body wall, gonad and intestine extracts of the *H. leucospilota*, a species found along the northern coasts of the Persian Gulf, Iran, was determined against *A.niger* and *Candida albicans*.

Materials and methods

Sample preparation

Samples of sea cucumber *H.leucospilota* (with an average weight and length of 450±5g and 15±3 cm, respectively) were collected from Persian Gulf, around the Lark Island, at the depth of 25-30 meters in January 2013. Samples were frozen after collection and transported to laboratory of Fisheries Department of Natural Resources at University of Tehran within 24 h and were stored at -20°C until next step.

Extractions of the samples

Sea cucumbers were washed with fresh water after defrosting. The samples were cut from the anus to the mouth. Then, sections of wall, intestine and gonad were cut into small pieces, separately. Samples were kept at 45 ° C for 2 days to dry completely. Then the dried samples were crushed by grinding machine (Worldstar) and were powdered thoroughly. Prepared powder was used for extraction with solvents of chloroform, methanol and hexane (Merck, Darmstadt, Germany) for 6 h using a soxhlet apparatus (Gaidi *et al*., 2001). The solvents were evaporated under vacuum conditions at each stage (Estrada *et al*., 2001). Finally, the extracts were dried by freeze dryer for complete removal of solvents; creating a solid form and increasing the purity of the extracts (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.

Antifungal assay

The antifungal activities of the *H. leucospilota* extracts were assessed against *A.niger* (ATCC 1105) and *Candida albicans* (ATCC 5027) by the Serial dilution method (McElroy, 1990). Minimum inhibitory concentrations (MIC) and Minimal fungicidal concentration (MFC) of the extracts were tested by Broth micro dilution method in the lowest concentration at which no growth was observed.

Statistical analyses

In this research the SPSS version 17 (SPSS Inc., Chicago, IL) was used for statistical analysis. Kolmogorov- Smirnov test was performed to determine the normality of the data. One-way analysis of variance (ANOVA) was used to determine significant differences. Averages were compared by Duncan test at 5% level.

Results

The effect of sea cucumber extracts were examined on *A.niger* and *C.albicans*. In this study, the OD of extracts was read at zero time and after 72 h with the ELISA reader at 630 nm. If the OD was reduced after 72 h, extracts have been inhibitory effect on fungal growth. Results of this study are given in Tables 1-5. All extracts have antifungal effect against *A.niger*, but Gonad methanol and intestine chloroform

extracts had no antifungal effect on *C.albicans*, and these extracts did not show MIC and MFC against *C.albicans*. Wall chloroform and intestine methanol extracts showed maximum inhibitory effect on the growth of *A.niger* and *C.albicans*,respectively.

Table 1: Effects of methanol extracts on *A.niger*.

concentration (mg/ml) / Extract	Wall body		Gonad		Intestine	
	Zero time	**After 72 h**	**Zero time**	**After 72 h**	**Zero time**	**After 72 h**
10	0.293±0.017 [a]	0.289±0.066 [a]	0.753±0.057 [a]	0.675±0.073 [b]	0.475±0.029 [a]	0.442±0.034 [b]
5	0.255±0.08 [a]	0.241±0.019 [b]	0.534±0.02 [a]	0.506±0.024 [b]	0.292±0.08 [a]	0.258±0.050 [b]
2.5	0.174±0.054 [a]	0.171±0.091 [a]	0.293±0.085 [a]	0.290±0.10 [a]	0.174±0.033 [a]	0.165±0.092 [b]
1.25	0.148±0.10 [a]	0.132±0.07 [b]	0.238±0.09 [a]	0.248±0.080 [a]	0.134±0.050 [b]	0.322±0.010 [a]
0.625	0.112±0.09 [a]	0.103±0.03 [b]	0.128±0.044 [a]	0.139±0.092 [a]	0.115±0.013 [a]	0.120±0.086 [a]
0.312	0.102±0.01 [a]	0.106±0.097 [a]	0.096±0.03 [a]	0.100±0.049 [a]	0.090±0.049 [a]	0.092±0.024 [a]
0.156	0.100±0.047 [a]	0.110±0.090 [a]	0.083±0.07 [a]	0.091±0.014 [a]	0.066±0.097 [a]	0.072±0.070 [a]
control	0.070±0.002[a]	0.070±0.002[a]	0.070±0.002[a]	0.070±0.002[a]	0.070±0.002[a]	0.070±0.002[a]

Values are the mean OD±SD of the three replicates. Different small letters in each column indicate significant differences between zero time and 72 hours (p<0.05).

