Original Article

Antioxidant and antimitotic activities of sulfated polysaccharide from marine brown algae *Padina tetrastromatica*

Geena Mariya Jose, R. Anitha, G. Muraleedhara Kurup*

Department of Biochemistry, University of Kerala, Karyavattom, Thiruvananthapuram, Kerala, India

*Address for correspondence:
G. Muraleedhara Kurup, Department of Biochemistry, University of Kerala, Karyavattom, Thiruvananthapuram, Kerala, India. E-mail: gmkbio@gmail.com

ABSTRACT

Antioxidants play a central role in the prevention of carcinogenesis. The most natural compounds exhibit their protective effects by eliciting antioxidant potential. A sulfated polysaccharide (SPS) was isolated from the brown algae *Padina tetrastromatica*, and then purified and evaluated for its composition and *in vitro* antioxidant and antimitotic activities. Both ethanolic sulfated polysaccharide (ESPS) and ethanolic sulfated polysaccharide-column purified (ESPS-CP) exhibited considerable amount of carbohydrates (11.2% and 17.6%), sulfate (11.4% and 7.4%), fucose (5.5% and 15.7%), uronic acid (4.7% and 11.8%), xylose (0.5% and 0.03%), and SPS (2.4% and 12.7%) content. The Fourier transform infrared analysis and phytochemical screening also confirmed the presence of SPS. In the *in vitro* antioxidant activity determination using 1-1-diphenyl 2-picryl hydrazyl radical scavenging activity, hydroxyl radical scavenging activity, superoxide anion scavenging activity, hydrogen peroxide scavenging activity, total antioxidant activity and reducing power, ESPS showed more activity than ESPS-CP. In the case of nitric oxide radical scavenging, ESPS-CP was found to be a more effective. At a concentration of 2 mg/ml, both samples were potent antioxidants with significant IC_{50} values. The antimitotic studies such as mitotic index in onion root tips and sprouting assay in green gram seeds also proved that both the extracts are able to prevent mitosis. The extrapolation of these results can find opportunities in therapeutic regiments of cancer.

KEY WORDS: Antioxidant activity, mitotic index, *Padina tetrastromatica,* sprouting assay, sulfated polysaccharide

INTRODUCTION

The exploitation of natural products for developing novel drugs has been highly appreciated. Although these biochemical face certain obstacles in bioavailability and isolation, they can overcome most of the difficulties evoked by synthetic drugs and therapeutics (da Rocha *et al.*, 2001). For instance, around 60% of the anticancer drugs are of natural derivatives and several are in clinical and pre-clinical trials (Patwardhan and Vaidya, 2010). The occurrences of the vast diversity of secondary metabolites, such as alkaloids and terpinoids, make phytomedicine a better choice for the management of diseases such as cancer, diabetes and heart attack. Vincristine, etoposide, paclitaxel, and irinotecan are some of plant-derived anticancer drugs that have been benefited millions of sufferers throughout the globe (Alam *et al.*, 2014)**.**

Along with terrestrial plants, the marine vegetation has also gained impact in pharmaceuticals and therapeutics (Schwartsmann *et al.*, 2001). Seaweeds from the richest renewable source of many bioactive compounds which makes them a vital candidate in drug development (Thomas and Kim, 2013). The prosperous and diverse marine vegetation consists of many bioactive compounds such as polyunsaturated fatty acids, proteins, polysaccharides, and pigments that form the basis of novel medicinal products (Senthilkumar *et al.*, 2013). Among the seaweed-born macromolecules, the sulfated polysaccharides (SPS) from marine macroalgae are reported to possess antioxidant, anti-inflammatory, anti-allergic, anticancer, antiviral, and anticoagulant activities despite their structural and nutritional roles (Ngo and Kim, 2013). Heterofucans from brown seaweed *Dictyopteris delicatula* was reported to have anticoagulant, antioxidant, and antitumor activities (Magalhaes *et al.*, 2011). The dependence of sulfate content on the bioactivity of SPS has been well-established (Li *et al.*, 2008).

Oxidative stress, due to the overproduction of reactive oxygen species (ROS) and reactive nitrogen species, can cause damage to nucleic acids, proteins, or lipids which eventually can lead to cardiovascular and neurodegenerative diseases, Alzheimer's and cancer (Dayem *et al.*, 2010). The most destructive one among these is cancer. These highly reactive species cause impairment in replication, transcriptional and translational errors and genomic instability, all of which finally result in cancer (Ogasawara and Zhang, 2009). Antioxidants are competent in scavenging these reactive species to prevent cancer development (Bennett *et al.*, 2012). Numerous genera of marine algae, including *Colpomenia*, *Gracilaria*, *Halymenia*, *Hydroclathrus*, *Laurencia*, *Padina*, *Polysiphonia*, and *Turbinaria*, have been reported to have antioxidant property (Kelman *et al.*, 2012). SPS from brown, green and red algae were found to have antioxidant property and can form candidates for cancer therapy (Ngo and Kim, 2013; Costa *et al.*, 2011; Qi *et al.*, 2005).

The brown algae *Padina tetrastromatica,* belongs to the order dictyotales of phaeophyta phylum, is found abundant in Indian coastal areas (Mohan *et al.*, 2014). *P. tetrastromatica* was proved to be a rich source of the most essential nutrients including vitamin B1, B2, B6, and minerals such as copper, manganese, zinc, lead, etc. (Sethi, 2012). However, the anticancer potential of SPS from *P. tetrastromatica* is yet to be identified. The present study aimed on the *in vitro* determination of antioxidant potential of crude and partially purified fractions of SPS from *P. tetrastromatica*.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grade obtained from Sisco Research Laboratories, Mumbai, Maharashtra, India.

Collection of Seaweed

Samples of *P. tetrastromatica* were collected from the Vizhinjam coast of Kerala, (Lat. 8°22′N; Long. 76°59′E on the west coast of India) during the month of January-February. The Professor and Botanist Dr. M.V.N Panikkar, Department of Botany, S.N. College, Kollam, Kerala, identified the algal material and a voucher herbarium specimen (No. KUBH 5804) was kept in the herbarium of Department of Botany, University of Kerala, India.

