Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.1.2013C477 

# Antioxidant and anti-inflammatory potential of the aqueous extract and polysaccharide fraction from brown marine macroalgae *Padina* sp. from Gulf of Mannar of Peninsular India

Nammunayathuputhenkotta Krishnankartha Praveen, Kajal Chakraborty*

*Marine Biotechnology Division, Central Marine Fisheries Research Institute, Ernakulam North P.O., P.B. No. 1603, Cochin-682018, Kerala, India*

PEER REVIEW

**Peer reviewer**

Prof. (Dr.) Ashim Chowdhury, Acharya P.C. Roy Professor of Chemistry, Fellow, National Academy of Agricultural Sciences, University of Calcutta, 35-Ballygunge Circular Road, Kolkata 700019, West Bengal.
Tel: +91-033-24615445/4959/5277 Ext 342;
+91-033-25299945 / 25798669 (Res)
E-mail: ashimchowdhry@gmail.com

**Comments**

This is a good study in which the authors evaluated the antioxidant and anti-inflammatory properties of brown seaweeds, *P. gymnospora* and *P. tetrastomatica* and their beneficial value as human food or as additives. This manuscript educates us about the type of molecules responsible for antioxidative and anti-inflammatory activities in the target seaweeds, pharmaceutical, and cosmetic industry.
Details on page 47

ABSTRACT

**Objective:** To evaluate the antioxidant and anti-inflammatory potential of the aqueous extract and polysaccharide fraction from two brown marine macroalga, *Padina gymnospora* (*P. gymnospora*) and *Padina tetrastomatica* (*P. tetrastomatica*) harvested from Gulf of Mannar of peninsular India.

**Methods:** The antioxidant activity was evaluated using different *in vitro* systems, *viz.*, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2′-azino-bis-3ethylbenzothiozoline-6-sulfonic acid diammonium salt (ABTS), $H_2O_2$/ HO• radical scavenging, $Fe^{2+}$ ion chelating ability, and reducing potential. Folin-Ciocalteu method was used to determine the total phenolic content, and the results were expressed as mg of gallic acid equivalents (GE). Thiobarbituric acid-reactive substance formation inhibition assay was employed to assess the ability of the samples to inhibit lipid oxidation in a model system. $COX_{II}$ and $LOX_V$ inhibition assays were employed to assess the anti-inflammatory potential of aqueous extract and polysaccharide fraction.

**Results:** The aqueous extract fraction of *P. tetrastomatica* realized high total phenolic content (288 mg GE/g), and its activity towards scavenging short-lived radicals (OH• and $H_2O_2$) (27.8% and 68.3%, respectively; 0.6 mg/mL) are higher than those registered for *Padina gymnospora*. Aqueous extract and polysaccharide fractions of *P. gymnospora* showed higher anti-inflammatory activities against $LOX_V$ (56% and 53%, respectively) and $COX_{II}$ (30% and 35%, respectively; 1 mg/mL) enzymes. The correlation studies confirmed that polysaccharides present with the *Padina* sp. are responsible for their anti-inflammatory potential. IR spectral data of polysaccharide fraction revealed the presence of polysaccharide in alginate form and also confirmed the presence of sulphated polysaccharides as principle bioactive constituents.

**Conclusions:** The study revealed that these seaweeds possess beneficial value as human food or health additives and can be used as a natural green remedy against oxidative stress induced inflammatory diseases.

KEYWORDS

*Padina tetrastomatica*, *P. gymnospora*, Antioxidant activity, Anti-inflammatory activity, Polysaccharide, Infra-red spectra

## 1. Introduction

Anti-inflammatory and antioxidant compounds are the substances, which can defend serious human diseases including melanoma, cardiac disorders, diabetes mellitus, inflammatory and neurodegenerative diseases[1] which are considered to be due to reactive oxygen species such as hydrogen peroxide ($H_2O_2$), hydroxyl radicals (HO•), superoxide anion ($O_2^{\bullet-}$) etc. These reasons explain their potential use in increasing shelf-life of food and as

*Corresponding author: Kajal Chakraborty, Marine Biotechnology Division, Central Marine Fisheries Research Institute, Ernakulam North P.O., P.B. No. 1603, Cochin-682018, Kerala, India.
Tel: +91 484 2394867; +91 484 2394909
E-mail: kajal_cmfri@yahoo.com
Foundation Project: Supported by the funding under the Science and Engineering Research Council (SERC) Scheme (SR/FTP/CS-63/2007) from Department of Science and Technology, New Delhi, India.

Article history:
Received 10 May 2013
Received in revised form 18 Jun, 2nd revised form 23 Jun, 3rd revised form 29 Jun 2013
Accepted 20 Jul 2013
Available online 28 Aug 2013

medicine[2,3]. Traditional drug treatments for relieving the pain and swelling of inflammation include non-steroidal anti-inflammatory drugs or NSAIDs which were reported to produce several side effects *viz.*, stomach ulcer if taken frequently[4]. Antioxidants which can counteract against these free radicals and inflammatory responses play significant roles in maintaining human health by protecting the cells from oxidative damage and inflammation.

Seaweeds constitute a major share of marine flora, and they were reported to possess structurally diverse compounds of various bioactivities endowed with antioxidant, anti-inflammatory, and anticarcinogenic activities[5]. These species were reported to grow in hostile saline habitats, and evolved a number of specialized biochemical mechanisms to withstand salt-induced oxidative stress in oceanic ecosystem. However, the absence of oxidative damage in the structural components, deterrence of predation, and the ability to reproduce successfully suggest that their cells are equipped with bioactive metabolites having anti-inflammatory and antioxidant properties, which provide competitive advantages to various oxidative stresses. Therefore these marine floras may be considered as valuable sources of natural antioxidant and anti-inflammatory ingredients, which could be used as food additives to provide nutrient stability and impart beneficial effect against inflammatory responses. *P. tetrastomatica* has been traditionally used as seasoning in dried flake form and as table salt replacement for high blood pressure patients and it was reported to contain alginic acid, a major polysaccharide which shows high anticoagulant and antiviral properties[6-8]. Antioxidant and anticoagulant activity of phenolics and sulfated polysaccharides have been identified from several brown seaweeds especially *Padina* sp.[9]. Alcoholic extracts from *Padina australis* were reported to possess DPPH radical scavenging activity[10]. There are published results showing *Padina* sp. to possess high phenolic content[11]. There are reports that the reducing power exhibited by *P. tetrastomatica* was higher than the standard antioxidant α-tocopherol[12]. Seaweed polysaccharides were also reported to be useful candidates to play an important role against inflammatory response and as free radical scavengers to prevent oxidative damage in living organisms[5]. Sulfated polysaccharides from brown seaweeds are known to have antioxidant importance and normalized lipid peroxidation status, and advocation of sulfated polysaccharides enhanced the antioxidant status, thereby preventing membrane injury and free radical formation[13].

Keeping in mind the adverse effects of synthetic NSAIDs and antioxidants, and the importance to explore novel sources to make a product useful against inflammatory diseases, it is imperative to target naturally available renewable sources to identify bioactive molecules for use against various deleterious stress-induced diseases *viz.*, inflammation and inflammation-induced oxidative stress without causing any serious adverse effects. The brown seaweeds contain a large assemblage of species that predominate in the coastal shelf areas of Gulf of Mannar region in southeastern coast of Indian subcontinent. Among various brown seaweeds, *Padina gymnospora* (*P. gymnospora*) (Kützing) Sonder and *P. tetrastromatica* (Hauck) are abundantly available in this area throughout different seasons, and therefore these species have been short listed for the present study to evaluate antioxidant and anti-inflammatory activities to understand their beneficial value as human food or health additives. This study therefore envisages evaluating the antioxidant and anti-inflammatory properties of the aqueous extract and polysaccharide fraction obtained from these species. We have also focused on using the infra red (IR) spectroscopic method as an effective tool to evaluate the structural features of polysaccharide fraction.

