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ANTILEISHMANIAL ACTIVITY IN THE CRUDE EXTRACT OF VARIOUS SEAWEED FROM THE COAST OF KARACHI, PAKISTAN

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Abstract

Crude seaweed extracts of *Caulerpa racemosa, Ulva fasciata, Caulerpa faridii, Codium flabellatum, Laurencia pinnatifida, Melanothamnus afaqhusainii, Gracilaria corticata* and *Scinaia hatei* exhibited significant results while *Codium iyengarii, Ulva reticulate, Ulva rigida, Scinaia indica, Centroceras clavulatum* and *Botryocladia leptopoda* showed good activity against leishmania *in vitro*. This unique characteristic of algae will help in the development of novel antileishmanial agents against the prevention of leishmania disease.

Introduction

Algae appear to be an interesting source for ethnomedicinal and phytochemical studies. The power of algal resources has been sought for thousands of years for their ability to prevent disease and prolong life. Algae contain minerals, an abundance of vitamins, variety of trace elements and have shown high potential in controlling antimicrobial, antitumor, anticoagulant and cytotoxic activity (Ali *et al*., 2000). A number of seaweeds from Karachi coast have been analyzed for their phycochemical investigation (Usmanghani *et al*., 1984; Qasim *et al*., 1986; Shameel *et al*., 1987; Aliya *et al*., 1991; Naqvi *et al*., 1992; Ahmad *et al.*, 1992; Rizvi *et al*., 2000; Khaliq-uz-Zaman *et al*., 2001). Algae also exhibit a distinct nutrient profile and a selective nature for their medicinal use (Dar *et al*., 2000). Leishmania, a trypanosometed protozoan which is transmitted by the female *Phlebotomus* sand fly causing leishmaniasis is prevalent in four continents (Fig. 1). It is considered to be endemic in 88 countries, 72 of which are developing countries. In the Asian and African region, the majority of visceral leishmania cases occur in Sudan, while cutaneous leishmania occurs mainly in Afghanistan, Saudi Arabia and Syria (Anon., 2004). Leishmania is found to be endemic in Pakistan, the worst affected region are the triabal area of NWFP and southern province of Sindh in the district Larkana, Dadu, Jacobabad, Meherjarh (Altaf *et al*., 2002). Some cases of Leishmania has also been found in Multan (Mujtaba & Khalid.1993) and Islamabad (Hassan *et al*., 1995). Leishmania is classified on the basis of symptomatology as cutaneous, visceral, muco cutaneous and diffused cutaneous leishmania. According to the WHO and UNAIDS reports there are two millions new cases of Leishmania per year (Anon., 2001).

Leishmania patients are highly susceptible to HIV infection. It is now considered a genuine AIDs related opportunistic disease largely due to latent infection by immuno suppression (Alvar *et al*., 1997, Anon., 1999). Many novel compounds isolated from various medicinal plants have been reported for their leishmanicidal activity (Fournet *et al.*, 1993; Hazra *et al.*, 1995; Gantier *et al*., 1996; Schmeda-Hirschman *et al.*, 1996; Lopes *et al.*, 1998; Waechter *et al*., 1998; Atta-ur-Rahman & Choudhary 1999; Kayser