Extraction, Isolation, and Purification of SPS

The collected *P. tetrastromatica* were cleaned in tap water and then with distilled water. The samples were dried, powdered in a blender and stored in dark within airtight containers. The SPS from the algae were extracted using cold acidic extraction method (Suresh *et al.*, 2013). Briefly, 100 g of the powdered algae was soaked in an acetone-methanol (7:3) mixture for 2 days under continuous shaking for removing pigments and other lipophilic constituents. After evaporating the solvents, 0.1N HCl added to the dried biomass left out and stirred for 24 h. This step repeated twice. The filtrates kept overnight at 4°C and precipitated by adding an equal amount of absolute ethanol. Ethanol was evaporated, and the precipitate was lyophilized in the freeze dryer (Labconco 2.5 Plus, Germany).

The SPS were purified using diethylaminoethyl (DEAE) cellulose anion exchange chromatography (Athukorala *et al.*, 2006). The crude ethanolic extract was applied to a DEAE-cellulose column (17 cm × 2.5 cm) equilibrated with 50 mM sodium acetate (pH 5.0). The SPS were eluted using sodium acetate buffer containing NaCl with graded concentrations (0.1 M, 0.5 M, 0.75 M, 1 M, 1.5 M and 2 M, respectively). 5 ml fractions were collected, and the polysaccharides content was determined by the phenol-sulfuric acid method. The fractions that showed highest values were pooled and dialyzed against distilled water for 2 days at 4°C and freeze-dried for further evaluations.

Phytochemical Screening

Test for reducing sugar
About 0.5 ml of the extract was mixed with 2 ml of an equal amount of Fehling's solution A and Fehling's solution B and the mixture was kept in a boiling water bath for 5 min. Appearance of brick red precipitate indicates the presence of reducing sugar (Wadood, 2013).

Test for flavonoids
To 1 ml of the extract few drops of 10% $FeCl_3$ was added and observed for the appearance of green or blue which indicates the presence of flavonoids (Ali Hassan and Abu Bakar, 2013).

Test for amino acids
1 ml of the extract was treated with few drops of ninhydrin reagent, heated in water bath for 1 min; purple color indicates the presence of amino acids (Ali Hassan and Abu Bakar, 2013).

Test for tannins
0.5 ml of 1% lead acetate was added to 0.5 ml of extract, and the presence of tannins is indicated by the appearance of a yellow precipitate (Ali Hassan and Abu Bakar, 2013).

Test for alkaloids

To 1 ml of test sample, few drops of con HCl and 1 ml Mayer's reagent were added and the turbidity or yellow precipitate indicates the presence of alkaloids (Ali Hassan and Abu Bakar, 2013).

Test for glycosides

Test sample was evaporated and dissolved in 1 ml glacial acetic acid. 1 drop of 10% $FeCl_3$ was added. Then 1 ml con H_2SO_4 was added through the sides. The occurrence of the brown ring at the interface indicates glycosides (Ali Hassan and Abu Bakar, 2013).

Test for steroids

The test sample was evaporated and dissolved in 2 ml chloroform, and 2 ml con H_2SO_4 was added along the sides of the test tube. Formation of a red ring indicates the presence of steroids (Ali Hassan and Abu Bakar, 2013).

Test for saponins

2 ml of distilled water and a pinch of sodium bicarbonate was added to the sample and shacked vigorously. Honey comb like appearance indicates the presence of saponins (Singh et al., 2012).

Test for terpenoids

2 ml chloroform was added to the test sample, and con H_2SO_4 was added along the sides of the test tubes. The appearance of a red ring indicates the presence of terpenoids (Wadood, 2013).

Determination of Chemical Composition

Estimation of total carbohydrates

Total carbohydrate content was estimated by the phenol–sulfuric acid method as described by Thinh et al. (2013). About 5% phenol and concentrated sulfuric acid were added to the test sample (10 mg/ml), incubated for 20 min and optical density (OD) was read at 490 nm. Dextrose was used as a standard.

Estimation of sulfate content

The sulfate content was determined by barium chloride-gelatin method using potassium sulfate as standard (Rocha de Souza et al., 2007). The reaction mixture was prepared by adding 2 g barium chloride to a solution of 0.6 g gelatin in 200 ml water which was kept overnight at 4°C. To the test solution (10 mg/ml) 4% TCA and 1 ml chloride – gelatin solution was added, and OD was read at 360 nm after 15 min incubation.

Estimation of uronic acid

Uronic acid content of the extract was estimated by carbazole method using glucuronic acid as standard

(Kumar et al., 2014). The test sample (10 mg/ml) was heated in a boiling water bath for 10 min with 0.025 M borax. Then 0.1% carbazole (in methanol) was added, and boiling was continued for 15 min. The OD was read at 540 nm.

Estimation of fucose

Fucose content was determined using cysteine hydrochloride (Sawke and Sawke, 2010). Concentrated sulfuric acid was added to the test sample (10 mg/ml) for 3 min. 3% cysteine hydrochloride was added, and the difference in the OD at 396 nm and 427 nm was calculated.

Estimation of SPSM

Total SPS were determined by metachromatic assay using heparin as standard (Liao et al., 2009). 0.005% toluidine blue solution and 0.2% NaCl were added to the test sample (10 mg/ml) and was mixed well for 30 s. Then n-hexane was added to the above mixture, and the 5 ml aqueous layer was separated. Equal volume of absolute ethanol was added and the OD was read at 631 nm.

Estimation of xylose

The monosaccharide xylose was estimated using orcinol method (Blackmore and Williams, 1974). 0.1 ml of sample (10 mg/ml) was heated in a boiling water bath for 30 min. OD was read at 670 nm.

Functional Group Analysis of Isolated Polysaccharide by infrared (IR) Spectroscopy

The functional groups present in the crude and purified samples were determined by Fourier transform infrared (FT-IR) spectral analysis. The freeze-dried samples were scanned using a KBr window using IR spectrophotometer (Nicolet 5700 FT-IR Spectrometer, Thermo Electron Corp., USA).

