Open Access Contents
Int. J. Drug Dev. & Res.
| April - June 2014 | Vol. 6 | Issue 2 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

In Vitro Antioxidant and Cytotoxic activity of Brown Alga *Padina Boergesenii*

KANAGARAJJEEVITHA,

JAGRUTI DAMAHE,

SAYANI DAS,

TEESTA ROY CHOWDHURY

SAMANTA S. KHORA*

School of Biosciences and Technology, VIT University, Vellore - 632014, Tamilnadu, India.

Corresponding Authors:
Email: sskhora@vit.ac.in

Abstract:
Brown alga, *Padina boergesenii* was collected from the Mandapam coast used for this present study. The phytochemical screening of the methanol extract of *P. boergesenii* revealed the presence of the Phenolic, flavonoids, tannins, and terpenoids. The total phenolic and flavonoid content were found to be 84.96±0.40 mg/g expressed as Gallic acid equivalent, 85.42 ± 0.97 mg/g expressed as Quercetin equivalent respectively. Antioxidant activity of the methanol extract was determined by the Phosphomolybdenum method and the scavenging activity on DPPH and Nitric oxide was evaluated. The methanol extract has shown the maximum antioxidant and scavenging activity among all other extract. Cytotoxicity of methanol extract against the liver cancer cell line Hep G2 was checked by MTT assay and the IC_{50} value were found to be 1.67 mg/ml. The present study has proved *P. boergesenii* has strong antioxidant and cytotoxic activity which may be utilized for the drug development.

Keywords: *Padina boergesenii,* antioxidant, cytotoxicity.

Introduction

Seaweeds are a group of photoautotrophic, multicellular algae occurring in marine environments. They can perform photosynthesis and the term includes some members of the red, brown and green algae. Seaweeds are commercially important renewable resource and they are used as food, medicine, fertilizer (1). Seaweeds also possess a wide application in food and pharmaceutical industry (2). It has become an untapped resource for the potential bioactive compounds as compared to terrestrial plants for food and health benefits. Among the diverse group of marine organisms, algae are considered as a most nutritious and possess wide range of bioactive compounds. Consumption of the marine algae is thought to ameliorate some inflammatory disorders; breast cancer and high cholesterol level. Seaweeds are considered as excellent sources of bioactive compounds such as carotenoids, dietary fibre, protein, vitamins (3) essential fatty acids and minerals (4). Seaweed lipids has been drawn attention because of the presence of important bioactive molecules like conjugated fatty acids and pigments that have wide range of physiological effects in the treatment of tumours and other cancer related problems (5). It possesses various pigments such as Fucoxanthin, Zeaxanthin, β Carotene and Anthocyanin derivatives (6). It considers as an important resource of long chain polyunsaturated fatty acid (PUFA) (n-3; n-6) which is fundamental for the formation of structural lipids and components of cell membrane (6). Excessive intake of PUFA n6:n3 ratio will promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune disease (7). Recent decades, Seaweeds have been recognized as valuable source of antibiotic production, could be an indicator for the synthesizing secondary

metabolites (8). *Padina boergesenii* -abrown alga, member of the class Phaeophyceae commonly found in coastal regions along continental shelf. Brown algae are said to be a rich source of various bioactive components like polyphenols, alkaloids, flavonoids, terpenoids etc. These secondary metabolites are helpful in studying various physiological effects i.e. either it is harmful or curative on human health (9). The main objective of the present study is to evaluate the antioxidant and cytotoxic activity of brown alga*P. boergesenii* collected from Rameswaram, Gulf of Mannar, India.

MATERIALS AND METHODS

Sample collection:

Brown alga*Padina boergesenii* (Allender & Kraft)were collected from Mandapam region, Gulf of Mannar lying along the longitude from 78°08 'N to 79°30'E and along the latitude 8°35´ to 9°25' during the low tide (temperature was 34-36°C) in the month of September 2013. The samples were cleaned and washed with sterile water to remove the dust particles, shade dried and then powdered with the help of a mechanical grinder.