Table 2: Effects of chloroform extracts on *A.niger*.

concentration (mg/ml) / Extract	Wall body		Gonad		Intestine	
	Zero time	After 72 h	Zero time	After 72 h	Zero time	After 72 h
10	1.222±0.017 [a]	0.167±0.054 [b]	0.302±0.10 [a]	0.238±0.084 [b]	0.271±0.072 [a]	0.123±0.065 [b]
5	1.080±0.08 [a]	0.923±0.069 [b]	0.252±0.022 [a]	0.216±0.062 [b]	0.192±0.053 [a]	0.129±0.047 [b]
2.5	0.559±0.01 [a]	0.531±0.031 [b]	0.150±0.086 [a]	0.143±0.048 [a]	0.132±0.045 [a]	0.116±0.099 [b]
1.25	0.469±0.031 [a]	0.391±0.086 [b]	0.123±0.015 [b]	0.444±0.022 [a]	0.109±0.078 [b]	0.125±0.086 [a]
0.625	0.332±0.092 [a]	0.314±0.042 [b]	0.085±0.092 [b]	1.077±0.090 [a]	0.085±0.089 [b]	0.105±0.043 [a]
0.312	0.123±0.011 [b]	0.541±0.013 [a]	0.069±0.033 [b]	0.783±0.089 [a]	0.081±0.010 [b]	0.860±0.026 [a]
0.156	0.120±0.078 [b]	1.119±0.090 [a]	0.067±0.071 [b]	0.881±0.035 [a]	0.068±0.031 [b]	1.032±0.061 [a]
control	0.073±0.006 [b]	1.411±0.028 [a]	0.073±0.006 [b]	1.411±0.028 [a]	0.073±0.006 [b]	1.411±0.028 [a]

Values are the mean OD ± SD of the three replicates. Different small letters in each column indicate significant differences between zero time and 72 hours (p<0.05).

Table 3: Effects of methanol extracts on *C.albicans*.

concentration (mg/ml) / Extract	Wall body		Gonad		Intestine	
	Zero time	After 72 h	Zero time	After 72 h	Zero time	After 72 h
10	0.550±0.061 [a]	0.374±0.100 [b]	0.666±0.043 [b]	0.751±0.056 [a]	0.486±0.075 [a]	0.444±0.100 [b]
5	0.127±0.052 [b]	0.158±0.029 [a]	0.471±0.87 [b]	0.483±0.038 [a]	0.278±0.083 [a]	0.240±0.016 [b]
2.5	0.374±0.076 [a]	0.094±0.037 [a]	0.303±0.100 [a]	0.299±0.049 [a]	0.245±0.019 [a]	0.255±0.033 [b]
1.25	0.094±0.030 [a]	0.094±0.050 [a]	0.194±0.070 [a]	0.197±0.065 [a]	0.620±0.043 [a]	0.391±0.081 [b]
0.625	0.081±0.023 [a]	0.088±0.031 [a]	0.132±0.046 [a]	0.138±0.080 [a]	0.532±0.071 [a]	0.463±0.092 [b]
0.312	0.093±0.042 [b]	0.198±0.083 [b]	0.100±0.068 [b]	0.683±0.045 [a]	0.093±0.026 [b]	0.740±0.032 [a]
0.156	0.079±0.012 [a]	0.085±0.033 [a]	0.088±0.054 [b]	0.099±0.013 [a]	0.086±0.041 [a]	0.086±0.023 [a]
control	0.068±0.002 [a]	0.068±0.004 [a]	0.068±0.002 [a]	0.068±0.004 [a]	0.068±0.002 [a]	0.068±0.004 [a]

Values are the mean OD±SD of the three replicates. Different small letters in each column indicate significant differences between zero time and 72 hours (p<0.05).