## 2. Materials and methods

### 2.1. Chemicals, reagents, and instrumentation

The solvents used to prepare samples were of analytical grade (E-Merck, Darmstadt, Germany) and were redistilled in an all-glass system. DPPH (1, 1-diphenyl-2-picrylhydrazyl), 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), lipoxidase extra pure ($LOX_V$), cycloxygenase-II (human recombinant, $COX_{II}$), carrageenan and linoleic acid were procured from Sigma-Aldrich Chemical Co. Inc. (St. Louis, MO). All other compounds were of analytical, spectroscopic or chromatographic reagent grade and were obtained from E-Merck (Darmstadt, Germany). The spectrophotometric measurements were performed using Varian Cary 50 conc UV-visible spectrophotometer (Varian Cary, USA). A Laboratory Freeze Dryer (Alpha 1-4 LD plus, Germany) was used for freeze-drying the samples. A bench top refrigerated centrifuge (Superspin PlastoCrafts R-V/Fm, Mumbai, India) was used for centrifugation. FT-IR was carried out by potassium bromide (KBr) pellet method and was recorded on Fourier transform-infrared spectrometer (type Shimadzu 8400S) in a range of 400-4000 $cm^{-1}$.

### 2.2. Aqueous extraction and polysaccharide fractionation

The seaweeds *P. tetrastomatica* and *P. gymnospora* were collected from Gulf of Mannar of Mandapam region located between 8°48′ N, 78°9′ E and 9°14′ N, 79°14′E on the southeast coast of India (Figure 1). The samples (2 kg) were thoroughly washed in fresh water to remove the epiphytes and other salts, and were shade dried and powdered. The dried seaweed powder (200 g) was thereafter extracted with hot water at 80-90 °C for 3-4 h to yield aqueous extract, which was cooled and centrifuged to remove the solid residues. One portion of the aqueous extract was freeze-dried to get the crude aqueous extract (yield 12% and 9% for *P.*

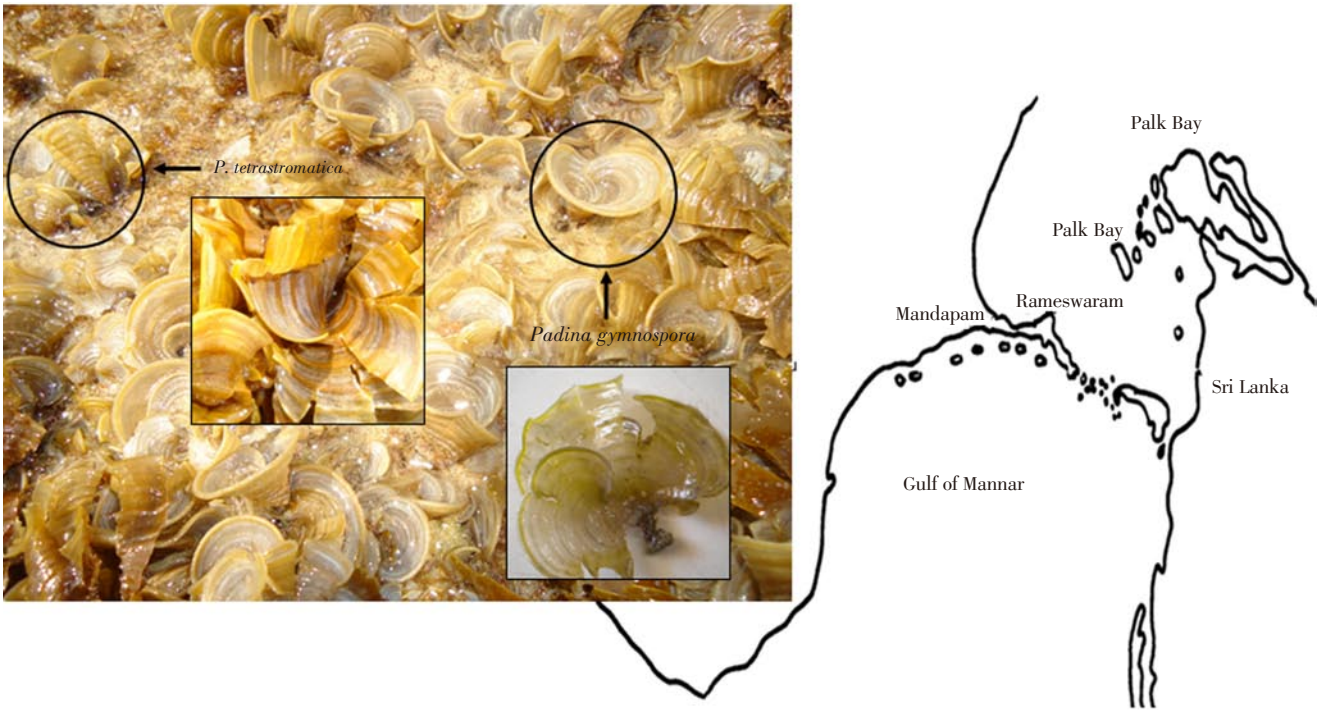


**Figure 1.** Seaweeds *P. tetrastomatica* and *P. gymnospora* were collected from Gulf of Mannar of Mandapam region located between 8°48′ N, 78°9′ E and 9°14′ N, 79°14′E on the southeast coast of India.

*tetrastomatica* aqueous extract and *P. gymnospora* aqueous extract, respectively). Another portion of the aqueous extract was concentrated (to 1/4$^{th}$), cooled, and precipitated with three volumes of ice cold ethanol. The precipitate was collected by centrifugation and lyophilized to get a dried brown crude polysaccharide fraction (yield 7% and 5% for *P. tetrastomatica* polysaccharide fraction and *P. gymnospora* polysaccharide fraction). This was then powdered and stored in a vacuum packed bag under refrigeration until further use.

### *2.3. Total phenolic contents and free radical scavenging activities of aqueous extract*

#### 2.3.1. Total phenolic contents

The total phenolic content of the aqueous extract was determined using the already established method with suitable modifications[14]. Gallic acid was used as a standard, and total phenolic content was expressed as mg of gallic acid equivalent (GE)/g sample.

#### 2.3.2. Evaluation of antioxidant activities by free radical scavenging assays

Free radical scavenging activity of the extracts were measured by 1,1-diphenyl-2-picryl- hydrazil (DPPH•) free radical using established method[15] with suitable modifications, and was calculated using the following equation: DPPH• scavenging effect (%)=100×($A_0$ − $A_1$/$A_0$), where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample. 2, 2′-Azino-bis-ethylbenzothiozoline-6-sulfonic acid diammonium salt assay was also used to assess the capacity of seaweed extract to scavenge free radicals following established method[15] with suitable modifications and the results were expressed as % radical scavenging ability. The ability of the aqueous extract to scavenge $H_2O_2$ was determined using established method[16]with suitable modifications. The percentage of scavenging of $H_2O_2$ of seaweed extracts was determined by the following formula: % scavenged ($H_2O_2$) = [($A_0$ − $A_1$)/A0]× 100, where $A_0$ was the absorbance of the control, and $A_1$ was the absorbance in the presence of the sample of the aqueous extract and standards. The HO• radical scavenging activity of the aqueous extract of the seaweeds was measured using established method[17] with suitable modifications. Percentage HO• radical scavenging activity was determined by comparing the results of the test and standard compounds.

#### 2.3.3. Evaluation of the ability of aqueous extract to inhibit lipid oxidation using thiobarbituric acid-reactive substances assay (TBARS)

This assay was based on the method described earlier with suitable modifications[18]. The model system used for TBARS assay was lyophilized mussel (*Perna viridis* L.) sample as a lipid source. The lipoperoxidation inhibitory activity was expressed as mmol/L of malonaldehyde equivalent compounds formed (MDAEC)/kg of sample.

#### 2.3.4. Evaluation of aqueous extract towards reducing ability and ferrous ion ($Fe^{2+}$) chelating activity

Total reduction capabilities and ferrous ($Fe^{2+}$) ion chelating activities of the crude aqueous extract of the seaweeds were estimated by using the established method with modifications [19,20].

### 2.4. Polysaccharide fraction of *Padina sp.*

#### 2.4.1. Chemical composition

Total carbohydrate was determined by the phenol-sulphuric acid colorimetric method[21] with D-glucose as standard at 490 nm. Sulfate content was measured with the gelatin-barium method, using $Na_2SO_4$ as the standard[22].

#### 2.4.2. IR spectral analysis

IR spectroscopy has been used to identifying the structural features based on the functional group of the compounds in a nondestructive manner. The aqueous extracts and polysaccharide fraction obtained from both the seaweed samples were analyzed as a KBr pellet.

#### 2.4.3. *In vitro* antioxidant potential of polysaccharide fraction

The antioxidant potential of the TCP was estimated by DPPH• radical scavenging, total reduction, and $Fe^{2+}$ ion chelating activities as illustrated in the earlier section. The ability to inhibit lipid oxidation in model systems was carried out by TBARS assay. All the above assays performed according to the procedure described under section 2.3.