Preparation of the crude extract

Ten grams of the powered sample was extracted with 100 mL of four different solvents petroleum ether, dichloromethane, ethyl acetate and methanol. The mixture was kept in the shaker at 150 rpm for 48 hours. The extracts were centrifuged for 3 minutes at 3000 rpm. The supernatant was stored at 4°C which was used for further analysis (10).

SCREENING OF PHYTOCHEMICALS

Phytochemicals present in the extract of *P. boergesenii* were carried out by following the standard method (11).

Test for alkaloids (Meyer's reagent)

3 mL of extract was stirred with 3 mL of 1% HCl on water bath. Mayer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloids.

Test for Tannins using ferric chloride

2 mL of extract was stirred with 2 mL of distilled water, and few drops of $FeCl_3$ were added. Formation of green precipitate indicates the presence of tannins.

Test for flavonoids

1 mL of lead acetate solution was added to 1 mL of extract. The Yellow precipitate formation was considered to be a positive test for flavonoids.

Test for Terpenoids

2 mL of extract was dissolved in 2 mL of chloroform and evaporated to dryness. 2 mL of conc. H_2SO_4 was then added and heated for about 2 minutes. A greyish colour development indicates the presence of terpenoids.

Test for Glycosides(Libermann's test)

2 mLof extract was dissolved in 2 mL of chloroform, followed by addition of 2 mL of acetic acid. The solution was then cooled in ice and few drops of H_2SO_4 were added. A colour change from violet to blue to green indicates the presence of glycosides.

Test for Steroids

2 mL of extract is combined with 2 mL of chloroform and 2mL of conc. H_2SO_4. A red colour produced in lower chloroform layer was taken as the positive test for presence of steroids.

Total Phenolic content

Total Phenolic content present in the extract was determined using the Folin Ciocalteau reagent

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., April - June 2014, 6 (2): 110-119

(12). 0.5 mL of sample was added with 1mL of Folin Ciocalteau (1:10) reagent followed by 3 minutes incubation at room temperature. 3 mL of 1% sodium carbonate solution was then added to the mixture. The content was thoroughly mixed and kept for incubation in dark for 2 hours. The absorbance was measured at 760 nm spectrophotometrically. Gallic acid was used as standard. Results were expressed as mg/g Gallic acid equivalents.

Total Flavonoid content

Total flavonoid content was estimated using the method of Ebrahimadeh (13). 0.5 mLof extract was mixed with 0.1 mL of 10% temperature for 30 minutes. The absorbance of aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8mL of distilled water. The contents were then incubated at room temperature and then the mixture was measured at 450nm using UV Spectrophotometer. Quercetin was used as a standard. Results were expressed as mg/g Quercetin equivalents.

Total Antioxidant activity

The total antioxidant activity wasmeasured using Phosphomolybdenum method (14). Different concentration of extract was prepared. 1mL of extracts was added to 3 mL of reagent(0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate) a reagent without the extract was used as the control. The reaction mixtures were incubated at 95^0C for 90 minutes. The absorbance was measured at 635nmspectrophotometrically.

Reducing potential

Different concentration of the extracts in 1mL of distilled water was prepared which were with 2.5 mL of 0.2 M phosphate buffer and 2.5ml of 1 % potassium ferric cyanide.The mixture was incubated for 20 minutes at 50^0C. After incubation 2.5 mL of 10% TCA was added to the mixtures and centrifuged for 10 minutes at 3000 rpm. 2.5 mL of supernatant was mixed with 2.5mL of distilled water and 0.5mL $FeCl_3$ (0.5mL,0.1%). The absorbance was measured at 700nm using spectrophotometer (15).

DPPH free radical scavenging test

The free radical scavenging activity of the extracts was studied using 1, 1-Diphenyl-2-picryl hydrazyl radical test (16).1mL of different concentration of the extract was added with 0.5mL of DPPH (0.16mM DPPH in methanol). Reagent without the extract was used as control.The mixture was Vortexed and then it was incubated at room temperature at 30 minutes. After incubation 2mL of distilled water was added to it. The absorbance was measured at 517nm spectrophotometrically.

