In-Vitro Antioxidant and Anticancer Activity of Selected Seaweeds of Kovalam Coast, Tamilnadu

Arulvasu C [*], Shiva Shakthi S, Rangasamy V, Hemavathi M
[*]Department of Zoology, University of Madras, Guindy Campus, Chennai-600 025, India
E-mail: carulvasu@gmail.com

Abstract: - **This study examines the antioxidant and anticancer activities of the aqueous extract of marine seaweeds *Gracillaria corticata, Ulva fasiata, Chaetomorpha antennina* and *Enteromorpha compressa*. The aqueous extracts of collected seaweeds were tested for its antioxidant and anticancer properties *in-vitro* against human breast adenocarcinoma cell line MCF 7. In the *in-vitro* antioxidant assay, *E. compressa* showed the highest DPPH free radical activity with an IC_{50} of 180 µg/ml. The Iron chelating activity was higher in the aqueous extract of *G. corticata* with 88 %. The reducing power of the all four selected seaweeds extract increased with the increase in concentrations in which the *U. fasiata* showed a significant IC_{50} values 1000 µg/ml. The anticancer activity of the seaweeds extract was observed at 72 h, in which *G. corticata* showed a greater activity with an IC_{50} value of 200 µg/ml. The morphology of the treated cells showed a great variation when compared to the control cells. The destruction of monolayer was observed with shrinkage and rupture of the cells. The morphological change and cell shrinkage was dose and time dependent. Thus the *in-vitro* assays indicate that the aqueous extracts of selected seaweeds are significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and a noble anticancer agent. This also infers that all the seaweeeds are potent antioxidant and anticancer agents, specifically *G. corticata* and *U. fasiata* could be a potential candidate for cancer therapy in the near future.**

Key words: Seaweeds; Aqueous extract; Antioxidant; Anticancer

I. INTRODUCTION

Cancer is due to failures of the mechanisms that usually control the growth and proliferation of cells. During normal development and throughout normal adult life genetic control system regulate the balance between cell birth and death in response to growth signals, growth inhibiting signals and death signals. The cancer forming process, called oncogenesis or tumorigenesis, is interplay between genetics and environment. Most of the cancer arises after genes are altered by carcinogens or by errors in the copying and repairs of genes. Cancer commonly results from mutation that arise during a life time is response to carcinogens which include certain chemicals, viruses and UV rays [1]. Cancer results highest mortality around worldwide, according to cancer report 2008, in 2005 over 7.6 million people died of cancer out of 58 million death worldwide. More than 70% of cancer death occurs in under developed countries, where resource available for prevention, diagnosis and treatment of cancer limited or non-existent. Based on projections cancer death will continue to rise with an estimated 9 million people dying from cancer in 2015 and 11.4 million dying in 2030. In 2005, cancer result's 826,000 people death in India, of which 5,19,000 were under the age of seventy.

Oxidative stress induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases such as cancer and other conditions [2,3]. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain polyphenols and flavonoids that act as free radical scavengers [4]. In modern medicine, the balance between antioxidation and oxidation is believed to be a critical concept of maintaining a healthy biological system [5,6,7,8,9].

Various components of the diet react may directly with carcinogens or free radical compounds such as hydroxyl radicals and some free atoms called antioxidants are unique component of some dietary food materials such as fruits, vegetables, herbs, spices, seeds, algae and fiber containing foods [10]. It may react directly with toxic oxygen (cause mutation in cells) and inactivate it, thus preventing the rate of DNA damage. The function of antioxidants is to remove free oxygen radicals from the body and as a result prevent the cells from damage or undergoing any carcinomatous change [11]. More over antioxidants are naturally occurring group of chemical compounds that can deviate the free radicals and prevent their formation [12] oxidants in the body have single unpaired electron can make molecular structure and destroy the cells and other molecules in their search for another electron [13].