In Vitro Antioxidant Activity of Crude and Purified Extracts

DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity

DPPH free radical scavenging activity was determined by the method described by Garcia et al. (Manochai et al., 2010). Different concentrations of samples were prepared in ethanol. The reaction mixture for test sample consists of 0.5 ml of sample, 3 ml of absolute ethanol and 0.3 ml of DPPH radical solution (0.5 mM in ethanol). A mixture of 3.3 ml ethanol and 0.5 ml sample served as blank and 3.5 ml ethanol and 0.3 ml DPPH radical solution used as a control. Ascorbic acid at different concentrations (5-25 µg/ml) was used as the standard. All the tubes were read at 517 nm after 100 min of reaction using a

UV-VIS spectrophotometer. The DPPH radical scavenging potential of the test sample was determined by using the formula:

$$\% \text{ of scavenging} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined using Fenton reaction (Corpuz *et al.*, 2013). The reaction mixture consists of 1 mM ferric chloride, 30 mM deoxyribose, 1 mM ascorbic acid, 1 mM EDTA and 20 mM H_2O_2 in 0.2 M phosphate buffer. 0.2 ml of gradient concentrations of test samples was incubated with 1 ml of the reaction mixture at room temperature for 1 h. 0.2 ml phosphate buffer instead of the test sample was used as control and 1.2 ml phosphate buffer served as blank. After incubation 1 ml thiobarbituric acid (TBA) trichloroacetic acid (TCA) reagent (1% TBA and 15% TCA in 0.25 N HCl) was added to all the tubes and incubated in boiling water bath for 20 min. The reduction in color due to the scavenging of hydroxyl radical was read at 532 nm. Ascorbic acid at different concentrations (50-300 µg/ml) was used as the standard. The hydroxyl radical scavenging ability was determined using the formula:

$$\% \text{ of scavenging} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

Superoxide anion scavenging activity

The superoxide anion scavenging activity was determined by using nitro blue tetrazolium (NBT) reduction method (Senthilkumar *et al.*, 2013). The reaction mixture contained 0.02 ml of different concentrations of extracts, 0.05 ml of riboflavin solution (0.12 mM), 0.2 ml of EDTA solution (0.1 M) and 0.1 ml NBT solution (1.5 mM) which is finally diluted up to 2.64 ml using phosphate buffer (0.067 M). Reaction mixture devoid of sample served as control. The difference in OD at 560 nm was taken after illuminating under fluorescent light for 5 and 30 min. Quercetin (20-100 µg/ml) served as standard. The scavenging percentage was determined using the formula:

$$\% \text{ of scavenging} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was determined by using Griess reaction (Shukla *et al.*, 2012). 0.5 ml of graded concentrations of the test sample in methanol was incubated with 3 ml sodium nitroprusside (5 mM) in phosphate buffer

at 25°C for 30 min. 0.5 ml of phosphate-buffered saline (PBS) instead of test sample was used as control. After incubation 1.5 ml Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthyl ethylene diaminedihydrochloride) was added to all the tubes and the absorbance was read at 546 nm using PBS as blank. Ascorbic acid (100-500 µg/ml) was used as the standard. The percentage of scavenging was measured using the formula:

$$\% \text{ of scavenging} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

Hydrogen peroxide scavenging activity

0.5 ml of graded concentrations of the test sample in methanol was added to 0.6 ml of 40 mM H_2O_2 solution in phosphate buffer and incubated for 10 min at room temperature. 0.5 ml of phosphate buffer instead of test served as control and phosphate buffer without H_2O_2 as blank. Reduction in OD was read at 230 nm. Ascorbic acid in different concentrations (20-100 µg/ml) was used as the standard (Ramani *et al.*, 2012). Percentage of H_2O_2 scavenging was determined by the formula;

$$\% \text{ of scavenging} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

Total antioxidant activity (TAA)

The TAA was determined by using the procedure described by Do QD *et al.* (2012). 0.3 ml of different concentrations of the test sample was mixed with 3 ml of the reagent (1 ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). 0.3 ml of distilled water instead of test sample was used as blank. All the tubes were incubated at 90°C for 90 min and the absorbance was read at 695 nm. The Gallic acid in different concentrations (20-100 µg/ml) was used as the standard. TAA is expressed as equivalent of ascorbic acid.

Reducing Power

Different concentrations of test samples (1 ml) were incubated with 2.5 ml 1% potassium ferricyanide and 2.5 ml phosphate buffer (0.2 M, pH 6.6) at 50°C for 20 min. 1 ml of distilled water instead of the test was used as blank. After incubation 2.5 ml 10% TCA was added and then centrifuged at 3000 rpm for 10 min. 2.5 ml of the upper layer was then mixed with 2.5 ml distilled water, and 0.5 ml of 0.1% ferric chloride was added to all the tubes. Absorbance was read at 700 nm (Zhao *et al.*, 2014). The Gallic acid in different concentrations (20-100 µg/ml) served as blank, and the reducing power is expressed as ascorbic acid equivalent.

Determination of Antimitotic Activity

Inhibition of water imbibition and sprouting in green gram seeds

Green gram seeds of equal weight were germinated in 500 µl of graded concentrations of crude extract in a 24-well microtiter plate. After 24 h, the seeds were dried on a dry tissue paper and weighed. Seeds germinated in distilled water served as the control and that in drug doxorubicin as the standard (Kumar and Singhal, 2009). For the morphological study, the length of the radical was observed. Percentage inhibition of water imbibition was determined by the formula:

$$\% \text{ of inhibition} = \frac{(Ww\text{-}Dw)_{control} - (Ww\text{-}Dw)_{test}}{(Ww\text{-}Dw)_{control}}$$

Ww - Wet weight of green gram seeds
Dw - Dry weight of green gram seeds

Inhibition of mitosis in onion root tips

Inhibition of mitosis by crude extract was determined using *Allium cepa* (onion) roots (Saboo *et al.*, 2012). Onion bulbs were allowed to sprout in water at room temperature for 48 h. Bulbs that developed uniform roots were then dipped in different concentration of the test sample for 3 h. Distilled water was used as the control and the drug doxorubicin as the standard. After 3 h root tips were transferred to a fixing solution (acetic acid:Ethanol in the ratio of 1:3 V/V) for 10-12 h. The roots were then stained using acetocarmine stain and observed under microscope (Leica DM IL LED, Germany). Mitotic index and inhibition of mitosis were calculated as follows:

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells}}{\text{Number of total cells}} \times 100$$

$$\% \text{ of mitotic inhibition} = \frac{MI_{control} - MI_{test}}{MI_{control}}$$

Statistical Analysis

All evaluations include six samples from each group. One-way analysis of variance was calculated by using online software for statistic calculations. The $P < 0.05$ were considered to be significant (Finosh and Jayabalan, 2015; Finosh *et al.*, 2015).

