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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

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PEER REVIEW

ABSTRACT

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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.

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Objective: To evaluate the antioxidant and anti-inflammatory potential of the aqueous extract and polysaccharide fraction from two brown marine macroalgae, *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-3ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), H₂O₂/HO• radical scavenging, Fe²⁺ 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 H₂O₂) (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 (H₂O₂), hydroxyl radicals (HO•), superoxide anion (O₂^{•-}) etc. These reasons explain their potential use in increasing shelf-life of food and as

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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 advocacy 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*) (Kützting) Sonder and *P. tetrastomatica* (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), cyclooxygenase-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.*

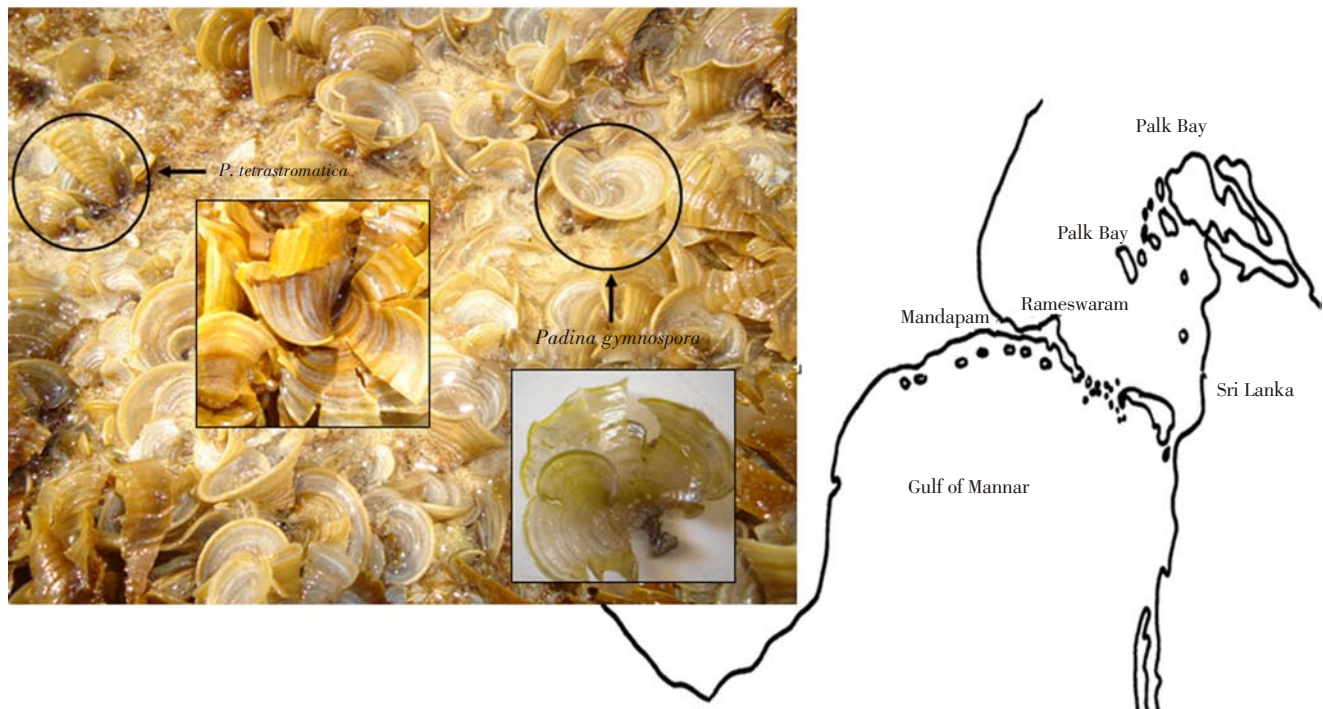


Figure 1. Seaweeds *P. tetrastomatica* and *P. gymnospora* were collected from Gulf of Mannar of Mandapam region located between 8°48' N, 78°9' E and 9°14' N, 79°14' E on the southeast coast of India.

