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IN VITRO CYTOTOXICITY OF SEAWEEDS FROM KARACHI COAST ON BRINE SHRIMP

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Abstract

Marine algal community signifies a huge source of compound endowed with ingenious structure and potential biological activities. Cytotoxicity of plant or fungal material is considered as the presence of antitumor compounds. In this study, ethanol extracts of seaweeds *Dictyota dichotoma* var. *velutricata*, *D. hauckiana*, *D. indica*, *Iyengaria stellata*, *Jolyna laminarioides*, *Melanothamnus afaqhusainii*, *Sargassum ilicifolium*, *S. lanceolatum* and *Ulva fasciata* occurring at Karachi coast were screened for the cytotoxic activity using brine shrimp lethality for larvae (nauplii). Out of 9 seaweeds tested, ethanol extract of eight species showed significant cytotoxicity (LC_{50} <1000µg) on brine shrimp. *Dictyota indica* showed highest cytotoxic activity (LC_{50} =143µg).

Introduction

Discovery of anticancer drugs that must kill or disable tumor cells in the presence of normal cells without undue toxicity is an extraordinary challenge (Hameed *et al*., 2009). Toxicity of plant or microbial material is considered as the presence of antitumor compounds. Brine shrimp bioassay has successfully been used as prescreening of bioactive compounds having antitumor activity (McLaughlin *et al*., 1993). This test has been established as a safe, practical and economic method for the determination of the bioactivity of synthetic compound (Almeida *et al*., 2002), mycotoxins of fungal pathogens (Schmidt *et al*., 1995), marine products (Ara *et al*., 1999, Manilal *et al*., 2009ab) as well as higher plant products (Stefanello *et al*., 2006; Nino *et al*., 2006). National Cancer Institute (NCI, USA) has found a significant correlation between the brine shrimp assay and *in vitro* growth inhibition of human solid tumor cell lines (Silva *et al*., 2007).

Global utilization of seaweed is a multi-billion dollar industry. Research and utilization of marine algae have increased markedly from last several decades (Jimenez-Escrig & Sanchez-Muniz, 2000). A number of products based on algae have been developed and applied in many fields like foods, pharmaceuticals, cosmetics and nutritional supplements. Much of this is based on farming of edible species or on the production of agar, carrageenan and alginate. Of all seaweed, hydrocolloids have had the biggest influence on modern western societies. They have attained commercial significance through their use in various industries which exploit their physical properties such as gelling, water retention and their ability to emulsify. However research towards the use of seaweeds for the treatment of various diseases has received less attention. In recent years, pharmacological firms have started looking towards seaweeds for new natural products for pharmacological benefits (Smit, 2004). In our previous studies, we

have reported antifungal and nematicidal (Ara *et al*., 1998), cytotoxic (Ara *et al*., 1999), antibacterial (Ara *et al*., 2002a) and hypolipidaemic (Ara *et al*., 2002b) activities of seaweed occurring at Karachi coast. The present report describes the screening of some other potential seaweeds of Karachi coast for cytotoxic activity on brine shrimp.

Materials and Methods

Seaweeds belonging to Phaeophyta (brown), Chlorophyta (green) and Rhodophyta (red) were collected from Buleji beach Karachi coast in different seasons at low tide. Seaweed species exposed on sand and rocks were collected in plastic bags and brought to the Laboratory. The voucher specimens and herbarium sheets were prepared. Each species was washed thoroughly with freshwater to remove salt, and epiphytes, dried under shade. Dried seaweeds were powdered in an electric grinder and stored in polyethylene bags at room temperature until used. Dry powder of seaweed (500 g) was extracted three times with ethanol (4 vol.) for 1 week. Extracts were pooled, filtered through cotton wool and concentrated to dryness on a rotary vacuum evaporator and weighed.

Brine shrimp lethality test: Brine shrimp lethality test for larvae nauplii was used to determine the toxicity of ethanol extract of seaweeds (McLaughlin *et al*., 1993). A concentration of 5, 10, 50, 100, 200, 500, 600, 700, 800, 900 and 1000 µg/ml of seaweed extracts were prepared in ethanol and 2 ml were transferred in glass vial and left open for 48 hours to evaporate the organic solvent before adding the nauplii.

Brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) were hatched in a shallow rectangular container (60 x 30 cm) filled one fourth with sea water at 27-30^{o}C with aeration. A plastic divider with holes was placed in the container to make two unequal compartments. The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 hours, the phototropic nauplii were collected from the illuminated side.

Ten brine shrimp nauplii were transferred in each vial. Two ml of sea water was added in each vial of ethanol extract before adding the nauplii. Observations were recorded after 24 hours and survivors were counted and percent death at each dose level was calculated. LC_{50} were calculated using probit analysis (Finney, 1971).

