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ORIGINAL PAPER

Antioxidant and cytotoxic activities of sulfated polysaccharides from five different edible seaweeds

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Abstract

In recent times, there has been a growing interest in the exploration of antioxidants and global trend toward the usage of seaweeds in the food industries. The low molecular weight up to 14 kDa sulfated polysaccharides of seaweeds (*Portieria hornemannii*, *Spyridia hypnoides*, *Asparagopsis taxiformis, Centroceras clavulatum* and *Padina pavonica*) were evaluated for in vitro antioxidant activities and cytotoxic assay using HeLa cell line and also characterized by FTIR. The high yield (7.74% alga dry wt.) of sulfated polysaccharide was observed in *P. hornemannii* followed by *S. hypnoides* (0.69%), *C. clavulaum* (0.55%) and *A. taxiformis* (0.17%). In the brown seaweed *P. pavonica,* the sulfated polysaccharide yield was 2.07%. High amount of sulfate was recorded in the polysaccharide of *A. taxiformis* followed by *C. clavulaum, P. pavonica, S. hypnoides* and *P. hornemannii* as indicative for bioactivity. The FTIR spectroscopic analysis supports the sulfated polysaccharides of *S. hypnoides, C. clavulatum* and *A. taxiformis* are similar to agar polymer whereas the spectral characteristics of *P. hornemannii* have similarities to carrageenan. The higher DPPH activity and reducing power were recorded in the polysaccharide of brown seaweed *P. pavonica* than the red seaweeds as follows: DPPH activities: *S. hypnoides* > *A. taxiformis* > *C. clavulatum* > *P. hornimanii*; Reducing power: *A. taxiformis* > *P. hornimanii* > *S. hypnoides* > *C. clavulatum.* The polysaccharide fractions contain up to 14 kDa from red seaweeds *P. hornemannii* and *S. hypnoides* followed by brown seaweed *P. pavonica* exhibit cytotoxic activity in HeLa cancer cell line (and are similar to structural properties of carrageenan extracted from *P. hornemannii*). The low molecular weight agar like polymer of *S. hypnoides* and alginate like brown seaweed *P. pavonica* showing better in vitro antioxidant activities that are capable of exhibiting cytotoxicity against HeLa cell line can be taken up further in-depth investigation for nutraceutical study.

Keywords Seaweeds · Sulfated polysaccharides · Antioxidants · Cytotoxic assay · HeLa cell lines

Introduction

Marine macroalgae (seaweeds) are the only resource for industrially important polymers such as agar and carrageenan from red seaweeds; alginate, fucoidan and laminarin from brown seaweeds. These polymers are extracted only from few seaweed species that meet certain industrial applications. This seaweed sulfated polysaccharides possess a variety of biological activities and immunomodulatory activities to mitigate associated negative effects including inflammation [1]. These polysaccharides have been used in food industries as gelling agents, thickening, and stable excipients for control release of drugs [2]. Recently, in a test of antiviral effectiveness against the virus which causes COVID-19, an extract from edible seaweeds substantially outperformed remdesivir, the current standard antiviral agent used to combat the disease. Further Heparin, a common blood thinner, and a heparin variant stripped of its anticoagulant properties, performed on par with remdesivir in inhibiting SARS-CoV-2 infection in mammalian cells [3]. Beside, this seaweed hydrocolloids have great economic importance because of their various bioactive (anticoagulant, antiviral, anticancer, antioxidant, antitumor,

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immunomodulating, antihyperlipidemic and antihepatotoxic activities) and unique rheological properties, hence they are being widely used in the pharmaceutical and biomedical sectors [4–6]. The structural features like the composition of monomers, sulfate level and its position in the sugar moiety, chain length, molecular weight etc. of the polymer make them ideal materials for various biomedical applications [7, 8]. Cholesterol and lipid-controlling properties of carrageenan have been demonstrated in a clinical trial and found significant reduction in the serum cholesterol and triglyceride levels [9]. Carrageenan is well known in controlling the inflammation and a complete review on carrageenan biological activities was reported [10, 11]. Recently, Zhong et al. [8] reviewed the cytotoxic and antioxidant activity in various seaweeds polysaccharides.

Among five seaweeds, the red *Portieria hornemannii*, *Spyridia hypnoides*, *Asparagopsis taxiformis* and *Centroceras clavulatum*; and brown seaweed *Padina pavonica* were taken up, a monoterpene possessing cytotoxicity against a diverse panel of human tumor cell line was isolated from the red alga *Portieria hornemannii* [12]. The antioxidant and in vitro cytotoxic activity of extracts prepared using methanol, chloroform, petroleum ether and ethyl acetate from red alga *Asparagopsis taxiformis* were demonstrated [13]. In another study, ethanol extract significantly exhibiting antibacterial activity against fish pathogenic bacteria was found [14]. Ethanolic extract of brown alga, *Padina pavonica* having antimicrobial, antioxidant, and anticancer activities was proved [15]. *Padina pavonica* extract contain phenolic, flavonoid and tannin which exhibits antioxidant activity [16] whereas there is no report on *Spyridia hypnoides* and *Centroceras clavulatum.* Further, in vitro antioxidant activity and cytotoxic assay using cancer cell-line from these five algae sulfated polysaccharides are significant. Because taping the potential of these seaweeds which are not exploited for polysaccharides of industrial quality and by considering its food value, they can be utilized for the source of compounds of biomedical and nutraceutical values. Hence, in the present study, results of sulfated polysaccharides extracted from these five seaweeds were evaluated also for antioxidant and cytotoxic properties.