Nitric oxide scavenging activity

Different concentration (250, 500, 750, and 1000) µg / mL of the extract were prepared. 100µl Griess reagent was taken and added to 300µl of sample. To this 2.6 mL of distilled water was added.Reagent without the extract was taken as control. The solution was incubated at room temperature for 30 minutes. Absorbance was measured at 548nm spectrophotometrically (17).

Cytotoxic activity against liver cancer Hep G2 cell line

Cytotoxic activity of the methanol extracts of *P. boergesenii was* screened against the liver cancer cell line Hep G2 by following the method of Zandi*et al.,*with little modifications (18). $5x10^3$ Hep G2 cells in 100µl DMEM medium with 10% FBS per well plated in a 96 well plate and incubated overnight at 37^0C in 5% CO_2 incubator. 10mg/ml stock of the test samples was prepared in DMEM. Samples were serially diluted from the stock using DMEM. 100 µl of these diluted samples were added to cells in triplicate wells containing 100 µl

of medium in accordance with the following layout as shown in the table 1.100 µl of DMEM was used as negative control and 5µg/ ml Doxorubicin was added as internal positive control for the assay. Wells without any cells are used as blank. Plate was gently shaken and incubated at 37◦C at 5% CO_2 for 48 hours. 20 µl of 5mg/ml MTT in PBS was added to each well and incubated at 37◦C at 5% CO_2 for 4 hours. Then the medium was aspirated out and 200µl of Dimethylsulfoxide (DMSO) to each well. The optical density of each well was measured using Microplate reader at 570 nm. Then, the inhibition of growth is a measure of cytotoxicity and the percentage inhibition is calculated as follows:

% Inhibition = 100 - ((Mean OD for test sample/ mean OD for the control) x 100)

Table 1: Layout of Cytotoxicity test

	1	2 (mg/ml)	3 (mg/ml)	4 (µg/ml)	5 (µg/ml)	6 (µg/ml)	7 (µg/ml)	8 (µg/ml)
A	No Cells	Dox	1.25	250	150	25	3	0.75
B	No Cells	5	1.25	250	100	25	3	0.375
C	No Cells	5	1	250	100	12.5	3	0.375
D	Medium	5	1	200	100	12.5	1.5	0.375
E	Medium	2.	1	200	50	12.5	1.5	
F	Medium	2.5	0.5	200	50	6.25	1.5	
G	Dox	2.5	0.5	150	50	6.25	0.75	
H	Dox	1.25	0.5	150	25	6,25	0.75	

Results

*P. boergeseniie*xtracts were screened for the qualitative phytochemical analysis. The results revealed the presence of alkaloids, phenolics, flavonoids, tannins, terpenoids, glycosides and sterol in the methanol extract as shown in the table 2.

Table 2: Phytochemical analysis of *P. boergesenii*

Contents	Methanol	Petroleum ether	Dichloromethane	Ethyl acetate
Alkaloids	+	-	+	-
Flavonoids	+	-	-	-
Tannins	+	+	+	+
Terpenoids	+	-	-	-
Glycosides	+	+	+	+
Sterol	+	-	-	+
Phenolics	+	-	+	_

The total phenolic content of the methanol extract *P. boergeseniiw*as determined by performing Folin-Ciocalteau reagent method spectrophotometrically. The phenolic content present in the extract was found to be 84.96±0.40 mg/g expressed as Gallic acid equivalent. The total flavonoid content of the extract *P.boergeseniiw*as estimated by aluminium chloride method spectrophotometrically. The flavonoid content present in the extract was found to be 85.42±0.97 mg/g expressed as Quercetin equivalent.The total antioxidant activity of *P. boergeseniiw*as determined using the Phosphomolybdenum method. It was found that the antioxidant activities increased with the increasing concentration. Higher antioxidant activity was exhibited at a concentration of 1000 µg/mL is (58.5 ± 0.48) % as shown in the figure 1.