Many plants have been identified as having potential antioxidant activities and their consumption recommended, present in various ecosystem such as marine, hills and lands. Recent findings evidenced that seaweeds contained antiviral [14], antibacterial [15], antifungal [16] and antitumoral [17] potentials, among numerous others. 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 2400

marine natural products have been isolated from seaweeds of subtropical and tropical populations [18]. According to existing literature, more than ten new experimental anti-tumor agents have been derived from marine sources and entered into clinical trials. Recent studies have suggested several possible pathways through which these antioxidant nutrients act to inhibit the development of cancer cells and to destroy then through apoptosis by their stimulation of cytotoxic cytokines, by their action on gene expression, by preventing the development of tumors necessary to blood supply (or) by cellular differentiation [19].

Many substances obtained from sea weeds such as alginate, carrageenan and agar as phycocolloids have been used for decades in medicine for a long time, so that antioxidant nutrients will be widely utilized and will play an important role in preventive medicine [20]. Most of the identified compounds belong to the classes of alkaloids, polyphenols, triterpens, steroid glycosides or small peptides [21,22,23,24,25,26,27]. The main objective of the present study is to examine the antioxidant and anticancer property of seaweeds potency (*Gracillaria corticata, Ulva fasiata, Chaetomorpha antennina* and *Enteromorpha compressa*) *in vitro* against human breast adenocarcinoma cell line MCF 7.

II. EXPERIMENTAL METHODS

A. Collection of samples

The all four seaweeds (*Gracillaria corticata, Ulva fasiata, Chaetomorpha antennina* and *Enteromorpha compressa*) were collected from Kovalam, Tamilnadu, Southeast coast of India. The identification of specimens were checked by Botanist, CAS in Botany, University of Madras, Chennai, India. All seaweed samples were washed several times with water, air dried in shaded area. The dried samples were grinded into fine particle by mortar pestle and stored in glass containers at room temperature for further experiments.

B. Preparation of seaweed extracts

Extraction from seaweed samples were done by taking 5 g of powdered seaweed samples mixed with 20 ml of distilled water and homogenized with glass homogenizer. After centrifugation at 1200 x g for 20 min, supernatants were collected and filtered with Whatman No.1 filter paper followed by 0.22 µm pore sized filter (Millipore, filter type GV) to obtain the water soluble extract. The remaining pellets were further mixed with 20 ml of distilled water, homogenized and centrifuged as described above. The process was repeated for the extraction of the unfiltered portion of the powdered seaweed samples. Finally the all aqueous extracts were subjected to lyophilization to make fine powder and stored at - 20°C for further experiments.

C. Antioxidant Analysis

a. DPPH Radical Scavenging Assay

The effect of marine algae extract on DPPH radical was estimated according to the method of Blois *et al*., [28]. The reaction mixture was prepared by tacking 200 µl of different concentration (200, 400, 600, 800 & 1000 µg/ml) of marine algae extract. Finally 2 ml of DPPH (0.1 mM) solution was added to each tube and sample keeps it for 30 min at 37ºC, under dark condition. The reaction mixture was observed at 517 nm against a blank. The DPPH solution without compound was used as a control. Gallic acid was used as a positive control. The radical scavenging activity was expressed as an inhibition percentage and the calculation as follows,

$$\text{Percentage (\%) of radical scavenging activity} = [A_{\text{Contorl}} - A_{\text{Sample}} / A_{\text{Control}}] \times 100$$

b. Chelating Assay

The chelating activity of the samples were measured after Dinis *et al*., [29], with slight modification. To take 600 µl of with different concentration of marine algae extract (200, 400, 600, 800 & 1000 µg/ml) was mixed with 3.5 ml of methanol and then mixture was reacted ferrous chloride (2 mM, 0.1 ml) and ferrozine (5mM, 0.2 ml) for 10 min at room temperature. The absorbance was measured at 562 nm against at blank. EDTA was used as a standard.

$$\text{Percentage (\%) of Inhibition} = [1 - AS/AC] \times 100$$

c. Reducing Power Assay

Reducing power of samples were determined following the method of Oyaizu *et al*., [30]. Reducing power of seaweed extracts was determined with different concentration (20, 40, 60, 80 and 100 µl) mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1 % potassium ferric cyanide. The mixture was incubated at 50ºC for 20 min. A volume of 2.5 ml 10 % Trichloroacidic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Aliquot of supernatant (1 ml) was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1 %) and absorbance was recorded at 700 nm. Increase in absorbance indicated increased reducing activity. Butylated Hydroxyl Anisole (BHA) was used as a standard.