### 2.5. *In vitro anti-inflammatory potential of aqueous extract and TCP*

#### 2.5.1. Determination of *in vitro* anti-inflammatory activity using cyclooxygenase ($COX_{II}$) inhibition assay

The COX inhibition assay was performed according to a modified method of[23]. The oxidation of leuco-dichlorofluorescein (1-DCF) in the presence of phenol by the hydroperoxide formed in the COX reaction can be used as a sensitive spectrophotometric assay for PGH synthase activity. Leuco-2,7-dichlorofluorescein diacetate (5 mg) was hydrolysed at RT in 1 mol/L NaOH (50 μL) for 10 min, then 1 mol/L HCl (30 μL) was added to neutralise excess NaOH before the resulting 1-DCF was diluted in 0.1 mol/L Tris-buffer, pH 8. COX enzyme ($COX_{II}$) was diluted in 0.1 mol/L Tris-buffer, pH 8, so that a known aliquot gave an absorbance change of 0.05/min in the test reaction. Test samples (or the equivalent volume of MeOH, 20 μL) were pre-incubated with enzyme at RT for 5 min in the presence of hematin. Premixed phenol, 1-DCF and AA were added to the enzyme mixture to begin the reaction, and to give a final reaction mixture of AA (50 μmol/L), phenol (500 μmol/L), 1-DCF (20 μmol/L) and hematin (1 μmol/L) in 1 mL final volume of 0.1 mol/L Tris-buffer, pH 8. The reaction was recorded spectrophotometrically over 1 min at 502 nm. A blank reaction mixture was analyzed in the spectrophotometer reference cell against each test reaction to account for any non-enzymatic activity attributed to the test sample.

#### 2.5.2. 5-lipoxygenase (LOXV) inhibition assay

The LOXV assay was used as an indication of the AI activity with suitable modification of the described method[24]. An aliquot of of the stock solution (50 μL, in DMSO and Tween 20 mixture; 29:1, w/w) of each test sample was placed in a 3-ml cuvette, followed by pre-warmed 0.1 mol/L potassium phosphate buffer (2.95 mL, pH 6.3) and linoleic acid solution (48 μL). Thereafter, ice-cold buffer (potassium phosphate) (12 μL) were mixed with of the thawed enzyme (100 U). The mixture was then transferred to the cuvette and the contents of the cuvette was shaken and placed into the spectrophotometer, before the absorbance was recorded at 234 nm. It is important to note that, prior to testing the sample; two samples were prepared as mentioned above but only with DMSO and Tween 20 mixture, to serve as controls (no enzyme inhibition).

### 2.6. *Statistical analysis*

Statistical analysis was done using SPSS 13.0 for windows package. Analyses were carried out in triplicate, and the means of all parameters were examined for significance ($P<0.05$) by analysis of variance (ANOVA) to assess significant protection in treatment groups. One-way ANOVA was performed followed by post-hoc LSD. The mean variance in the data set was detected using principal component analysis. All data were mean centered and scaled to equal unit variance prior to principal component analysis.

## 3. Results

### 3.1. *Total phenolic contents and antioxidant activities of aqueous extract of Padina sp.*

#### 3.1.1. *Total phenolic contents*

In the present study it was observed that aqueous extract of *P. tetrastomatica* realized a total phenolic content of 288.2 GE/g that is significantly higher than that of *P. gymnospora* (170.6 mg GE/g; ($P<0.05$).

#### 3.1.2. *Antioxidant activities of aqueous extract by ABTS and DPPH• radical scavenging assays*

In the present study ABTS+ free radicals scavenging activity (%) of aqueous extract obtained from *P. gymnospora* was higher (6.7%-29.1% at 0.1-0.6 μg/mL) as compared to that in *P. tetrastomatica* (3.9% and 27.3%, respectively) (Table 1), but were not significantly different. At lower doses (0.1 and 0.2mg/ml) only aqueous extract of *P. gymnospora* was effective to scavenge DPPH free radicals. But no significant differences in DPPH radical scavenging activities were apparent between the aqueous extract of *P. gymnospora* & *P. tetrastomatica* ($P<0.05$) at higher tested concentrations (45-58.4%, at 0.5 and 0.6 mg/mL) (Table 1). Total phenolic content assay registered correlation with DPPH scavenging activities as realized by PCA analyses (Figure 2).

**Table 1**
Antioxidant potential of aqueous extract of *P. tetrastomatica* and *P. gymnospora* at different concentrations.

| Concentrations ( mg/mL) | | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 |
|---|---|---|---|---|---|---|---|
| ABTS radical scavenging ability (%)# | *P. gymnospora* | $6.70^{ap}$±0.96 | $12.18^{aq}$±2.06 | $14.31^{aqr}$±0.35 | $14.75^{ar}$±1.00 | $22.14^{at}$±0.78 | $29.14^{au}$±1.60 |
| | *P. tetrastomatica* | $3.96^{ap}$±0.34 | $4.81^{bp}$±0.11 | $10.33^{aq}$±0.51 | $14.27^{aqr}$±0.83 | $20.95^{as}$±0.09 | $27.37^{at}$±0.99 |
| DPPH radical scavenging ability (%) | *P. gymnospora* | $6.90^{ap}$±0.01 | $11.48^{aq}$±0.72 | $22.78^{a}$±0.40 | $38.81^{as}$±0.88 | $47.52^{at}$±2.26 | $58.40^{au}$±0.55 |
| | *P. tetrastomatica* | ND | ND | $13.04^{bq}$±1.14 | $31.86^{br}$±0.33 | $45.22^{as}$±1.22 | $55.85^{at}$±0.56 |
| HO• radical scavenging activity (%) | *P. gymnospora* | $5.77^{ap}$±0.55 | $11.27^{aq}$±1.51 | $12.90^{aqr}$±0.86 | $17.92^{as}$±0.89 | $20.03^{ast}$±0.73 | $23.38^{atu}$±1.38 |
| | *P. tetrastomatica* | $14.12^{bp}$±1.54 | $19.14^{bq}$±0.79 | $19.87^{bqr}$±0.78 | $24.14^{bs}$±0.22 | $24.36^{bst}$±1.40 | $27.83^{asu}$±0.49 |
| $H_2O_2$ scavenging activity (%) | *P. gymnospora* | $7.19^{ap}$±0.39 | $8.38^{ap}$±0.64 | $16.21^{aq}$±1.50 | $33.43^{ar}$±0.88 | $45.09^{as}$±1.26 | $60.76^{at}$±1.33 |
| | *P. tetrastomatica* | $12.86^{bp}$±0.52 | $18.42^{bq}$±0.81 | $31.66^{br}$±0.98 | $49.02^{bs}$±1.21 | $56.18^{bt}$±0.85 | $68.30^{bu}$±1.40 |
| TBARS formation inhibition assay (MDAEC/kg)** | *P. gymnospora* | $51.12^{ap}$±1.51 | $43.08^{aq}$±0.57 | $35.44^{ar}$±1.53 | $15.28^{as}$±0.30* | $8.76^{at}$±0.41** | $6.09^{atu}$±0.56*** |
| | *P. tetrastomatica* | $44.00^{bp}$±1.50 | $41.69^{ap}$±1.37 | $18.89^{bq}$±0.71 | $13.37^{ar}$±0.50 | $10.26^{as}$±0.36 | $7.96^{ast}$±0.18 |
| Reducing capacity($Ab_{700}$ nm) | *P. gymnospora* | $0.19^{ap}$±0.01 | $0.21^{aq}$±0.00 | $0.26^{ar}$±0.00 | $0.29^{as}$±0.00 | $0.35^{at}$±0.01 | $0.41^{au}$±0.00 |
| | *P. tetrastomatica* | $0.20^{ap}$±0.00 | $0.22^{aq}$±0.00 | $0.22^{aqr}$±0.00 | $0.31^{as}$±0.00 | $0.37^{bt}$±0.00 | $0.42^{au}$±0.00 |
| $Fe^{2+}$ ion chelating activity(%) | *P. gymnospora* | $44.09^{ap}$±0.80 | $62.31^{aq}$±0.39 | $66.97^{ar}$±2.09 | $81.59^{as}$±0.76 | $82.62^{ast}$±0.56 | $85.34^{asu}$±0.84 |
| | *P. tetrastomatica* | $25.24^{bp}$±0.04 | $34.12^{bq}$±0.23 | $41.64^{br}$±0.69 | $49.70^{bs}$±0.41 | $57.36^{bt}$±0.95 | $63.61^{bu}$±2.61 |

