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BIOCHEMICAL CHARACTERIZATION, HAEMAGGLUTINATING ACTIVITY AND CYTOTOXIC ACTIVITY OF *PADINA GYMNOSPORA* (KUTZING) SONDER

Neeta V.M. Joshi and S. Srisudha

Department of Botany and Microbiology, Lady Doak College, Madurai, Tamil Nadu, India.

ABSTRACT

Extracts of the brown seaweed *Padina gymnospora* were examined for ash, lipid, carbohydrate, protein, nitrogen, sodium, potassium, calcium, sulphate, flavanoid, alginic acid, sodium, calcium alginate, phenol, mannitol, alkaloid, glycoside, tannin, sterol, fat contents. The study determined the presence of high carbohydrate (76 mg/g), protein (58.66 mg/g), flavanoid (70.66 mg/g), alginate (25.46%), mannitol (9.87%) and phenol (30.33 mg/g) contents. Qualitative test indicated the presence of major metabolites. *Padina gymnospora* have been screened for agglutinic activity by using human erythrocytes of A, B, AB, O positive blood groups and also for anticancer activity. The extracts from *Padina gymnospora* revealed high haemagglutinating activity against the B, AB and O positive human erythrocytes. The results showed that the extracts of brown seaweed *Padina gymnospora* have remarkable antitumor activity against HEpG2 cell line.

Key Words: Haemagglutinins, HEpG2 cell line, Human erythrocytes, Anticancer, Immunomodulatory.

INTRODUCTION

Marine seaweeds have been repeatedly recognized as producers of biologically active substances. Specific studies on seaweeds carried out in the Atlantic, Pacific and Indian oceans have demonstrated haemagglutinic and anticancer property (Ballesteros *et al*., 1992).

Since the discovery of haemagglutinating activity in marine algal extracts in 1966, several algal haemagglutinins (lectins) have been detected, isolated and characterized. However, information is slowly emerging, concerning the biochemical characteristics of lectins from Asian marine algae. Lectins are proteins or glycoproteins which bind, reversibly to carbohydrates. Algal lectins differ from higher plant lectins in a variety of properties. In general, algal lectins have low molecular weight than higher plant lectins and have no affinity for simple sugars but are more specific for complex oligosaccharides, often glycoproteins (Suttisrisung *et al*., 2011).

The nutritive value of seaweeds is mainly due to their proteins, polysaccharides, minerals and vitamin content. The high levels of non-digestible polysaccharide in their cell wall make seaweeds a rich-source of dietary fibre (Ruperez and Calixto, 2001). Thus brown seaweeds contains alginates, fucans and laminarans as a soluble dietary fibre, while the red ones have the galactans agar and a carragenan. The green seaweed consists mainly of cellulose (Carvalho *et al*., 2009).

The haemagglutinins, agglutinins or lectins are carbohydrate binding proteins having specific binding for some types of mono or polysaccharides, widely distributed in nature and constitute a group of proteins or glycoproteins present in a wide range of organisms (Liener *et al*., 1986). This carbohydrate binding specificity has made them an interesting tool in a variety of biochemical and biomedical research areas. In basic and medical sciences, lectins are useful for detection of disease related

Corresponding Author

S. Srisudha
Email: srisudha2009@ymail.com

alterations of glycan synthesis, blood group typing, quantification of abberations of cell surface glucan presentation, eg., in malignancy, cell markers for diagnostic purposes including infectious agents (viruses, bacteria, fungi, parasites) and they may also be used to target therapeutic agents (Rudiger *et al*., 2001).

The occurrence of agglutinins in extracts of marine algae was first described by Boyd *et al*. (Boyd *et al*., 1966). Scientists have isolated chemical compounds in brown algae that have antitumour and anticancer properties. The compound that was isolated in greatest abundance named bromophycolide A by the researchers killed human tumour cells by inducing programmed cell death (apoptosis), a mechanism that is promising for development of new anti-cancer drugs (Anonymous 1). This phenomenon takes place when the DNA digesting enzymes are produced by the cell itself to cleave the DNA molecule into fragments and this will induce the cell death. Many seaweed species also have been used as herbal medicine in China.