Results

Total nine seaweeds, seven belong to Phaeophyta (brown), one each belongs to Chlorophyta (green) and Rhodophyta (red) were screened for the cytotoxic activity. Out of nine species, eight have showed LC_{50} <I000 µg in ethanolic extracts. Ethanolic extracts of seaweed were evaluated for their cytotoxicity at different concentrations and were classified as non-cytotoxic (NCT<50% death), mild cytotoxic (MCT>50% death but <75% death) and highly cytotoxic (HCT>75% death) at 1000 µg/ml based on their lethality to brine shrimp (Table 1). Among these seaweeds, *Dictyota indica*, *Iyengaria stellata* and *Melanothamnus afaqhusainii* showed the LC_{50}=141µg, 186 µg & 190 µg respectively. While *Dictyota hauckiana* was found to be moderately effective (MCT>50% death but <75% death) on nauplii of brine shrimp (LC_{50}= 524 µg). Whereas *Ulva fasciata*, *Sargassum lanceolatum*, *Jolyna laminarioides*, and *Dictyota dichotoma* var. *velutricata*, were found to be less cytotoxic with LC_{50}=724µg, 800µg, & 812µg respectively (Table 1).

Table 1. Percent death of brine shrimps at different concentrations of ethanolic extracts of seaweeds after 24 hours.

S. No.	Seaweed species	5µg	10µg	50µg	100µg	200 µg	500µg	600 µg	700µg	800µg	900µg	1000µg	LC50
1.	*Iyengaria stellata*	0	0	0	6	56	72	74	76	80	86	93	186 µg
2.	*Ulva fasciata*	0	0	0	6	20	30	36	43	80	90	96	724 µg
3.	*Dictyota dichotoma* var. *velutricata*	0	0	0	3	6	10	25	30	46	70	70	812 µg
4.	*Dictyota indica*	0	0	0	30	70	100	100	100	100	100	100	141 µg
5.	*Dictyota hauckiana*	0	0	0	6	30	43	80	86	100	100	100	524 µg
6.	*Melanothamnus afaqhusainii*	0	0	0	0	60	65	73	80	80	83	86	190 µg
7.	*Jolyna laminarioides*	0	0	0	20	26	30	30	33	43	90	96	812 µg
8.	*Sargassum ilicifolium*	0	0	0	0	0	0	20	20	40	40	50	1000 µg
9.	*Sargassum lanceolatum*	0	0	0	0	0	20	30	40	50	60	70	800 µg

Discussion

Marine algae (seaweeds) contain a number of biodynamic compounds of therapeutic value. These compounds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations (Selvin & Lipton, 2004). Many of these secondary metabolites biosynthesized by the marine plants are well known for their cytotoxic property (Manilal *et al*., 2009a). Pakistan has a rich algal flora in the coastal and inshore waters of Arabian Sea. Of the 9 seaweed extracts examined for brine shrimp cytotoxicity, *Dictyota indica*, *Iyengaria stellata* and *Melanothamnus afaqhusainii* were found effective and showed considerable activity and their LC_{50} were 141µg, 186 µg & 190 µg respectively. Several diterpenes from *Dictyota* species exhibit significant cytotoxicity have been reported (Tringali *et al*., 1984ab). Results of this study showed that among the seven seaweeds of Phaeophyta group (brown seaweed) six possessed the cytotoxic activity. Prenylated aromatics with small side chains are relatively common in brown algae (Sun *et al*., 1980). There are reports that marine macroalgae belonging to Phaeophyta group possess antitumor activity, and sterols from *Sargassum carpophyllum* exhibited cytotoxic activity against several cultured cell lines (Tang, *et al*., 2002). Significant antiviral activity against *Vaccinia virus* was found in brown algae *Sargassum myriocystum* (75%) and *S. weightii* (50%) (Kamat *et al*., 1994). Many researches have revealed that bioactive compounds including fucoidans, terpenes, stypoldione, sterols, polyunsaturated fatty acids and phenolic compounds have anticancer and cytotoxic activity (Gerwick *et al*., 1993, Carte *et al*., 1996, Manilal *et al*., 2009b; Synytsya *et al*., 2010). The brown algae *Iyengaria stellata* showed adrenergic activity (Bhakuni & Rawat, 2005). The genera *Halimeda, Penicillus* and *Udotea* are found to contain highly active but unstable sesquiterpenoids and diterpenoids. Some of these diterpenoids exhibited cytotoxic and antimicrobial activities (Paul & Fenical, 1984ab; Tillekeratne & Schimitz, 1984). *Ulva fasciata* in this study have shown cytotoxic activity with LC_{50}=724µg. Alcoholic extracts of *Ulva fasciata* and *Ulva lactuca* exhibited antiviral and antiimplantation activities (Bhakuni & Rawat, 2005). *Ulva fasciata* has been reported to produce a novel sphingosine derivative with antiviral activity (Jha & Rong, 2004). Seaweeds exhibit a high level of fatty acid diversity and many of which possess potential bioactivity (Ara *et al*., 2005). Several cytotoxic compound such as fucoidans, laminarians, and terpenoids stated to posses anticancer, antitumor and antiproliferative properties are reported to be abundant in seaweeds (Smit, 2004). These compounds could be further explored as novel leads to cancer chemoprevention and complementary chemotherapy and necessitates further investigation (Vinayak *et al*., 2010).

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