tetrastomatica aqueous extract and *P. gymnospora* aqueous extract, respectively). Another portion of the aqueous extract was concentrated (to 1/4th), 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 (%) = $100 \times (A_0 - A_1) / A_0$, where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample. 2, 2'-Azino-bis-ethylbenzothiazoline-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 H₂O₂ was determined using established method^[16] with suitable modifications. The percentage of scavenging of H₂O₂ of seaweed extracts was determined by the following formula: % scavenged (H₂O₂) = $[(A_0 - A_1) / A_0] \times 100$, 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 (Fe²⁺) chelating activity

Total reduction capabilities and ferrous (Fe²⁺) 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 Na₂SO₄ 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 Fe²⁺ 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. tetrastratica* 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. tetrastratica* (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. tetrastratica* ($P < 0.05$) 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 1Antioxidant 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 (%)#	<i>P. gymnospora</i>	6.70 ^{ap} ±0.96	12.18 ^{al} ±2.06	14.31 ^{ar} ±0.35	14.75 ^{at} ±1.00	22.14 ^{al} ±0.78	29.14 ^{au} ±1.60
	<i>P. tetrastomatica</i>	3.96 ^{ap} ±0.34	4.81 ^{bp} ±0.11	10.33 ^{al} ±0.51	14.27 ^{ar} ±0.83	20.95 ^{as} ±0.09	27.37 ^{al} ±0.99
DPPH radical scavenging ability (%)	<i>P. gymnospora</i>	6.90 ^{ap} ±0.01	11.48 ^{al} ±0.72	22.78 ^{al} ±0.40	38.81 ^{as} ±0.88	47.52 ^{al} ±2.26	58.40 ^{au} ±0.55
	<i>P. tetrastomatica</i>	ND	ND	13.04 ^{bl} ±1.14	31.86 ^{br} ±0.33	45.22 ^{al} ±1.22	55.85 ^{al} ±0.56
HO• radical scavenging activity (%)	<i>P. gymnospora</i>	5.77 ^{ap} ±0.55	11.27 ^{al} ±1.51	12.90 ^{ar} ±0.86	17.92 ^{as} ±0.89	20.03 ^{al} ±0.73	23.38 ^{al} ±1.38
	<i>P. tetrastomatica</i>	14.12 ^{bp} ±1.54	19.14 ^{bl} ±0.79	19.87 ^{br} ±0.78	24.14 ^{bs} ±0.22	24.36 ^{bsl} ±1.40	27.83 ^{asl} ±0.49
H ₂ O ₂ scavenging activity (%)	<i>P. gymnospora</i>	7.19 ^{ap} ±0.39	8.38 ^{ap} ±0.64	16.21 ^{al} ±1.50	33.43 ^{ar} ±0.88	45.09 ^{as} ±1.26	60.76 ^{al} ±1.33
	<i>P. tetrastomatica</i>	12.86 ^{bp} ±0.52	18.42 ^{bl} ±0.81	31.66 ^{br} ±0.98	49.02 ^{bs} ±1.21	56.18 ^{bl} ±0.85	68.30 ^{bl} ±1.40
TBARS formation inhibition assay (MDAEC/kg)**	<i>P. gymnospora</i>	51.12 ^{ap} ±1.51	43.08 ^{al} ±0.57	35.44 ^{ar} ±1.53	15.28 ^{as} ±0.30 ^e	8.76 ^{al} ±0.41 ^{**}	6.09 ^{au} ±0.56 ^{***}
	<i>P. tetrastomatica</i>	44.00 ^{ap} ±1.50	41.69 ^{ap} ±1.37	18.89 ^{bl} ±0.71	13.37 ^{ar} ±0.50	10.26 ^{as} ±0.36	7.96 ^{al} ±0.18
Reducing capacity (A _{700 nm})	<i>P. gymnospora</i>	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
	<i>P. tetrastomatica</i>	0.20 ^{ap} ±0.00	0.22 ^{aq} ±0.00	0.22 ^{ar} ±0.00	0.31 ^{as} ±0.00	0.37 ^{at} ±0.00	0.42 ^{au} ±0.00
Fe ²⁺ ion chelating activity(%)	<i>P. gymnospora</i>	44.09 ^{ap} ±0.80	62.31 ^{al} ±0.39	66.97 ^{ar} ±2.09	81.59 ^{as} ±0.76	82.62 ^{al} ±0.56	85.34 ^{au} ±0.84
	<i>P. tetrastomatica</i>	25.24 ^{bp} ±0.04	34.12 ^{bl} ±0.23	41.64 ^{br} ±0.69	49.70 ^{bs} ±0.41	57.36 ^{bl} ±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