Materials and methods

Collection of seaweeds

The fresh and healthy specimens of red seaweeds about 3 kg of *Portieria hornemannii* (Lyngbye) P.C. Silva, *Spyridia hypnoides* (Bory) Papenfuss, *Asparagopsis taxiformis* (Delile) Trevisan and *Centroceras clavulatum* (C. Agardh) Montagne; and brown seaweed *Padina pavonica* (Linnaeus) Thivy occurring along the Coast of Pamban (9.2798° N, 79.2291° E), Gulf of Mannar, Tamil Nadu, India were collected during the month of September 2018.

Extraction and fractionation of polysaccharides

The extraction procedure of Distantina et al. [17] was followed with minor modifications. The collected five seaweeds were cleaned with fresh water to remove epiphytes and sand particles. The samples were shade dried under air in dark for 5 days. Thirty gram of dried seaweeds was soaked in 250 mL of 0.3 N KOH solutions for 12 h and then heated at 60 °C for 30 min. The alkaline digested sample was washed thrice in 250 mL of tap water and then 0.1 N HCl was added into digested sample until the pH was neutral (7.0). Then distilled water was added to sample to reach 1:50 ratio (30:1500 w/v) and extracted by heating at 80 °C for 1 h. The hot water extract was filtrated through muslin cloth followed by Whatman No. 1 filter paper and cooled. The crude polysaccharide in the filtered extract was precipitated by adding cold (4 °C) 90% ethanol in 1:150 ratio (30:4500 sample: 90% ethanol w/v). The precipitate was collected and stored at 4 °C over night and thawed further, the crude polysaccharide was separated by centrifugation at 704×*g* for 5 min to remove cell debris. Then the supernatant was collected and again centrifuged at 7826×*g* for 10 min to obtain pellets of crude polysaccharides which were stored at 4 °C. These pellets were dialyzed using dialysis tubing of 14 kDa (Sigma Aldrich D9652) molecular weight cut off against two volumes of distilled water for 24 h at room temperature then freeze-dried and weighed (Fig. 1. Flow chart).

Estimation of sulfate in fractionated polysaccharides

The total sulfate [18] content of dialyzed polysaccharides were estimated.

Characterization of polysaccharides by FTIR spectra

The extracted polysaccharides were characterized by FTIR spectroscopy (Perkin-Elmer Version 10.5.1 spectrometer, Boston, USA). The polysaccharide (5 mg) of each seaweeds were ground with spectroscopic grade potassium bromide (KBr) powder and pressed into 1 mm pellet for FTIR measurement in the wavelength that ranges from 400 to 4000 cm^{-1} [19]. The FTIR spectrum was finalized on the basis of spectral consistency recorded from triplicate KBr powder samples from each seaweed.

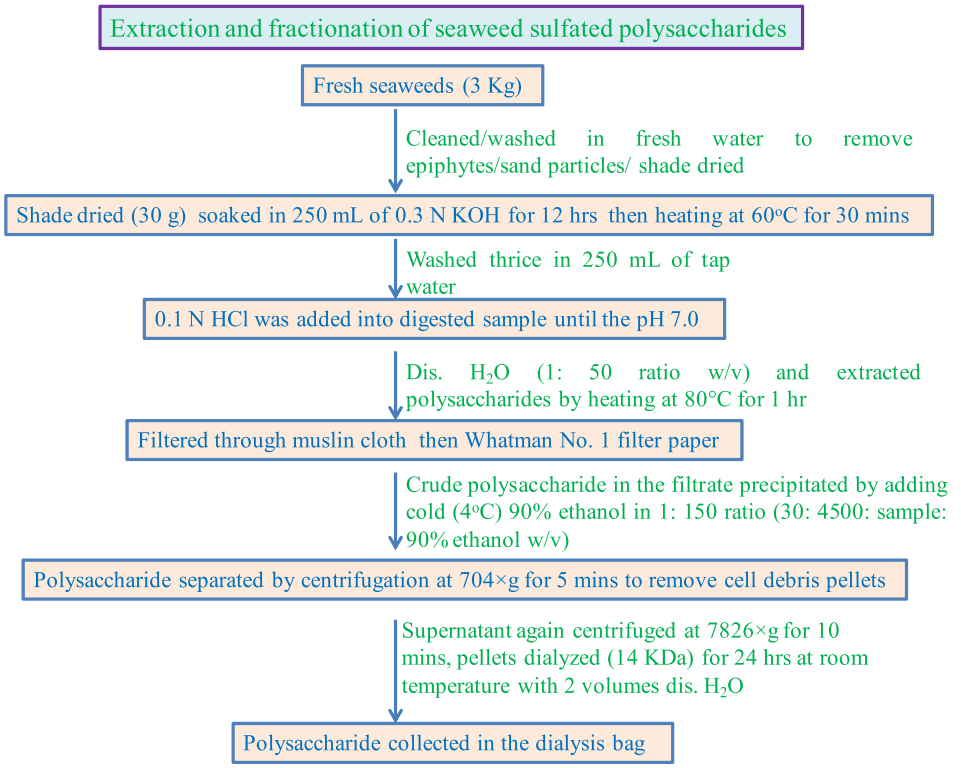

Fig. 1 Flow chart for the extraction and fractionation of seaweed sulfated polysaccharides