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., April - June 2014, 6 (2): 110-119

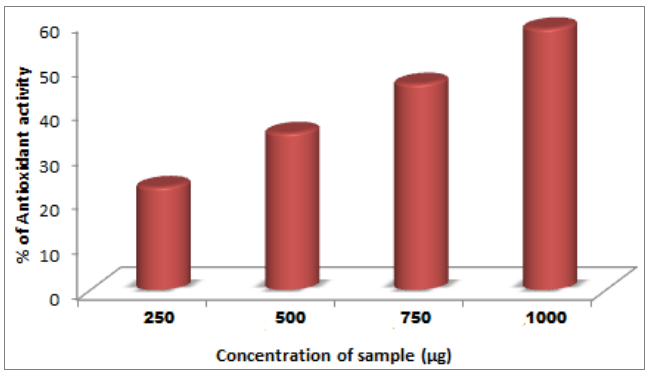

Fig.1 Total Antioxidant activity

The reductive capability of the methanol extracts of *P.boergesenii* the reductionof ferric to ferrous iontransformation. Reducing power assaywas an electron donor and it terminates the oxidation chainreaction by reducing the oxidized intermediates in to the stable form (19). Increasing absorbance indicates the increasing reducing power. The reducing power increased with the increase in concentration.as shown in the figure 2.

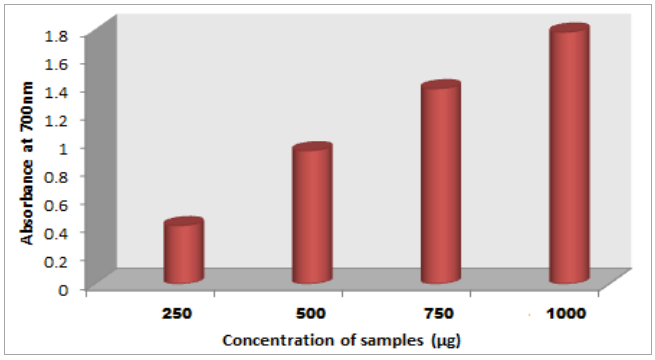

Fig. 2 Reducing power

The DPPH assay is a quick and cost effective method which has frequently been used for the estimation of the ant oxidative potential of different natural products. It shows that by using DPPH radical scavenging assay methanol extracts of brownalga *P. boergesenii* exhibited potent antioxidant activity in a dose-dependent manner. It also reveals that the methanol extract of *P. boergesenii* also contains a high amount of phenolic compounds. The higher scavenging activity of *P. boergesenii* may be attributed to hydroxyl groups in the phenolic compounds, which might provide the essential component (20). It was found that the scavenging effect increased with the increase in concentration of the sample. The value obtained was 59.70 ± 0.21 % at 1000 µg/ml.

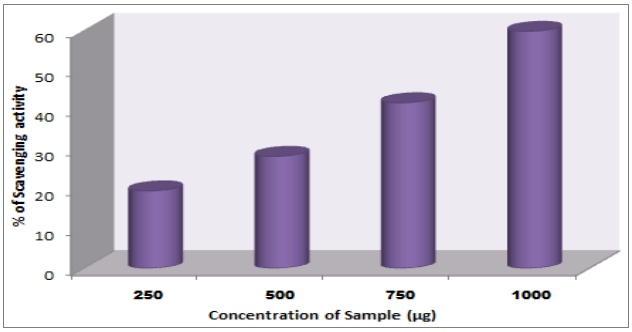

Fig. 3 DPPH Radical scavenging activity

Nitric oxide plays a major role in promoting inflammatory response and the toxicity when they react with oxide radicals to form peroxynitrite which can damage the biomolecules. Nitric oxide generates when sodium nitroprusside reacts with oxygen to form nitrite. Seaweeds reduce the formation of nitrite by competing with oxygen to react with nitric oxide (21). The scavenging activity of *P. boergesenii* was found to be 54.3 ± 0.34 % at a maximum concentration of 1000 µg/ml as shown in the figure 4.

