International Journal of Microbiological Research 10 (1): 16-23, 2019
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2019.16.23

Antioxidant Activities of Sulfated Polysaccharide Obtained from Green Seaweed *Ulva lactuca* L. in Tuticorin Coast, Gulf of Mannar, South East Coast of India

[1]S. Palpperumal, [1]B. Harinathan, [1]S. Sankaralingam, [2]T. Shankar, [3]S. Mahendran, [1]D. Kathiresan and [4]N. Sivakumar

[1]PG and Research Department of Botany, Saraswathi Narayanan College, Madurai, Tamilnadu, India
[2]Department of Microbiology,
Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tamilnadu, India
[3]Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India
[4]Department of Molecular Microbiology, School of Biotechnology,
Madurai Kamaraj University, Madurai, Tamilnadu, India

Abstract: To evaluate the *in vitro* antioxidant activities of sulfated polysaccharide from *Ulva lactuca* in Tuticorin coast, Gulf of Mannar, South east coast of India. The *in vitro* antioxidant activities of sulfated polysaccharide were determined using total antioxidant capacity, reducing power, Hydrogen peroxide, DPPH radical scavenging assay. Sulfated polysaccharide 5.5 ± 0.6% was extracted from green seaweed *Ulva lactuca* through ethanol precipitation and purified through DEAE cellulose column and sephadex G -50. The sulfated polysaccharide extracted from *Ulva lactuca* exhibits 65.71 ± 0.28% carbohydrate, 23.92± 0.32% sulfate and 5.98 ± 0.19% uronic acid. The Total antioxidant and DPPH activity of sulfated polysaccharide was 79.97 ± 0.13% and 75.32 ± 0.31% respectively. The Hydrogen peroxide scavenging ability and reducing power of sulfated polysaccharide was 78.17 ± 0.39% and 1.71 ± 0.21% respectively. These results indicate the potential *in vitro* antioxidant activity of sulfated polysaccharide obtained from *Ulva lactuca.* This may also justify the frequent use natural antioxidants in a variety of food products.

Key words: *Ulva lactuca* · Seaweed · Polysaccharide · Antioxidant · DEAE Cellulose · Sephadex

INTRODUCTION

In recent years, marine resources have engrossed attention in the search for bioactive compounds to develop new drugs and healthy foods [1, 2]. Seaweed constitutes a commercially important renewable resource. *Sargassum*, *Padina*, *Dictyota* and *Gracilaria* sp. can be used as fertilizers, food additives and animal feed [3]. Although seaweeds acquire wide application in food and in the pharmaceutical industry, the antioxidant activities of many types of seaweed in the South Indian coastal area are still unexplored. Moreover, sulfated polysaccharides from marine algae are known to exhibit many biological and physiological activities including anticoagulant, antiviral, antitumor, anti-inflammatory and antioxidant [4-7]. Most of these bioactive substances isolated from marine algae are chemically classified as brominates, aromatics, nitrogen-heterocyclic, nitro sulphuric heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides [8]. The polysaccharides are important component of algae [9]. The immense interest in them is because of their broad spectrum biological activity. Antioxidants play an important role in inhibiting and scavenging radicals and thus providing protection to humans against infections and degenerative diseases. A number of marine algae were reported to possess antioxidant properties [10].

The antioxidant activity of sulfated polysaccharides has been investigated by different *in vitro* methods, including hydrogen per-oxide, superoxide anion and hydroxyl radical scavenging assays [11]. DPPH radical scavenging assay is recurrently used for the analysis of

Corresponding Author: S. Sankaralingam, PG and Research Department of Botany,
Saraswathi Narayanan College, Madurai-22, Tamilnadu, India.

food and substances obtained from natural sources. The most commonly used antioxidants at the present time arebutylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propyl gallate (PG) and tertbutylhydroxytoluene (TBHQ). However, most of the antioxidants used have been suspected of being responsible for liver damage and carcinogenesis. Thus, it is essential to develop and utilize effective natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases. There is ample evidence that reactive oxygen species (ROS) generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis and coronary heart diseases. To overcome these problems a wide range of synthetic antioxidants (butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propyl gallate (PG) and butylatedhydroquinone) have been used as food preservatives [12]. However, these synthetic antioxidants have side effects such as liver damage and are suspected to be mutagenic and neurotoxic. Hence, most consumers prefer additive-free foods or a safer advance like the utilization of more effective antioxidants of natural origin [13]. Recently, various phytochemicals like polyphenols, which are widely circulated in plants, have been reported to act as free radical scavengers [14].