RESULTS

The ethanolic sulfated polysaccharide (ESPS) obtained by ethanol precipitation was designated as ESPS and its

yield was found to be 17% (w/w). Further, purification was done using DEAE cellulose column chromatography. Column purification of ESPS showed the highest carbohydrate yield in fractions 7-11 of 0.5 M NaCl. These fractions were pooled and lyophilized to obtain ethanolic sulfated polysaccharide-column purified (ESPS-CP) and its yield was found to be 9% (w/w). The elution profile of DEAE cellulose column chromatography is shown in Figure 1. The chemical composition of ESPS and ESPS-CP are given in Table 1. The percentage of total carbohydrate was greater in purified sample than that of ethanolic one. However, the sulfate and xylose content were less in ESPS and ESPS-CP. At the same time, percentage of fucose, uronic acid, and SPS are a higher in purified sample.

FT-IR spectral analysis was used to determine the major functional groups present in ESPS and ESPS-CP (Figure 2). The broad peak around 3200-3500 indicates the presence
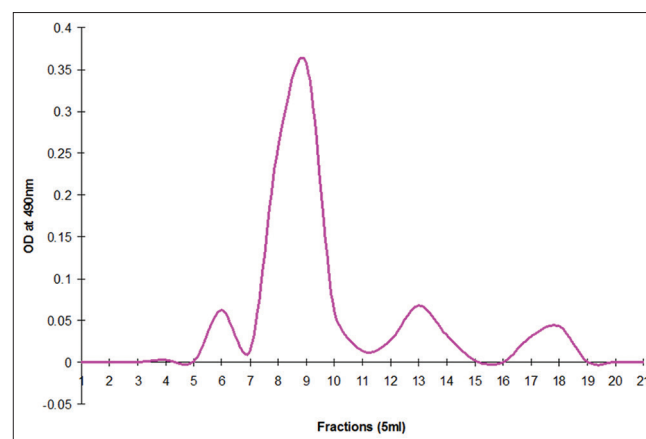

Figure 1: Ion-exchange chromatography (DEAE-cellulose) of sulfated polysaccharide isolated from brown marine algae *P. tetrastromatica*. Fractions 7 to 11, eluted with 0.5M NaCl, showed highest amount of carbohydrate and were pooled to obtain Ethanolic Sulfated polysaccharide-Column Purified (ESPS-CP).
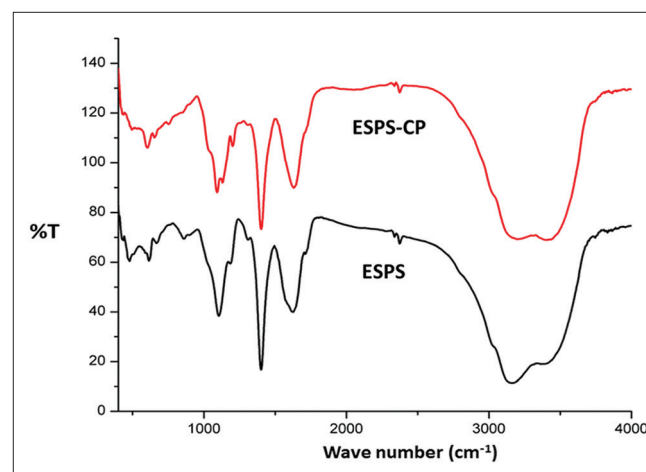

Figure 2: FT-IR spectral analysis to determine the functional groups present in the extracts.

of ample amounts of -OH functional groups on both the extracts. This may also be due to the presence of primary -NH$_2$ groups. The peak appearing ~1620/cm is an indication of double bonds, which might be contributed by the secondary metabolites. The sharp peak at 1402/cm was an indication of bending of C-H bonds present in SPS and in secondary metabolites. The peak at 1100/cm indicates the -C-O- stretch of glycosidic bonds of the SPS and the S=O stretch. The intensities of most of the peaks were found to be reduced in ESPS on comparing with ESPS-CP, which may be due to the removal of phytochemicals, by purification. The feeble peak around 700/cm also indicates the presence of thioester groups. From these, it can be concluded that the active component of both the extracts can be SPS. The results were compared with the standard SPS for confirmation as reported elsewhere (Badrinathan *et al.*, 2012; Saboural *et al.*, 2014).

Table 2 represents the phytochemical composition of both ESPS and ESPS-CP. The result shows that crude extract

Table 1: Chemical composition ESPS and ESPS-CP

Composition	ESPS	ESPS-CP
Carbohydrates (%)	11.2	17.6
Sulfate (%)	11.4	7.4
Fucose (%)	5.5	15.7
Uronic acid (%)	4.7	11.8
Sulfated polysaccharide (%)	2.4	12.7
Xylose (%)	0.5	0.03

ESPS: Ethanolic sulfated polysaccharide, ESPS-CP: Ethanolic sulfated polysaccharide-column purified

contains other metabolites such as flavonoids steroids and terpenoids. The column-purified sample contains only reducing sugar and glycosides, the normal constituents of polysaccharides, which in turn accounts for the purity of the isolated polysaccharide.