D. Cancer cell line and chemicals

Cancer cell line MCF-7 was purchased from National Centre for Cell Science (NCCS), Pune, India. Dulbecco's Modified Eagle's Medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum (FBS), 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT), sodium bicarbonate, Dimethyl sulphoxide (DMSO) and antibiotic solution were purchased from Himedia. 96 well plates, 6 well plates, Tissue culture flasks (25 and 75 mm^2), centrifuge tubes (15 and 50 ml) were purchased from Hi media.

a. Screening of drug for Antiproliferation Activity - MTT Method

The antiproliferative effect was assessed by MTT method as described by Mosmann [31]. Cancer cells were plated in 96 well plates with DMEM medium containing 10% FBS. The cells were incubated for 24 h under 5% CO_2 95% at 37ºC. The medium was removed, washed with PBS and then serum free medium was added and kept for 1 h in the incubator. Then, serum free medium was removed and the control plates received serum free medium and treatment plates received 200, 400, 600, 800, 100 µg/ml of marine algae extract. The cultures were again incubated as above. After 72 h, 100 µl of 5 mg/ml MTT solution was added to each well and the cultures were further incubated for 4 h and then 100 µl of DMSO was added and the crystals formed were dissolved gently be pipetting 2 to 3 times. A micro plate reader was used to measure absorbance at 570 nm for each well. Growth inhibition rate was calculated as follows.

$$\text{Percentage of Growth inhibition} = \frac{A_{570\ of}\ \text{treated cells}}{A_{570}\ \text{of control cells}} \times 100$$

b. Morphological studies

Cancer cells line (MCF-7) was tripsinized and 5 ml of growth medium was added to arrest the tripsinized enzyme and the cell suspension was mixed well. Then 2 ml of cell suspension was added to the sterilized test tubes containing cover slip. The test tubes were placed in a slanting position and kept in CO_2 incubator for two days. The monolayer of cells formed in the cover slip was observed under light microscope and photographed.

E. Statistical analysis

Data obtained in the present study was expressed as mean ± standard deviation (SD) to find out the variation between the control and experimental group.

III. RESULTS

A. Antioxidant activity

a. DPPH free radical scavenging activity

The percentage of DPPH radical scavenging activity of aqueous extract of sea weeds are presented in table1. The Aqueous extract of *E. compressa* exhibited a maximum DPPH scavenging activity of 60% at 1000 µg/ml whereas for Gallic acid (standard) was found to be 70% at 1000 µg/ml. The IC_{50} of the aqueous extract of *E. compressa* and Gallic acid were found to be 180 µg/ml and 160 µg/ml respectively.

Table 1. DPPH free radical scavenging activity of aqueous seaweeds extract

S. No	Concentration (µg/ml)	% of activity (±SD)*				
		Standard (Gallic acid)	*G. corticata*	*U. fasciata*	*C. antennina*	*E. compressa*
1	200	63.0 ± 2.0	52 ± 3.6	52 ± 4.0	53.0 ± 4.0	52 ± 4.0
2	400	66.0 ± 2.6	53 ± 3.7	53 ± 4.0	54.0 ± 4.0	53 ± 4.1
3	600	68.0 ± 2.6	54 ± 3.6	54 ± 4.0	55.0 ± 3.6	54 ± 3.6
4	800	69.0 ± 5.2	56 ± 4.0	54 ± 4.5	57.0 ± 3.0	57 ± 3.0
5	1000	70.0 ± 4.5	58 ± 3.6	54 ± 3.6	59.0± 3.6	60± 3.6

b. Iron chelating activity

Iron binding capacity of the aqueous extract of seaweeds and the metal chelator EDTA at various concentrations (200, 400, 600, 800, 1000 µg/ml) were examined and the values were presented in table 2. Maximum chelating of metal ions was seen in *G. corticata* at 1000 µg/ml with 84 %. The IC_{50} value of *G. corticata* extract and EDTA was recorded as 360 µg/ml and 120 µg/ml respectively.