Anticancer alkaloids from marine floras namely fucoidans are the polysaccharides containing substantial percentages of L-fucose and sulfate ester groups which are the main constituents of brown seaweed. Fucoidans isolated from different species have been extensively studied due to their varied biological activities, including anticoagulant and antithrombotic, antivirus, antitumour and immunomodulatory, anti-inflammatory, blood lipids reducing, antioxidant and anticomplementary properties, gastric protective effects and therapeutic potential in surgery (Boopathy and Kathiresan, 2010). Fucoidan a gift from the sea is improving health for many, many people (Anonymous 2).

The evaluation of the anticancer potential of crude extracts from different sea organisms has been carried out by *in vitro* cytotoxicity test in malignant cell cultures (Russell, 1963). Cancer is a dreadful human disease, increasing with changing life style, nutrition and global warming. Cancer treatments do not have potent medicine as the currently available drugs are causing side effects in some instances. In this context, the natural products derived from medicinal plants have gained significance in the treatment of cancer (Boopathy and Kathiresan, 2010).

The present study focuses on the chemically mediated bioactivity of *Padina gymnospora* (Kutzing) Sonder by examining its haemagglutinating activity on human erythrocytes and by performing cytotoxic tests.

MATERIALS AND METHODS

Collection of seaweed samples

The seaweed species *Padina gymnospora* (Kutzing) Sonder, collected from the Mandapam coast, Tamil Nadu was used in this study. After collection, the material was cleaned, washed with tap water and then with distilled water to eliminate salt and salt particles, and finally stored at -20°C until use.

Phytochemical analysis

The alkaloids, glycosides, tannin, carbohydrates, steroids, flavanoid, cardiac glycosides (Srivastava *et al*., 2010),ash (Benevidas *et al*.,1999), lipid (Bligh and Dyer., 1959), carbohydrate (Hedge and Hofrieter, 1962), protein (Lowry *et al*., 1951), nitrogen (Umbriet *et al*. 1974), sodium, potassium, calcium, sulphate (Mannivanan *et al*., 2008), flavanoid (Meenakshi *et al*., 2009), alginic acid, sodium and calcium alginate (Haug, 1974), phenol (Meenakshi *et al*., 2009), mannitol (Black, 1951a) was determined as per the standard protocol.

Preparation of seaweed extract

Ten grams of each species was homogenized with 10 mL of phosphate-buffered saline (PBS, pH 7.2). It was centrifuged at 1000 rpm for 20 min. The supernatant was removed, filtered through a 0.45-pm Millipore filter, and kept at -20°C. The resultant solution was made up to 20 ml of volume and used as a test solution. The stock concentration of the seaweed extract was 1 g/ml (Alam *et al*., 1994).

Preparation of 2% suspension of erythrocytes

Human blood groups A, B, AB, and O were obtained from authorized blood banks in Madurai. Blood samples were maintained at 4°C in Alsevier's solution. A 2% erythrocyte suspension in PBS (pH 7.2) was prepared from each type of blood group by centrifugation at 1000g for 20 min and supernatant was removed. The blood was again centrifuged with PBS (pH 7.2). The supernatant was removed and the erythrocytes were obtained. About 2 ml of erythrocyte was added to 98 ml of phosphate buffer to obtain a 2% suspension of erythrocytes (Alam *et al*., 1994).

Preparation of hemagglutinins

Dilutions were prepared in a 96-well microtiter plate: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024 by adding 50 µl of phosphate buffer (pH 7.2) in each well. Then, 25µl of algal extract was added into the 1:2 dilution and there after transferred 25 µl into the 1:4 dilution and 25 µl from the 1:4 to 1:8 and 25 µl from the 1:8 to 1:16 to obtain serial dilutions. Ten dilutions of seaweed extract were prepared for each A, B, AB, and O positive human blood groups (Alam *et al*., 1994).