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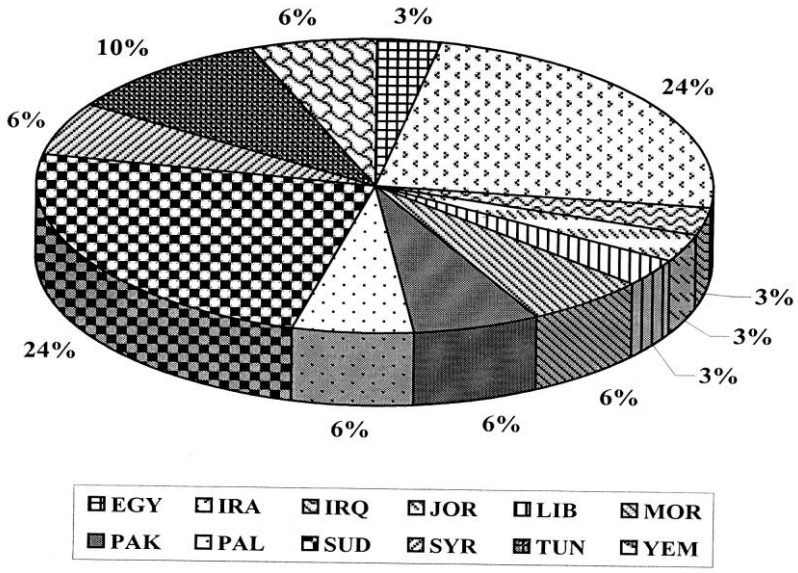

Fig. 1. Research on Leishmaniasis in EMR countries funded by SGS, 1992-2001.

et al., 2001). The current therapy against leishmaniasis is unsatisfactory (Nielsen *et al*., 1998). The recommended drugs for both visceral and cutaneous leishmania are the pentavalent antimonials sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime) drugs that have been used for over 50 years, but they require long courses of parenteral administration for treating leishmania. The use of these recommended drugs also exhibit therapeutic failure, side effects and long duration for healing of lesions (Croft *et al.*, 1988; Ribeiro *et al.*, 1999). Some of the drugs used from the natural sources are Iriodoid isolates from *Nyctanthes arbortristis* (Oleaceae) (Tandon *et al*., 1991), Diterpen16, 17 dihydrobrachycalyxolide from *Vernonia brachycalyx*, Asteraceae (Oketch *et al.*, 1998), Triterpenes and Lignans from *Doliocarpus dentatus* have shown general toxicity against Leishmanial disease (Sauvain *et al.*, 1996). These pharmaceutical problems point towards the need to develop novel chemotherapeutic agents through the identification of biochemical pathways and develop mechanical based structural modification of chemicals from seaweed which exhibit the most promising activity against leishmaniasis. The present report describes the antileishmanial activity in the crude extract of various seaweed from the coast of Karachi, Pakistan.

Materials and Methods

Seaweed, were collected as drift as well as benthic forms from mid, lower and littoral rocks of Buleji Karachi coast from October 2003 to March 2004. Approximately 500g of Seaweed was washed with the help of tap water to remove sand particles, epiphyte and animal castings. Each species of the genus was preserved in 4% formalin seawater solution for identification and herbarium specimens were prepared for taxonomic studies. All plants were chopped into small pieces and soaked in 1 liter of ethyl alcohol for 3-4 days at room temperature. The extract was filtered and then evaporated. The seaweed extract was then examined for antileishmanial Bioassay with the help of 96 - well plate serial dilution method.

Antileishmanial bioassay: Leishmanial promastigotes was aseptically sedimented down at 3000rpm for 10 min, counted with the help of improved Neubauer chamber under the microscope and diluted with the fresh medium to a final concentration of 1×10^6 parasite. In a 96 well microtiter plate, 180µl of the parasite culture (1×10^6 parasite/ml) was added in different wells in which 20µl of the experimental compound was added in culture and serially diluted so that minimum concentration of the compound was 1µg/ml. Negative control received medium with a parasite density1×10^6 cells/ml. The positive control contained varying concentration of standard antileishmanial compound e.g., Amphotericine B, Pantamidine. The plate was incubated between 21-22^{o}C for 72hrs. The culture was examined microscopically on an improved Neubauer chamber and IC50 values of compound possessing antileishmanial activity were counted.