Table 2. Iron-chelating effect of the sea weeds aqueous extract

S. No	Concentration (µg/ml)	% of activity (±SD)*				
		Standard (EDTA)	*G. corticata*	*U. fasciata*	*C. antennina*	*E. compressa*
1	200	82 ± 2.6	21.0 ± 3.0	10.0 ± 3.0	19.0 ± 5.0	25.0 ± 4.5
2	400	84 ± 4.3	59.0 ± 3.6	15.0 ± 3.0	64.0 ± 3.6	36.0 ± 5.1
3	600	86 ± 2.0	72.0 ± 5.5	27.0 ± 4.3	69.0 ± 6.0	39.0 ± 6.0
4	800	88 ± 4.5	76.0 ± 4.0	38.0 ± 5.1	71.0 ± 3.0	64.0 ± 6.0
5	1000	90 ± 4.5	84.0 ± 3.5	44.0 ± 7.0	72.0 ± 5.5	69.0 ± 3.6

c. Reducing power assay

The reductive capabilities of the aqueous extracts of seaweeds were compared with BHA for the reduction of the Fe^{3+}- Fe^{2+} transformation. Table 3 shows the reducing power of extract of seaweeds at various concentrations. The reducing power of the extract increased with the increase in concentrations. The IC_{50} value of the extract of *U. fasciata* and BHA was found to be 1000 µg/ml and 240 µg/ml respectively.

Table 3. Reducing power of sea weeds aqueous extract

S. No	Concentration (µg/ml)	% of activity (±SD)*				
		Standard (BHA)	*G. corticata*	*U. fasciata*	*C. antennina*	*E. compressa*
1	200	42.0±3.6	27.0 ± 4.5	25.0 ± 4.5	20.0 ± 3.0	26.0 ± 3.4
2	400	78.0±3.0	35.0 ± 4.3	34.0 ± 3.6	23.0 ± 5.2	32.0 ± 5.0
3	600	92.0±3.3	37.0 ± 2.0	36.0 ± 4.0	24.0 ± 3.6	33.0 ± 4.3
4	800	93.0±3.4	41.0 ± 3.0	38.0 ± 5.2	28.0 ± 2.6	34.0 ± 3.6
5	1000	94.0±3.6	47.0 ± 6.2	50.0 ± 6.0	29.0 ± 3.5	39.0± 6.0

B. Anticancer activity

The antiproliferative effect of seaweeds with various concentrations (200, 400, 600, 800 and 1000 µg/ml) were tested by MTT assay, against human breast adenocarcinoma cell line MCF 7. The treatment of seaweeds inhibited the growth of MCF7 cells in a concentration and time dependent manner. Figure 1 indicate decrease in the cell viability of the results at concentration ranging from 200 to 1000 µg/ml. The IC_{50} value of *G. corticata, U. fasciata, C. antennia* on MCF 7 was 200 µg/ml, 300 µg/ml and 1020 µg/ml respectively.

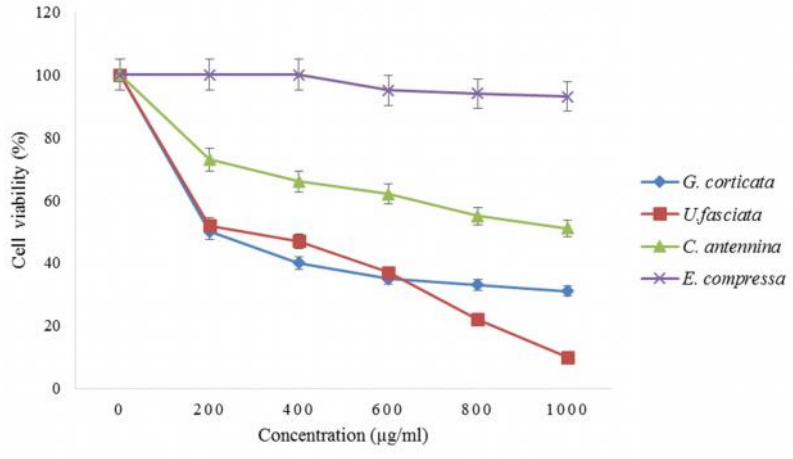

Figure 1. Effect of seaweeds aqueous extract on human breast cancer cell line MCF 7 by MTT assay (72 h)