Assay of hemagglutinating activity

Hemagglutinating activity was investigated serially in different dilutions of algal extract against all the four positive human blood groups. Twenty-five microliters of 2% erythrocyte suspension, of the respective blood sample group, was added to all dilutions; shaken for a few seconds; and then allowed to stand for 3 hrs. Control was also run simultaneously. An extract in PBS was made as a control. Agglutination was observed after 3 hrs (Alam *et al*., 1994).

Observation of agglutination

Smooth button formation due to settling of erythrocytes at the bottom showed negative activity, whereas a rough granular deposition in the tube due to agglutination of erythrocyte, indicated positive reaction. Low, medium and high agglutinations were determined on the basis of the amount of granular deposition at the bottom of the tube (Alam *et al*., 1994).

Preparation of seaweed extracts for anticancer activity

Seaweeds were rinsed with distilled water. One gram of dried *Padina gymnospora* was homogenized in 100 ml of cold double-distilled water. Then, it was filtered through Whatman paper No. 1. The clarified crude seaweed extract was sterilized by Millipore filter with 0.45-pm pore size and was stored at -20°C, until the date of use **(**Zandi *et al*.,2010).

Cell line and culture

HEpG2 (Liver hepatocellular cells) cell lines was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All the other chemicals and reagents were obtained from Sigma, Mumbai.

In vitro assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of the samples on HEpG2 was determined by the MTT assay (Mossman *et al*., 1983). Cells (1×10^5/well) were plated in 100µl of medium/well in 96-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48hrs at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4hrs incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm.

Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HEpG2 cell line was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100%.

RESULTS

The phytochemical analysis showed the presence of alkaloids, glycosides, tannin, carbohydrates, steroids, flavanoid in chloroform, methanol and water extract of *Padina gymnospora*. Salkowski test for the detection of steroids indicated the presence of steroids in the chloroform extract and triterpenoids in the methanol and water extracts. But Keller Killani test showed the presence of cardiac glycosides only in the chloroform extract.

The ash content in *Padina gymnospora (Kutzing) Sonder*was found to be 1.301 mg/g. El-Sarraf and El-Shaarawy., (1994) observed that the ash content was 53.0 mg g-1 for *Ulva fasciata* and 350.0 mg g-1 for *Corallina mediterranea*. The low ash content showed that there was little non-combustible residue such as minerals in the seaweed samples. The low percentage of ash also indicated that the samples were not contaminated by any calcareous organisms. The carbohydrate content was found to be 76 mg/g and the protein content was 58.66 mg/g. These results reveal the presence of high amount of sugars like fucose, glucose, maltose, galactose, arabinose and proteins like phycoerythrin, lectin in this brown seaweed.

The nitrogen content was very low and it was found to be 19.6 mg/g. The sulphate content was 7.66 mg/g and the mannitol content was 9.87%. The alginic acid and the alginate content was found to be 10.26% and 25.46%. The alginate content was higher in the brown seaweed as the cell wall contain large amount of anionic polysaccharides (Salgado *et al*., 2011). The total flavanoid and phenol content was 70.66 mg/g, 30.33 mg/g. Flavonoids are the largest group of phenolic compound and are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties (Kahkonen *et al*., 1999). Santoso *et al*., (2002) and Yoshie *et al*., (2000) reported that series of flavonoid compounds such as catechin (e.g gallocathecin, epicathecin, catechin gallate), flavonols and flavonol glycosides have been identified from methanol extracts of red and brown algae.

The haemagglutinic activity of the brown seaweed *Padina gymnospora (Kutzing) Sonder*predicted positive result for all the four A, B, AB, O positive blood groups. But higher haemagglutinating activity was observed in B, AB and O positive blood group. Chu *et al*., (2006) reported that the different specificities for antigens or carbohydrates receptors on the erythrocytes may account for the variation in the agglutinating activity.