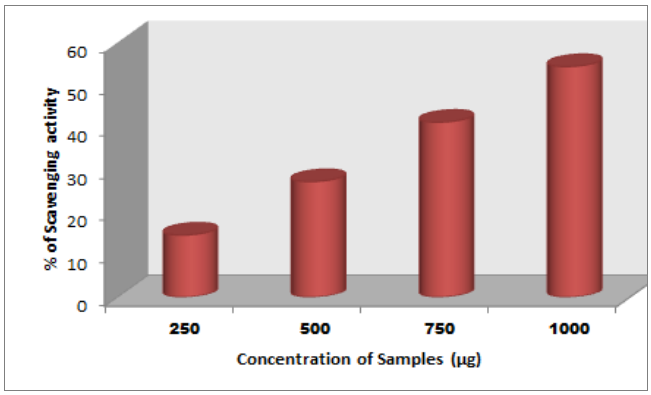

Fig. 4 Nitric oxide scavenging activity

Marine algae have been used from ancient times in Chinese herbal medicine for the treatment of cancer (22).The activity againstcancer cell lines is one of the major activities of marine algae, and many algae have showed cytotoxic and antitumor activities (23). The viability of Hep G_2 liver cancer cell line treated with the methanolic extract of *P. boergesenii* wasdetermined by MTT assay. It has shown the strong cytotoxic activity as shown in the table 3 and the IC_{50} value was found to be 1.67 mg/ml.

Table 3: Results of Cytotoxic activity on Hep G2cell lines treatment with different concentration of methanol extract of *P. boergesenii.*

Concentration	Mean Absorbance	Sample-Blank	% Inhibition
5 mg/ml	0.296	0.281	86.9
2.5mg/ml	0.425	0.401	81.1
1.25 mg/ml	1.986	1.971	11.9
1.0 mg/ml	1.852	1.842	17.8
500 µg/ml	1.787	1.773	20.7
250 µg/ml	1.717	1.759	24.0
200 µg/ml	1.617	1.602	28.2
150 µg/ml	1.649	1.631	26.8
100 µg/ml	1.751	1.739	22.3
50 µg/ml	1.972	1.956	12.5
25 µg/ml	2.020	2.015	10.3
12.5 µg/ml	2.067	2.052	8.3
6.25 µg/ml	2.061	2.041	8.5
3 µg/ml	2.050	2.033	9.1
1.5 µg/ml	2.161	2.151	4.1
0.75 µg/ml	2.124	2.108	5.7
0.375 µg/ml	1.969	1.951	12.7

Discussions

Recent decades, seaweeds have been considered as a rich source of reactive oxygen species (ROS) inhibitors and can be used as additives in food and also provide a protection against oxidative damage in tissue induced by ROS(24). The present study has shown that methanol extract of *P. boergesenii* possess antioxidant activity and potential to scavenge free radicals. Few evidences are also available that seaweeds have certain bioactive compounds with a high antioxidant and anti-proliferative activity. Seaweeds contain some bioactive compound which is not found in the terrestrial plants (25).

Antioxidant compounds has an ability to scavenge free radicals leads to the reduces the level of risk associated with oxidative stress related disease (26). Seaweeds have been attracted attention as a rich natural antioxidant source due to the toxic and mutagenic effects caused by the synthetic antioxidants (27).

This method used for the analysis of phenolic content, flavonoid content, antioxidant activity and scavenging assays such as DPPH, Nitric oxide and reducing potential refers as a convenient and accurate method for the detection of potential source of antioxidant compounds (28).The total phenolic content of *P. boergesenii*was found to be84.96±0.40mg gallic acid equivalents/g extract.The total flavonoid content of *P. boergesenii* was found to be 85.42±0.97 mg/g expressed as Quercetin equivalent. Phenolic compounds are having potential to acts as free radical scavengers. It's commonly found in the edible marine algae in which the antioxidant property has been correlated to their phenolic content (29). Previous studies were reported phenolic compounds play an important role in preventing the oxidative damage to tissue (30). The relationship between the total phenols and antioxidant activity has been observed in many seaweed species (31-32). Flavonoid acts as an antioxidant and free radical scavenger due to its unique chemical structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are very important for their antioxidant and free radical scavenging activities (33). Hence, the potential

scavenging activity of the *P. boergeseniic*ould be linked to the phenolics and flavonoids present in it.