The antioxidant potential of both ESPS and ESPS-CP were determined using various methods and compared with standard antioxidants. The crude samples (ESPS) showed more activity than purified sample (ESPS-CP) except in the case of nitric oxide radical scavenging. The DPPH radical scavenging activity of ethanolic (ESPS) and purified (ESPS-CP) samples are depicted in Figure 3. Both ESPS and ESPS-CP exhibited a dose-dependent increase in the DPPH scavenging activity. The IC$_{50}$ values of ESPS, ESPS-CP, and ascorbic acid were found to be 1.6, 2.5, and 0.2 mg/ml respectively. The hydroxyl radical scavenging potential of ESPS and ESPS-CP is given in Figure 3. All samples exhibited a dose-dependent increase in the percentage of free radical scavenging. The IC$_{50}$ values of ESPS and ESPS-CP were found to be 1.9, and 2.1 mg/ml respectively. At the same time, ascorbic acid exhibited 50% scavenging ability at 0.27 mg/ml. At a concentration of 1.5 mg/ml, ESPS exhibited 50%, and ESPS-CP showed 44% inhibition of superoxide radicals. In the case of standard quercetin, 50% inhibition was observed at 0.9 mg/ml (Figure 3). The nitric oxide scavenging efficiency of ESPS and ESPS-CP showed a reverse trend than that of the other
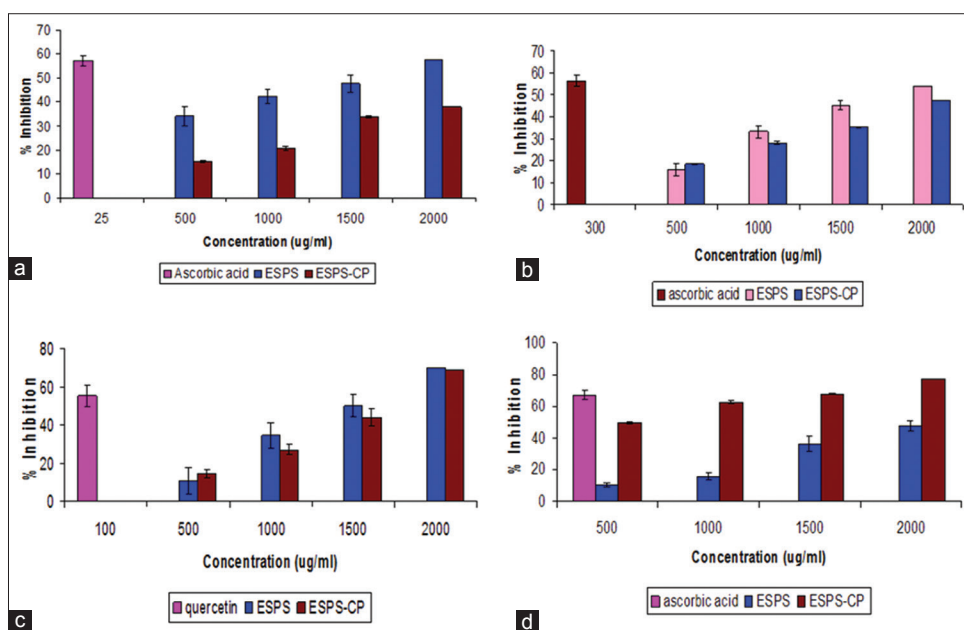

Figure 3: a) DPPH radical scavenging activity of ethanolic (ESPS) and purified (ESPS-CP) samples using ascorbic acid as reference standard. b) Hydroxyl radical scavenging potential of ESPS and ESPS-CP. Ascorbic acid was used as the positive control. c) Super oxide radicals scavenging activity of ethanolic (ESPS) and purified (ESPS-CP) samples using quercetin as the reference standard. d) The nitric oxide scavenging efficiency of ESPS and ESPS-CP. Ascorbic acid was used as the positive control.

antioxidant parameters (Figure 3). Here, the purified sample ESPS-CP showed 50% inhibition at 0.5 mg/ml concentration, whereas the crude sample showed less activity (50% at 2.3 mg/ml). Ascorbic acid showed 50% scavenging at 0.3 mg/ml. The hydrogen peroxide scavenging potential of ESPS and ESPS-CP proved ESPS to be a potent inhibitor of hydrogen peroxide. The IC_{50} values of ESPS, ESPS-CP, and ascorbic acid were found to be 0.9, 14.1, and 0.04 mg/ml, respectively. A dose-dependent increase in the percentage of H_2O_2 scavenging was observed (Figure 4). The TAA of ethanolic (ESPS) and purified (ESPS-CP) samples are shown in Figure 4.

1 mg/ml of ESPS showed 30.8 µg/ml equivalence of ascorbic acid where as for the same concentration of ESPS-CP it was 15 µg/ml equivalence of ascorbic acid. For the positive control, gallic acid 100 µg/ml was equivalent to 83 µg/ml equivalence of ascorbic acid. The reducing power of both the samples is shown in Figure 4. Here both ESPS and ESPS-CP showed extremely low activity when compared to gallic acid standard. 1 mg/ml of ESPS showed 18 µg/ml equivalence of ascorbic acid where as for the same concentration of ESPS-CP it was 4 µg/ml equivalence of ascorbic acid. The purified sample ESPS-CP showed very less reducing power when
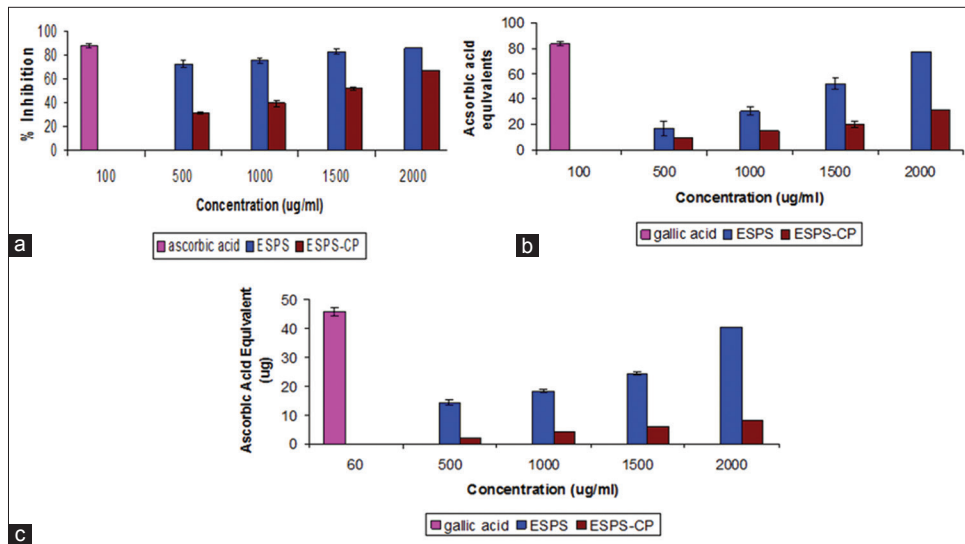