A morphological observation of the MCF 7 shows polygonal cell morphology. Upon treatment with four different sea weeds, the polygonal cells begin to shrink and appear spherical in shape (Fig. 2). The cell shrinkage increased progressively with dose and time and this shrinkage may be due to the growth inhibitory effect of seaweeds.

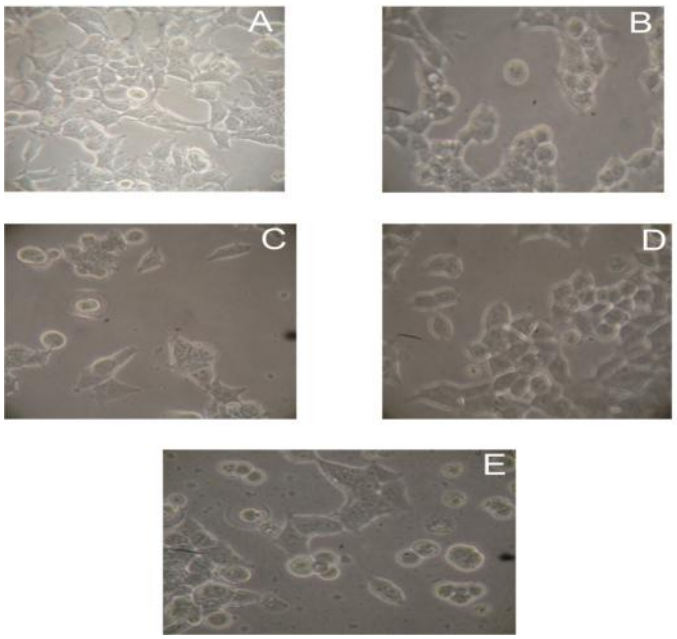

Figure 2. Morphological changes of human breast cancer cell line after 72 h incubation. A. Control, B. *Gracillaria corticata;* C. *Ulva fasciata*; D. *Chaetomorpha antennina;* E. *Enteromorpha compressa*

IV. DISCUSSION

In this study, the antioxidant and anticancer activity *in vitro* from four different marine algae samples (*Gracillaria corticata, Ulva fasiata, Chaetomorpha antennina* and *Enteromorpha compressa*), were determined. Some metabolites such as phenols, carotene and steroids were isolated and purified in some algae and their activity against free radical scavenging as an antioxidant. 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 [32].

The DPPH radical has been widely used to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. As the concentration of each seaweed aqueous extract increased, the DPPH radical scavenging activity also increased. Decreased concentration of DPPH radical due to the scavenging ability of the soluble constituents in the aqueous extract of all seaweeds and the standard Gallic acid as s reference compound presented the highest activity at all concentrations. This shows that all selected seaweeds possess hydrogen donating capabilities and acts as an antioxidant. A possible explanation of the free radical scavenging activity is the neutralization of DPPH free radical by the antioxidant components of crude extract/fractions, either by transfer of hydrogen or of an electron [33].

Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion [34]. The iron-chelating capacity test measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion [35]. Ferrozine can quantitatively from complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is decreased. The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [36]. As shown results chelating capacity of the aqueous extracts of seaweeds increased with increase in concentration.

The reducing capacity of a compound Fe^{3+}/ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity [37,38]. The existence of reductions are the key of the reducing power, which exhibit their antioxidants activities through the action of breaking the free radical chain by donating a hydrogen atom [39]. The reductive capabilities of the aqueous extract of selected four seaweeds were compared with BHA as a standard compound for the reduction of the Fe^{3+}- Fe^{2+} transformation in the presence of the seaweed aqueous extract. The reducing capacity of a compound may serve as a significant indicator of its

potential antioxidant activity and it was found that the reducing power of the extract increased with the increase in concentrations. This show the aqueous extracts of seaweed possess minimum reducing capabilities and acts as an antioxidant. Similar antioxidant activity has been reported to be related to reducing power by some investigators [40,41].