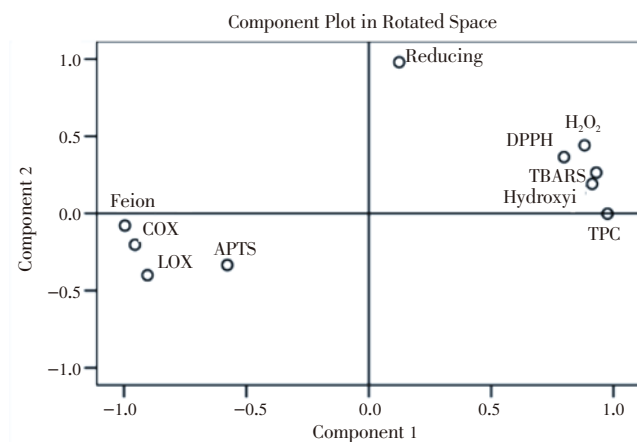


Figure 2. PCA loading plot showing the correlation between antioxidant activity assays of *in vitro* anti-inflammatory and antioxidant potential of aqueous extract and polysaccharide fraction of both the brown seaweeds *P. tetrastomatica* and *P. gymnospora*.

3.1.3. Assays for detection of scavenging of transiently stable radicals of aqueous extract from *Padina* sp.: H₂O₂ 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 H₂O₂ (>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. Fe²⁺ chelating and reducing abilities of aqueous extract of *Padina* sp.

Aqueous extract of *P. gymnospora* (0.6 mg/mL) showed significantly higher ($P<0.05$) Fe²⁺ 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 % K₂SO₄ 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

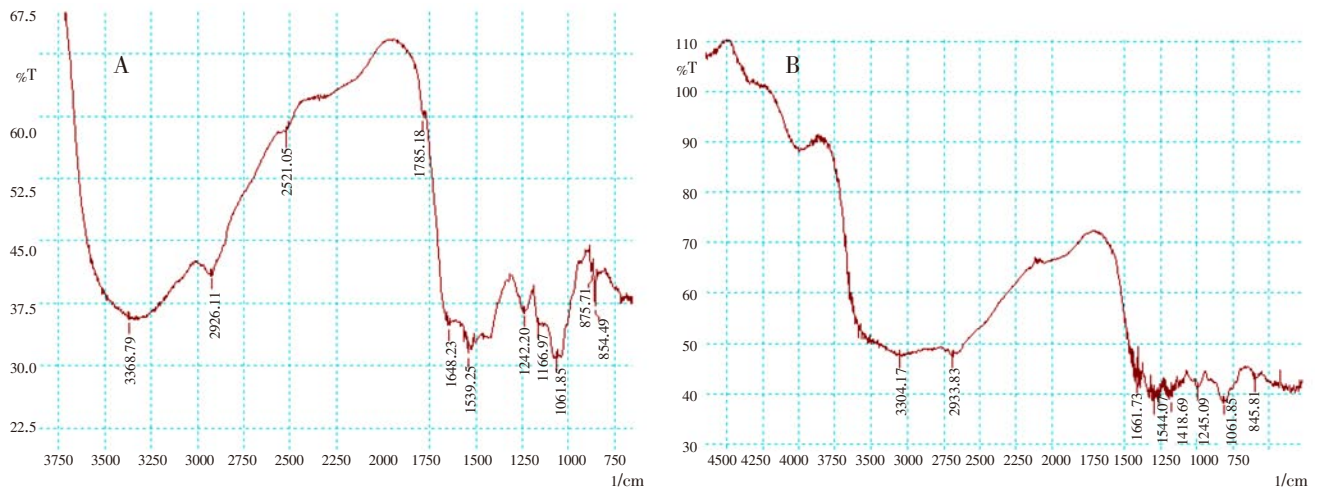


Figure 3. Infra red spectra of (A) aqueous extract and (B) polysaccharide fraction of *P. tetrastomatica*.