Data are the mean values of triplicate and expressed as mean± standard deviation. Row (p–u) and column (a–b); values with different letters are significantly different ($P<0.05$). *, ** and *** are having concentrations 1, 1.5 and 2 mg/mL, respectively. # for ABTS assay the concentrations used were 0.1 to 0.6μg/mL

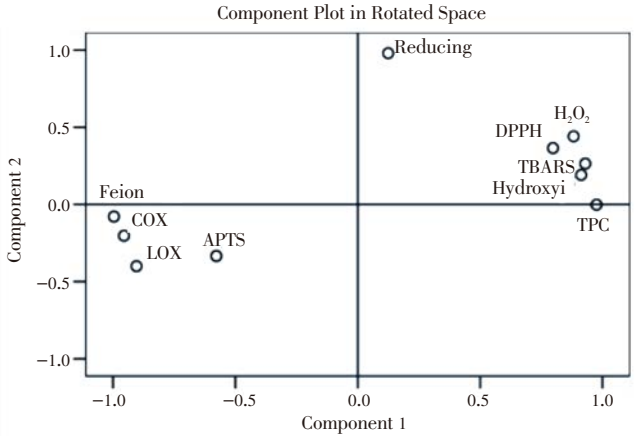


**Figure 2.** PCA loading plot showing the correlation between antioxidant activity assays of *in vitro* anti-inflammatory and antioxidant potential of aqueous extract and polysaccharide fraction of both the brown seaweeds *P. tetrastomatica* and *P. gymnospora*.

### *3.1.3. Assays for detection of scavenging of transiently stable radicals of aqueous extract from Padina sp.: $H_2O_2$ and HO• scavenging activities*

Aqueous extract of *P. tetrastomatica* realized significantly higher ($P<0.05$) HO• radical scavenging activities (>27%, 0.1–0.6 mg/mL) than that of *P. gymnospora* (>23%) (Table 1). The activities were found to be proportionately decreased with decrease in concentration. It is of note that aqueous extract of *P. tetrastomatica* also found to be significantly effective ($P<0.05$) to scavenge $H_2O_2$ (>68.3 %) than that of *P. gymnospora* (>60.7%) in all the tested doses (0.1–0.6 mg/mL) (Table 1).

### *3.1.4. Ability of aqueous extract of Padina sp. to inhibit lipid oxidation: thiobarbituric acid-reactive substances assay*

The aqueous extract fraction of *P. gymnospora* showed higher TBARS formation inhibition ability (6–8.7 mmol/L MDA equivalent compound formed/kg of the sample (MDAEC/kg)) than *P. tetrastomatica* (7–10.2 MDAEC/kg) at higher concentrations (1.5 and 2 mg/mL) (Table.1). The activities were found to be proportionately decreased with concentrations (Table 1). It is of note that at lower doses (0.1–1 mg/mL) aqueous extract of *P. tetrastomatica* (13–44 MDAEC/kg) was superior to that of *P. gymnospora* (15–51 MDAEC/kg) towards inhibiting TBARS formation.

### *3.1.5. $Fe^{2+}$ chelating and reducing abilities of aqueous extract of Padina sp.*

Aqueous extract of *P. gymnospora* (0.6 mg/mL) showed significantly higher ($P<0.05$) $Fe^{2+}$ chelating activity (85.34%) than that of *P. tetrastomatica* (63.61%). The same trend was followed for the lower concentrations. The aqueous extract of both *P. gymnospora* and *P. tetrastomatica* were equally effective in their reducing potential values ($A_{700\ nm}$ 0.19 and 0.42, 0.1–0.6 mg/mL) as they were not significantly different ($P>0.05$) among each other in each tested concentrations.

## *3.2. Polysaccharide fraction of Padina sp.*

### *3.2.1. Chemical composition*

The polysaccharide fraction obtained from *P. tetrastomatica* and *P. gymnospora* appeared to be sulfated as a total sulfate content of 3.60 and 5.66 % $K_2SO_4$ equivalence were recorded. The total carbohydrate content (%) found with the polysaccharide fraction from *P. tetrastomatica* and *P. gymnospora* were 33.16% and 33.28%.

### *3.2.2. IR spectroscopic analyses of polysaccharide fraction*

The IR spectra with respect to the aqueous and polysaccharide fractions of *P. tetrastomatica* and *P. gymnospora* have been illustrated under Figure 3 and Figure 4. The IR spectrum of common and dominant brown seaweed polysaccharides, sodium alginate and alginic acid were