The lower incidence of breast cancer in the Indian populations has intrigued researchers. Researchers have found that dietary marine algae and their extracts inhibit carcinogen- induced breast cancers, lung metastases and leukemia in animal models [42]. Similarly, tests on the seaweed extracts in bacterial systems revealed that the extracts had a profound antimutagenic quality. Most recently Funahashi *et al.*, [43] have shown that wakame extracts have a potent inhibitory effect on the progression of mouse mammary tumors. Similar extracts produced an equally profound apoptotic effect on breast cancer cells *in vitro* while the extracts were nontoxic to ordinary breast cells. Likewise in this study, the aqueous crude extracts of *G. corticata, U. fasiata, C. antennina* and *E. compressa* were studied for its probable antitumor activity against human breast cancer cell line (MCF7). Based on previous experience, filtration method is the best way for algae extract sterilization [44]. The water extract of *G. corticata, U. fasiata* and *C. antennina* showed reasonable activity against tumor cells replication. The results showed that the three algae species (*G. corticata, U. fasiata* and *C. antennina*) had high growth inhibitory activity against MCF7 cells. The results here are in agreement with that obtained by Bhaskar *et al.*, [45] who found that the total lipid and lipid classes of marine algae S*argassum marginatum* possessed an inhibition effect against human pro-melocytic leukemia HL60. Zandi *et al* [46] demonstrated the activity against cancer cell lines is one of the most important specificities of marine algae, and many algae have showed cytotoxic and antitumor activities.

Based on the results obtained in the present study, we can conclude that the *G. corticata, U. fasiata* and *C. antennina* aqueous extract did exhibit considerable cytotoxic effect against human breast cancer cells. Therefore, more studies for final application of this alga could be important in the field of natural antitumor investigation.

REFERENCES

[1] M. Harvey, H. Vogel, D. Morris, A. Bradley and L.A. Donehower. A mutant p53 transgene accelerate tumor development in heterozygous but not nullizyous p53 deficient mice. Nature genet. 9: 305-311, 1995

[2] C. Smith, M.J. Mitchinson, O.I. Arouma and B. Halliwell. Stimulation of lipid peroxidationand hydroxyl radical generation by the contents of human atherosclerosis lesions. Biochem. J., 15: 910-905. 1992.

[3] N. Akhtar, M. Ali and M.S. Alam. Chemical constituents from the seeds of Zanthoxylum alatum. J. Asian Nat. Prod. Res. 11: 91-5, 2009.

[4] S.M. Sabir and J.B.T. Rocha. Water extractable Phytochemical from *Phyllanthus niruri* exhibit distinct *In vitro* antioxidant and *In vivo* hepato-protective activity against paracetamol induced liver damage in mice. Food Chemistry, 111: 845-851, 2008.

[5] I.E. Dreosti. Trace elements, micronutrients, and free radicals. Totowa. Human Press 1991, pp. 149-199.

[6] S. Ahmad. Oxidative stress and antioxidant defences in biology. New York: Chapman and Hall, 1995.

[7] K.J. Davies. Oxidative stress, antioxidant defenses and damage removal, repair, and replacement systems. IUBMB Life. 50: 279–289, 2000.

[8] A.K. Tiwari. Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidant therapy. Curr. Sci., 8: 1179-1187, 2001.

[9] V. Katalinic, M. Milos, T. Kulisic and M. Jukic. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 94: 550–557, 2006.

[10] B. Trock, E. Lanza and B. Greenwald. Dietary fiber vegetables and colon cance:critical review and meta-analysis of the epidemiological evidence. J. Natl. Cancer inst., 82: 650-661, 1990.

[11] M. Valko, D. Leibfritz, J. Moncol, M. Cronin, M. Mazur and J. Telser. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology, 39(1): 44-84, 2007.