In vitro antioxidant activities of the polysaccharide

DPPH radical scavenging activity

The scavenging effects of polysaccharide were determined using ascorbic acid as a positive control [20]. For the assay, 2 mL of 0.16 mM DPPH in methanol was added into the test tube containing 2 mL aliquot of test sample at various concentrations (0.1, 0.5 and 1 mL from stock of 1 mg mL^{-1}). The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance was measured at 517 nm in a UV–visible spectrophotometer (Shimadzu UV-2600). The capacity to scavenge the DPPH radical was calculated by the following equation:

$$\text{Scavenging effect (\%)} = \left[1 - \left(A_{\text{sample}} - A_{\text{sample blank}}\right)/A_{\text{control}}\right] \times 100$$

where, A_{control} is the absorbance of the control (DPPH solution without sample); A_{sample} is the absorbance of the test sample (DPPH solution plus test sample); $A_{\text{sample blank}}$ is the absorbance of the sample (sample without DPPH solution).

Reducing power

The reducing power of fractioned polysaccharide was determined by following the method described by Yen and Chen [20]. One mL of polysaccharide solution in various concentrations (0.1–1 mg mL^{-1}) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min and rapidly cooled. A 2.5 mL of trichloroacetic acid (10%) was added into the mixture and centrifuged at 704×*g* for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL $FeC1_3$ (0.1%) was measured at 700 nm absorbance. An increased absorbance of the reaction mixture indicated the increased reducing power activity. The ascorbic acid (1 mg mL^{-1}) was used as a control.

MTT assay

The cytotoxic activities of the five algal polysaccharides were examined using colorimetric 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole (MTT) assay [21]. Formation of purple formazan by reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. The effectiveness of this agent in causing cell death can be deduced, through the production of dose–response curve. The resulting purple solution is spectrophotometrically measured.

HeLa cells cultured in a complete DMEM (10% FBS, 1% antibiotic–antimycotic solution) were seeded 5000 cells per well in 96-well plate and incubated in 5% CO_2 for 24 h. After attachment of cells, 100 µL of polysaccharide of increasing

dilutions (starting from 1 mg mL^{-1} to 0.0019 mg mL^{-1}) were added to the wells and incubated for 24 h. Blank wells containing DMEM alone and control wells with cells and no added drug were maintained for each plate. MTT reagent at 0.5 mg mL^{-1} was added followed by incubation for 3 h. Formazon crystals were dissolved in DMSO and the absorbance was read at 570 nm with a reference wavelength of 630.

Statistical analysis

The differences in the biological activities among the polysaccharides were tested for significance ($p < 0.05$) by one-way analysis of variance (ANOVA) by the Tukey post hoc comparison test using SPSS14.0.

Results and discussion

The seaweed polysaccharides were extracted from *Portieria hornemannii*, *Spyridia hypnoides*, *Asparagopsis taxiformis, Centroceras clavulatum* and *Padina pavonica* and their sulfate content was analyzed (Table 1). Agar and carrageenan are present in red seaweeds and glugan is found in green seaweeds whereas brown seaweeds contain alginate and fucoidan [22]. The red seaweed species such as *Gelidium, Gracilaria, Gelidiella, Ahnfeltia, Pterocladia*, *Acanthopeltis*, and *Annfeltia* are the major source for agar collectively called agarophytes [22] and the agar content is varied depending on the species for example, 53% in *Gracilaria* sp. whereas in *Gelidium* 44% [23]. These seaweed polysaccharides are commercially utilized worldwide in the processed foods, cosmetics, pharmaceutical products and medicine as gelling and stabilizing agents [24].

Carrageenans, an another major cell wall polysaccharides extracted from certain species of red seaweeds (*Rhodophyceae*) belong to the family members of *Solieriaceae, Rhabdoniaceae, Hypneaceae, Phyllophoraceae, Gigartinaceae, Furcellariaceae* and *Rhodophyllidaceae* [22]. This group of algae constitutes carrageenan up to 75% (dry wt.) in *Kappaphycus* with 15 to 40% of ester-sulfate content [25] and these species polymers are being used in various food industries as texturing and gelling agents [26, 27]. It also exhibits various biological and biomedical properties [4, 6]. The brown seaweeds (*Phaeophyceae*) contain alginate up to 40% in some species, example *Ascophylum nodosum* 22–30%, *Laminaria digitata* 25–44%, *Sargassum* sp. 17–45% [28, 29]. Agar extracted in red seaweeds in the molecular weight range of 100–30,000 kDa [30]. Among the different fraction of polysaccharides (3.2, 10.5, 29.0, and 48.8 kDa) isolated from red seaweed *Pyropia yezoensis,* 3.2 kDa found as effective against stress protection [31]. The yield of carrageenan varies depending on species and up to 70% (dry basis) was recorded from some species such as *Betaphycus gelatinum* and *Kappaphycus alvarezii*. Other species like *Eucheuma denticulatum* and *Chondrus crispus* had 30% yields. Sulfate content of carrageenans varied depends on the types (20% in κappa, 33% in ioda and 41% in λ) [32]. The molecular weight of commercially values carrageen in the range of 100 to 800 kDa was recorded [25] and low molecule weights possess more bioactivity properties [33]. Sulfated galactan of 16 kDa showing anticoagulant and antioxidant activities was purified from red *Spyridia hypnoides* [34]. Sulfated polysaccharide of 60 to 500 kDa isolated from Red seaweed *Asparagopsis taxiformis* exhibit anticoagulant activity [35].