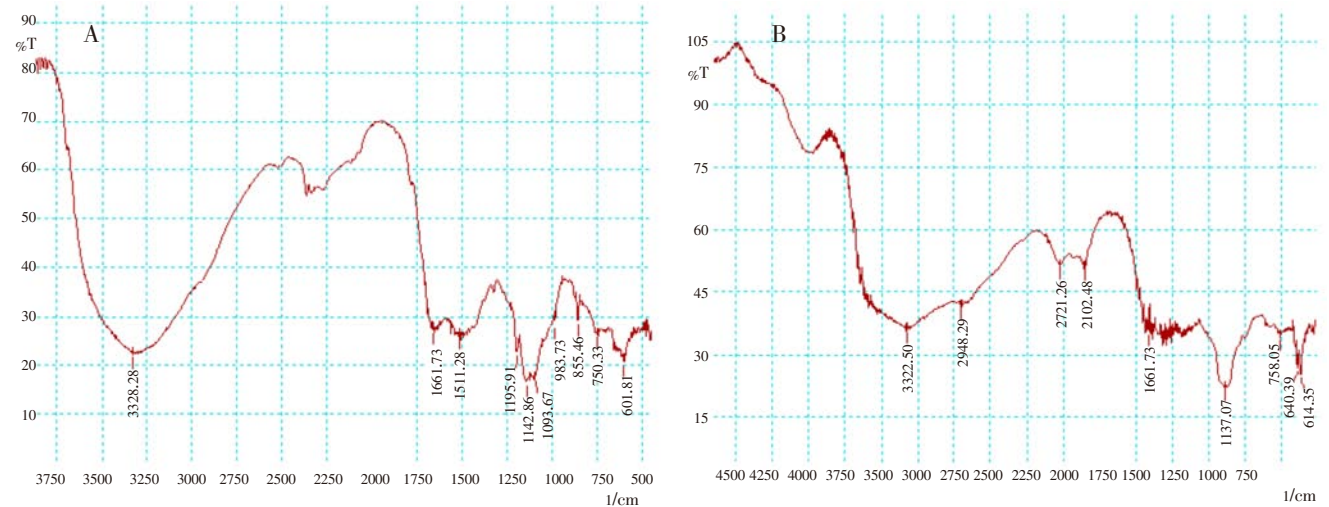


Figure 4. Infra red spectra of (A) aqueous extract and (B) polysaccharide fraction of *P. gymnospora*.

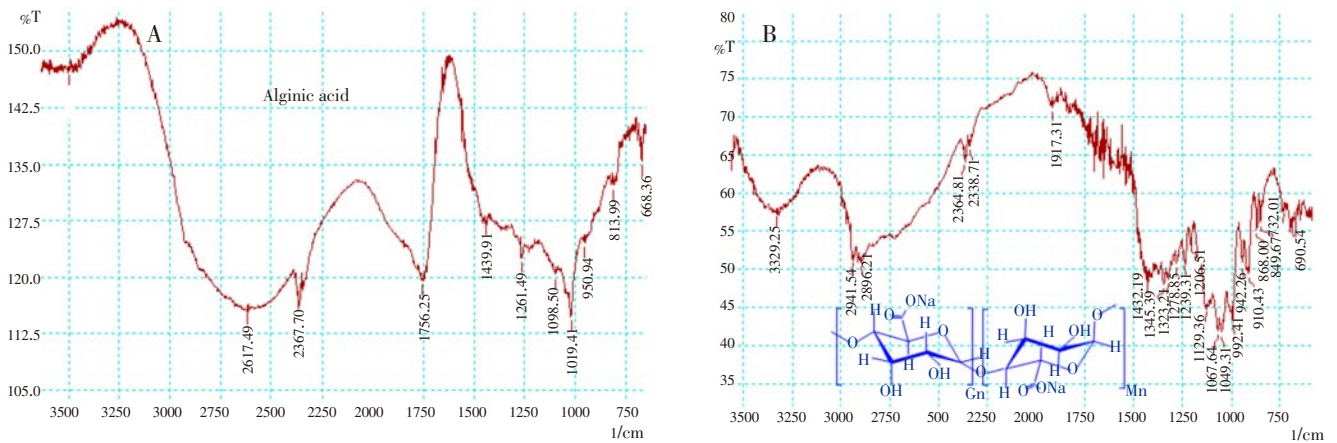


Figure 5. Infra red spectra of alginic acid and sodium alginate standards (A and B, respectively).