[12] I. Chapple. Role of free radicals and antioxidants in the pathogenesis of the inflammatory periodontal diseases. Clinical Molecular Pathology. 49(5):M247, 1996.

[13] J. McCord. The evolution of free radicals and oxidative stress. The American journal of medicine. 108(8): 652-9, 2000.

[14] B. Matsuhiro, A.F. Conte, E.B. Damonte, A.A. Kolender, M.C. Matulewicz, E.G. Mejias, C.A. Pujol and E.A. Zuniga. Structural analysis and antiviral activity of a sulfated galactan from the red seaweed *Schizymenia binderi* (Gigartinales Rhodophyta). Carbohydr. Res. 340: 2392-2402, 2005.

[15] N. Xu, X. Fan, X.J. Yan, X. Li, R. Niu and C.K. Tseng. Antibacterial bromophenols from the marine red alga *Rhodomela confervoides*. Phytochemistry, 62: 1221-1224, 2003.

[16] X.C. Li, M.R. Jacob, Y. Ding, A.K. Agarwal, T.J. Smillie, S.I. Khan, D.J. Nagle, D. Ferreira and A.M. Clark. Capisterones A and B, which enhance fluconazole activity in *Saccharomyces cerevisiae*, from the marine green alga, *Penicillus capitatus*. J. Nat. Prod. 69: 542-546, 2006.

[17] H. Harada, T. Noro and Y. Kamei. Selective antitumor activity in vitro from marine algae from Japan coast. Biol. Pharm. Bull. 20: 541-546, 1997.

[18] A. Manilal, S. Sujith, G.S. Kiran, J. Selvin, C. Shakir, R. Gandhimathi and M.V.N. Panikkar. Bio-potentials of seaweeds collected from Southwest coast of India. J. Mar. Sci. & Technol. 17: 67-73, 2009.

[19] G. Shklar. Mechanisms of cancer inhibition by antioxidant nutrients. Oral Oncology, 34: (1): 24-9, 1998.

[20] H.S. Garewal and S. Schantz. Emerging role of beta-carotene and antioxidant nutrients in prevention of oral cancer. Arch Otolaryngol Head Neck Surg. 121(2):141-4, 1995.

[21] O. Nakamura, Y. Mimaki, H. Nishino and Y. Sashida. Steroidal saponins from the bulbs of *Lilium speciosum* L. nobilissimum 'Star Gazer' and their antitumour-promoter activity. Phytochemistry, 36: 463-467, 1994.

[22] T. Inoue, Y. Mimaki, Y. Sashida, A. Nishino, Y. Satomi and H. Nishino. Steroidal glycosides from *Allium macleanii* and *A. senescens*, and their inhibitory activity on tumour promoter-induced phospholipid metabolism of HeLa cells. Phytochemistry. 40: 521-525, 1995.

[23] Y. Mimaki, T. Kanmoto, M. Kuroda, Y. Sashida, Y. Satomi, A. Nishino and H. Nishino. Steroidal saponins from *Hosta longipes* and their inhibitory activity on tumour promoter-induced phospholipid metabolism of HeLa cells. Phytochemistry, 42: 1065-1070, 1996a.

[24] Y. Mimaki, T. Kanmoto, Y. Sashida, A. Nishino, Y. Satomi and H. Nishino. Steroidal saponins from the underground parts of Chlorophytum comosum and their inhibitory activity on tumour promoter-induced phospholipid metabolism of HeLa cells. Phytochemistry, 41: 1405-1410, 1996b.

[25] Y.H. Kuo and L.M. Kuo. Antitumour and anti-AIDS triterpenes from *Celastrus hindsii*. Phytochemistry, 44: 1275-1281, 1997.

[26] S. Javed, N.K. Mehrotra, Y. Shukla. Chemopreventive effects of black tea polyphenols in mouse skin model of carcinogenesis. Biomed. Environ. Sci., 11: 307-313, 1998.

[27] K.N. Rao and S.R. Venkatachalam. Inhibition of dihydrofolate reductase and cell growth activity by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine: the in vitro cytotoxicity of these plant alkaloids and their potential as antimicrobial and anticancer agents. Toxicol. In Vitro, 14: 53-59, 2000.