The main objective of the present study is to evaluate the antioxidant activity of sulfated polysaccharide obtained from green seaweed (*Ulva lactuca*) Tuticorin coast, Gulf of Mannar, South east coast of India.

MATERIALS AND METHODS

The green algae, *Ulva lactuca* was collected from Tuticorin coast (Lat 08°45'; Long78°12'E), Gulf of Mannar, South east coast of India. Gulf of Mannar is a marine biosphere which harbour biodiversity of global significance and unique for coral reef, seaweed and sea grass ecosystems.

Extraction of Crude Polysaccharide: The polysaccharide from the green seaweed *Ulva lactuca* was extracted by the method followed by Subash *et al*. [15]. About, 100g of dried seaweed powder was extracted with three volumes of water at 90-95°C for 16 hrs. The brown coloured syrup was then filtered through Whatman No. 3 filter paper and concentrated to 1/4th of the original volume; it was cooled and precipitated with three volumes of ethanol overnight at 4°C. The obtained precipitate was collected by centrifugation and the pellet dehydrated with diethyl ether to yield 10% green crude polysaccharide (10% yields).

Purification of Polysaccharide: The crude polysaccharide was further purified by column chromatography. Fifty milligrams of crude polysaccharide was dissolved in 10 ml of distilled water, it was applied to a DEAE cellulose column (3×45 cm) pre-equilibrated with water and eluted in NaCl gradient (0-3 M) until no carbohydrate is detected. Each fraction was assayed for carbohydrates content by phenol sulfuric acid method of Dubois *et al*. [16]. The Carbohydrate-positive fractions were pooled together and dialyzed (MWCO 14, 000) for 24 hours against distilled water and then lyophilized.

Analysis of Biochemical Composition: The total carbohydrate content was estimated calorimetrically by the phenol-sulfuric acid method using d-glucose as a standard [16]. Sulfate content was determined by barium chloride gelatin method according to the procedure of Lloyd *et al.* [17]. The carbazole reaction which is the most satisfactory method for estimating uronic acid was employed for quantification [18]. The glucuronolactone was used as a standard. The results were expressed as % for sulfated polysaccharide.

Free Radical Scavenging Activity of Polysaccharide

Determination of Total Antioxidant Capacity (TAC): Total antioxidant activity of seaweed polysaccharide was determined according to the method of Prieto *et al.* [19]. Briefly, 0.3 ml of sample was mixed with 3.0 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min under water bath. Finally the antioxidant property of the sample was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

Determination of Reducing Power: Reducing power of the polysaccharide was determined by following the method of Yamaguchi *et al.* [20]. Briefly, 4 ml of reaction mixture, containing samples of different concentration in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1% w/v) at 50°C for 20 min. The reaction was terminated by TCA solution (10% w/v). The solution was then mixed with distilled water and ferric chloride (0.1% w/v) solution and the absorbance was measured at 700 nm.

Hydrogen Peroxide Scavenging Assay: The free radical scavenging activity of the polysaccharide was determined by hydrogen peroxide assay Gulcin *et al*. [21]. Hydrogen peroxide (10 mM) solution was prepared in phosphate buffered saline (0.1M, pH 7.4). 1ml of the extract containing samples of different concentration (100, 250, 500, 750 and 1000 µg) was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer after 10 min of incubation at 37°C against a blank (without hydrogen peroxide). The percentage of scavenging of hydrogen peroxide was calculated using the formula;

Percentage scavenging ($H_2 O_2$) = ((A_o-A_1) / A_o) x 100

A_o - Absorbance of control; A_1 - Absorbance of sample

DPPH Radical Scavenging Assay: The free radical scavenging activity of polysaccharide was measured by 1-1- Diphenyl-2-picryl-hydrazyl (DPPH) following the method of Blois [22]. DPPH was used as a reagent which evidently offers a convenient and accurate method for titrating the oxidizable groups of natural (or) synthetic antioxidants. 0.1mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3ml of seaweed extracts of different concentration (100, 250, 500, 750 and 1000 µg). After 10 minutes, absorbance was measured at 517 nm. The percentage scavenging activity values were calculated using following formula:

Percentage of Scavenging = ((A_o-A_1) / A_o) x 100

where A_o is absorbance of control and
A_1 is absorbance of sample turbidity factor

RESULTS

Analysis of Biochemical Composition of the Purified Sulfated Polysaccharide: Biochemical composition of the purified polysaccharide from *Ulva lactuca*was determined as carbohydrate content (65.71± 0.28%), sulfate content (23.92± 0.32%) and uronic acid content (5.98± 0.19%) (Fig. 1).