Total antioxidant activity of *P. boergesenii* was determined by the Phosphomolybdenum method. The results observed that *P. boergeseniih*ave the maximum antioxidant activity of (58.5 ±0.48) %. Oxidative damage within the tissues is a prolonged process involving free radical chain initiation and propagation steps (34). One of the mechanisms by which antioxidants bring about their action is by scavenging free radicals (35). So, it becomes necessary to assess the scavenging ability of the brown alga *P. boergeseniie*xtract. Reducing potential of methanol extracts of *P. boergeseniiw*as evaluated usingpotassium cyanoferrate. The reducing ability of a compound widely varies based on the presence of reductones, which have exhibit antioxidative potential by breaking the free radical chain by donating a hydrogen atom (36). The reducing potential of the extracts is a significant indicator of antioxidant and scavenging activity. Similar reports have also been suggested by Kumaran and Karunakaran (37), Kumar*et al.*, (38), Vijayabhaskar (3) in methanolic extracts of higher plants, brown seaweeds from India.

In this study, DPPH, Nitric oxide free radical assays were done for the evaluation of scavenging potential of methanol extract of *P. boergesenii*. 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) is stable nitrogen centered free radical which can be effectively scavenged by antioxidants (39-40). Hence, it has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors (41).The present study has shown that the *P. boergesenii* extract has exhibited maximum DPPH scavenging activity (59.70 ± 0.21) % at 1000 µg/ml. It indicates the hydrogen donating ability of *P.boergesenii*.Since the effect of antioxidant on DPPH might be due to their hydrogen donating capability (42). Nitric oxide radicals plays an vital role in inducing inflammatory response and their toxicity increases when they react with O_2 radicals to form peroxynitrite, which leads to the damage in biomolecules such as proteins, nucleic acids, lipids. (21). Nitric oxide is generated when sodium nitroprusside reacts with oxygen to form nitrite. The methanolic extract of *P.boergeseniih*as exhibited higher scavenging activity as (54.3 ± 0.34) % at 1000 µg/ml. These results suggest that *P. boergesenii* could be a capable and novel therapeutic agent for scavenging of NO^- and the regulation of pathological conditions caused by excessive generation of NO^- and its oxidation product peroxynitrite. It is believed that the antioxidant activity of phenolics is a result of their ability to act as reducing agents, free radical scavengers (43). It may be possible that the antioxidant activity of *P.boergesenii* could be the result of their high concentration of phenolic compounds.

The cytotoxic activity of the *P.boergesenii's* methanol extract against Hep G2 liver cancer cell line was studied and the IC_{50} was found to be 1.67 mg/ml. Brown algae represent a rich source of polysaccharides and glycosides and phenolics, the activity could be connected with these compounds. *P. pavonica* aqueous extract showed cytotoxic activity against breast cancer cell line *in vitro* (44).

Conclusion

In the present study, the extracts of *P. boergesenii* showed high antioxidant activity as

well as cytotoxic activity to the best of our knowledge. Very few works have been carried out with this seaweed from the region of Gulf of Mannar, Rameswaram. Hence, *P. boergesenii* can be used as a potential therapeutic intervention due to its bioactive properties.

Acknowledgement

We are thankful to the authority of VIT University for the facilities and their constant support.