Table 4: Effects of chloroform extracts on *Candida albicans.*

concentration (mg/ml) / Extract	Wall body		Gonad		Intestine	
	Zero time	After 72 h	Zero time	After 72 h	Zero time	After 72 h
10	1.352±0.078 [a]	0.709±0.043 [b]	0.709±0.043 [b]	0.666±0.043 [b]	0.252±0.064 [b]	0.315±0.050 [a]
5	1.217±0.100 [a]	0.982±0.098 [b]	0.982±0.098 [b]	0.471±0.87 [b]	0.175±0.047 [a]	0.183±0.028 [a]
2.5	0.978±0.038 [a]	0.948±0.076 [b]	0.948±0.076 [b]	0.303±0.100 [a]	0.130±0.044 [a]	0.132±0.069 [a]
1.25	0.570±0.045 [a]	0.437±0.080 [b]	0.437±0.080 [b]	0.194±0.070 [a]	0.114±0.047 [a]	0.105±0.038 [a]
0.625	0.623±0.033 [b]	0.820±0.095 [a]	0.820±0.095 [a]	0.132±0.046 [a]	0.083±0.076 [b]	0.593±0.060 [a]
0.312	0.265±0.030 [b]	1.036±0.076 [a]	1.036±0.076 [a]	0.100±0.068 [b]	0.088±0.018 [b]	0.686±0.019 [a]
0.156	0.166±0.092 [b]	0.986±0.045 [a]	0.986±0.045 [a]	0.088±0.054 [b]	0.078±0.032 [b]	0.652±0.048 [a]
control	0.077±0.010 [b]	0.733±0.028[a]	0.077±0.010 [b]	0.733±0.028[a]	0.077±0.010 [b]	0.733±0.028 [a]

Values are the mean OD±SD of the three replicates. Different small letters in each column indicate significant differences between zero time and 72 hours (p<0.05).

Table 5: Minimum inhibitory concentrations and minimum fungicidal concentrations (mg/ml).

Fungi	Extracts	MIC	MFC
Aspergillus niger	Wall methanol	0.625	10
	Gonad methanol	5	5, 10
	Intestine methanol	2.5	5, 10
	Wall chloroform	0.625	5, 10
	Gonad chloroform	2.5	2.5, 5, 10
	Intestine chloroform	2.5	2.5, 5, 10
Candida albicans	Wall methanol	2.5	2.5, 5, 10
	Gonad methanol	-	-
	Intestine methanol	0.625	10
	Wall chloroform	1.25	10
	Gonad chloroform	2.5	2.5, 5, 10
	Intestine chloroform	-	-

Discussion

In the present study, antifungal effect of methanol and chloroform extracts of parts of the wall, gonads and intestine of the sea cucumber, *H. leucospilota*, were examinedon *A.niger* and *C.albicans*. The extracts showed antifungal activity at certain concentrations, but gonad methanol and intestine chloroform extracts did not show any inhibitory effect on *C.albicans*. Each microorganism strain has unique characteristics that is different from others.

So there are some differences in the structure of these strains that caused differences in the results.

The rationale for searching drugs from the marine environment stems from the fact that marine plants and animals have adapted to all sorts of marine environments and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction (Kumaravel *et al*., 2010).

Several chemical and pharmacological studies have been conducted on several species of sea cucumber suggesting that these invertebrates have some compounds as triterpene glycosides (Mokhlesi *et al*., 2011). Chludil *et al* (2002) have isolated two new sulfated glycosides, hemoiedemosides A and B from the sea cucumber, *Hemioedema spectabilis* which exhibited considerable antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum* (Mokhlesi *et al*., 2011). Yuan *et al*. (2009) have isolated antifungal activity from the sea cucumber species, *Bohadschia marmorata*. Triterpene glycosides are secondary metabolites predominantly in the sea cucumber, which show a range of biological activities such as antifungal activity, cytotoxic, hemolytic, etc. (Mokhlesi *et al*., 2011). Ridzwan *et al.* (1995) reported the evaluation of *H. atra*, *H.scabra* and *Bohadschia argus* against seven species of bacteria and found that lipid and methanolic extracts had no inhibitory activity; while a phosphate buffered saline extract showed inhibitory activity. Antimicrobial activity of extracts derived from sea cucumber is caused by secondary metabolites and bioactive compounds in these organisms. Saponins are one of major isolated products from sea cucumber (Bhakuni and Rawat, 2005). This glycoside is soluble in water and showed hemolytic and cytotoxic activity in vitro and natural conditions, these compounds can be used to treat cancer and fungal infections (Kelly, 2005). Several triterpene glycosides were isolated from the sea cucumber, *Psolus patagonicus*, which had antifungal effects (Muniain *et al*., 2008). So triterpene glycosides can be a more important factor in antimicrobial activity of the sea cucumber extracts.

In this study, all of the extracts showed antifungal effect except gonad methanol and intestines chloroform extracts. Also, gonad chloroform extract had more fungicidal effect. Based on this study, sea cucumber (*H.leucospilota*) extracts can be recommended for antifungal medication that should be considered in future research.

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