Free Radical Scavenging Activity of Sulfated Polysaccharide

Total Antioxidant Activity: The total antioxidant capacity of polysaccharide from *Ulva lactuca*was measured by phosphomolybdenum method. The antioxidant activities increased with increasing concentration of the sample. At the concentration of 1000 µg/ml, the polysaccharide of *Ulva lactuca* exhibited higher antioxidant activity (79.97 + 0.13) % as compared with the standard, ascorbic acid (Fig. 2).

DPPH Radical Scavenging Activity: The effect of seaweed extracts and standard on DPPH radical was compared and shown in Figure 3. The scavenging effect increases with the concentration of standard and samples. At 1000 µg/ml concentration, *Ulva lactuca* possessed (75.32+ 0.31) % scavenging activity on DPPH. All the concentration of *Ulva lactuca* showed higher activity than the standard gallic acid.

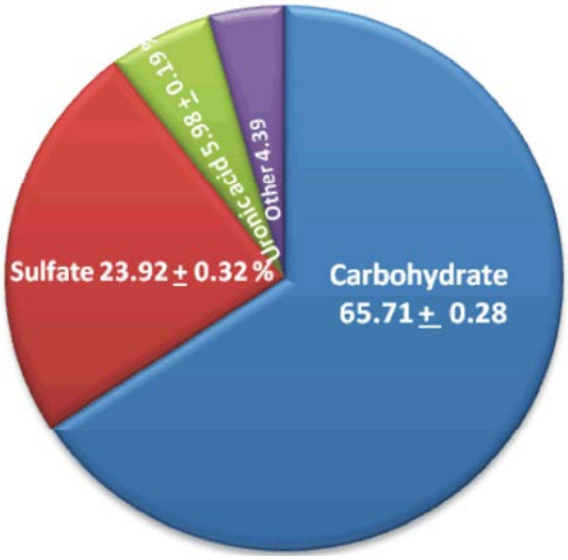

Fig. 1: Analysis of biochemical composition

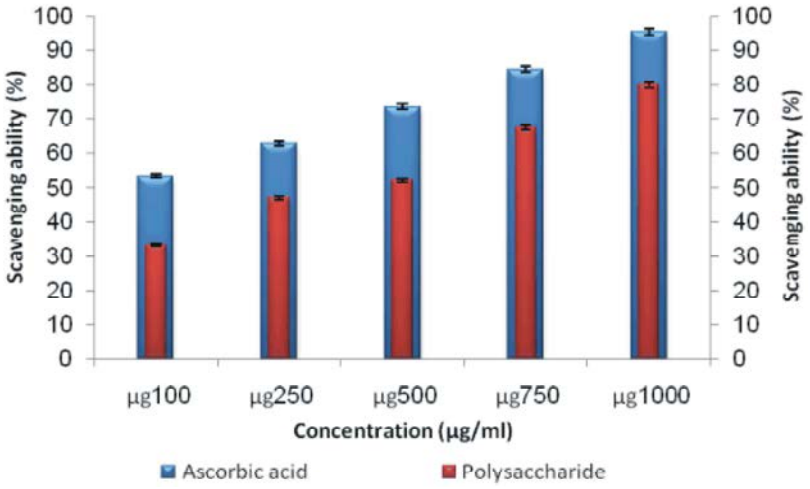

Fig. 2: Total antioxidant activity of *Ulva lactuca* extract compared with standard Ascorbic acid

