RESEARCH ARTICLE

Screening for *in vitro* Cytotoxic Activity of Seaweed, *Sargassum sp*. Against Hep-2 and MCF-7 Cancer Cell Lines

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Abstract

Discovery of anticancer drugs that kill or disable tumor cells in the presence of normal cells without undue toxicity is a potential challenge for therapeutic care. Several papers in the literature have emphasized the potential implications of marine products such as seaweeds which exhibit antitumor activity. Study attempts to screen the antitumor effect of *Sargassum sp*, against chosen cell lines such as MCF-7 (Breast cancer) and Hep-2 (Liver Cancer). Ethanol extract of *Sargassum sp*. was concentrated using a Soxhlet apparatus and dissolved in DMSO. In vitro cytotoxic activity of *Sargassum sp* at various concentrations (100 µg/ml-300 µg/ml) screened for antitumor effect against the chosen cell lines using MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole). The study documented that the percentage of cell viability has been reduced with increased concentration, as evidenced by cell death. *Sargassum sp* extract shows potential cytotoxic activity (P≤0.05) with IC_{50} of 200 µg/ml and 250 µg/ml against Hep-2 and MCF-7 cell lines respectively. The ethanol fraction of *Sargassum sp* induced cell shrinkage, cell membrane blebbing and formation of apoptotic bodies with evidence of bioactive components as profound influencing factors for anti-tumor effects. Further research need to be explored for the successful application of *Sargassum sp* as a potent therapeutic tool against cancer.

Keywords: MTT - *Sargassum sp* - apoptosis - HEP 2 - MCF 7

Asian Pacific J Cancer Prev, **13 (12)**, 6073-6076

Introduction

Cancer is one of the most serious threats to human health in the world and chemotherapy is still the standard treatment method. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also aggravates patient's recovery. (Zandi et al., 2010). The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology (Xu et al., 2009). With this aim, many attentions have been paid to natural compounds in plants, marine organism and microorganisms. Regarding the low side effects of plants and other natural compounds, scientists are interested in working on them to find new medications.

Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as source of food, feed and medicine. Until now, more than 2,400 marine natural products have been isolated from seaweeds of subtropical and tropical populations (Manilal et al., 2009). Recent findings evidenced that seaweeds contained antiviral, antibacterial, antifungal and antitumoral (Harada et al., 1997) potentials, among numerous others. Seaweeds have caused an emerging interest in the biomedical area due to the presence of potent pharmacologically bioactive substances with wide arrays of potential health benefits (Blunden et al., 1993; Smit et al., 2004). According to existing literature, more than ten new experimental anti-tumor agents derived from marine sources have entered clinical trials, including bryostatin-1, aplidine, ecteinascidin-743 (ET-743), Kahalalide F, as well as derivatives of dolastatin such as TZT-1027 and LU 103793 (Song et al., 2008). *Sargassum sp*., is a brown macro algae belongs to the class phaeophyceae and order fucales. *Sargassum sp* has the biological effects of natural marine products that include antitumour, antioxidant, antibacterial, antifungal, anti inflammatory, anti viral activity. The Atlantic oceans Sargassum sea was named after the algae as it hosts a large amount of *Sargassum sp*. citede by Booth (1964). The present study is to determine the Cytotoxic activity of *Sargassum sp* against Hep-2 (Liver Cancer) and MCF-7 (Breast Cancer) cancer cell lines using MTT assay.

Materials and Methods

Sample collection and preparation

Seaweeds (*Sargassum sp*.) were collected from the Tuticorin sea. The algal sample was identified by

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following standard procedure (Dharallear and Kavleekas, 2004; Sambarmurty, 2005)

Algae samples were washed twice with distilled water to remove the sand, salt and other extraneous materials and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried in till constant weight obtained and ground in an electric mixer. The powdered samples subsequently stored in refrigerator.

Preparation of extracts

20 gms of powdered algal *Sargassum sp* were added to 150 ml of ethanol and the extract was obtained using Soxhlet apparatus for 3 hrs. The solvent extract were then filtered and the filter was concentrated by rotary evaporater at 45-50˚C. The resulting extract were then dissolved in dimethylsulfoxide and kept for further use.

Cell lines and culture condition

MCF-7 and Hep-2 were kindly provided by the Department of Endocrinology, Dr ALM PG Institute of Basic Medical Science, Chennai. Cells were cultured in DMEM medium and supplemented with 10% of fetal bovine serum (FBS) then the culture flasks were incubated for 3-4 days at 37˚C in 5% CO_2 incubator.

MTT assay

Tumour cells like MCF-7 and Hep-2 were seeded in 24 well plates at the concentration of $2x10^4$ cells/ml using DMEM and incubated for 24-48 hrs. When cells reached >80% confluence the medium was replaced and the cells were treated with the algal extract at 100, 150, 200, 250 and 300 μg/ml which is dissolved in dimethyl sulfoxide (DMSO) at a maximum concentration of 15 mg in 150 μl of DMSO and was incubated for 24 hrs.Then MTT solution were prepared by adding 7.5mg of MTT in 15 ml of DMEM medium. 2 μl of the MTT stock solution were added in each well and incubated at 37˚C for 3 hrs. The MTT stock solution was replaced by DMSO 200μl to each well and observe the colour in each well. The amount of MTT-formazan that is directly proportional to the number of living cells was determined by measuring the optical density (OD) at 540 nm using reader. MTT assay was performed in the Department of Endocrinology, Dr ALM PG Institute of Basic Medical Science, Chennai.