Results and Discussion

Various seaweed showed a significant Antileishmanial activity viz., *Caulerpa faridii* (IC50<34 µg/ml), *Codium flabellatum* (IC50<34 µg/ml), *Caulerpa racemosa* (IC50<37.5 µg/ml), *Ulva fasciata* (IC50<50 µg/ml), *Laurencia pinnatifida* (IC50<6.25 µg/ml), *Scinaia hatei* (IC50<14.10 µg/ml), *Melanothamnus afaqhusaini* (IC50<32.6 µg/ml), *Gracilaria corticata* (IC50<37.5 µg/ml) exhibited significant results while other species like *Scinaia indica* (IC50 <59.6 µg/ml), *Centroceras clavulatum* (IC50 <57.89 µg/ml), *Botryocladia leptopoda* (IC50<60.81 µg/ml), *Codium iyengarii* (IC50<60.40 µg/ml), *Ulva reticulata* (IC50<64.75 µg/ml) and *Ulva rigida* (IC50<65.69 µg/ml) showed good Antileishmanial activity against Promastigotes (extracellular) of parasite *Leishmania major in vitro*. Results indicated that among the 14 genera analysed *Laurencia pinnatifida* displayed the most significant activity against promastigote stage of *Leishmania major* with an IC50 value of 6.25 µg/ml (standard drug for Amphotericin B with an IC50 Value 0.19 µg/ml). Table 1 shows the average IC50 values for standard antileishmanial drugs against promastigotes form is less than 50-25 µg/ml of variance in seaweed extract which reduces the viability of cultured promastigotes stage of the parasite *in vitro*. The results of antileishmanial activity from seaweed belonging to two different Division like Chlorophyta (*Codium flabellatum, Caulerpa racemosa, Ulva fasciata, Codium iyengarii, Caulerpa faridii, Ulva rigida, Ulva reticulata*) and Rhodophyta (*Laurencia pinnatifida, Melanothamus afaqhusainii, Centroceras clavulatum, Gracilaria corticata, Scinaia hatei, Botryocladia leptopoda, Scinaia indica*) is given in Table 1. Both these division are extremely different to one another and are classified on the basis of chlorophyll containing pigments, carotenes, xanthophylls, biliprotein, main food reserves and cell wall composition substances which have different type of chemical composition such as fatty acid, sterol, terpenes and phycocolloids and their effects in biological function vary in between phylla and from species to species. Many novel compounds isolated from various medicinal plants have been reported for their leishmanicidal activity (Fournet *et al.,* 1993; Hazra *et al.,* 1995; Gantier *et al*., 1996). The use of the recommended drug exhibited therapeutic failure, side effects such as renal, neural cardiac toxicity, pancreatitis, risk of diabetics (Ribeiro *et al*., 1999; Becker *et al.,* 1999) and change in the period of healing and lesions. The results obtained by bioassay method as given in the present report will make it possible to classify the extracts with respect to their chemical compound which gives preventation against leishmania disease.

Table 1. Percentage inhibition test of *Leishmania*.

S. No.	Plants name	STD. Drug Amphotericin B µg/ml	% Inhibition test or IC50 µg/ml	Results
	Division: Chlorophyta			
1.	*Codium flabellatum*	0.19	34.0	Significant
2.	*Caulerpa faridii*	0.19	34.0	Significant
3.	*Caulerpa racemosa*	0.19	37.5	Significant
4.	*Ulva fasciata*	0.19	50	Significant
5.	*Codium iyengarii*	0.19	60.40	GOOD
6.	*Ulva reticulata*	0.19	64.75	GOOD
7.	*Ulva rigida*	0.19	65.69	GOOD
	Division: Rhodophyta			
8.	*Laurencia pinnatifida*	0.19	*6.25	Significant
9.	*Melanothamnus afaqhusainii*	0.19	32.6	Significant
10.	*Gracilaria corticata*	0.19	37.5	Significant
11.	*Scinaia hatei*	0.19	14.10	Significant
12.	*Scinaia indica*	0.19	59.6	GOOD
13.	*Centroceras clavulatum*	0.19	57.89	GOOD
14.	*Botryocladia leptopoda*	0.19	60.81	GOOD

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