References

1) Rasmussen RS, Morrissey MT. Marine biotechnology for production of food ingredients. Advances in Food and Nutrition Research 2007; 52:237–292.
2) Abdala Diaz RT, Cabello-Pasini A, Perez-Rodriguez E, Conde Alvarez RM, Figueroa FL. Daily and seasonal variations of optimum quantum yield and phenolic compounds in *Cystoseiratamariscifolia*(Phaeophyta). Marine Biology 2006; 148: 459-465.
3) Vijayabaskar P and Shiyamala V. Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J. Agardh, 1848. Asian Pacific Journal of Tropical Biomedicine 2012; 90-98.
4) Holt S. Seaweed for healing and weight loss. Natural. Benefit News 2008;2: 1-4.
5) Bhaskar N and Miyashita K. Lipid composition of *Padina tetratomatica*(Dictyotales, Phaeophyta), brown seaweed of the west coast of India. Indian Journal of Fisheries 2005; 52: 263-268.
6) Hosokawa M, Wanezaki S, Miyauchi K, Kurihara H, Kohno H, Kawabata J, Odashima S, Takahashi K. Apoptosis inducing effect of fucoxanthin on Leukemia cell lines Hl 60. Food Science Technology Research 1999; 5: 243-246.
7) Rodríguez-Bernaldo de Quirós A, Frecha FS, Vidal PA, López HJ. Antioxidant compounds in edible brown seaweeds, European Food Research and Technology 2010; 231, 495–498.
8) Dawczynski C, Schubert R, JahreisG. Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chemistry 2007; 103: 891-899.
9) RajamaniKarthikeyanST, Somasundaram T, Manivasagam T, Balasubramanian T, Anantharaman P. Hepatoprotective activity of brown alga *Padina boergesenii* against CCl_4 induced oxidative damage in Wistar rats. Asian Pacific Journal of Tropical Medicine 2010; 696-701.
10) Chiheb I, Hassane R, Martinez – L. Joes, Francisco DSJ, Antonio GVJ, Hassan B. Mohamed K. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. African Journal of Biotechnology 2009; 8(7): 1258 – 1262.
11) Harborne JB.Phyto-chemical methods. London: Chapman and Hall;1998.
12) Senevirathene MS, Hyun Kim N, Siriwardhana J, Hwan HK, Wan L, Jin Jeon Y. Antioxidant potential of *Eclinia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. Food and Science Technology International 2006; 12: 27-38.
13) Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Jafari M. Free Radical Scavenging Activity and Antioxidant capacity of *Eryngium caucasiumtrautv* and *Froripiasubpinna*.Pharmacologyonline 2008; 3: 19-25.
14) Prieto P, Pineda M, Aguilar MM, Spectrophotometric quantification of antioxidant capacity through the formation of a Phosphomolybdenum complex; specific application to the determination of vitamin E. Analytical Biochemistry1999; 269: 337-341.
15) Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition 1986; 44: 307-15.

16) Mensor LL, Menezes FS, Leitao GG, Reis AS, Dos Santos TC, Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotheraphy Research 2001; 15:127-130.

17) Gulcin I. Antioxidant and antiradical activities of L- Carnitine. Life Sciences 2006; 78: 803-811.

18) KeivanZandi, SaeedTajbakhsh, IrajNabipour, Zahra Rastian, ForoughYousefi , SaminSharafian, KohzadSartavi. 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(40): 6787-6790.

19) Chung Y, Chang C, Chao T, Lin CF, Chou ST. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis*. Journal of Agriculture and Food Chemistry 2002; 50: 2454-2458.

20) Palanisamy Senthil Kumar, SellappaSudha. Evaluation of Antioxidant Activity and Total Phenolic Content of *Padina boergesenii* from Gulf of Mannar.Drug Invention Today 2012; 4(12): 635-639.

21) Singh G, Maurya S, De Lampasona MP, Catalan C. Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract. Food Chemistry 2006; 17: 745-75.

22) Yamamto I, Takahashi M, Tamura E, Maruyama H, Mori H. Antitumor activity of edible marine algae: Effect of crude fucoidan fractions prepared from edible brown seaweeds against L-1210 leukemia. Hydrobiologia 1984;116:145-148.

23) Xu N, Fan X, Yan X, Tseng C .Screening marine algae from China for their antitumor activities. Journal of Applied Phycology 2004;16:451-456.