Statistical analysis

Each data point was obtained by making at least 3 independent measurements. All data are expressed as means±S.D. Data were analyzed by an analysis of variance ($p<0.05$) and the means separated by one way ANOVA.

Results

In vitro cytotoxic activity of seaweed, *Sargassum sp*. extract at various concentration against Hep-2 and MCF-7 cancer cell lines were studied using MTT assay. Antitumour activity of seaweed *Sargassum sp*. extract at various concentrations against Hep-2 cancer cell lines was represented in Figure 1. With increase in concentration of seaweed extract from 100, 150, 200, 250, 300 μg/ml. documents reduced percentage of cell viability respectively. Figure 2 depicts the cytotoxic effect of *Sargassum sp* extract with increased concentration of 100 μg/ml-300 μg/ml against MCF-7 cancer cell lines. Then the percentage of cell density has been decreased evident the cell death.

IC_{50} value were calculated from the extract treated OD value of *Sargassum sp*. extract against Hep-2 and MCF-7 cancer cell lines were 200 μg/ml to 250 μg/ml respectively. Promising cytotoxic activity was observed in Hep-2 cell lines. One way ANOVA shows significant ($p<0.05$) difference for the various concentration of *Sargassum sp*. extract against Hep-2 and MCF-7 cancer cell lines (Table 1).

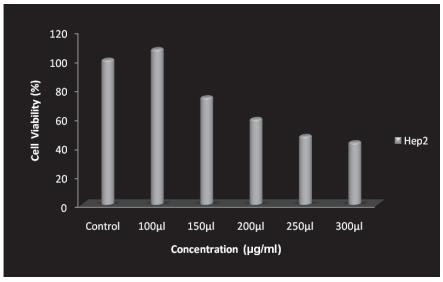

Figure 1. Cytotoxic Effect of *Sargassum sp* Extract at Various Concentration (100 μg/ml-300 μg/ml) Aganist Hep2 Cancer Cell Lines

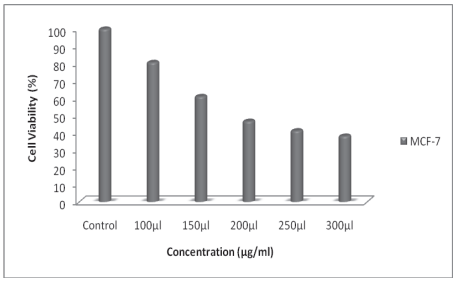

Figure 2. Cytotoxic Effect of *Sargassum sp* Extract at Various Concentration (100 μg/ml-300 μg/ml) Aganist MCF-7 Cancer Cell Lines

Table 1. One Way Analysis of Variance of *Sargassum sp*. Extract at Various Concentration Against MCF-7 and Hep2 Cancer Cell Lines

Source of variation		SS	Df	MS	F	Table value at 5% level	Level of Significance
MCF-7	Concentration of *Sargassum sp*.	0.047768	5	0.009554	91.8611111	3.105875	**Significant**
		0.001248	12	0.000104			
	Total	0.049016	17				
Hep2	Concentration of *Sargassum sp*.	0.040767	5	0.008153	121.364606	2.772853	**Significant**
		0.001209	18	6.72E-05			
	Total	0.041976	23				

Discussion

Discovery of anticancer drugs that must kill or disable tumor cells in the presence of normal cells without undue toxicity is potential challenge for therapeutic care (Hameed et al., 2009). Several literature emphasize the potential implications of marine products such as seaweeds which exhibit antitumour activity (Ayesha et al., 2010). Our study document the potential influence of the seaweed *Sargassum sp* extract against Hep2 and MCF cancer cell lines using MTT assay. The intensity of Hep2 and MCF-7 cell density were decreased while increasing the concentration of *Sargassum sp* extract from 100 μg/ml to 300 μg/ml. This infers the existence of dose dependent properties of *Sargassum sp* extract against cancer cell lines which was found effective and the IC_{50} value of seaweed extract against Hep2 and MCF-7 were 200µg/ml, and 250µg/ml respectively. Study reveals that the ethanol fraction of *Sargassum sp* induced cell shrinkage, cell membrane blebbing and formation of apoptotic bodies which evidence the existence of cytotoxic effect and also document the evidence of bioactive compounds which profound to be the influencing factor for the antitumour effect. Sodium alginate is one of the bioactive compound present in *Sargassum sp*. which exhibit various biological effects like removal of heavy metal particle from body, function of heavy metal detoxin antitumour and anti-inflammatory property (Hu et al., 2004).

