Abstract. – Background and Objectives: Leishmaniasis is a protozoan parasitic disease which is transmitted by the female Phlebotomus sandfly and is prevalent in four continents. The first-choice treatment for the leishmaniasis is pentavalent antimonials, which are potentially toxic and often ineffective and use of them exhibit therapeutic failure. These pharmaceutical problems point towards the need to develop novel chemotherapeutic agents. Seaweeds are considered as source of bioactive metabolites characterized by a broad spectrum of biological activities.

Materials and Methods: In this experimental study, cold and hot water crude extracts of four species of green, brown and red marine algae "Caulerpa sertularioides, Gracilaria corticata, Gracillaria salicornia and Sargassum oligocystum" collected along the Bushehr coast of the Persian Gulf (southwest of Iran), prepared and their in vitro activities against Leishmania major promastigote were evaluated by using the MTT assay test.

Results: The cold and hot water crude extracts of four algae species exhibited different anti-Leishmanial activities. The minimum inhibitory concentration of hot water extracts calculated as IC50 was as follows: Caulerpa sertularioides (IC50 ≤ 85 µg/ml), Gracilaria corticata (IC50 ≤ 38 µg/ml), Gracillaria salicornia (IC50 ≤ 46 µg/ml) and Sargassum oligocystum (IC50 ≤ 78 µg/ml), while these values for cold water extracts were (IC50 >125 µg/ml) for Caulerpa Sertularioides (IC50 >65 µg/ml) for Gracilaria corticata (IC50 >74 µg/ml) for Gracillaria salicornia and (IC50 >105 µg/ml) for Sargassum oligocystum, IC50 values for reference drug (Amphotericin B) was (0.16-0.2 µg/ml).

Discussion: According to the results, inhibitory effects of the crude extracts from these four species algae specially hot water crude extracts from "Gracilaria corticata, Gracillaria salicornia and Sargassum oligocystum" are significant and in accordance with other studies that has been done on different algae species. So these results are sufficiently promising to be followed with further studies on isolation and characterization of pure compounds from these algae species as well as in vivo experiments, a work that is already under way in our laboratory.

Key Words: Leishmania, Brown algae, MTT assay, Persian Gulf.

Introduction

Leishmaniasis is a protozoan parasitic disease which is transmitted by the female Phlebotomus sandfly and is prevalent in four continents. It is considered to be endemic in 88 countries, 72 of which are developing countries. Leishmaniasis is classified on the basis of symptomatology as cutaneous, visceral, muco cutaneous and diffused cutaneous forms. There are two millions new cases of Leishmania per year. The current therapy against leishmaniasis is ineffective and unsatisfactory. The first-choice treatment for the several forms of leishmaniasis is pentavalent antimonials, which are potentially toxic and often ineffective and use of these recommended drugs exhibit therapeutic failure, side effects and long duration for healing of lesions. Second-line compounds used in treatment of Leishmaniasis generally include pentamidine and amphotericin B, which may be very toxic and in some area are ineffective.
These pharmaceutical problems point towards the need to develop novel chemotherapeutic agents. The interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during the last years. To date, many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new pharmaceuticals.

Seaweeds are considered as source of bioactive metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anti-helminthic, antifungal and antibacterial activities have been detected in green, brown and red algae. Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world. The present report describes the anti-Leishmania activities in the crude extract of various seaweed from the Persian Gulf, Bushehr (Southwest of Iran).

**Materials and Methods**

**Parasite Culture**

Leishmania major promastigotes (received as a gift from the Parasitology Department of Isfahan University of Medical Sciences) were cultivated in RPMI 1640 (Sigma, St. Louis, MO, USA) medium supplemented with 10% fetal calf serum (FCS) (Sigma, St. Louis, MO, USA) and 50 mg/ml Ampicillin (Sigma, Steinheim, Germany) at 25°C. Promastigote forms from logarithmic phase were suspended in fresh RPMI 1640 medium to a final concentration of 4×10^6 cells/ml. The test was performed in 96-well microtitre plates and each well was filled with 100 µl of culture medium and the plates were incubated at 27°C for 1 h before algae extracts addition.

**Preparation of the Extracts**

*Caulerpa sertularioides*, *Gracilaria corticata*, *Gracillaria salicornia* and *Sargassum oligocystum* were collected along the Bushehr coast of the Persian Gulf (Southwest of Iran). Algae extracts were prepared as previously described by Zandi et al. Briefly, about 10 g of each fresh algae, corresponding to 1 g of dry alga material was homogenized in 100 ml cold double distilled water. The mixture was clarified by filtration using Whatman No. 1 filter paper. Then the crude extract was sterilized by filtering (0.22 µm). The sterilized extracts were stored in –80°C until the date of use.

**Antileishmanial Bioassay**

The stock water extracts of algae (10 mg/ml) were serially diluted with phosphate buffered saline (PBS), and each prepared algae extracts was added to each well of 96-well Nunc microtitre plate (final concentrations of 1-281 µg/ml) and incubated at 25°C for 72 hours, all tests were performed in triplicate. Negative control only received RPMI medium with a parasite density of 4×10^6 parasites/ml and the positive control contained varying concentrations of standard anti-Leishmania compound, Amphotericine B (Sigma, Steinheim-Germany). MTT (Sigma, St. Louis, MO, USA) solution was prepared as 5 mg/ml in RPMI-1640 without phenol red and filtered through a 0.2 µm filter and 20 µl of this concentration was added to each well and incubated at 25°C for 24 hours. After this incubation and in order to solving the formazan crystals, 150 µl of acidic isopropanol was added to each well. The plate was Read on an ELISA reader (Biotech, Highland Park, Winooski, VT, USA) using 540 nm as test wavelength and 630 nm as the reference wavelength. The results were expressed as the half maximal inhibitory concentration (IC_{50}).