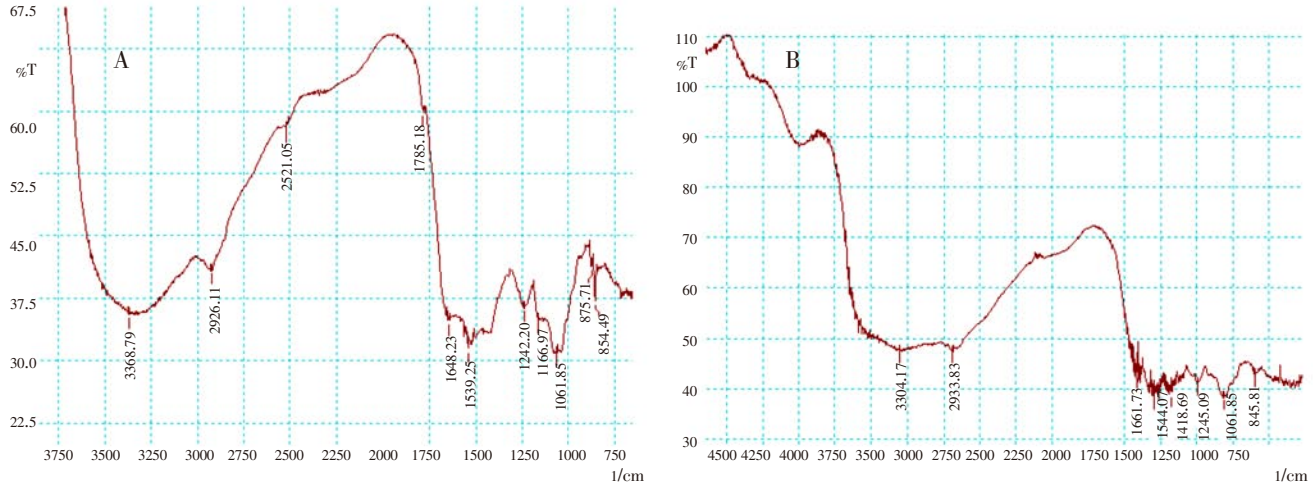


**Figure 3.** Infra red spectra of (A) aqueous extract and (B) polysaccharide fraction of *P. tetrastomatica*.

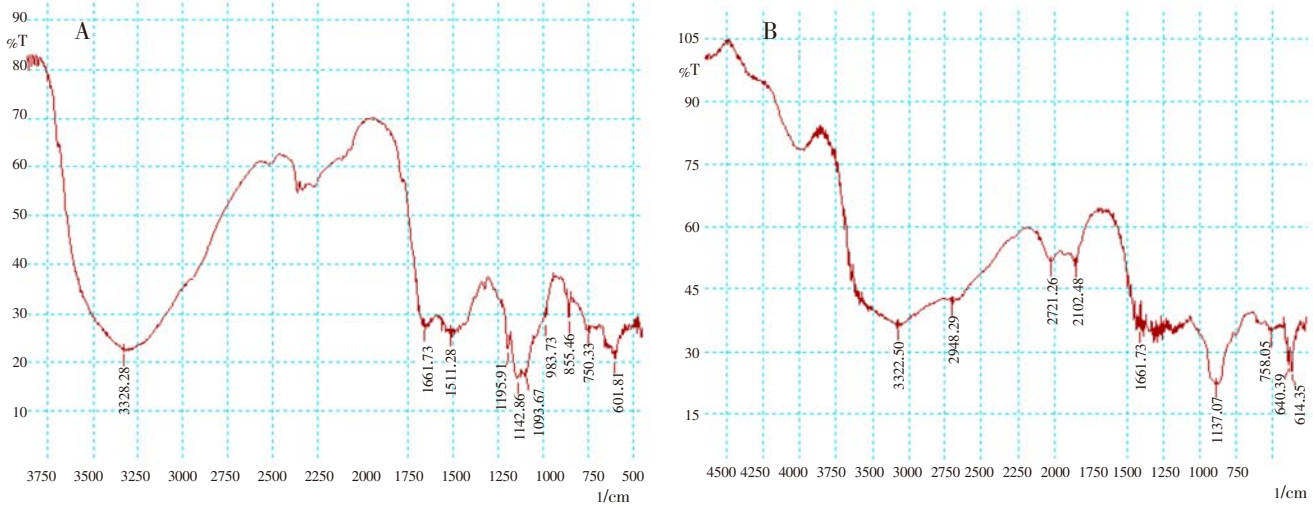


**Figure 4.** Infra red spectra of (A) aqueous extract and (B) polysaccharide fraction of *P. gymnospora*.

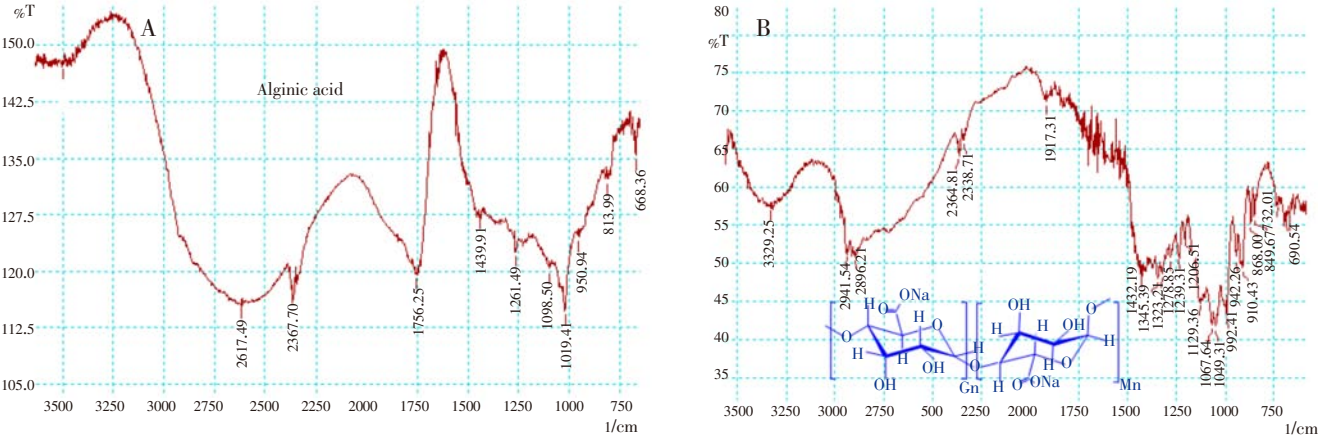


**Figure 5.** Infra red spectra of alginic acid and sodium alginate standards (A and B, respectively).

given for comparison (Figure 5). The IR spectrum indicates the presence of polysaccharide units and glycosidic bonds along with common functional groups present in aqueous extract (Figure 3A, 4A). On the IR spectrum, broad absorption peaks around at 3200–3500 $cm^{-1}$ was observed. Absorption peaks are also found between 2800–2900 $cm^{-1}$. Characteristic bands are also observed at 1600 $cm^{-1}$. The IR spectra of both the polysaccharide fractions exhibited a broad band around 1220–1260 $cm^{-1}$, and bands at 850 $cm^{-1}$, 1025 and 1100 $cm^{-1}$ are also observed as appeared in (Figure 3B, 4B).

### *3.2.3. Antioxidant potential of polysaccharide fraction obtained from Padina sp.*

The results obtained with total polysaccharide fraction from *Padina* sp indicated *P. tetrastomatica* was found to be more effective in scavenging free radicals (DPPH•) than *P. gymnospora*. polysaccharide fraction of *P. tetrastomatica* was able to inhibit DPPH to the tune of 51% at a low concentration of the active principle (0.5 mg/mL) than 32%

realized by *P. gymnospora*. At higher tested concentration of *P. tetrastomatica* polysaccharide fraction, (1 mg/mL) the inhibitory effect reached to 77.5% whereas only 44.5% DPPH. scavenging ability was observed with the polysaccharide fraction of *P. gymnospora*. Although the scavenging activity was found to be proportionately decreased with concentrations, *P. tetrastomatica* polysaccharide fraction exhibited significantly higher OH. scavenging activities (8-65%) than that of *P. gymnospora* (3-20%, $P<0.05$) at all tested concentrations (0.25-1 mg/mL). Among all the tested doses (0.25-1 mg/mL) *P. tetrastomatica* polysaccharide fraction exhibited a significantly higher ($P<0.05$) reducing ability ($A_{700\ nm}$ 0.34-0.90) than those realized by *P. gymnospora* ($A_{700\ nm}$ 0.14-0.35) (Table 2). *P. tetrastomatica* polysaccharide fraction registered significantly higher ($P<0.05$) $Fe^{2+}$ chelating activity to the tune of 43% at 1 mg/mL than 39% recorded by *P. gymnospora*. All the test doses except the lowest one (0.25 mg/mL) a significantly higher ($P<0.05$) $Fe^{2+}$ chelating activity was observed with the polysaccharide fraction of *P. tetrastomatica* than that exhibited by *P. gymnospora*. *P. tetrastomatica* polysaccharide fraction also showed marked ($P<0.05$) ability to inhibit TBA-MDA adduct formation (14.9 MDAEC/kg, 2 mg/mL) than *P. gymnospora* (21.2 MDAEC/kg) (Table. 2) and the same trend was observed with all the tested concentrations. *P. tetrastomatica* polysaccharide fraction also showed marked ability to inhibit TBA-MDA adduct formation (14.9 MDAEC/kg, 2 mg/mL) than *P. gymnospora* (21.2 MDAEC/kg) (Table 2).

**Table 2**
Antioxidant activity exhibited by polysaccharide fraction of *P. tetrastomatica* and *P. gymnospora* in different concentrations.

| Concentrations (mg/mL) | | 0.25 | 0.50 | 0.75 | 1.00 |
|---|---|---|---|---|---|
| DPPH radical scavenging ability (%) | A | $34.9^{ap}\pm0.35$ | $50.89^{bp}\pm0.42$ | $65.1^{cp}\pm0.86$ | $77.55^{dp}\pm0.4$ |
| | B | $20.58^{aq}\pm0.56$ | $31.96^{bq}\pm0.59$ | $40.98^{cq}\pm0.38$ | $44.52^{dq}\pm0.05$ |
| Reducing ability ($Ab_{700}$) | A | $0.34^{ap}\pm02$ | $0.46^{bp}\pm01$ | $0.65^{cp}\pm0.01$ | $0.90^{dp}\pm02$ |
| | B | $0.14^{aq}\pm01$ | $0.22^{bq}\pm01$ | $0.29^{cq}\pm0.01$ | $0.35^{dq}\pm02$ |
| $Fe^{2+}$ ion chelating ability (%) | A | $8.66^{ap}\pm0.59$ | $17.09^{bp}\pm1.37$ | $33.39^{cp}\pm0.83$ | $42.53^{dp}\pm0.46$ |
| | B | $8.09^{ap}\pm0.6$ | $11.79^{bq}\pm0.54$ | $25.77^{cq}\pm0.41$ | $39.16^{dq}\pm0.12$ |
| Hydroxyl radical scavenging ability (%) | A | $8.45^{ap}\pm0.4$ | $34^{bp}\pm0.15$ | $47.91^{cp}\pm0.35$ | $62.54^{dp}\pm0.15$ |
| | B | $3.4^{aq}\pm0.24$ | $12.51^{bq}\pm0.26$ | $18.02^{cq}\pm0.37$ | $20.91^{dq}\pm0.53$ |
| TBARS formation inhibition assay (MDAEC/kg) | A | $89.76^{ap}\pm0.53$ | $51.02^{bp}\pm0.62^{*}$ | $27.19^{cp}\pm0.08^{**}$ | $14.92^{dp}\pm0.66^{***}$ |
| | B | $98.37^{aq}\pm0.91$ | $85.32^{bq}\pm0.75^{*}$ | $66.34^{cq}\pm1.77^{**}$ | $21.22^{dq}\pm0.75^{***}$ |

Data are the mean values of triplicate and expressed as mean± standard deviation. Row (a-d) and column (p-q) values with different letters are significantly different ($P<0.05$). *, ** and *** are having concentrations 1, 1.5 and 2 mg/mL, respectively.
A: *P. tetrastomatica*; B: *P. gymnospora*.