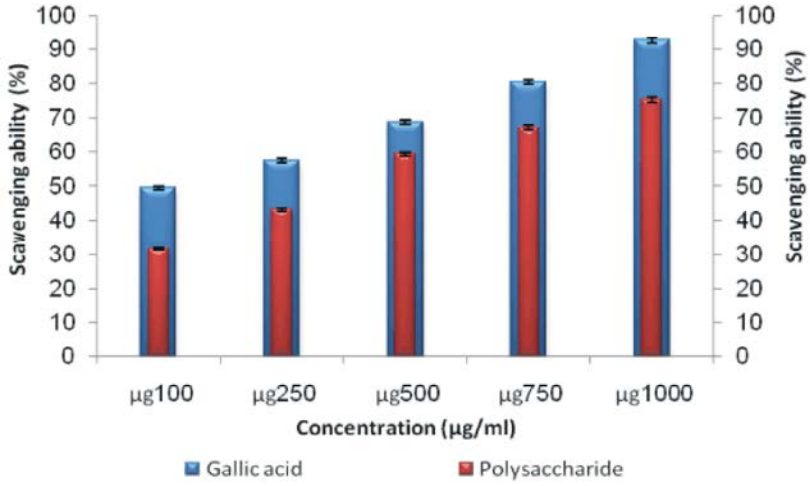

Fig. 3: DPPH radical scavenging activity of *Ulva lactuca* extract compared with standard Gallic acid

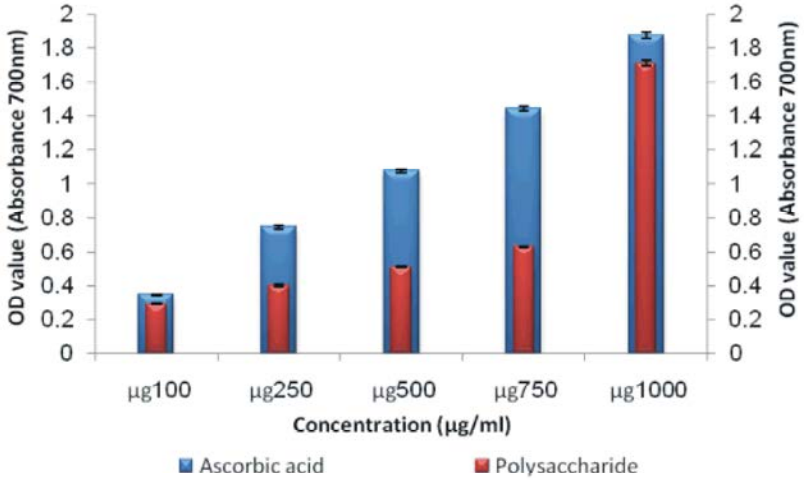

Fig. 4: Reducing power of *Ulvalactuca*extract compared with standard Ascorbic acid

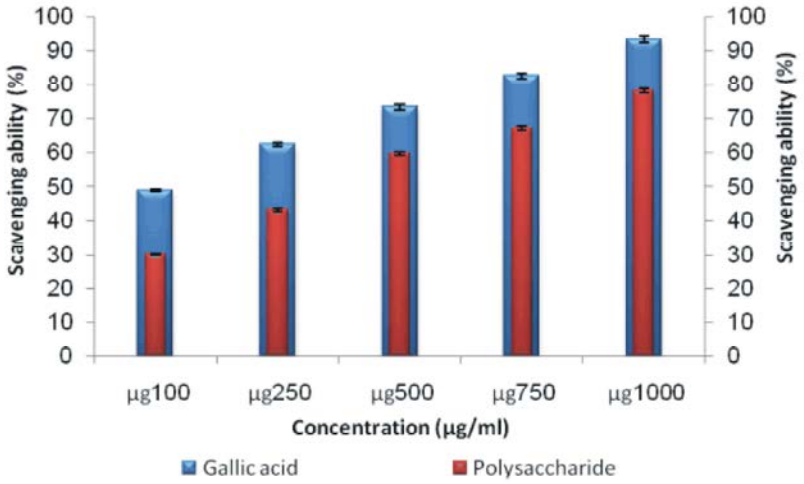

Fig. 5: Hydrogen peroxide scavenging activity of *Ulva lactuca* extract compared with standard Gallic acid

Reducing Power: The reducing power of *Ulva lactuca* was compared with the standard ascorbic acid. The reducing power increases with the increasing concentration. The reducing power of the samples was shown in Fig. 4 and it was found to be in the following order: ascorbic acid (0.34± 0.18) - (1.87± 0.16), *Ulva lactuca* (0.29± 0.11) - (1.71± 0.21).