[28] M.S. Blois. Antioxidant determinations by the use of a stable free radical. Nature, 26: 1199-1200, 1958.

[29] T.C.P. Dinis, V.M.C. Maderia and Almeida. Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavaengres. Arc Biochem Biohys, 315: 161-169, 1994.

[30] M. Oyaizu. Studies on product of browning reaction prepared from glucose amine. J. Nutr, 44: 307-315, 1986.

[31] T. Mosmann. Rapid colorimetroc assay of celluar growth and survival: An application to proliferation and cytotoxicity assays. J. Immuno. Meth., 65: 55-63, 1983.

[32] B.M. Ly, N.Q. Buu, N.D. Nhut, P.D. Thinh, T. Thi and T. Van. Studies on fucoidan and its production from Vietnamese brown sea weeds. AJSTD. 22: 371-380, 2005.

[33] K. Shimada, K. Fujikawa, K.Yahara and T. Nakamura. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. Journal of Agricultural and Food Chemistry. 40, 945–948, 1992.

[34] C.L. Hsu, W. Chen, Y.M. Weng and C.Y. Tseng. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chemistry. 83: 85-92, 2004.

[35] M. Elmastas and I. Glucin. Radical scavenging activity and antioxidant capacity of bay extracts. J. Iran, chem.soc. 3:258-266, 2006.

[36] A.M. Aboul-Enein, F.K. El-Baz, G.S. El-Baroty, A.M. Youssef and H.H. Abd El-Baky. Antioxidant activity of algal extracts on lipid peroxidation. J. Med. Sci., 3: 87-98, 2003.

[37] A. Yildrim and A. Mavi. Comparison of antioxidant and antimicrobial activity of tilia (*Ttilia argentea* deset ex DC) (*Salvia triloba*) and (*Lamellia sinensis*) extracts. J. Argic food chem. 48: 5030-5034, 2004.

[38] S. Meir and J. Kanner. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. J. Agric. Food Chem. 43: 5030-5034, 1995.

[39] R.E. Xing, S. Liu, Z.Y. Guo, H.H. Yu, P.B. Wang, C.P. Li, Z.E. Li and P.C. Li. Antioxidant activity of differently regioselective chitosan sulfates in vitro. Bio org. Med. Chem. 13 (4): 1387-1392, 2005.

[40] P.D. Duh, Y.Y Tu and G.C. Yen. Antioxidant activity of the aqueous extract of *Chrysanthemum morifolium* Ramat. Lebensmitt. Wiss.Technol. 32: 269-277, 1999.

[41] Yen, G and Chen, H. 1995. Antioxidant activity of various tea extract in relation to their antimutagenicity. J. Agric. Food. Chem. 43: 7-32.

[42] H. Itoh, H. Noda, H. Amano and H. Ito. Immunological analysis of inhibition of lung metastases by fucoidan (GIV-A) prepared from brown seaweed *Sargassum thunbergii*. Anticancer Res. 15(5B):1937-1947, 1995.

[43] H. Funahashi, T. Imai and T. Mase. Seaweed prevents breast cancer. Jpn J Cancer Res. 92(5):483–487, 2001.

[44] K. Zandi, M.A. Fouladvand, P. Pakdel and K. Sartavi. Evaluation of in vitro antiviral activity of a brown alga from Persian Gulf, *Cystoseira myrica* against herpes simplex virus type 1. Afr. J. Biotechnol. 6: 2511-2514, 2007.

[45] N. Bhaskar, M. Hosakawa and K. Miyashita. Growth inhibition of human pro-myelocytic Leukemia (HL-60) cells by lipid extracts of marine algae *Sargassum marginatum* (Fucales, phaeophyta) harvested off Goa (West Coast of India) with special reference to fatty acid composition. Indian journal of MarineSciences. 33(4): 335-360, 2004.

[46] K. Zandi. Anticancer activity of *Sargassum oligocystom* water extract against human cancer cell lines. Eur Rev Med Pharmacol Sci. 14(8):669-673, 2010.