In this study, polysaccharide from five seaweeds (*P. hornemannii*, *S. hypnoides*, *A. taxiformis, C. clavulatum* and *P. pavonica*) up to 14 kDa was isolated by considering the significance of high bioactivity recorded in low molecular weight polysaccharides [36]. Whereas the alginate polymer of brown seaweeds in the range of 300–1000 kDa is commercial valuable [37, 38] but low molecule weight alginate polymers have potential bioactive properties [39]. Among the four red seaweeds, highest yield of 7.74% dry wt. of sulfated polysaccharide was extracted from *P. hornemannii* followed by *S. hypnoides* (0.69%), *C. clavulaum* (0.55%) and *A. taxiformis* (0.176%). Whereas the yield of sulfated polysaccharide extracted from the brown seaweed, *Padina pavonica* was 2.07% dry wt. (Table 1). Comparing the polysaccharides of terrestrial plant origin, seaweed polysaccharides constitute unique monomers like galactose, mannose, fucose, xylose etc. with varying degree of sulfation in their sugar residues [40] that influence the structure and biological properties of polymer isolated from each seaweed species [41]. This sulfate group of sugar moieties is found responsible for exhibiting anionic charge to the polymers [27, 39, 40]. Antibacterial and antiviral activity of anionic seaweed sulfated polysaccharides exhibit by binding the positively charged glycoprotein virus envelope and bacteria cell surface thereby integrating with virus or bacteria thus it prevent the pathogens entry into host cells [42–44]. Positive charge glycoprotein receptor of bacteria cell surface bind sulfate polysaccharides thereby inhibiting the bacteria [44]. So, high sulfate content in seaweed polysaccharides proportionally display more bioactivity [45, 46]. This study results showing high amount of sulfate in the polysaccharides of *A.*

Table 1 Yield and sulfate content of seaweed polysaccharides

S.No	Seaweed	Total yield (% alga dry wt.)	Sulfate content (μg/mg)
1	*P. hornemannii*	7.74	1.08 ± 0.38
2	*S. hypnoides*	0.69	2.31 ± 0.47
3	*C. clavulaum*	0.55	17.12 ± 1.37
4	*A. taxiformis*	0.18	48.51 ± 4.83
5	*P. pavonica*	2.07	3.18 ± 0.71

taxiformis (48.51 ± 4.83 μ mg^{-1}) followed by *C. clavulaum, P. pavonica, S. hypnoides* and *P. hornemannii* (Table 1) indicate the potential of these polysaccharides for biological activity.

FTIR spectroscopy characterization of polysaccharides

Structure of agar, carageenan and alginate

Agar and carrageenan extracted from Rhodophyceae (red seaweeds), the latter is highly sulfated ones. Thus, it constitutes repeating disaccharide units of 3-linked β-D-galactose (G-units) and 4-linked α-galactose (D-units) or 3,6-anhydro-α-galactose (AnGal-units) whereas agar is less sulfated polymer composed of agarose, agaropectin, fibre, protein and ash. The agaropectin of agar is a charged sulfated non-gelling polymer constitutes D-glucuronic acid, and small amounts of pyruvic acid. The gelling part of the agar, agarose is a neutral polymer with molecular weight of 120 kDa free of sulfate constitute repeating unit of agarobiose. It is a disaccharide made by β-1,3-linked- D-galactose and α-1,4-linked 3,6-anhydro-L-galactose [47] that is constituted by repeating units of D-galactose and L-galactose [48, 49]. The specific band at 890 cm^{-1} for agar attributed to anomeric C–H of β-galactose residues generally absent in carrageenan [50]. At least fifteen different types of carrageenan categorized on the basis of structural characteristics, they are: sulfation patterns and presence or absence of AnGal on D-units are reported from various red seaweed species and most prevalent are ioda, lamda and kappa [51] with molecular weight in the range of 100 to 800 kDa was recorded [25]. The cell wall of brown seaweeds mainly contain alginate polymer which constitute the blocks of β-l,4-linked D-mannuronic acid and blocks of α-l,4-1inked L-guluronic acid or blocks of D-mannuronic acid alternatively by L-guluronic acid [52].

Red seaweed polysaccharides

The FTIR spectroscopy remains a best tool to characterize the seaweed polysaccharides by identifying their functional groups [53]. The broad band between 3500 and 3200 cm^{-1} due to stretching vibrations of OH group is recorded in the FTIR spectra of all seaweed polysaccharides and standard spectra of agar, carrageenan and alginate (Figs. 2 and 3). The FTIR spectral characters of polysaccharides of red seaweeds *Portierea hornemanii, Spiridia hypnoides, A. taxiformis* and *C. clavulatum* were compared with both agar and carrageenan standards (Fig. 2). A peak at 930 cm^{-1} shows the presence of C–O–C of glycosidic bond for 3,6-anhydro-L-galactose of agar and 3,6-anhydro-D-galactose of carrageenan is found in the spectra of both standards (agar and carrageenan), *Spyridia hypnoides, A. taxiformis* and *C. clavulatum* [53, 54]. A band at 1650 cm^{-1} for carbonyl group of a carboxylic acid; a peak at 1375 cm^{-1} and a broad band in between 1210 and 1280 cm^{-1} indicate the presence of sulfate esters and a peak at 1140 cm^{-1} for C–O glycosidic bond are recorded in all red seaweed polysaccharide samples as well as both agar and carrageenan standards [Fig. 2; 53, 54].