24) Alekseyenko TV, Zhanayeva SY, Vendiktova AA, Zvyagintseva TN, Kuznetsova TA, Besednova N. Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk sea *Fucusevanescens* brown alga. Bulletin Experimental Biology and Medicine 2007; 147: 730-732.

25) Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Science Technology 2008; 41: 1067-1072.

26) Wu XJ, Hansen C. Antioxidant capacity, phenolic content, polysaccharide content of *Lentinusedodes* grown in Whey permeate based submerged culture. Journal of Food Science 2008; 73: 434-438.

27) Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R. Chemica Moldavian balm *(Dracocephalummoldavica*L) extract. Food Science and Technology 2007; 40: 239-248.

28) Stratil P, Klejdus B, Kubanhacek V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. J of Agri and Food Chemistry 2006; 54: 607-616.

29) Ganesan P, Kumar CS, Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresource Technology 2009; 99: 2717-272.

30) Yuan VY, Bone ED, Carrington FM. Antioxidant activity of Dulse (*Palmaria palmate)* extract evaluated *in vitro*. Food Chemistry 2005; 91: 485-94.

31) Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. Food Chemistry 2006; 95: 37-43.

32) Heo SJ, Park EJ, Lee KW, Jeon YJ. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresource Technology 2005; 96: 1613-1623.

33) Meenakshi S, Manicka Gnanambigai D, Tamil Mozhi S, Arumugam M, Balasubramaniam T, Total Flavanoid and in vitro Antioxidant Activity of Two Seaweeds of Rameshwaram Coast. Global Journal of Pharmacology 2009; 3: 59-62.

34) Valko M, Leibfritz D, Moncol J, Cronin M, Milan Mazura, Joshua Telser. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology 2007; 39: 44-54.

35) Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R. Chemical composition and *in vitro* antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalummoldavica* L) extract. Food Science and Technology 2007; 40: 239-248.

36) Kumaran A, Karunakaran RJ. *In vitro* antioxidant properties of methanol extracts of five *Phillanthus*species from India. LebensWissen and Technologie 2007; 40: 344-352.

37) Kumar Chandini S, Ganesan P, Bhaskar N. In vitro antioxidant activities of three selected brown seaweeds of India. Food Chemistry 2008; 107: 707-713.

38) Tao Wang, Rosa Jonsdottir, GuorunOlafsdottir. Total phenolic compounds, radical scavenging and metal cheatation of extracts form Icelandic seaweeds. Food Chemistry 2009; 116: 240-2.

39) Cumashi A, Ushakova NA, Preobrazhenskaya ME, D-Incecco A, Piccoli A, Totani L. Comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology 2007; 17(5): 541-552.

40) Jao CH, Ko WC. 1,1-Diphenyl-2- picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. Food Science 2002; 68: 430-435.

41) Porto C, Calligaris S, Celotti E, Nicoli MC. Antiradical properties of commercial cognags assessed by the DPPH test. Journal of Agriculture and food Chemistry 2000; 48: 4241-4245

42) Conforti F, Loizzi MR, Statti GA, Menichini F. Comparative radical scavenging and antidiabetic activities of methanolic extract and fractions from *Achillealigustica*. Biological and pharmaceutical bulletin 2005; 28: 1791-1794.

43) Koppenaol WH, Liebman JF. The oxidizing nature of the hydroxyl radical. A comparison with the ferryl ion (FeO^{2-}). Journal of physical chemistry 1984; 88: 99-101.

44) Tatjana P. Stanojkovic, Katarina Savikin, GordanaZdunic, ZoranKljaji, NadjaGrozdanic,Jadranka Antic.In vitro antitumoral activities of *Padina pavonia*on human cervix and breast cancer cell lines.Journal of Medicinal Plants Research 2013; 7(8): 419-424.

Article History: ----------

Date of Submission: 24-04-2014

Date of Acceptance: 29-04-2014

Conflict of Interest: NIL

Source of Support: NONE