

BIOACTIVE ANALYSIS OF CHLOROPHYCOTA SPECIES VIA 96-WELL MICRO TITER PLATE TECHNIQUE

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Abstract

The 96- well Micro Titer Plate technique was conducted for various species of the phylum Chlorophycota against promastigote stage of *Leishmania major*. Among selected species of seaweeds *Codium flabellatum* ($IC_{50}/72h = 34.4 \pm 6.2/\mu g/mL$) *Caulerpa faridii* ($IC_{50}/72h = 34.0 \pm 0.15/\mu g/mL$) *Caulerpa racemosa* ($IC_{50}/72h = 37.6 \pm 0.45/\mu g/mL$) and *Ulva fasciata* ($IC_{50}/72h = 50 \pm 0.65/\mu g/mL$) displayed significant values, while the other tested species like *Ulva lactuca* $IC_{50}/72h = 64.75 \pm \mu g/m$, *Ulva rigida* ($IC_{50}/72 = 65.69 \pm 6.8/\mu g/m$) and *Codium iyengarri* ($IC_{50}/72h = 60.40 \mu g/m$) presented good activity. This result indicates that seaweeds of the phylum Chlorophycota possess antileishmanial activity which was conducted for the first time in Pakistan that could be utilized and employed beneficially.

Introduction

Parasitic infections are a serious threat to the human inhabitants and serve as a main cause of morbidity and mortality in both tropical and subtropical regions and they cause more misery when they are compared to bacterial and viral diseases (Godfrey, 1984). In 1960 Leishmaniasis was reported for the first time in Pakistan (Bhutto *et al.*, 2008). Both visceral and cutaneous leishmaniasis overcome over several endemic foci with different climatic and geographical conditions (Ayub *et al.*, 2003), but cutaneous leishmaniasis is prevalent in Pakistan and is second most endemic vector-borne syndrome in the country, after malaria (Ejaz *et al.*, 2007).

There is no perfect treatment for cutaneous appearances which may be topical, effective, inexpensive, and safe. Drug treatment is the way to tackle leishmaniasis (Ghaffarifar *et al.*, 2006), therefore effectively restricted curative trials are necessary to appraise the effectiveness of any new healing (Khan & Muneeb, 2005). Different manners of treatment are used for cutaneous leishmaniasis such as antimonials, parenteral pentamidine, amphotericin B (Fatima *et al.*, 2005).

Natural products derived from plants still provide the incomparable chemical ingredients and bioactivities, which have lead to the expansion of thousands of pharmaceutical drugs (Shu, 1998; Gulfaraz *et al.*, 2011; Sarwat *et al.*, 2012). Seaweed derived natural products are a potentially prolific resource of highly bioactive secondary metabolites that might correspond to useful leads in the expansion of new pharmaceutical agents (Dar *et al.*, 2000). This study therefore aims to investigate the potential of antileishmanial activity of seaweeds.

Materials and Methods

Different varieties of seaweeds belonging to the phylum Chlorophycota were collected from different spots along the coast of Karachi in different seasons at low tides, but most of the collections were made from the hilly and rocky ledges at the wall bounded and protected area near Haji Ali Goth, Buleji (French Beach) during November 2004. The voucher specimens have been deposited in the Botany department which were preserved in 4% formalin seawater solution. About 1 kg of fresh and clean fresh algal material was dried with blotting paper to absorb extra water, then chopped into small pieces and soaked in 1 liter

of ethyl alcohol for 2 weeks at room temperature. Later on 3 time extracts was filtered, concentrated and evaporated to obtain the thick ethanol extract.

Technique of 96-Well Micro titer plate bioassay:

Seaweed extracts were examined for their antileishmanial bioassay with the help of 96 - well plate serial dilution method (Sabina *et al* 2005, 2006; Sabina & Aliya *et al.*, 2011).

Results and Discussion

This study indicates that these extracts significantly reduced the viability of *Leishmaniaia major* *In vitro*. Among the selected species of the phylum Chlorophycota *Codium flabellatum* ($IC_{50}/72h = 34.4 \pm 6.2/\mu g/mL$), *Caulerpa faridii* ($IC_{50}/72h = 34.5 \pm 6.25/\mu g/mL$), *Caulerpa racemosa* ($IC_{50}/72h = 37.6 \pm 6.7/\mu g/mL$) and *Ulva fasciata* ($IC_{50}/72h = 50 \pm 0/\mu g/mL$) exhibited the significant values and rest of green seaweeds like *Ulva lactuca* ($IC_{50}/72h = 64.75 \pm \mu g/mL$), *Ulva rigida* ($IC_{50}/72 = 65.69 \pm 6.8/\mu g/mL$) and *Codium iyengarri* $IC_{50}/72h = 60.40 \mu g/mL$) showed good activity. *Caulerpa scalpelliformis* and *Halimada tuna* showed week leishmanicidal activity.

The details of results in comparison with the activity of reference drug (standard drug for Amphotericin B/Pantamidine with an IC_{50} Value $0.19 \mu g/mL$) used against *Leishmania major* is presented in (Table 1), which indicates the variation among species to species. The average IC_{50} value for standard antileishmanial drugs against promastigotes stage of *Leishmania major* form is less than 50-25 $\mu g/mL$ of variance in seaweed extracts which induce significantly and inhibit the growth of cultured promastigotes stage of the parasite *In vitro*.

Due to the prolonged therapy, side effects and drug resistance to the available drugs against leishmaniasis has paved a path which has led to the urgent need for new therapeutic agents against this disease. In this regard many Angiospermic plants have been reported for their antileishmanial activity in the last few years (Fournet *et al.*, 1993; Schmeda-Hirschman *et al.*, 1996; Lopes *et al.*, 1998; Fatima *et al.*, 2005) and most of them also have been used as drug against this disease, e.g. Iridoid isolates from *Nyctonthes arbrtristis* (Oliaceae) (Tandon *et al.*, 1991), triterpenes and lignans from *Doliocarpus dentatus* plant, but they indicated general toxicity.

Table 1. Chlorohycota species showing activity against promastigote form of *Leishmania major*.

Seaweeds species	STD drug amphotericin B µg/mL	% of inhibition test or IC ₅₀ µg/mL	Result
<i>Codium flabellatum</i> Silva et Nizamuddin	0.19	34	Significant
<i>Caulerpa faridii</i> Nizamuddin	0.19	34	Significant
<i>Caulerpa racemosa</i> (Forsskål) J. Agardh	0.19	37.5	Significant
<i>Caulerpa scalpelliformis</i> (R. Brown ex Turner)	0.19	92.3	no activity
<i>Caulerpa taxifolia</i> (Vahl) C. Agardh	0.19	100	no activity
<i>Ulva fasciata</i> Delile	0.19	50	Significant
<i>Codium iyengarii</i> Børgesen	0.19	60.4	Good
<i>Ulva lactuca</i> Linnaeus	0.19	64.75	Good
<i>Ulva rigida</i> C. Agardh	0.19	65.69	Good
<i>Halimeda tuna</i> (Ellis et Solander) Lamouroux	0.19	94.4	No activity

On the basis of this result it is concluded that, most of the Chlorophycota species which show promising activity against this pathogen are capable to inhibit this syndrome and aim of this investigation opens the possibility to examine the naturally occurring bioactive compounds against this pathogenic disease which can be used as a suitable treatment for most infections, with least side effects.

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