Table 1. **Phytochemical analysis of seaweed *Padina gymnospora***

	Padina gymnospora			
Test for alkaloids		**C**	**M**	**W**
	Wagners test	+	+	+
	Tannic acid	+	+	+
	Hagers test	+	+	+
Test for Glycosides	Legals test	+	+	+
Test for Tannin	Alkaline reagent test	+	-	-
Test for Carbohydrates	Benedicts test	+	+	+
	Fehlings test	+	+	+
	Molischs test	+	+	+
Test for steroids and triterpenoids	Salkowski test	Steroids +	Triterpenoids +	Triterpenoids +
Test for cardiac glycosides	Keller Killani test	+	-	-
Test for flavanoid	Alkaline reagent test	+	+	+

Table 2. Biochemical characterization of *Padina gymnospora*

S.no	Parameters	Total amount (Mean±SD)
1	Ash(mg/g)	1.301±0.2322
2	Lipid(mg/g)	1.172±0.1028
3	Carbohydrate(mg/g)	76±7.549
4	Protein(mg/g)	58.66±4.163
5	Nitrogen(mg/g)	19.6±0.9165
6	Calcium(mg/l)	79.0±3.6041
7	Sodium(mg/l)	35.56±11.35
8	Potassium(mg/l)	228.1±16.65
9	Sulphate(mg/g)	7.66±1.5275
10	Flavanoid(mg/g)	70.66±3.2145
11	Alginic acid(%)	10.26±6.9895
12	Alginate(%)	25.46±2.470
13	Phenol(mg/g)	30.33±0.577
14	Mannitol(%)	9.87±0.0984

Table 3. Haemagglutinic activity of *Padina gymnospora* against human A, B, AB, O (+) blood groups

	Dilutions									
Blood groups	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
A(+)	+++	+++	+++	+++	+++	+++	+++	+++	-	-
B(+)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AB(+)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
O(+)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 4. Antitumour effect of seaweed *Padina gymnospora* on HEpG2 cell lines

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.06	11.5
2	500	1:1	0.13	25.0
3	250	1:2	0.21	40.3
4	125	1:4	0.28	53.8
5	62.5	1:8	0.34	65.3
6	31.2	1:16	0.39	75.0
7	15.6	1:32	0.42	80.7
8	7.8	1:64	0.46	88.4
9	Cell control	-	0.52	100

The cytotoxicity of *Padina gymnospora* extracts on the HEpG2 cells using MTT assay is represented in Table 4. To determine the cytotoxic effects of *Padina gymnospora*, the HEpG2 cell lines were exposed to various concentrations of seaweed extracts. As a result of incubation it was found that as the seaweed concentrations increases, the number of viable cells decreases. The cytotoxic compounds present in the seaweed *Padina gymnospora (Kutzing) Sonder*arrest further growth of HEpG2 cell lines and decreases the cell viability. Thus marine seaweeds was found to be effective in the treatment of cancerous cells.

DISCUSSION AND CONCLUSION

Seaweeds has been a source of a variety of major metabolites such as polysaccharides, lipids, proteins, carotenoids, vitamins, sterols, enzymes, antibiotics and many other fine chemicals. Marine organisms are a rich source of structurally novel and biologically active metabolites like laminarin, fucoxanthin, fucoidan etc... These bioactive compounds from the sea are very attractive. Secondary or primary metabolites produced by these organisms may be of importance in pharmaceutical industry. 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 medicines against cancer and also various other diseases. Lectins with haemagglutinating activity occur in a variety of red, brown and green seaweeds (Smit, 2004). The activities range from properties of pharmacological interest, like cytotoxic activity against tumoral cells, antiviral, immunosuppressive activity etc. In this perspective, results of the present study on the biochemical, phytochemical, haemagglutinic activity and anticancer activity of seaweeds collected from Mandapam coast are discussed.

Seaweeds contain the anticancer compounds selenium, iodine and omega-3 fatty acids. It also contains U-fucoidan, which is a complex polysaccharide. U-fucoidan essentially breaks down and destroys cancer cells while leaving the healthy, normal tissue alone. Different types of seaweed contain a variety of cancer-fighting powers. For example, the brown seaweed *Laminaria japonica* contains a concentrated extract called modifilan. Modifilan has a very high content of soluble polysaccharides like fucoidan, laminarin and alginate. These compounds assist in killing cancer cells and removing toxins from the body. Seaweed therapy is also being examined for its impact on cancer. Seaweed therapy is researched in Japan because of its anti-inflammatory and antitumour properties, and some scientists have already reported promising results. Seaweed therapy can be used externally where the patient is wrapped in pure seaweed for a cleansing effect, or can be taken internally in food or pill form.