Figure 4: a) The hydrogen peroxide scavenging potential of ESPS and ESPS-CP using ascorbic acid as the standard. b) The total antioxidant activity of ethanolic (ESPS) and purified (ESPS-CP) using gallic acid as the positive control. c) The reducing power of ESPS and ESPS-CP. Gallic acid was used as the positive control.

Figure 5: Anti mitotic activity of ESPS and EASPS-CP by inhibiting sprouting in green gram seeds. a&G) Distilled water, b&H) Doxorubicin, c) ESPS 0.5 mg/ml, d) ESPS 1mg/ml, e) ESPS 1.5mg/ml, f) ESPS 2mg/ml, I) ESPS CP 0.5mg/ml, j) ESPS CP 1mg/ml, k) ESPS CP 1.5 mg/ml, l) ESPS CP 2 mg/ml.
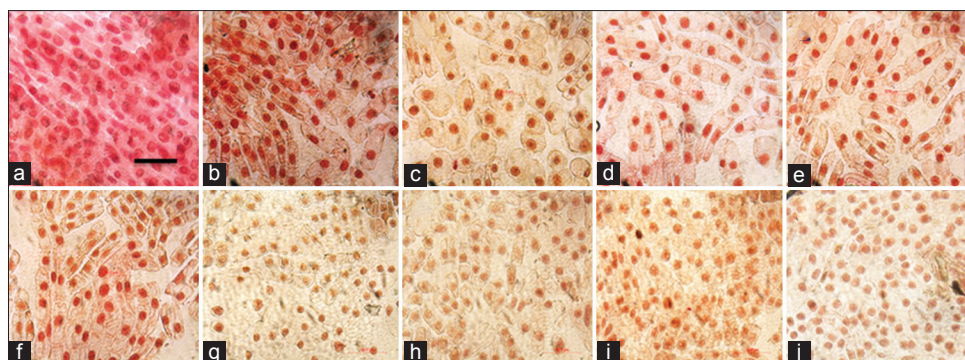
Figure 6: Anti mitotic activity of ESPS and ESPS CP by inhibiting cell division in onion root tip. a) Distilled water, b) Doxorubicin, c) ESPS 0.5 mg/ml, d) ESPS 1mg/ml, e) ESPS 1.5 mg/ml, f) ESPS 2 mg/ml, g) ESPS CP 0.5 mg/ml, h) ESPS CP 1 mg/ml, i) ESPS CP 1.5 mg/ml, j) ESPS CP 2mg/ml,

compared to crude sample. For the positive control, gallic acid 100 μg/ml was equivalent to 85 μg/ml equivalence of ascorbic acid.

Both ESPS and ESPS CP showed inhibition of water imbibition (Table 3). At a concentration of 1.5 mg/ml both extracts showed a comparable effect to that of 0.1 mg/ml doxorubicin. Here, also the crude sample is better than purified as in the case of antioxidant activity. The morphological examination revealed that extracts also showed a dose-dependent inhibition of seed germination (Figure 5).

The mitotic index of the onion root tips treated with both ESPS and ESPS CP is given in Table 4. There is a decrease in the mitotic index with increase in the concentration of the drug. At a concentration of 2 mg/ml both extracts showed a comparable effect to that of 0.1 mg/ml doxorubicin. Here, also the crude sample showed more activity when compared to the purified one (Figure 6).

DISCUSSION

The present study is aimed at the extraction, purification, and characterization of SPS with antioxidant potential from brown algae, *P. tetrastromatica*. Here, we used direct ethanolic precipitation to obtain the ethanolic sample. Various reports are already available regarding the isolation of SPS from brown algae. In the hot acidic extraction of fucose-containing sulfated polysaccharides from brown algae *Sargassum* sp. using HCl, the yield was reported to be 7.1% (Ale *et al.*, 2011). Relatively higher yield was obtained for our samples. However, the purified sample yields lower SPS content than the crude one. This is because the DEAE column is specific for the isolation of negatively charged compounds and thus, the purified fraction contained only the negatively charged SPS devoid of other contaminants like phytochemicals (Thinh *et al.*,

Table 2: Phytochemical analysis of ESPS and ESPS-CP

Parameters	ESPS	ESPS-CP
Reducing sugar	++	++
Flavanoids	++	-
Amino acids	-	-
Tannins	+	-
Alkaloids	-	-
Glycosides	++	++
Steroids	+	
Saponins	-	-
Terpenoids	+	-

ESPS: Ethanolic sulfated polysaccharide, ESPS-CP: Ethanolic sulfated polysaccharide-column purified

Table 3: Inhibition of water imbibition by ESPS and ESPS-CP in Green gram seeds

Concentration (mg/ml)	% inhibition of water imbibition			
	0.5	1	1.5	2
ESPS	14.3	25.45	36.8	42.45
ESPS-CP	23.41	32.25	38.3	41.1
Doxorubicin (0.1 mg/ml)	34.5			

ESPS: Ethanolic sulfated polysaccharide, ESPS-CP: Ethanolic sulfated polysaccharide-column purified

Table 4: MI showing inhibition of mitosis in onion root tips by ESPS and ESPS-CP

Concentration (mg/ml)	MI in %			
	0.5	1	1.5	2
ESPS	96.7	92.2	90.7	86.5
ESPS-CP	93.5	97.5	91.7	86.9
Doxorubicin (0.1 mg/ml)	87.7			
Distilled water	100			

ESPS: Ethanolic sulfated polysaccharide, ESPS-CP: Ethanolic sulfated polysaccharide-column purified, MI: Mitotic index

2013). In short, the column fractionation is a feasible method for the isolation of SPS from brown algae as it shows specificity toward negatively charged SPS.