A peak at 840 cm^{-1} show the presence of galactose sulfated at C4 is found in the spectra of carrageeenan standard and polysaccharides of *Spyridia hypnoides* and *A. taxiformis* (Fig. 2b). Agar differs from carrageenan by having L-configuration for the 4-linked galactose residue; nevertheless, they have some structural similarities with carrageenan. Peaks at 770 cm^{-1} and 740 cm^{-1} for pyranose ring are recorded in all the spectra (Figs. 2ab, 4). Agar specific peak at 890 cm^{-1} due to anomeric CH in the β-galactose residues and a band at 930 cm^{-1} correspond to 3,6-anhydro-L-galactose recorded in the spectra of agar standard, *Spyridia hypnoides* and *Centroceras clavulatum* belong to the order *Ceramiales* and *Asparagopsis taxiformis* of the Order *Florideophyceae* confirmed the presence of polysaccharides in agar forms [34, 50] and the spectral characteristics of *P. hornemannii* of order *Gigartinales* shows more spectral similarities with the carrageenan standard. The FTIR spectral characteristics found the presence of agar form of low molecular weight up to 14 kDa polysaccharides in *S. hypnoides, C. clavulatum* and *A. taxiformis* and carrageenan form in *P. hornemannii*, among the four red seaweeds.

Brown seaweed polysaccharides

The FTIR spectral characteristics of polysaccharide of brown seaweed, *Padina pavonica* show spectral similarities with alginate standard (Fig. 3). Peaks at 3250 cm^{-1} assigned for OH groups; 1620 cm^{-1} for S=O; 1420 cm^{-1} for C–O and 1025 C–O–C groups are recorded from the spectra of both alginate standard and sample [55]. Peaks at 1290 cm^{-1} for M/G block (Mannose/Galactose) and at 808 cm^{-1} assigned to M block are recorded from the spectra of both alginate standard and sample [7, 56]. The anomeric region of fingerprint (950–750 cm^{-1}) showed three characteristic absorption bands in alginate standard and sample. A band at 950 cm^{-1} is assigned to the C–O stretching vibration of uronic acid residues, the one at 880 cm^{-1} is assigned to the C1–H deformation vibration of β-mannuronic acid residues and the band at 808 cm^{-1} is characteristic of mannuronic acid residues [57]. From this spectral data the extracted polysaccharide of *Padina pavonica* shows similar characteristics of alginate polymer.