In this study *Padina gymnospora (Kutzing) Sonder*showed high cytotoxic activity against the HEpG2 cell lines. The most effective concentration in which the number of viable cells decreased were 500µg/ml of algal extract. As the concentration of the seaweed extract increases, the number of viable cell decreased. Joshi and Srisudha., (2012) reported that extracts of *Caulerpa scalpelliformis* exhibited cytotoxic activity against HEp2 cells *invitro* with a CD50 of 250µg/ml. Thus the bioactive compounds of present study with *Padina gymnospora (Kutzing) Sonder*can be considered as a chemotherapeutic agent against cancer. 7.8µg/ml of the seaweed extract exihibited 88.4% cell viability whereas 1000µg/ml of the seaweed extract showed 11.5% cell viability. Increasing concentration of seaweed extract from 7.8µg/ml to 1000µg/ml leads to 76.9% decrease in cell viability of HEpG2 cell lines.

In conclusion, the present study showed high heamagglutinating activity and a decrease in cancer cell count as a confirmatory evidence for protection against HEpG2 cells. The low molecular weight lectin present in the seaweed *Padina gymnospora (Kutzing) Sonder*are responsible for haemagglutinating activity.

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REFERENCES

Alam MT, Usmanghani K, Studies on marine algae for haemagglutinic activity. *Pakistan Journal of Pharmaceutical Sciences*. 1994; 7:1-15.

Anonymous 1. http://www.hyscience.com/archives/2005/10/seaweed_from_fi.php

Anonymous 2. http://EzineArticles.com/5644846

Ballesteros E, Martin D, Uriz Biological MJ, Activity of Extracts from Some Mediterranean Macrophytes. *Botanica Marina*. 1992; 35:481 -485.

Benevidas NMB, Stelio RMO, Marjory LH, Fabio RH, Ana LP, Frietas AHS, Seasonal variations in heamagglutinating activity and chemical composition of two red marine algae *Gracilaria domigensis* and *Gelidium pusillum. Revista Brasileria Fisiologia Vegetal*. 1999; 11:91-95.

Black WAP, Dewar ET, Woodward FN, Manufacture of algal chemicals.II. Laboratory scale isolation of mannitol from brown algae. *Journal of Applied Chemistry*. 1951a; 1:414-424.

Bligh EG, Dyer WJ, A rapid method for total lipid extraction and purification. *J Biochem Physiol.* 1959; 37:911-917.

Boopathy SN, Kathiresan K, Anticancer Drugs from Marine Flora: An Overview. *Journal of Oncology*. 2010; 10:18.

Boyd WC, Almodovar LR, Boyd LG, Agglutinins in marine algae for human erythrocytes. *Transfusion*. 1966; 6:82–83.

Carvalho AFU, Portela MCC, Sousa MB, Martins FS, Rocha FC, Farias DF, Feitosa JPA, Physiological and physico-chemical characterization of dietary fibre from the green seaweed *Ulva fasciata* Delile. *Braz. J. Biol*. 2009; 69:969-977.

Chu YC, Rang H, Lin LP, Analysis of agglutinating activity from unicellular algae. Journal of agglutinating activity from unicellular algae. *Journal of Applied Phycology*. 2006; 19:401-408.

El-Sarraf W.M, El-Shaarawy G, Chemical composition of some marine algae from the Mediterranean sea of Alexandria, Egypt. *The Bulletin of the H.I.P.H.* 1994; 24:525-530.

Haug A, Composition and properties of alginate. *Rep Norw Inst Seaweed Res*. 1974; 30:123-128.

Hedge JE, Hofrieter BT, In Carbohydrates Chemistry: 17 (Eds. Whistler RL and Be Miller JN) Academic Press, New York. 1962.