Hydrogen Peroxide Scavenging Assay: The hydrogen peroxide scavenging effect increased with the concentration of standard and samples. The polysaccharide from *Ulva lactuca* possessed (78.17± 0.39%) scavenging activity. The hydrogen peroxide scavenging effect of samples is shown in Fig. 5.

DISCUSSION

The antioxidant activity of several naturally occurring compounds has been known for decades. Recently, many types of seaweed have been measured as source of reactive oxygen species inhibitors. They can be used as food additives and can also provide protection against tissue oxidation [23]. The present investigation has also proved that seaweed sulfated polysaccharide (*Ulva lactuca*) possess antioxidant activity to scavenge free radicals. Dietary natural antioxidants are reported to help in preventing aging and other diseases. There are some evidences that seaweeds contain compounds with a relatively high antioxidant activity. Seaweeds are low in fat but contain vitamins and bioactive compounds like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants [24].

In the present investigation *Ulva lactuca* consisted total carbohydrate content (65.71%), sulfate content (23.92%) and uronic acid content (5.98%) of the polysaccharide determined. Ye *et al.* [25] reported that chemical composition of the purified water soluble polysaccharide from *Capsosiphon fulvescens* composed of neutral sugars (49.8%), sulfate (5.7%) and uronic acid (4.8%). Nirmal *et al.* [26] reported the carbohydrate content of green seaweeds *Cladophora fascicularis* (43.4%), *Caulerpa racemosa* (41.0%) and *Ulva lactuca* (36.2%). Similarly, Vriesmann *et al*. [27] found that the uronic acid content increases with decreasing extraction pH; furthermore, uronic acid decreases when there is an increase in temperature. These results were found during the extraction of pectin from cacao pod husks.

In this study, the total antioxidant activity of sulfated polysaccharide was determined by phosphomolybdenum assay and compared with L-ascorbic acid (standard antioxidant). The results demonstrated that the isolated sulfated polysaccharide exhibited the total antioxidant activity (79.97 ± 0.131%) than L-ascorbic acid (95.45 ± 0.28%). The above finding coincides with earlier reports that the total antioxidant activity of isolated fucoidan shows somewhat similar activity to the fucoidan isolated from *Padina tetrastomatica*, *Turbina riaconoides* and *Sargassum tenerrimum* [28, 29].

Reducing power assay is one of the important antioxidant assays that provide significant reflection on the antioxidant activity. In this assay, the bioactive compound serves as an electronic donor that reduces the oxidized intermediates of lipid per oxidation, so is that can be performed as a primary and secondary antioxidant. The presence of antioxidant properties of the Fe^{3+} ferricyanide

complex was reduced and changed into ferrous form. In the present study, the polysaccharide exhibited the maximum reducing power activity at 1.71+0.21. However, the reducing power activity of standard L-ascorbic acid was 1.873 ± 0.27%. Captivatingly, the reducing power scavenging activity of the extracted fucoidan was higher compared to the previous report of *Hypnea valentiae* (42.63% at 50 mg/ml) [30].

The extent of H_2O_2 scavenging activity is one of the useful methods for determining the ability of antioxidants to decrease the level of pro-oxidants [31]. In the present study hydrogen peroxide scavenging activity of polysaccharide of *Ulva lactuca* was found to be 78.17±0.396%. Antonio *et al.* [32] reported that the H_2O_2 production by *Caulerpa taxifolia* was significantly higher (52%).

DPPH was widely used to examine the free radical scavenging activity of compounds. In this assay, the reduction of DPPH into DPPH-H was measured based on the ability of antioxidant compounds to lose hydrogen [33, 34]. In this study, the DPPH radical scavenging activity of fucoidan was determined by comparing with standard gallic acid and the obtained results with highest scavenging activity that was 75.32 ± 0.31% and 92.63± 63%, respectively. The DPPH scavenging activity was significantly higher than the previously accounted on fucoidan of *Laminaria japonica* [35]. Subash *et al.* [15] accounted that the total antioxidant ability of crude polysaccharide (TCP) from a brown alga *Turbina riaornata*was compared with Trolox standard.