The SPS from brown algae are mainly hetero fucans composed of fucose, sulfate, xylose, glucose, galactose and uronic acid (Thinh *et al.*, 2013). Several reports are available which deals with the chemical composition of

SPS from brown algae (Costa *et al.*, 2011; Magalhaes *et al.*, 2011). Some reports substantiate the presence of high percentage of total carbohydrates (above 50%) in brown seaweeds (Yang *et al.*, 2011; Melo *et al.*, 2013). Still, some others reported lower carbohydrate contents in brown algae like *Sargassum tenerrimum* (8.2%) (Vijayabaskar and Vaseela, 2012). The total carbohydrate content of ESPS was found to be toward the lower limit (11.2%), and there is an increase in the carbohydrate content after purification (17.6%) which is higher than that of fucoidan (12.8%). In the present study, the crude sample ESPS contain only less fucose but after purification, the percentage was found to be increased which is comparable to that of fucoidan. The sulfate content of the brown algae was reported to vary from 5% to 17% (Magalhaes *et al.*, 2011; Vijayabaskar and Vaseela, 2012; Melo *et al.*, 2013) and our results were also within this range. The total SPS content was also high in ESPS-CP than fucoidan. All the results of chemical composition revealed that the purified fraction is more effective than crude one on comparison with fucoidan. Moreover, the composition of biomolecules varies with species, maturity, growth environments, geographical locations, climate and harvesting seasons (Matanjun *et al.*, 2009). In short, the column purification has removed much of the contaminants, which is evident from relative higher carbohydrate content in ESPS-CP. Still, ESPS-CP could not be claimed as the purest form and more purification strategies are needed to achieve the maximum purity. In the present study, we analyzed the bioactivity of ESPS-CP.

The phytochemical analysis of isolated crude polysaccharide revealed the presence of other active components also. *Ulva lactuca,* a green algae bears ample amounts of flavonoids, terpenoids, glycosides and alkaloids (Abd Elmegeed *et al.*, 2014). A study by Govindappa *et al.* relates the phytochemical composition and the antioxidant property and revealed that the total phenol and flavanoid content contribute much to the free radical scavenging ability (Govindappa *et al.*, 2013). Even though the phytochemicals are pharmacologically active, we removed much of them from our sample during the purification step and the purified sample showed only the presence of glycosides and reducing sugar.

DPPH radical (2,2-diphenyl-1-picrylhydrazyl radical) is a stable free radical, which is widely used to determine the radical scavenging activity of natural compounds. The method is based on the decrease of absorbance at 517 nm due reduction of purple colored DPPH radical to non-radical form (Do QD *et al.*, 2014). In the present study, both our samples (ESPS and ESPS-CP) showed a considerable decrease in the absorbance at 517 nm with graded increase in the concentration. The proton donor groups like -COOH and -SO$_3$H present in our SPS can contribute to the DPPH reducing the capacity of both samples. However, the samples are found to be effective between 2 and 2.5 mg/ml and similar results were reported elsewhere for many other antioxidant compounds too. In the case of SPS, most of the studies reported to possess activity at higher concentration (Yang *et al.*, 2011). The SPS fraction from the brown algae *Sargassum horneri,* the effective concentration was reported to be 2.5 mg/ml (Shao *et al.*, 2014). On comparing with ascorbic acid, the scavenging ability of both the samples was found to be lower. However, most SPS were reported to possess IC50 values around this range (Hu *et al.*, 2010).

Hydroxyl radicals are the most reactive form of free radicals responsible for DNA damage and lipid peroxidation which can pave way to cancer and related complications (Senthilkumar *et al.*, 2013). Thus, the ability to quench the hydroxyl radical is significant for an antioxidant compound. The method used in the current study was based on the scavenging of hydroxyl radical generated through Fenton reaction (ferric-ascorbate–EDTA–H$_2$O$_2$ system) by antioxidant compound. Both ESPS and ESPS-CP showed a dose-dependent decrease in the absorbance with high IC$_{50}$ values. A sulfated fucan from the brown algae *D. delicatula* showed moderate hydroxyl radical scavenging activity. At 0.5 mg/ml, around 15% inhibition was observed (Magalhaes *et al.*, 2011). At the same time ESPS and ESPS-CP exhibited higher activity at 0.5 mg/ml. The recent reports revealed that the hydroxyl radical scavenging is not the major mechanism for scavenging ROS by polysaccharides (Melo *et al.*, 2013). But the ability of SPS to suppress hydroxyl radical generation and removal of the hydroxyl radical can be correlated to their hydroxyl radical scavenging potential (Wang *et al.*, 2008).

Metabolic processes in the body produce the superoxide anions. They are relatively weak oxidants but can interact with other molecules to produce secondary ROS such as hydroxyl radical, hydrogen peroxide and singlet oxygen and can leads to the oxidative damage to lipids, proteins and DNA (Wang *et al.*, 2008). The results showed a considerable increase in the inhibition of superoxide radical with respect to the increasing concentration of both the samples. Furthermore, at 1.5 mg/ml both sample showed a significant superoxide radical scavenging effect. A heterofucan from the brown algae *D. delicatula* possess a very low superoxide radical scavenging activity of 5.7% at 0.5 mg/ml concentration (Magalhaes *et al.*, 2011). The SPS from another brown algae *S. horneri* showed 65%

inhibition of superoxide radical at 2.5 mg/ml (Shao *et al*., 2014). When compared to these results, both ESPS and ESPS-CP study evoked a significant superoxide radical scavenging ability. It was also reported that the superoxide scavenging ability of vitamin C at a concentration of 2.0 mg/ml is only 68.19% (Wang *et al*., 2008), which is lower than that of both the samples in our study.