### 3.3. In vitro anti-inflammatory potential of aqueous extract and TCP of Padina sp

The anti-inflammatory properties of aqueous extract and TCP were evaluated *in vitro* to determine their $COX_{II}$ and $LOX_V$ inhibitory potencies, and were compared to synthetic NSAID aspirin used as positive control (Figure 6). Particularly, *P. gymnospora* aqueous extract showed excellent inhibitory activity against LOXV (56%, 1 mg/mL), and its inhibitory activity was found to be more than *P. tetrastomatica* (52%, 1 mg/mL). aqueous extract and TCP of *P. gymnospora* also exhibited potent inhibitory action on $COX_{II}$ (31 and 35%, respectively, 1 mg/mL or 0.01%), and a higher anti-$COX_{II}$ activitiy was reported with respect to synthetic NSAID (aspirin 50%).

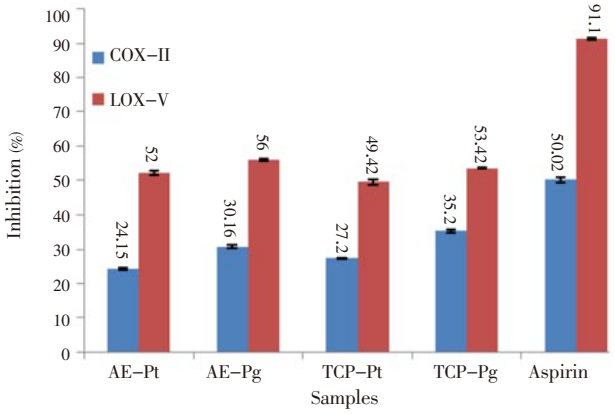


**Figure 6.** *In-vitro* anti-inflammatory potential (percent inhibition of COXII and LOXV enzymes) exhibited by aqueous extract (AE) and polysaccharide fraction of *P. tetrastomatica* (Pt) and *P. gymnospora* (Pg). Data are the mean values of triplicate. Inhibition (%) has been recorded at 1 mg/mL.

### 3.4. Correlations between total phenolic contents in aqueous extract and TCP with anti-inflammatory and antioxidant activities

The similarities and differences of the total phenolic content and different antioxidant activity indicators of aqueous extract, and the relationships among them were statistically analyzed using PCA. The first two principal components explained 88.46% (PC 1-78.19%; PC 2-10.27%) of the total variance in the data set (Figure 2). The results of the various parameters studied shows that total phenolic content to significantly correlate with DPPH/$H_2O_2$/OH. scavenging potential and inhibition of TBA-MDA adduct formation. A significant but negative correlation was observed between the ability of the seaweeds to chelate $Fe^{2+}$ ion and total phenolic content/DPPH/$H_2O_2$/OH. scavenging potential and inhibition of TBA-MDA adduct formation. Interestingly, the polysaccharide fraction of *P. tetrastomatica* demonstrated higher antioxidative activities than *P. gymnospora*; whereas the latter exhibited higher anti-inflammatory potential than the former. A negative correlation was realized between $Fe^{2+}$ ion chelating ability of aqueous extract and other antioxidative indicators as evident from the principle component analyses plot (Figure 2). Also, it was observed that these antioxidative indicators are found to be negatively correlated with $COX_{II}$ and $LOX_V$ inhibition.

## 4. Discussion

Phenolic antioxidants apparently disrupt free-radical chain reaction by donating H· to fatty acid radicals to terminate chain reactions, and, therefore have roles to

inhibit lipid peroxidation. The presence of phenolic compounds is indicative of potential antioxidant activities in terms of their ability to scavenge free radicals, inhibiting lipoprotein oxidation, efficient metal chelators, and reducing oxidation of low-density lipoproteins[25,15]. There are other reports to support the present study that brown seaweeds and *Padina* species are rich resources of antioxidant phenolic compounds[11]. The results of the various antioxidant parameters studied demonstrated a significant correlation between the phenolic content and the antioxidant activities by different *in vitro* systems. From these observations it can be inferred that aqueous extract of both the seaweed species selected for the present study are enriched with polyphenolic compounds endowed with antioxidative potential.

A direct idea about the antioxidant competence of a compound can be obtained by ABTS and DPPH assays. The assay is base on the principle that the ABTS free radical generated by the reaction between ABTS• and $K_2S_2O_8$ reduces to ABTS• by the antioxidant compounds, which can be easily detected spectrophotometrically by decrease in absorbance at 734 nm. DPPH has a nitrogen free radical in its structure, and is readily destroyed by a free radical scavenger, with a capacity to donate a H to DPPH, and is commonly used to evaluate free radical scavenging activities of extracts/ compounds. This high antioxidant potential demonstrated by the aqueous extract of both the selected *Padina* sp showed that aqueous extract of these seaweeds could be potential rich sources of natural antioxidants. There are reports suggesting that the *Padina* sp are good reservoirs of potentially active antioxidant compounds[12] capable of deactivating free radicals such as ABTS and DPPH[9,26].

Hydrogen peroxide, a reactive non radical compound, is very important as it can penetrate biological membranes. Although $H_2O_2$ itself is not very reactive, it may convert into more reactive species such as singlet oxygen and HO• radicals. The results obtained by $H_2O_2$ and HO• Assays from our present study showed significant positive correlation with DPPH. scavenging and total phenolic content as realized by PCA analyses indicate the fact that the polyphenolic compounds present in aqueous extract may be the reason for its antioxidant potential. There are earlier studies which showed that brown seaweeds are good scavengers of $H_2O_2$ and HO• radical which is in support with our present observation[25].

The TBA value is an index of lipid peroxidation measuring malondialdehyde content. Higher TBARS formation inhibition activity exhibited by fraction aqueous extract of the seaweeds were found to be significantly correlated with their high phenolic contents and TBARS as realized by PCA analyses. This observation indicates that the inhibition in lipid peroxidation may be due to the presence of polyphenolic antioxidants that were reported to disrupt free-radical chain reaction by donating proton to fatty acid radicals to terminate chain reactions, may have roles to inhibit lipid peroxidation[5].

The reduced form of iron ($Fe^{2+}$) can stimulate and accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract H- and perpetuate chain reaction of lipid peroxidation[25]. The negative correlation realized between total phenolic content and ion chelating potential lead us to conclude that the chelation potential exhibited by the aqueous extract may be due to the presence of polysaccharides like alginate/ fucoidan present with the aqueous extract. An earlier study demonstrated that seaweed-derived polysaccharides are more effective than phlorotannins (phenolics) to detoxify transition metal accumulation by chelation[27]. Metal ($Fe^{2+}$) chelating agents, by virtue of their capacities to stabilize the oxidized form of $Fe^{2+}$ to reduce the redox potential, are effective secondary antioxidants.

The high reducing potential exhibited by the aqueous extract of both the seaweeds indicates the presence of potential antioxidant principles harbored in aqueous extract, and may function against various parameters responsible for oxidative stress. Earlier studies too support the present observation that brown seaweeds possess high reducing ability[25]. A correlation between reducing potential and antioxidant activities, as established by PCA analyses, indicated the presence of similar groups of compounds responsible for the reducing potential and chelating ability. The results have been supported by earlier studies to infer that these seaweed species may harbour phenolics as potential reductants by breaking the free radical chain by donating a hydrogen atom to reduce the reactive free radicals thereby inhibiting oxidation of biomolecules[28].

IR is a fast analytical technique which requires meager amount of sample, and is non-destructive. Polysaccharide fraction/complexes were reported to be one of the principle active ingredients responsible for anti-inflammatory activities in brown seaweeds[1]. These polymers received considerable attention because of their anticoagulant, antithrombotic, anti-proliferative/antitumor/ anticancer, immunomodulatory, anti-inflammatory, and other biological activities[29]. It is of note that soluble alginate in salt form (stable in solution between pH 6 and 9) is converted into insoluble alginic acid in free acid form (at acid pH) and this conversion of soluble alginate into insoluble alginic acid also happens naturally in seaweeds[30]. The band around 1600 $cm^{-1}$ is due to the existence of carboxylate group as carboxylate anion form (COO-) in alginate, than carboxylic acid ester form (C=O, around 1700 $cm^{-1}$) as in alginic acid[30]. This observation substantiates the fact that the polysaccharide present with *Padina* sp is in alginate form than alginic acid form. The IR spectra of polysaccharide fractions exhibited a broad band around 1220-1260 $cm^{-1}$, which has been assigned to be due to the presence of sulphate ester groups (S=O) characteristic component in fucoidan and homogenous sulphated polysaccharides other than alginates in brown seaweeds[31,32]. An additional sulphate absorption band around 850 $cm^{-1}$ (C-O-S, secondary axial sulphate) indicated that the sulphate group is located

at position 4 of the fucopyranosyl residue[33]. Bands around 1025 and 1100 $cm^{-1}$ as appeared in Figure 4B arise from guluronic and mannuronic acid residues of sodium alginate respectively[34].

