ANTIBACTERIAL, ANTIFUNGAL AND CYTOTOXIC
ACTIVITIES OF **BOHADSKHA MARMORATA**, A SEA
CUCUMBER FROM NORTH COASTAL OF PERSIAN
GULF

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**Summary**

Ethyl acetate, methanol and water-methanol extracts of the cuvierian organ, coelomic fluid and body wall of the sea cucumber, *Bohadschia marmorata*, collected from Persian Gulf, were evaluated for their antibacterial and antifungal activities against *Aspergillus niger*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The activity was determined using the disc diffusion test. Cytotoxic activities of the extracts were assessed by *Artemia salina* larvae. Results showed that methanol extract of body wall and water-methanolic extract of cuvierian organ (Minimum inhibitory concentration (MIC/disc) < 1 mg against *A. niger*) and methanol extract of body wall and water-methanolic extract of cuvierian organ (MIC/disc = 3 mg against *C. albicans*) showed significant antifungal activities but no inhibitory effect of the extracts against bacteria was observed. Significant inhibitory effect was observed in cytotoxic assays which was highest in cuvierian organ methanol extract (LC$_{50}$ = 12 µg/ml).

**Keywords:** antibacterial, antifungal, cytotoxic, *Bohadschia marmorata*

**Introduction**

Many publications regarding the bioactivity of marine extracts although several promising reports were published, for example on anti-inflammatory and analgesic effects (Villasin and Pomory, 2000); antioxidative effects (Kuznetsova et al., 1982); immunostimulating activity (Ridzwan et al., 1995); antidermopert activity (Tian et al., 2005); in vitro bioactivity (Fredalina et al., 1999); antifungal activity (Hawa et al., 1999); antiviral (Bakus, 1973; Heding, 1940); antimicrobial activity (Fenica et al., 1973; Toral-Granda, 2006) and anti-HIV-1 activity (Mamelona et al., 2007). Sea cucumber is a marine invertebrate of the phylum Echinoderm and the class Holothuroidea, which is found on the sea floor worldwide. There are about 1,200 holothurian species in the world (McElroy, 1990). Among marine organisms, sea cucumbers are a large and diverse group of organisms from which a wide range of secondary metabolites have been isolated.
Many bioactive compounds have been reported from different species of sea cucumber. A number of these compounds possess biological activity (Bryan et al., 1992; Villasin and Pomory, 2000). Some of sea cucumber species in Malaysia water are being used in traditional medicine to treat wound, eczema, arthritis or hypertension (Farouk et al., 2007). Several papers support the multiple biological activities of their extracts as wound healing promoter and exhibiting antimicrobial, anticancer, and immunomodulatory properties (Aminin et al., 2001; Kuznetsova et al., 1982; Fredalina et al., 1999; Ridzwan et al., 1995; Tian et al., 2005). They are also rich in saponin glycosides exhibited anticancer activity. Their antioxidant properties have been recently reported from coelomic fluid of three species (Bohadschia marmorata vitiensis, Stichopus variegatus, Stichopus badionotus) (Hawa et al., 1999).

The Persian Gulf is a unique environment with a rich biodiversity which make this environment as an excellent host for marine bioactive research. Heding recorded 17 species of holothurians found in the waters around Iran (Heding, 1940). Despite several worldwide studies about the efficacy of some sea cucumber species as the antimicrobial, antifungal and cytotoxic sources, there is no information about the bioactivity of the most Persian Gulf cucumber species. Altogether, in our last study we surveyed biological activity on Holothuria leucospilota (Mokhlesi et al., in press). Those results showed relatively high antifungal and cytotoxic activities, while not a significant antibacterial activity.

In the present study, cytotoxic, antibacterial and antifungal activities of the body wall, cuvierian organ and coelomic fluid extracts of the Bohadschia marmorata, a species found along the north coastal of Persian Gulf, Iran, was determine.

**Materials and Methods**

**Sample collection**

Samples of the sea cucumber *B. marmorata* were collected from the Persian Gulf, around the sandy shore of the Bostaneh, Iran in low tide time, in June 2009. The collected samples were cleaned by rinsing with seawater and distilled water and transported in cool box to the laboratory where dissected to remove internal organs (cuvierian organ, coelomic fluid and body wall), and packed immediately with ice prior to send to the lab and kept at −20°C until extracted. The taxonomic identity of the samples was confirmed based on the studies of Heding (1940).
Extractions of the samples

The samples of cuvierian organ, coelomic fluid and body wall were defrosted before evaluation. The coelomic fluid was homogenized with stirring using the magnetic stirrer for 15 min, and filtered using some cotton wool followed by passage through a Whatman filter paper, after centrifugation (15 min, 30,000 × g, 4º C). The body wall was cut into small pieces (about 2 cm). Cuvierian organ and the body wall 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. Then, the centrifugated and filtered extracts were evaporated under vacuum at 45º C by a rotary evaporator. The powdered extracts of each sample were obtained by freeze dryer and stored at -20 ºC (Mokhlesi et al., in press).

Antibacterial and antifungal assay

The antibacterial and antifungal activities of the B. marmorata extracts were assessed against Staphylococcus aureus (ATCC 29737), Escherichia coli (ATCC 8739), Candida albicans (ATCC 14053) and Aspergillus niger (ATCC 16404) by the Disc Diffusion Susceptibility method triplicates (Gohari et al., 2010). Minimum inhibitory concentrations (MIC) of the extracts were tested in the lowest concentration at which no growth was observed. Gentamycin and Fluconazole were used as positive controls.