Joshi NVM, Srisudha S, Haemagglutinin activity of a few selected seaweeds from Mandapam coast, TamilNadu. *International Journal of Current Research*. 2012; 4:49-55.

Kahkonen MP, Hopia AI, Vuorela HJ, Raiuha JP, Pihlaja K, Kujala TS, Heinonen M, Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*. 1999; 47:3954-3962.

Liener IE, Sharon N, Goldstein IJ, The Lectins: Properties, Functions, Applications in Biology and Medicine.Academic press. Orlando. 1986.

Lowry OH, Rosebrough NJ, Fair AL, Randell RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951; 193:265-275.

Mannivanan K, Devi G.K, Thirumaran G, Anantharaman P, Mineral composition of marine macroalgae from Mandapam coastal regions, Southeast coast of India. *American Eurasian Journal of Botany*. 2008; 1:58-67.

Meenakshi S, Gnambigai DM, Tamilmozhi S, Arumugam M, Balasubramanian T, Total flavanoid and *in vitro* antioxidant activity of two seaweeds of Rameswaram coast. *Global Journal of Pharmacology*. 2009; 2:59-62.

Rudiger H, Gabius HJ, Plant lectins: occurrence, biochemistry, functions and application. *Glycoconjucate Journal*. 2001; 18:589-613.

Ruperez P, Calixto F S, Dietary and physicochemical properties of edible Spanish seaweeds. *European Food Research and Technology.* 2001; 212:349-354.

Russell FE, Advances in marine biology. Academic press. New York. 1963; 3:255-256.

Salgado LT, Filho GMA, Fernandez MS, Arias JL, Farina M, The effect of alginates, fucans and phenolic substances from the brown seaweed *Padina gymnospora* in calcium carbonate mineralization in vitro. *Journal of Crystal Growth*. 2011; 321: 65–71.

Sampaio AH, Rogers DJ , Barwell CJ, Saker-Sampaio S, Nascimento KS, Nagano CS, Farias, WRL, New affinity procedure for the isolation and further characterization of the blood group B specific lectin from the red marine alga *Ptilota plumosa*. *J. Appl. Phycol*. 2002; 14:489–495.

Sampaio AH, Rogers DJ, Barwell CJ, A galactose specific lectin from the red marine alga *Ptilota filicina. Phytochemistry*. 1998; 48:765-769.

Sampaio AH, Rogers DJ, Barwell CJ, Saker-Sampaio S, Costa FHF , Ramos MVA, New isolation procedure and further characterisation of the lectin from the red marine alga Ptilota serrata. *J. Appl. Phycol*. 1999; 10:539–546.

Santoso JY, Yoshie T, Suzuki T, The distribution and profile of nutritions and catechins of some Indonesian seaweed. *Fisheries Science*. 2002; 68: 1647-1648.

Smit AJ, Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*. 2004; 16: 245–262.

Souza BWS, Teixeira DIA, Andrade AK, Melo MRS, Munoz AM, Frietas ALP, A survey of Antartic algae for agglutinins. *Oecol Bras*. 2007; 11:122-130.

Srivastava N, Saurav K, Mohanasrinivasan V, Kannabiran K, Singh M, Antibacterial Potential of Macroalgae Collected from the Madappam Coast, India. *British Journal of Pharmacology and Toxicology*. 2010; 1: 72-76.

Suttisrisung S, Senapin S, Withyachumnarnkul B, and Wongprasert K, Identification and characterization of a novel legume-like lectin cDNA sequence from the red marine algae *Gracilaria fisheri*. *J. Biosci.* 2011; 36: 1–11

Yoshie Y, Wang W, Petillo D and Suzuki T, Distribution of catechins in Japanese seaweeds. *Fisheries Science*. 2000; 66 :998-1000.

Zandi K, Tajbakhsh S, Nabipour I, Rastian Z, Yousefi F, Sharafian S, Sartavi K, *In vitro* antitumor activity of *Gracilaria corticata* (a red alga) against Jurkat and molt-4 human cancer cell lines. *African Journal of Biotechnology*. 2010; 9:6787-6790.