*P. tetrastomatica* polysaccharide fraction also showed marked ability to inhibit TBA-MDA adduct formation than *P. gymnospora* thereby indicating that this polysaccharide fraction fraction of *P. tetrastomatica* can be effective towards inhibiting lipid peroxidation. There are other research works showing that the polysaccharides possess potent antioxidant and lipoperoxidation activity[11]. There are reports indicating that polysaccharides play major role to deter the formation of lipid oxidation products in biological system[35].

Prostagalndins and leukotrienes are potent mediators of inflammation that results in edema, pain, and vasodilation, and are derived from arachidonic acid metabolism by cycloxygenases (COXs) and lipoxidases (LOXs), respectively, which play important roles in modulating a number of pathophysiological conditions, including inflammatory and allergic immune response[36]. In particular, the enzymes $COX_{II}$ and $LOX_V$ catalyze the oxygenation of n-6 polyunsaturated fatty acids, preferably arachidonate (20:4 n-6, AA), to form the biologically active prostagalndins and leukotriene metabolites. It was reported that seaweed extracts decrease the production of inflammatory prostagalndins and leukotrienes, by acting as powerful inhibitors of $COX_{II}$ and/or $LOX_V$[37]. The anti-inflammatory potential of both aqueous extract and polysaccharide fraction of both the seaweeds as revealed from its $COX_{II}$ and $LOX_V$ inhibition values indicate the fact that they are the reservoirs of potential anti-inflammatory agents for use in food and pharmaceutical products in place of synthetic ones to decrease the adverse effect caused by the usage of NSAIDs.

The results of the various parameters studied using PCA showed that total phenolic content to significantly correlate with DPPH/$H_2O_2$/OH· scavenging potential and inhibition of TBA-MDA adduct formation, indicating the presence of phenolic compounds in aqueous extract, capable of inhibiting the radical formation and lipid peroxidation. This finding is in agreement with the earlier reports indicating a positive correlation between total phenolic contents and antioxidant activities of seaweed extracts[26]. A significant but negative correlation was observed between the ability of the seaweeds to chelate $Fe^{2+}$ ion and total phenolic content/ DPPH/$H_2O_2$/OH· scavenging potential and inhibition of TBA-MDA adduct formation, thereby demonstrating the presence of other non-phenolic compounds like polysaccharides capable of chelating metal ions and with antioxidant potential. The variation observed between the activities exhibited by the aqueous extract and polysaccharide fraction of the selected seaweeds led us to conclude that the antioxidant and anti-inflammatory activities in polysaccharide fraction and aqueous extract are due to different sets of bioactive molecules. The results of the various parameters studied shows fraction *P. tetrastomatica* aqueous extract and TCP as effective reducing agent, reactive against TBA-MDA adduct formation, and were effective in stabilizing the OH/$H_2O_2$ radicals. Also, we note that these antioxidative indicators are found to be negatively correlated with $COX_{II}$ and $LOX_V$ inhibition. These observations further demonstrate the presence of other non-phenolic compounds like polysaccharides capable of chelating metal ions and are good anti-inflammatory agents. It was reported that seaweed extracts decrease the production of inflammatory prostagalndins and leucotrienes, by acting as powerful inhibitors of $COX_{II}$ and/or $LOX_V$[37]. There are studies reporting that polysaccharides present with the brown seaweeds were identified to possess potent metal binding and antioxidant capacities[11,38] and may be because of the same reason in our present study a negative correlation was realized between antioxidant potential and ion chelating ability. All these results apparently indicate that several states of reduced oxygen and short-lived free radicals involved in the oxidative events are well scavenged by the seaweed derived polyphenolics. A high negative correlation between total phenolic content and $COX_{II}$ and $LOX_V$ inhibition values inferred that the compounds other than phenolics like polysaccharides present with the seaweeds belonging to *Padina* sp. are good anti-inflammatory agents, therefore could be used as a natural green alternative of the synthetic non-steroidal anti-inflammatory drugs available in the market.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgements

This work is supported by the funding under the Science and Engineering Research Council Scheme (SR/FTP/CS-63/2007) from Department of Science and Technology, New Delhi, India. The authors are thankful to Indian Council of Agricultural Research, New Delhi for providing necessary facilities and encouragements to carry out the work. The authors thank Dr. G. Syda Rao, Director, Central Marine Fisheries Research Institute for his guidance and support. Thanks are due to Dr. K.K. Vijayan, Head, Marine Biotechnology Division, Central Marine Fisheries Research Institute for facilitating the research activity under the project.

## Comments

### *Background*

Antioxidant and anti-inflammatory compounds play vital roles against various diseases such as chronic inflammation, atherosclerosis, cancer, cardiovascular disorders, and ageing processes that explains their potential use in medicine, food

production, and the cosmetic industry. The adverse effects of synthetic anti-inflammatory drugs and antioxidants, and the importance to explore novel sources to make a product useful against inflammatory diseases. Antioxidant and anti-inflammatory activity of phenolics have been identified from several brown seaweeds, and seaweeds as a source of phenolic compounds shall spell out their importance as novel sources of antioxidative and anti-inflammatory molecules.

### *Research frontiers*

Although antioxidant and anti-inflammatory properties of seaweeds were proved by numerous studies from past two decades there is scanty information regarding the antioxidant potential from *Padina* spp from the coastal shelf areas of Gulf of Mannar region in southeastern coast of Indian subcontinent. In the present study *P. gymnospora* and *P. tetrastomatica* abundantly available along with the south-east coast of Indian subcontinent have been evaluated for antioxidant and anti-inflammatory activities, and the results revealed the potential of *Padina* spp. to isolate valuable molecules including polysaccharides and phenolic compounds with respect to anti-inflammatory and antioxidant activities to be used as new generation alternatives to synthetic anti-inflammatory molecules, as food supplements/functional foods, to increase shelf-life of food items, and nutraceuticals to deter deleterious free radical-induced inflammatory disease cascades.

### *Related reports*

Brown seaweeds were reported to possess antioxidative and anti-inflammatory molecules (Pavia *et al*., 1986). Results obtained in the present study are in accordance with the earlier reports suggesting that brown seaweeds collected from different regions were found to be endowed with potential reducing abilities and antioxidant properties (Cho *et al.* 2011). The positive correlation observed between total phenolic content and radical scavenging activities of seaweed extracts is in agreement with the earlier literature data (Rajauria *et al.* 2010).

### *Innovations and breakthroughs*

This study evaluated the antioxidant and anti-inflammatory potential of the aqueous extract and polysaccharide fraction from two brown marine macroalga, *P. gymnospora* and *P. tetrastomatica* harvested from Gulf of Mannar of peninsular India by different *in vitro* systems. It was found from the present study that aqueous extract fraction of *P. tetrastomatica* realized high phenolic contents, and its activity towards scavenging short-lived radicals are higher than those registered for *P. gymnospora*. Aqueous extract and polysaccharide fractions of *P. gymnospora* showed higher anti-inflammatory activities against LOXV and $COX_{II}$ enzymes.

### *Applications*

The present study focussed to evaluate the antioxidant and anti-inflammatory activities and total phenolic contents of these seaweed species to understand their beneficial value as human food or as additives. The role of polysaccharides in the aqueous extracts of *P. gymnospora* and *P. tetrastomatica* is valuable information, which will lead to isolate phenolic compounds as new generation green antioxidants for increasing the shelf-life of food products, as nutraceuticals and/or functional foods, and in combating carcinogenesis and inflammatory diseases.

### *Peer review*

The brown seaweeds contain a large assemblage of species that predominate in the coastal shelf areas of Gulf of Mannar region in southeastern coast of Indian subcontinent. Among various brown seaweeds, *P. gymnospora* and *P. tetrastomatica* are abundantly available in this area throughout different seasons. The present study provides insights regarding the antioxidant and anti-inflammatory properties of these two brown seaweed species. The present study propose a path to further research on characterization of the phenolic and polysaccharide compounds accountable for their antioxidant and anti-inflammatory activity, for application in food, pharmaceutical, and cosmetic industry.