**Statistical Analysis**

To compare the effects of different algae extracts and control, statistical analysis were performed using the non parametric Kruskal-Wallis test.

**Results**

The crude extracts of four brown, green and red marine algae species were evaluated for their in vitro activities against the Leishmania major promastigotes. Various algae showed different anti-Leishmania activities. Leishmanicidal effects (mean growth inhibition of triplicate for each algae) measured as IC_{50} values for hot water extracts were as follows: *Caulerpa sertularioides* (IC_{50} ≤85 µg/ml), *Gracilaria corticata* (IC_{50} ≤38 µg/ml), *Gracillaria salicornia* (IC_{50} ≤46 µg/ml) and *Sargassum oligocystum* (IC_{50} ≤78 µg/ml),
while these values for cold water extracts were, *Caulerpa Sertularioides* (IC<sub>50</sub> >125 µg/ml), *Gracilaria Corticata* (IC<sub>50</sub> >65 µg/ml), *Gracilaria salicornia* (IC<sub>50</sub> >74 µg/ml) and *Sargassum oligocystum* (IC<sub>50</sub> >105 µg/ml). Amphotericin B was tested as control drug and IC50 values (mean growth inhibition of triplicate) for this drug ranged from 0.16 to 0.02 µg/ml.

Table 1 displays the IC<sub>50</sub> values determined for each algae extract, as well as for the reference drug, Amphotericine B. Although, the effect of algal extracts in comparison with control were not significant but statistical analysis proved that hot water crude extracts of *Gracilaria corticata* and *Gracilaria salicornia* are significantly stronger Leishmania growth inhibitors, compared to other algae (*p* =0.016). They showed 38 and 46 mean of growth inhibition respectively and also lower mean differences with the control, compared to other algae. This fact should be considered that the materials used in this study are crude extracts and further purification and fractionation to isolate and characterize bioactive metabolites can lead to promising results.

**Discussion**

Anti-Leishmania therapy is based on pentavalent antimony compounds. The toxicity of these agents and the persistence of side effects are severe, even after modification of the dose level and the duration of treatment. So, search to find more effective drugs with less side effects is necessary. Some studies have been done on different alga species but a few researches have been carried out on the anti-Leishmania activities of green, brown and red algae and there are only a few papers available on this subject. Also in Iran no research has been done about Leishmanicidal activities of *Persian Gulf algae*. However, these few studies showed promising results regarding the anti-Leishmania activities of algae in particular red and brown algae. Different algae species widely distributed along the coast of the Persian Gulf (Southwest of Iran). So, this study was designed to respond to this need and this will be the first report of anti-Leishmanicidal activities of *Persian Gulf algae* from Iran. All crude extracts of studied brown, green and red algae displayed anti-Leishmania activities with variations in their IC<sub>50</sub> values. However, hot water crude extracts from all four species of studied algae exhibited stronger inhibition of *Leishmania* growth, compared to cold water crude extracts. *Gracilaria corticata* displayed stronger Leishmanicidal effect compared to *Gracilaria salicornia*, *Sargassum oligocystum* and *Caulerpa sertularioides*.

Studies about anti-Leishmanicidal effects of Mediterranean red algae showed that halogenated compounds from these algae has strong Leishmanicidal activities, besides, study of Jasmine et al. on brown algae from British and Irish water has showed that all studied brown algae displayed anti-Leishmania activities, but some species showed more potent Leishmanicidal activity. Moreover, Sabina et al. have reported the promising anti-Leishmanicidal effects of brown and green algae from the coast of Karachi, Pakistan.

Our results shows that red and brown algae from *Persian Gulf* (*Gracilaria corticata*, *Gracilaria salicornia* and *Sargassum oligocystum*) have strong anti-Leishmania effects and are very promising. Therefore, more accurately evaluation of these algae, through further fractionation in order to isolate and characterize their bioactive metabolites can lead to promising results.

**Acknowledgements**

The Author would like to thank the Vice-Chancellor of Research of Bushehr University of Medical Sciences for his financial support, Bushehr (Iran).

**Table 1.** Anti-Leishmanial activity of *Persian Gulf algae*.

<table>
<thead>
<tr>
<th>Seaweed species</th>
<th>Cold water extract (IC&lt;sub&gt;50&lt;/sub&gt;) µg/ml</th>
<th>Hot water extract (IC&lt;sub&gt;50&lt;/sub&gt;) µg/ml</th>
<th>Amphotericin B (IC&lt;sub&gt;50&lt;/sub&gt;) µg/ml</th>
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</thead>
<tbody>
<tr>
<td><em>Caulerpa sertularioides</em></td>
<td>125</td>
<td>85</td>
<td>0.2</td>
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<tr>
<td><em>Gracilaria corticata</em></td>
<td>65</td>
<td>38</td>
<td>0.16</td>
</tr>
<tr>
<td><em>Gracilaria salicornia</em></td>
<td>74</td>
<td>46</td>
<td>0.18</td>
</tr>
<tr>
<td><em>Sargassum oligocystum</em></td>
<td>105</td>
<td>78</td>
<td>0.18</td>
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</tbody>
</table>
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