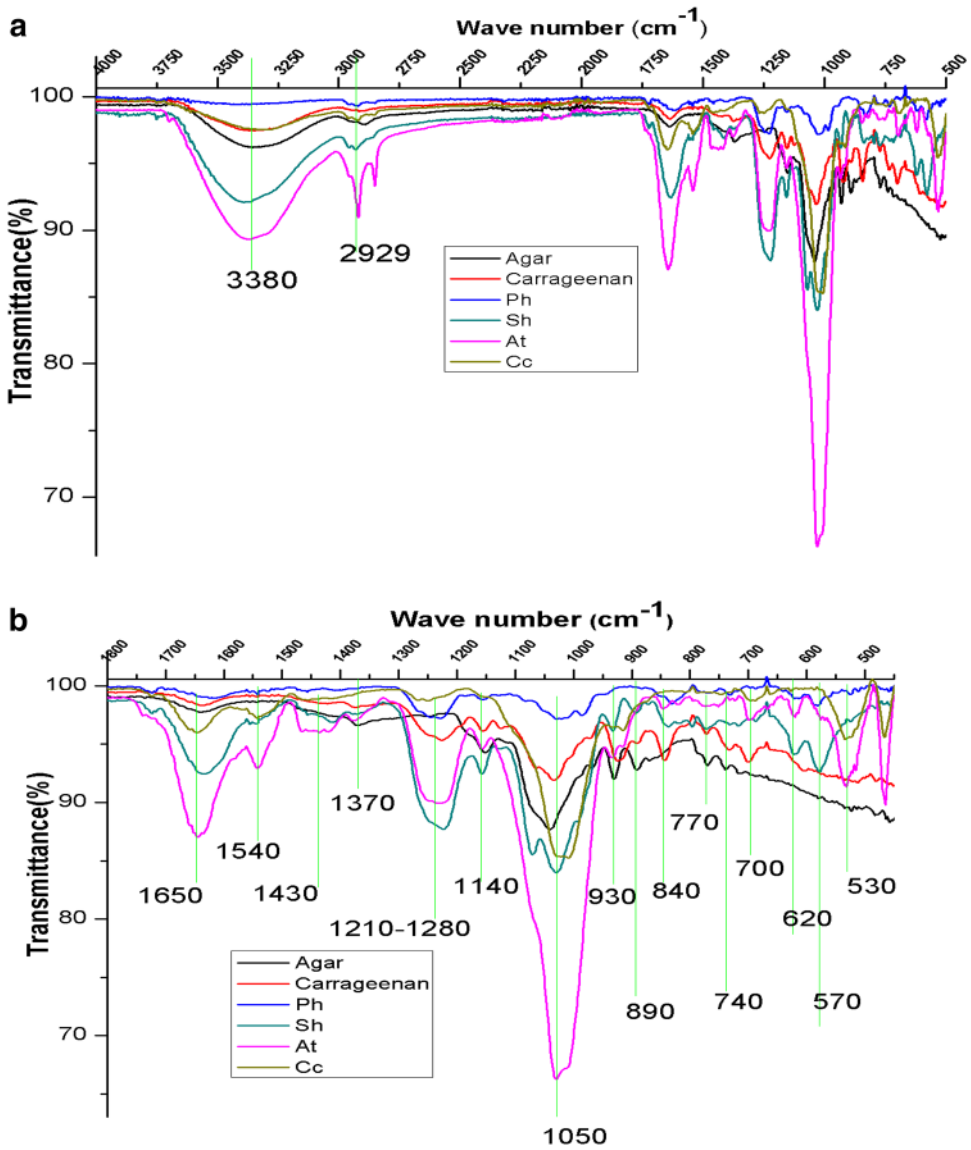

Fig. 2 FTIR spectra of sulphated polysaccharide of red seaweeds (*Portieria hornemannii*-Ph, *Spyridia hypnoides*-Sh, *Asparagopsis taxiformis*-At, *Centroceras clavulatum*-Cc and *Padina pavonica*-Pp) by comparing agar and carrageenan standards

Antioxidant activities

In vitro antioxidant examinations are used to measure the degree of protection against free radicals [58]. Seaweed polysaccharides having antioxidant properties are the promising source for manufacture of food products and pharmaceuticals [58]. The molecular weight, type of monomer residues, degree of sulfation and sulfate position significantly influence their antioxidant activity of seaweed polysaccharides [8]. In this study, among the in vitro antioxidant activities, DPPH hydroxyl radical scavenging and reducing power of the sulfated polysaccharides were measured.

DPPH assay

The free radical scavenging activities of different sulfated polysaccharides assessed by DPPH assay based on the hydrogen ion donating ability of the polysaccharide are presented (Fig. 4). DPPH is a stable free radical appears in purple color in methanol/ethanol turns colorless by reduction in the presence of hydrogen donating antioxidants [59]. The DPPH, a stable free radical shows maximum absorbance at 517 nm in methanol. When DPPH encounters a proton donating substance like antioxidant, radical would be scavenged and the absorbance reduced. For every sulfated polysaccharide tested, there was a concentration dependent increase in scavenging activity where 1 mg mL^{-1} polysaccharide of *P. pavonica* exhibited an increase in DPPH free radical scavenging activity up to 63%. Khaled et al. [60] explained that the ethyl acetate fraction of the algae *Padina pavonica* showed the highest antioxidant activity (42.5%); those activities may be due to phenolic compounds present in significant amounts in this fraction (8.98 GAE/g). In another study, Nouf et al.

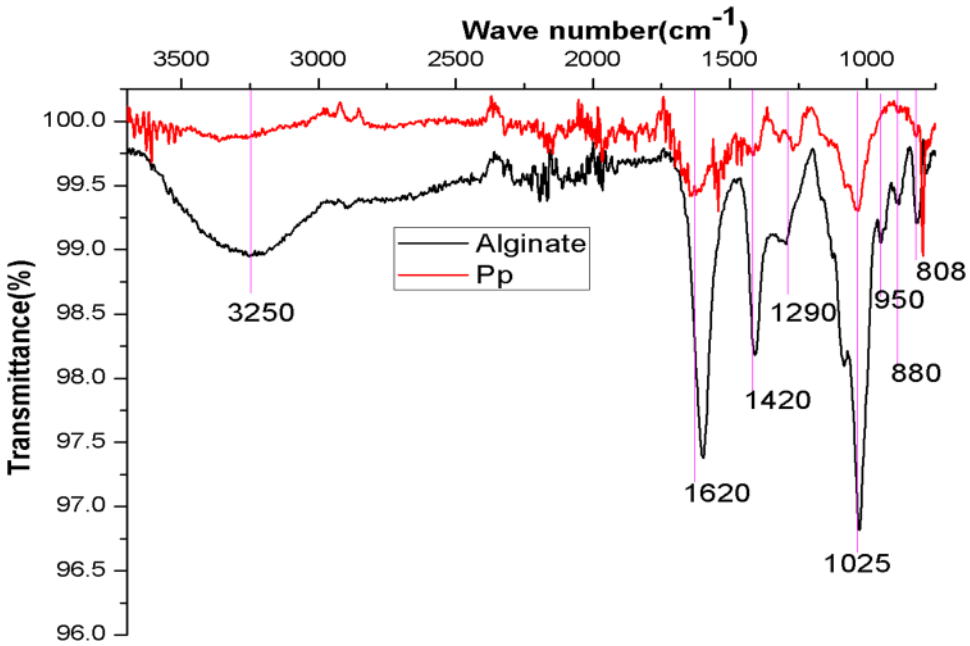

Fig. 3 FTIR spectrum of sulfated polysaccharide of brown seaweed *Padina pavonica* (Pp) by comparing alginate standard