CONCLUSION

The sulfated polysaccharide from green alga *Ulva lactuca* was effective in *in vitro* condition as an antioxidant activity. These results recommend that *Ulva lactuca* sulfated polysaccharide is a promising agent to be estimated for the application in the natural antioxidants in a variety of food industry, pharmaceutical industries and that it presents a significant antioxidant activity. Future work may focus on the biological activities such as immune modulation, antitumor and anti-inflammatory activities.

REFERENCES

1. Qi, H., T. Zhao, Q. Zhang, Z. Li and Z. Zhao, 2005. Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa* Kjellm (Chlorophyta). Journal of Applied Phycology, 17: 527-534.
2. Palanisamy, S., M. Vinosha, T. Marudhupandi, P. Rajasekar and N. Marimuthu Prabhu, 2017. *In vitro* antioxidant and antibacterial activity of sulfated polysaccharides isolated from *Spatoglos sumasperum*. Carbohydrate Polymers, 170: 296-304.
3. Abdala Diaz, R.T., A. Cabello-Pasini, E. Perez-Rodriguez, R.M.Conde Alvarez and F.L. Figueroa, 2006. Daily and seasonal variations of optimum quantum yield and phenolic compounds in *Cystoseira tamariscifolia* (Phaeophyta). Mar. Biol., 148: 459-465.
4. Becker, C.F., J.A. Guimaraes, P.A.S. Mourao and H. Verli, 2007. Conformation of sulphated galactan and sulphated fucan in aqueous solutions: implications to their anticoagulant activities. Journal of Molecular Graphics and Modelling, 26: 391-399.
5. F-Tischer, P.C.S., L.B. Talarico, M.D. Noseda, S.M.P.B. Guimaraes and M.E.R. Duarte, 2006. Chemical structure and antiviral activity of carrageenans from Meristiellagelidium against herpes simplex and dengue virus. Carbohydrate Polymers, 63: 459-465.
6. Souza, M.C.R., C.T. Marques, C.M.G. Dore, F.R.F. Silva, H.A.O. Rochaand and E.L. Leite, 2007. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. Journal of Applied Phycology, 19: 153-160.
7. Ye, H., K. Wang, C. Zhou, J. Liu and X. Zeng, 2008. Purification, antitumor and antioxidant activities *in vitro* of polysaccharides from the brown seaweed *Sargassum pallidum*. Food Chem., 111: 428-432.
8. Oktay, M., I. Gulcin and O. Kufrevioglu, 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Leben Wissen and Technologie, 36: 263-271.
9. Hanan, M., A. Khairy and Mohamed A. El-Sheikh, 2015. Antioxidant activity and mineral composition of three Mediterranean common seaweeds from Abu-Qir Bay, Egypt. Saudi Journal of Biological Sciences, 22: 623-630.
10. Yasantha, A., K.N. Kimand and Y.J. Jeon, 2006. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown algae *Ecklonia cava*. Food Chem. Toxicol., 44: 1065-1074.
11. Mahendran, S. and S. Saravanan, 2013. Purification and *in vitro* antioxidant activity of Polysaccharide isolated from green seaweed *Caulerpa racemosa*. Int. J. Pharm. Bio. Sci., 4(4): 1214-1227.