Nitric oxide is a diffusible free radical generated by macrophages and neurons that involves in various physiological processes such as smooth muscle relaxation and neuronal signaling (Shukla *et al*., 2012). The antioxidants can compete with oxygen for nitric oxide generated from sodium nitroprusside and can reduce the formation of nitrite ions, which can be estimated by Griess reaction (Corpuz *et al*., 2013). In the present study, a dose-dependent decrease in the absorbance was observed for both samples. The column fraction showed a comparable activity to that of standard ascorbic acid. Only a very few reports are available on the nitric oxide scavenging efficiency of SPS. DEAE CP SPS from the marine algae *Caulerpa racemosa* showed 50% inhibition of nitric oxide production at a concentration of 250 µg/ml (Mahendran and Saravanan, 2013). This is comparable to the result obtained for purified sample (ESPS-CP) of the present study. Hydrogen peroxide is a weak oxidant produced during metabolic processes and can produce hydroxyl radicals in the cell, which will lead to cell damages. The addition of antioxidants to H_2O_2 solution causes fast decrease in the absorbance at 230 nm (Ramani *et al.,* 2012). In the present study, the crude sample (ESPS) showed a noticeable capability to scavenge. The results obtained for both samples are much higher than the hydrogen peroxide scavenging potential reported for fucoidan and hetero fucans from the algae *Fucus vesiculosus* and *Padina gymnospora* (Rocha de Souza *et al*., 2007).

The TAA shows the ability of a sample to donate electrons. The method is based on the reduction of molybdenum VI to molybdenum V to form a green colored phosphate/molybdenum V complex (Senthilkumar *et al*., 2013). Highly active compounds show an increase in the absorbance. In the present study, there was a dose-dependent increase in the absorbance for all samples at 695 nm. However, when compared to standard gallic acid, both sample showed only moderate result. Furthermore, a fucan from brown algae *Dictyopteris justii*, showed much higher antioxidant activity (1 mg/ml sample contains 82.9 µg/ml equivalence of ascorbic acid) when compared to our samples (Melo *et al*., 2013)**.** Thus, we concluded that the TAA contributes less to the antioxidant potential of the extracts. Reducing the power of a compound is

the significant indicator of its antioxidant activity (Liu *et al*., 2013). It is related to their electron transfer ability and they can act as primary and secondary antioxidants (Chaudhuri *et al*., 2014). An increase in the absorbance at 700 nm indicates high reducing ability. But the obtained result was found to be reduced when compared to the reducing capacity of SPS from other algal sources (Magalhaes *et al*., 2011).

The antioxidant activity of purified sample (ESPS-CP) was found to be less than that of crude one (ESPS). This can be explained by several reasons. The presence of polyphenols such as flavonoids in the crude sample contributes to its high antioxidant capacity when compared to the purified sample which is devoid of compounds other than SPS (Mak *et al*., 2013). The low sulfate content of the purified sample may also contributes to its low scavenging effect (Yang *et al*., 2011). In most of the antioxidant properties analyzed in our study, our SPS showed less activity when compared to ascorbic acid or gallic acid.

The biochemical similarity between plants and animals can be exploited to use plants as an alternative system for animal experiments for the development of new drugs (Nayak and Lakshmi, 2014). The chromosomal mutations in higher plant system can be directly correlated to the mammalian system (James *et al*., 2014; Nielsen and Rank, 1994). Growth inhibition assays in plants provide another better way of detecting the antimitotic potential of various drugs (Alamgir *et al*., 2014). Germination of green gram seeds and *A. cepa* test are the two common methods used to test antimitotic activity (Nayak and Lakshmi, 2014; Sadananda, 2013). The relative germination ratio of the seed will decrease with increase in the extract concentration (Mohamed and El-Ashry, 2012). It is also proven that that higher doses of extracts were much more effective in inhibiting seed germination (Alamgir *et al*., 2014). A dose-dependent decrease in the water imbition and seed sprouting was observed in the current study. The inhibition of germination can be associated with reduction in water imbition (Kumar and Singhal, 2009). This can further affect the osmotic potential to reduce the turgor pressure within the seed and hence prevent the radicle protrusion (Kumar and Singhal, 2009). The results show the non-selective cytotoxic effect of both the extracts. Potent anticancer drugs such as Lapachol and Quercetin were also showed a similar trend on seed germination (Sadananda, 2013).

Most of the chemotherapeutic drugs act by interrupting cell division (mitosis) in fast-dividing cells. The inhibition of mitosis in onion root tip is considered as a sensitive and

easy method for the determination of cytotoxicity of drugs (Thenmozhi *et al.*, 2011) and *Allium* test shows an excellent relationship with mammalian test systems. A standard anticancer drug such as methotrexate also showed a decrease in the mitotic index of onion root tip. A study about the inhibition of mitosis in onion root tip proved that there is a disturbance in the mitotic spindle formation and also inhibition of cell plate formation which may be due to the arrest of cell division at G2 phase or S phase (Yuet Ping *et al.*, 2012). The inhibition of mitosis by both the extracts is beneficial for their possible applications for life-threatening diseases such as cancer. Thus, we suggest that the cytotoxic action of both ESPS and ESPS-CP can involve disturbance of mitotic processes in the fast-dividing cancer cells which will be beneficial for cancer management (Mohamed and El-Ashry, 2012). Our samples, being capable of arresting the mitotic progression of plant cells; it may have the potential to find opportunities in arresting the cancer cell proliferation. More studies are required on the cellular and the molecular levels of animals (both *in vitro* and *in vivo*) to correlate these aspects with the cancer biology.

CONCLUSION

The present study signifies the SPS isolated from the brown algae *P. tetrastromatica* as a bioactive compound with antioxidant and antimitotic activity. Both the unpurified and ethanolic column purified fraction of the SPS can be used as an antioxidant and antimitotic compound. The isolated SPS fractions can be a good candidate for antioxidant therapies especially as the supplements in cancer therapeutics.

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