## References

[1] Ananthi S, Rao H, Raghavendran B, Sunil AG, Gayathri V, Ramakrishnan G, et al. *In vitro* antioxidant and *in vivo* anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (marine brown alga). *Food Chem Toxicol* 2010; **48**: 187-192.

[2] Liu L, Heinrich M, Myers S, Dworjanyn SA. Towards a better understanding of medicinalusesof the brown seaweed *Sargassum* in traditional Chinese medicine: A phytochemical and pharmacological review. *J Ethnopharmacol* 2012; **142**: 591-619.

[3] Uzuner H, Bauer R, Fan TP, Guo DA, Dias A, El-Nezami H, et al. Traditional Chinese medicine research in the post-genomic era: good practice, priorities, challenges and opportunities. *J Ethnopharmacol* 2012; **140**: 458-468.

[4] Modi CM, Mody SK, Patel HB, Dudhatra GB, Kumar A, Avale M, et al. Toxicopathological overview of analgesic and anti-inflammatory drugs in animals. *J Appl Pharm Sci* 2012; **2**(1): 149-157.

[5] Gupta S, Abu-Ghannam N. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. *Innov Food Sci Emerg Technol* 2011; **12**: 600-609.

[6] Novaczek I, Athy A. *Sea vegetable recipes for the Pacific islands*. Fiji: The University of the South Pacific: Secretariat of Pacific Community; 2001.

[7] Prasada Rao NVSAV, Sastry KV, Venkata Rao E. Carbohydrates of *Padina tetrastromatica*. *Phytochem* 1984; **23**: 2531-2533.

[8] Chatterji A, Dhargalkar VK, Sreekumar PK, Parameswaran PS, Rodrigues R, Kotnala S. Anti-influenza activity in the Indian seaweeds-A preliminary investigation. In: Abidi SAH, Ravindran M, Venkatesan R, Vijayakumaran M, editors. *Proceedings of*

*the National Seminar on New Frontiers in Marine Bioscience Research*. Chennai: National Institute of Ocean Technology; 2004, p. 11-16.

[9] Matanjun P, Mohamed S, Mustapha NM, Muhammad K, Ming CH. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *J Appl Phycol* 2008; **20**: 367-373.

[10] Gunji S, Santoso J, Yoshie-Stark Y, Suzuki T. Effects of extracts from tropical seaweeds on DPPH radicals and caco-, cells treated with hydrogen peroxide. *Food Sci Technol Res* 2007; **13**(3): 275-279.

[11] Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT-Food Sci Technol* 2008; **41**: 1067-1072.

[12] Chandini SK, Ganesa P, Bhaskar N. *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chem* 2008; **107**: 707-713.

[13] Veena CK, Josephine A, Preetha SP, Varalakshmi P. Beneficial role of sulfated polysaccharides from edible seaweed *Fucus vesiculosus* in experimental hyperoxaluria. *Food Chem* 2007; **100**: 1552-1559.

[14] Kumar M, Gupta V, Kumari P, Reddy CRK, Jha B. Assessment of nutrient composition and antioxidant potential of *Caulerpaceae* seaweeds. *J Food Comp Anal* 2011; **24**: 270-278.

[15] Chakraborty K, Paulraj R. Sesquiterpenoids with free radical scavenging properties from marine macroalga *Ulva fasciata* Delile. *Food Chem* 2010; **122**: 31-41.

[16] Fu W, Chen J, Cai Y, Lei Y, Chen L, Pei L, et al. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. Et Sav.) Ching. *J Ethnopharmacol* 2010; **130**: 521-528.

[17] Cho M, Lee HS, Kang ILJ, Won MH, You SG. Antioxidant properties of extract and fractions from *Enteromorpha prolifera*, a type of green seaweed. *Food Chem* 2011; **127**: 999-1006.

[18] Doolaege EHA, Vossen E, Raes K, Meulenaer BD, Verhé R, Paelinck H, et al. Effect of rosemary extract dose on lipid oxidation, colour stability and antioxidant concentrations, in reduced nitrite liver pâtés. *Meat Sci* 2012; **90**: 925-931.

[19] Shiyamala VP. Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J. Agardh, 1848. *Asian Pac J Trop Biomed* 2012; **2**: S90-S98.

[20] Sivasothy Y, Hadi AHA, Mohamad K, Leong KH, Ibrahim H, Sulaiman SF, et al. Spectaflavoside A, new potent iron chelating dimeric flavonol glycoside from the rhizomes of *Zingiber spectabile* Griff. *Bioorg Med Chem Lett* 2012; **22**: 3831-3836.

[21] Mak W, Hamid N, Liu T, Lu J, White WL. Fucoidan from New Zealand *Undaria pinnatifida*: Monthly variations and determination of antioxidant activities. *Carbohyd Polym* 2013; **95**: 606- 614.

[22] Redouan E, Emmanuel P, Philippe M, Bernard C, Josiane C, Cedric D, et al. Synthesis of new glycosaminoglycans-like families by regioselective oxidation followed by sulphation of glucoglucuronan from *Rhizobium* sp. T1. *Carbohyd Polym* 2012; **89**: 1261- 1267.

[23] Larsen LN, Dahl E, Bremer J. Peroxidative oxidation of leucodichlorofluorescein by prostaglandin H synthase in prostaglandin biosynthesis from polyunsaturated fatty acids. *Biochim Biophys Acta* 1996; **1299**: 47-53.

[24] Demirci B, Temel HE, Portakal T, Kırmızıbekmez H, Demirci F, Başer KHC, et al. Inhibitory effect of *Calamintha nepeta* subsp. Glandulosa essential oil on lipoxygenase. *Turkish J Biochem* 2011; **36**(4): 290-295.

[25] Chakraborty K, Praveen NK, Vijayan KK, Rao GS. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to *Turbinaria* spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar. *Asian Pac J Trop Biomed* 2013; **3**(1): 8-16.

[26] Wang T, Jónsdóttir R, Liu H, Gu L, Kristinsson HG, Raghavan S, et al. Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. *J Agric Food Chem* 2012; **60**(23): 5874-5883.

[27] Toth G, Pavia H. Lack of phlorotannin induction in the brown seaweed *Ascophyllum nodosum* in response to increased copper concentrations. *Mar Ecol Prog Ser* 2000; **192**: 119-126.

[28] Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agricl Food Chem* 1995; **43**: 27-32.

[29] Wijesinghe WAJP, You-Jin J. Enzyme-assistant extraction (EAE) of bioactive components: A useful approach for recovery of industrially important metabolites from seaweeds: A review. *Fitoterapia* 2012; **83**: 6-12.

[30] Gómez-Ordóñez E, Rupérez P. FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. *Food Hydrocolloid* 2011; **25**: 1514-1520.

[31] Chandía NP, Matsuhiro B. Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties. *Int J Biol Macromol* 2008; **42**(3): 235-240.

[32] Synytsya A, Kim WJ, Kim SM, Pohl R, Synytsya A, Kvasnicka F, et al. Structure and antitumour activity of fucoidan isolated from sporophyll of Korean brown seaweed *Undaria pinnatifida*. *Carbohyd Polym* 2010; **81**(1): 41-48.

[33] Ghosh T, Auerochs S, Saha S, Ray B, Marschall M. Anti-cytomegalovirus activity of sulphated glucans generated from a commercial preparation of rice bran. *Antivir Chem Chemoth* 2010; **21**: 85-95.

[34] Karmakar P, Pujol CA, Damonte EB, Ghosh T, Ray B. Polysaccharides from *Padina tetrastromatica*: Structural features, chemical modification and antiviral activity. *Carbohyd Polym* 2010; **80**: 513-520.

[35] Damasceno SRB, Rodrigues JC, Silva RO, Nicolau LAD, Chaves LS, Freitas ALP, et al. Role of the NO/KATP pathway in the protective effect of a sulfated-polysaccharide fraction from the algae *Hypnea musciformis* against ethanol-induced gastric damage in mice. *Rev Bras Farmacogn* 2013; **23**(2): 320-328.

[36] Tilley SL, Coffman TM, Koller BA. Mixed message: gene: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest* 2001; **107**: 191-195.

[37] James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* 2000; **71**: 343-348.

[38] Davis TA, Volesky B, Mucci M. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res* 2003; **37**: 4311-4330.