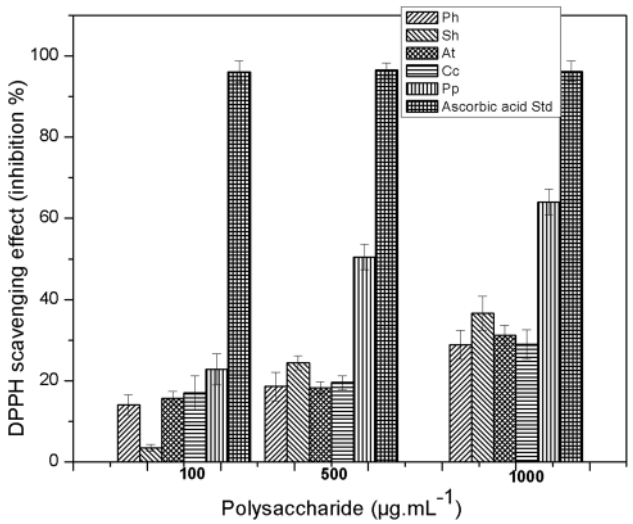

Fig. 4 DPPH free radical scavenging activity of sulfated polysaccharides (*Portieria hornemannii*-Ph, *Spyridia hypnoides*-Sh, *Asparagopsis taxiformis*-At, *Centroceras clavulatum*-Cc and *Padina pavonica*-Pp). Error bars indicate replicates standard deviation of each analysis

[61] described the extracts of *Padina pavonica* showed a concentration-dependent manner with maximum scavenging activity of 77.6%, IC_{50} = 5.59 µg/mL. Among the red seaweeds, high DPPH activity was recorded by polysaccharide (1 mg mL^{-1}) of *Spyridia hypnoides* followed by *A. taxiformis* (31%) and *C. clavulatum* (28%) compared to ascorbic acid as standard (96%). Results from previous studies [62] are in accordance with the present study that polysaccharides of *P. pavonica* followed by red seaweeds *Spyridia hypnoides*, *A. taxiformis*, *C. clavulatum* and *P. hornimanii* exhibit antioxidant power in their capacity to scavenge the DPPH radical and reduce ferric Fe^{3+} ions to Fe^{2+} by decreasing order.

Reducing power assay

The presence of antioxidant compounds with reducing ability exerts antioxidant activity by breaking the free radical chain or by donating a hydrogen atom. By reducing power assay, the ability of the polysaccharides capable of reducing Fe^{3+} to Fe^{2+} measured was recorded based on the increasing absorbance (Fig. 5). From the measurements of the reductive ability of the polysaccharides, the reducing power of all the five sulfated polysaccharides increased with increasing concentration although not as much as ascorbic acid exhibited. As reported by Jinzhe et al. [63], polysaccharide of *P. pavonica* had the greatest reducing power closely followed by red seaweeds *A. taxiformis* then *P. hornimanii, Spyridia hypnoides* and *C. clavulatum* implicated on the basis of sulfate content and monosugar constituents [64]. The Ferric reducing antioxidant power (FRAP) of *P.pavonica* reveals a higher antioxidant activity with IC50 = 0.4 mg mL^{-1} [65]. El-Shazoly and Fawzy [66] described that the ethyl alcohol extract of *Padina pavonica* showed significant activity (339.92 mg g^{-1} dry wt.).

In vitro cytotoxic assay using HeLa cell-line

The five sulfated polysaccharides were assayed for the cytotoxic effect in HeLa cell line at different concentrations from 0.0019 mg mL^{-1} to 1 mg mL^{-1}. The effect has measured

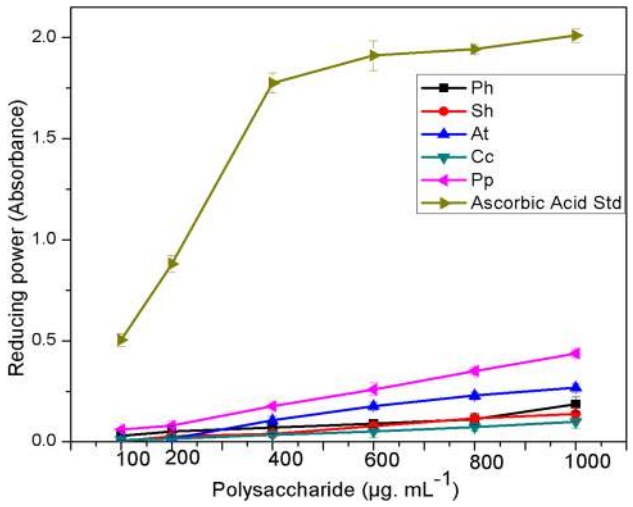

Fig. 5 Reducing power assay of sulfated polysaccharides (*Portieria hornemannii*-Ph, *Spyridia hypnoides*-Sh, *Asparagopsis taxiformis*-At, *Centroceras clavulatum*-Cc and *Padina pavonica*-Pp). Error bars indicate replicates standard deviation of each analysis