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 $3200\text{--}3500\text{ cm}^{-1}$ was observed. Absorption peaks are also found between $2800\text{--}2900\text{ cm}^{-1}$. Characteristic bands are also observed at 1600 cm^{-1} . The IR spectra of both the polysaccharide fractions exhibited a broad band around $1220\text{--}1260\text{ cm}^{-1}$, 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 ($A_{700\text{ nm}}$ 0.34–0.90) than those realized by *P. gymnospora* ($A_{700\text{ nm}}$ 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} ±0.35	50.89 ^{bp} ±0.42	65.1 ^{cp} ±0.86	77.55 ^{dp} ±0.4
	B	20.58 ^{am} ±0.56	31.96 ^{bm} ±0.59	40.98 ^{cm} ±0.38	44.52 ^{dm} ±0.05
Reducing ability (A_{700})	A	0.34 ^{ap} ±0.02	0.46 ^{bp} ±0.01	0.65 ^{cp} ±0.01	0.90 ^{dp} ±0.02
	B	0.14 ^{am} ±0.01	0.22 ^{bm} ±0.01	0.29 ^{cm} ±0.01	0.35 ^{dm} ±0.02
Fe^{2+} ion chelating ability (%)	A	8.66 ^{ap} ±0.59	17.09 ^{bp} ±1.37	33.39 ^{cp} ±0.83	42.53 ^{dp} ±0.46
	B	8.09 ^{am} ±0.6	11.79 ^{bm} ±0.54	25.77 ^{cm} ±0.41	39.16 ^{dm} ±0.12
Hydroxyl radical scavenging ability (%)	A	8.45 ^{ap} ±0.4	34 ^{bp} ±0.15	47.91 ^{cp} ±0.35	62.54 ^{dp} ±0.15
	B	3.4 ^{am} ±0.24	12.51 ^{bm} ±0.26	18.02 ^{cm} ±0.37	20.91 ^{dm} ±0.53
TBARS formation inhibition assay (MDAEC/kg)	A	89.76 ^{ap} ±0.53	51.02 ^{bp} ±0.62*	27.19 ^{cp} ±0.08**	14.92 ^{dp} ±0.66***
	B	98.37 ^{am} ±0.91	85.32 ^{bm} ±0.75*	66.34 ^{cm} ±1.77*	21.22 ^{dm} ±0.75***

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 LOX_V (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} activity was reported with respect to synthetic NSAID (aspirin 50%).

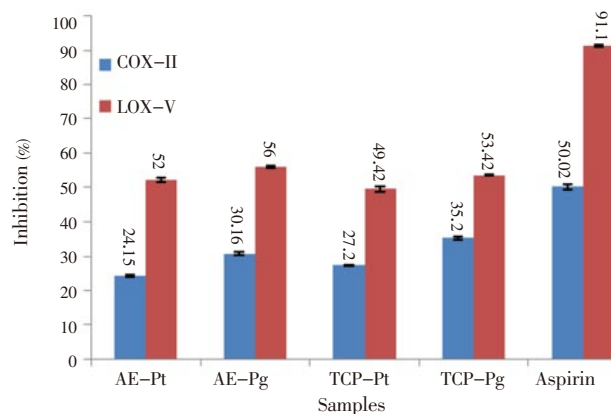


Figure 6. In-vitro anti-inflammatory potential (percent inhibition of COXII and LOXV enzymes) exhibited by aqueous extract (AE) and polysaccharide fraction of *P. tetrastomatica* (Pt) and *P. gymnospora* (Pg). Data are the mean values of triplicate. Inhibition (%) has been recorded at 1 mg/mL.

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/H₂O₂/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/H₂O₂/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 based 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 peroxy and alkoxy 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 $1220\text{--}1260\text{ cm}^{-1}$, 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 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 cyclooxygenases (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/H₂O₂/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 Fe²⁺ ion and total phenolic content/ DPPH/H₂O₂/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/H₂O₂ 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 leukotrienes, 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.

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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.

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