Brine Shrimp Lethality Assay (BSA)

Artemia salina was used for cytotoxic activities of the B. marmorata extracts according to modified Mongelli method described by Saeidnia et al (2009). According this way, brine shrimp (Artemia salina) eggs were hatched in flask containing 300ml artificial seawater made by dissolving distilled water in 29–30 ºC temperature. The flask was well aerated with the aid of an air pump. Four concentrations of each extract were prepared with 10, 100, 500 and 1000 µg/ml and dissolved in normal saline water obtained by serial dilution. Ten to 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. There was used of seawater and berberine hydrochloride (LC_{50} =26 µg/ml) as controls. Lethality percentage was determined and LC_{50}
calculated based on Probit Analysis with 95% of confidence interval (Saeidnia et al., 2009).

Results

Results of antibacterial and antifungal assay

Results of the all concentrations of ethyl acetate, water-methanol and methanol extracts from cuvierian organ, coelomic fluid and body wall were not showed antibacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli* and no inhibition zone observed for these tests. Altogether, antifungal activity of ethyl acetate extracts did not see, but results of other screening test for water-methanol and methanol extracts are summarized in Table 1.

Table 1: Selected antifungal activity of the effective extracts of *B. marmorata*.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Extracts</th>
<th>MIC/disc (mg)</th>
<th>8 mg</th>
<th>4 mg</th>
<th>2 mg</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>Cuverian organ (water-methanol)</td>
<td>&lt;1</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>Body wall (Methanol)</td>
<td>&lt;1</td>
<td>17</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>Cuverian organ (water-methanol)</td>
<td>3</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>Body wall (Methanol)</td>
<td>3</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are presented by the diameter of the inhibition zones (mm)

Results of cytotoxic assay

Such as antibacterial and antifungal tests no inhibitory effects of the ethyl acetate extracts were observed in cytotoxic assays on *B. marmorata* organs.
Unlike to the ethyl acetate, rather powerful inhibitory effects in some tests were observed on Water-methanol and methanol extracts. Based on this the higher cytotoxic activity was observed for Cuverian organ methanolic followed by Body wall methanolic and Coelomic fluid methanolic. Results of BSA assays are summarized in Table 2.

**Table 2:** Brine Shrimp Cytotoxicity of the extracts of *B. marmorata*.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Extracts</th>
<th>Concentrations (µg/ml)</th>
<th>LC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1000  500  100  10</td>
<td></td>
</tr>
<tr>
<td>Cuverian organ</td>
<td>Ethyl acetate</td>
<td>14 *  15  15  15</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>1     2    5    8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Water-methanol</td>
<td>13    13   12   13</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Body wall</td>
<td>Ethyl acetate</td>
<td>15    15   15   15</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>5     5    6    8</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Water-methanol</td>
<td>14    14   13   14</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Coelomic fluid</td>
<td>Ethyl acetate</td>
<td>14    14   14   15</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>2     9    11   12</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>Water-methanol</td>
<td>14    15   14   14</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

* Number of live larvae.

**Discussion**

Results of all the antibacterial assays on different organ extracts of *B. marmorata* showed no inhibition. This is similar to antibacterial assays on *H. leucospilota*, another sea cucumber in this area of Persian Gulf. Also antifungal activity of ethyl acetate extracts was not observed in both species. Results of this study confirm antifungal activity of Cuverian organ and body wall extracts of *B. marmorata* on *A. niger* and *C. albicans*. As shown in literature antifungal activity of Cuverian organ and coelomic fluid extracts of *H. leucospilota* on *A.*
niger and C. albicans were indicated. Another difference was between antifungal activities of Cuvierian organ extracts from both species. Methanolic extract of H. leucospilota, while was active water-methanolic extract of B. marmorata showed antifungal activity (Mokhlesi et al. in press).

Various studies were carried out on antifungal and antibacterial properties of several species of sea cucumbers. Interestingly, the extract of Parastichopus parvimensis has been reported that was not inhibited bacterial growth compared to Tetracycline and Ampicillin (Villasin and Pomory, 2000). Ridzwan et al. (1995) reported the evaluation of H. atra, H. scabra and Bohadshia 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. Anyhow, unlike results of the present study, antibacterial activity was reported in several sea cucumber species such as Strongylocentrotus droebachiensis (Echinoidea), Cucumaria frondosa (Holothuroidea), and Asterias rubens (Asteroidea) (Haug et al., 2002).

There are some studies confirmed the antifungal activity of various sea cucumbers. Antifungal activity of C. japonica against C. albicans and C. tropicalis is reported (Batrakov et al., 1980). The antifungal activities of two triterpene glycosides isolated from H. axiloga against three strains: C. albicans, Cryptococcus neoformans and A. fumigates have been reported (Wei-Huete al., 2008).

There are some more studies on the cytotoxic activity of the sea cucumbers. In this study, the higher amount of cytotoxic activity of B. marmorata was observed in methanolic extracts of Cuvierian organ followed by Body wall and Coelomic fluid. While, methanolic extract of the Body wall for H. leucospilota showed the highest activity (Mokhlesi et al. in press).

**Conclusion**

Significant antifungal activity was showed only in methanol and water-methanol extracts of Cuvierian organ and Body wall of B. marmorata in the present study. This indicates that the active compound(s) which are responsible at least in part, for the antifungal activity of both extracts is located in the mentioned organs, unlike the Coelomic fluid for the H. leucospilota. Sea cucumbers might be one of the appropriate sources of antifungal and cytotoxic natural compounds in the future.
This benthic organism deserves much more interest in marine natural products as its antifungal and cytotoxic properties. Its potential application in nutraceutical and medicinal products needs to be studied.

Acknowledgments

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