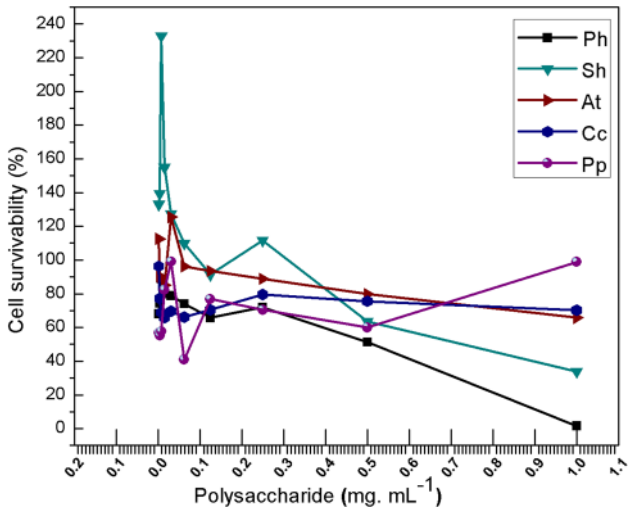

Fig. 6 Cell survivability of sulfated polysaccharides (*Portieria hornemannii*-Ph, *Spyridia hypnoides*-Sh, *Asparagopsis taxiformis*-At, *Centroceras clavulatum*-Cc and *Padina pavonica*-Pp) tested by MTT Assay using HeLa cell line

based on the cell survivability percent (Fig. 6) and IC_{50} values. Figure 6 evidently shows the concentration of polysaccharide increases the cell survivability decreases by the red seaweeds *P. hornemannii* and *S. hypnoides*. Ktari and Guyot [67] evaluated the cytotoxic activity of dichloromethane extract of *Padina pavonica* against KB cells and the results showed significant activity (IC50 10 µg mL^{-1}). The antitumor activity of *Padina pavonica* against breast cancer cells (MCF-7) and several strains of prostate cancer (DU-145, LNCaP and PC3) by in vitro cytotoxicity assay with methanolic extract found that *P. pavonica* has more toxicity against cell strains tested according to Taskin et al. [68]. The cell survivability was decreased by low concentrations of *P. pavonica* polysaccharide but at high concentrations it promotes the cell survivability (Fig. 6) since the IC50 value was, 1.059.

Indeed polysaccharides of *P. hornemannii* and *S. hypnoides* showed cytotoxic effects at higher concentrations but at low concentrations promote cell growth hence, IC50 values recorded were 0.428 and 0.755, respectively (Fig. 7). Instead IC50 values for polysaccharides of *C. clavulatum* and *A. taxiformis* were 1.27 and 3.27, respectively indicate these polysaccharides have nutracetical values. This investigation results suggest that crude polysaccharides at high dose of red seaweeds *P. hornemannii* and *S. hypnoides* and at low dose of brown seaweed *P. pavonica* exhibit cytotoxic activity in HeLa cancer cell line whereas polysaccharides of *C. clavulatum* and *A. taxiformis* have nutraceutical values.

Conclusion

The sulfate recorded in the polysaccharide of *A. taxiformis* was higher followed by *C. clavulaum, P. pavonica, S. hypnoides* and *P. hornemannii*. The FTIR spectroscopy characteristics support that this low molecular weight sulfated polysaccharide fractions up to 14 kDa extracted from *S. hypnoides, C. clavulatum* and *A. taxiformi* were similar to agar polymer whereas *P. hornemannii* similar to carrageenan and polysaccharide of *P. pavonica* shows similarity to alginate possessing varying biological activities. Higher DPPH activity and reducing power were recorded in the polysaccharide

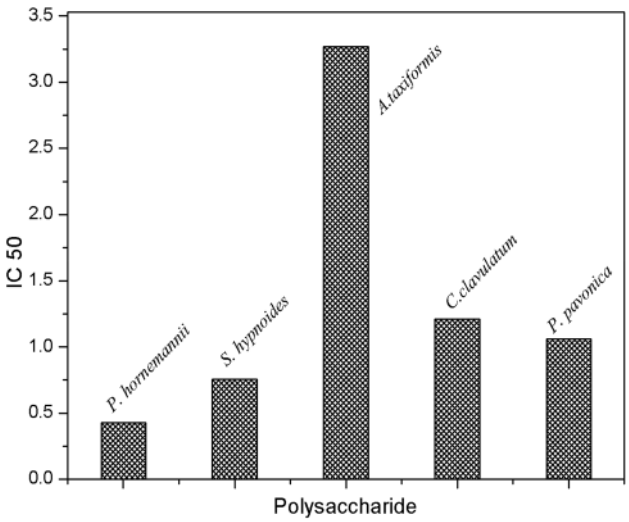

Fig. 7 IC50 value of cell survivability of different concentrations of polysaccharides (*Portieria hornemannii*-Ph, *Spyridia hypnoides*-Sh, *Asparagopsis taxiformis*-At, *Centroceras clavulatum*-Cc and *Padina pavonica*-Pp) tested by MTT Assay using HeLa cell line

of brown *P. pavonica* than red seaweeds. Thus, the study clearly suggest that low molecular weight carrageenan like polysaccharide extracted from *P. hornemannii,* agar like from *S. hypnoides* and alginate polymer from *P. pavonica* shows in vitro antioxidant activities which are capable of inhibiting HeLa cell line could be used for biomedical applications whereas polysaccharides of *C. clavulatum* and *A. taxiformis* for nutraceutical products.

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