Many studies have been developed in order to determine the bioactive compounds produced by marine algae (Albano et al., 1990; Berlinck et al., 1996). Some metabolites such as bromophenols, carotene and steroids were isolated and purified in some algae and their activity against some cancer cell lines were demonstrated (Xu et al., 2004). Also, in another study, it was shown that the sulfated compounds such as fucoidans which were extracted from Sargassum polycystum and some other brown algae exhibited important roles against some human cancer cell lines (Ly et al., 2005). 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). 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, 1996; Synytsya et al., 2010). One way analysis shows significant ($p<0.05$) difference for the various concentration of *Sargassum sp*. extract against Hep2 and MCF-7 cell lines. Present study infers that the *Sargassum sp* extract exhibit effective antitumor activity and seems to have no side effects. They are less cost effective, easy in production and purification. In future it can be recommended to the patients as a effective therapeutic tool in form of food or drug. Further research need to be explored to study the bioactive compounds of *Sargassum sp* and for the successful implication of them as a potent therapeutic tool against cancer.

Acknowledgements

This research is supported by Dr.Ravi Shankar, Prof and Head Department of Endocrinology and Mr. Benson research scholar Dr ALMPG Indian Basic Medical Science Chennai, for providing the human cancer cell lines and for his supposssrt to complete the MTT assay successfully in his laboratory.

References

Albano RM, Pavao MS, Mourao PA, Mulloy B (1990). Structural studies of a sulfated L-galactan from *Styela plicata* (Tunicate): analysis of the smith-degraded polysaccharide. Carbohydr. *Res*, **208**, 163-74.

Ayesha, Hira, Sultana V, Ara J, Ehteshamul-Haque S (2010). *In vitro* cytotoxicity of seaweeds from Karachi coast on brine shrimp. *Pak J Bot*, **42**, 3555-60.

Berlinck RG, Ogawa CA, Almeida AM, et al (1996). Chemical and pharmacological characterization of halitoxin from *Amphimedon viridis* (Porifera) from the southeastern Brazilian coast. *Comp Biochem Physiol Clin Pharmacol Toxicol Endocrinol*, **115**, 155-63.

Blunden G (1993). Marine algae as sources of biologically active compounds. *Interdiscip Sci Rev*, **18**, 73-80.

Booth E (1964). Trace elements and seaweeds. In: De Virville, A.D., Feldmann, J. (Eds.), Proceeding of the 4th International seaweed symposium. Macmillan, London, pp. 385-393.

Carte BK (1996). Biomedical potential of marine natural products. *Bioscience*, **46**, 271-86.

Dharallear VK, Kavleekas D (2004). Seaweed – a field manual national insititute of oceanography Dunapoula Goa.

Gerwick WH, Bernart MW (1993). In: Marine Biotechnology. (Eds.): D. Attaway and O. Zaborsky. Plenum Press, New York. pp. 101-152.

Hameed S, Sultana V, Ara J, Ehteshamul-Haque S, Athar M (2009). Toxicity of fusarium solani strains on brine shrimp (Artemia salina). *Zoological Res*, **30**, 468-72.

Harada H, Noro T, Kamei Y (1997). Selective antitumor activity *in vitro* from marine algae from Japan coast. *Biol Pharm Bull*, **20**, 541-6.

Hu X, Jiang X, Hwang H, Liu S, Guan H (2004). Antitumour activities of alginate-derived oligosaccharides and their sulphated substitution derivatives. *Eur J Phycol*, **39**, 67-71.

Ly BM, Buu NQ, Nhut ND, et al (2005). Studies on fucoidan and its production from Vietnamese brown sea weeds. *AJSTD*, **22**, 371-80.

Manilal A, Sujith S, Kiran GS, Selvin J, Shikar C (2009). Cytotoxic potentials of red alga, laurencia brandenii collected from the Indian Coast. *Global J Pharmacol*, **3**, 90-4.

Sambarmurty AVSS (2005). A text book of algae I.K International pvt Ltd, New delhi, 97-65.

Smit AJ (2004). Medicinal and pharmaceutical uses of seaweed natural products: a review. *J Appl Phycol*, **16**, 245-62.

Song L, Ren S, Yu R, et al (2008). Purification, characterization and in vitro anti-tumor activity of proteins from arca subcrenata lischke. *Marine Drugs*, **6**, 418-30.

Synytsya A, Kim WJ, Kim SM, et al (2010). Structure and antitumor activity of fucoidan isolated from sporophyll of Korean brown seaweed *Undaria pinnatifida*. *Carbohydrate Polymers*, **81**, 41-8.

Tang HF, Yang-Hua Y, Yao XS, et al (2002). Bioactive steroids from the brown alga *Sargassum carpophyllum*. *J Asian Nat*

Prod Res, **4**, 95-105.
Xu N, Fan X, Yan X, Tseng C (2004). Screening marine algae from China for their antitumor activities. *J Appl Phycol*, **16**, 451-6.
Xu H, Yao L, Sung H, Wu L (2009). Chemical composition and antitumor activity of different polysaccharides from the roots *Actinidia eriantha*. *Carbohydr Pol*, **78**, 316-22.
Zandi K, Tajbakhsh S, Nabipour I, et al (2010). In vitro antitumor activity of *Gracilaria corticata* (a red alga) against jurkat and molt-4 human cancer cell lines. *African J Biotechnology*, **9**, 6787-90,