12. Seedevi, P., M. Moovendhan, S. Sudharsan, S. Vasanthkumar, A. Srinivasan, S. Vairamani and A. Shanmugam, 2015. Structural characterization and bioactivities of sulphated polysaccharide from *Monostroma oxyspermum*. International Journal of Biological Macromolecules, 72: 1459-1465.
13. Lekameera, R., P. Vijayabaskar and S.T. Somasundaram, 2008. Evaluating antioxidant property of brown alga *Colpomenia sinuosa* (Derb. Et sol). African J. Food Sci., 2: 126-130.
14. Lekameera, R., P. Vijayabaskar and S.T. Somasundaram, 2007. Potential antioxidant activity of brown alga *Loboohora varie*. Seaweed Res. Utiln, 29(1&2): 55-61.
15. Ananthi, S., H.R.B. Raghavendran, A.G. Sunil, V.G.G. Ramakrishnan and H.R. Vasanthi, 2010. *In vitro* antioxidant and *in vivo* anti-inflammatory potential of crude polysaccharide from *Turbinaria ornate* (Marine Brown Alga). Food and Chemical Toxicology, 48: 187-192.
16. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Analytical Chem., 28: 350-356.
17. Lloyd, A.G., K.S. Dodgson, R.G. Price and F.A.I. Rose, 1961. Polysaccharide sulphates. Biochimica Biophysica Acta, 1: 108-115.
18. Bitter, T. and H.M. Muir, 1962. Analytical Biochem., 4: 330-334.
19. Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry, 269: 337-341.
20. Yamaguchi, T., H. Takamura, T. Matoba and J. Terao, 1998. HPLC method for evaluation of the free radical - scavenging activity of foods by using 1, 1-Diphenyl - 2 - Picrylhydrozl. Biosci. Biotechnal. Biochem., 62: 1201-1204.
21. Gulcin, T., K.O. Irfan, M. Kufrevioglu, M. Oktayand, M.E. Buyukokuroglu, 2004. Antioxidant, antimicrobial, antiuclcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol., 90: 205-215.
22. Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.
23. Alekseyenko, T.V., S.Y. Zhanayeva, A.A. Vendiktova, T.N. Zvyagintseva, T.A. Kuznetsova and N. Besednova, 2007. Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk sea *Fucus evanescens* brown alga. Bull. Exp. Biol. & Med., 147: 730-732.
24. Chew, Y.L., Y.Y. Lim, M. Omarand and K.S. Khoo, 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci. Technol., 41: 1067-1072.
25. Ye, S.N., J.K. Woo, M.K. Sung, K.P. Jae, M.L. Sae, O. Sung, Ki, S. Andriyand and I.P. Yong, 2010. Purification, characterization and immunostimulating activity of water-soluble polysaccharide isolated from *Capsosiphon fulvescens*. International Immunopharmacology, 10: 364-370.
26. Nirmal Kumar, J.I., Ritankumar, K.A.M.B. Manmeet, Anubhutibora and Sudeshnachakraborty, 2010. Variation of biochemical composition of eighteen marine macroalgae collected from OKHA Coast, Gulf of Kutch, India. EJEAF Che., 9(2): 404-410.
27. Vriesmann, L.C., R.F. Teofilo and C.L. De Oliveira Petkowicz, 2011. Optimization of nitric acid-mediated extraction of pectin from cacao pod husks (*Theobroma cacao* L.) using response surface methodology. Carbohydrate Polymers, 84(4): 1230-1236.
28. Chandini, S.K., P. Ganesan and N. Bhaskar, 2008. *In vitro* antioxidant activities of three selected brown seaweeds of India. Food Chem., 107: 707-713.
29. Marudhupandi, T., T.T.A. Kumar, S. Lakshmanasenthil and K.N. Devi, 2014. *In vitro* antioxidant properties of fucoidan fractions from *Sargassum tenerrimum*. Pakistan Journal of Biological Sciences, 17: 402-407.
30. Revathi, D., K. Baskaran and R. Subashini, 2015. Antioxidant and free radical scavenging capacity of red seaweed *Hypnea valentiae* from Rameshwaram coast, Tamil nadu, India. International Journal of Pharmacy and Pharmaceutical Sciences, pp: 232-237.
31. Czochra, M.P. and A. Widensk, 2002. Spectrophotometric determination of hydrogen peroxide scavenging activity. J. Anlitica. Chem. Acta 452: 177-184.
32. Antonio Box, Antoni Sureda, Jorge Terrados, Antoni Pons and Salud Deudero, 2008. Antioxidant response and caulerpenyne production of the alien *Caulerpa taxifolia* (Vahl) epiphytized by the invasive algae *Lophocladia lallemandii* (Montagne). Journal of Experimental Marine Biology and Ecology, 364: 24-28.

33. Fukumoto, L.R. and G. Mazza, 2000. Assessing antioxidant and pro oxidant activities of phenolic compounds. Journal of Agricultural and Food Chemistry, 48: 3597-3604.
34. Marudhupandi, T. and T.T.A. Kumar, 2013. Antibacterial effect of fucoidan from *Sargassum wightii* against the chosen human bacterial pathogens. International Current Pharmaceutical Journal, 2(10): 156-158.
35. Wang, J., Q. Zhang, Z. Zhangand and Z. Li, 2008. Antioxidant activity of sulfated polysaccharide fractins extracted from *Laminaria japonica*. Int. J. Biol. Macromol., 42: 127-32.