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ORIGINAL ARTICLE

Polysaccharides of red alga *Gracilaria intermedia*: structure, antioxidant activity and rheological behavior

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Abstract

A sulfated polysaccharide fraction from the red alga Gracilaria intermedia (PLS) was obtained by papain digestion (60 °C, 30 min). The extract was subjected to colorimetry and turbidimetry analysis, Fourier transform infrared (FTIR) spectroscopy, ^{1}H, ^{13}C and ^{2}D ^{1}H COSY nuclear magnetic resonance (NMR) and gas chromatography/mass spectrometry analysis. Antioxidant activity tests were performed (chelation of ferrous ion, total antioxidant capacity, and scavenging of DPPH radicals); significant activity of the extract indicated that these polysaccharides may be used as non-synthetic antioxidants. The rheological behavior of aqueous polysaccharide solutions was studied at 25 ± 1 °C using steady-shear and dynamic oscillatory measurements. All the solutions analyzed showed pseudoplastic behavior and potential to act as a thickening agent, as proved through a preliminary comparison with a commercial product used for this application.

Keywords: *antioxidant activities, Gracilaria intermedia, polysaccharides, rheological behavior, structure.*

1. Introduction

Polysaccharides are polymers that show a wide range of properties depending on their monomeric composition and have applications in different types of industries. Applications include the use as a stabilizer, thickener, flocculant, and water-retaining agent in the textile, food, pharmaceutical, and biotechnology industries[1-4].

Carrageenans and agarans are sulfated polysaccharides obtained from red algae. The industrial applications of these polymers are closely dependent on their rheological properties. Such properties make these two polymers important gelling agents and thickeners, especially in the food industry[5]. Sulfated polysaccharides also exhibit various biological activities, including antioxidant activities, among others[6-9]. Therefore, they also have potential application as agents to reduce cellular damage and to prolong the shelf time of foods; these are areas where compounds with antioxidant activity are currently in great demand[10-12]. Non-synthetic compounds, particularly polysaccharides, with antioxidant potential are currently under investigation by several research groups[13-18] as an alternative to synthetic antioxidant compounds traditionally used in the food and pharmaceutical industries such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl hydroquinone (TBHQ) due to their suspected damage to liver tissue and carcinogenic potential[9,13,19].

Red algae from the genus Gracilaria are economically important in the phycocolloids industry[20] and are currently the source of about 65% of the 7.5 tons of agar produced annually throughout the world[21]. Sulfated polysaccharides obtained from species of the genus *Gracilaria* contain 3-linked-β-D-galactopyranose (G unit) and 4-linked-3,6-anhydro-α-L-galactopyranose (LA unit)[22-25] with substitution of hydroxyl groups by ester sulfate, methyl groups, and pyruvic acid[23-26] (Figure 1). Gracilaria is very common in the Northeast Brazilian coast and its extraction has been pointed as an alternative for the economic inclusion of part of the population of this region[27].

Yield of extraction and final properties of agarans depend strongly on the seaweed species, the methodology of extraction of polysaccharides, seasonal variations and the place of origin of the specimens. So the chemical, structural, and rheological characterization of polysaccharydes extracted from seaweeds is necessary[26,28]. In the specific case of the

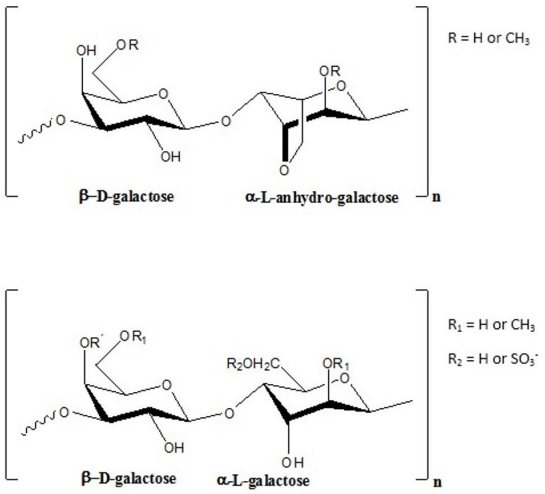

Figure 1. Chemical structure of agar type molecules with the different types of sugar units and substituents.

species *Gracilaria intermedia*, the related scientific research *Gracilaria intermedia* is still scarce. To the knowledge of the authors, only studies on its taxonomy[28-31] and ecological aspects[32] have been reported.

In this context, the goal of this paper was to characterize sulfated polysaccharides extracted from the red seaweed *Gracilaria intermedia*. This characterization was performed by spectroscopy, turbidimetric and colorimetric methods. The antioxidant activity of the polymer and its rheological behavior in solution were also evaluated.

2. Materials and Methods

2.1 Materials

Papain, cysteine and cetylpyridinium chloride (CPC) were obtained from Vetec (Brazil), ammonium molybdate was purchased from Dinâmica (Brazil) and D-galactose from Acros Organics (USA). Ferrozine, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (USA). Ascorbic acid was obtained from Synth (Brazil) and Carboxymethyl cellulose was obtained from Mix (Brazil). All other solvents and chemicals were of analytical grade.

2.2 Extraction of sulfate polysaccharides

Specimens of *G. intermedia* were collected in April 2013 on the Atlantic coast of Brazil (Taiba beach, São Gonçalo - Ceará). The collected seaweed were cleaned of epiphytes, washed with distilled water, and stored at -20 °C. A voucher specimen (n° 2386) was deposited in the phycological Herbarium of the Laboratory of Marine Sciences, Universidade Federal do Ceará, Brazil.

The enzymatic extraction of polysaccharides was performed according to the methodology of Farias et al.[33], with some modifications. The dried tissue (5 g) was suspended in 250 mL of 0.1 mol.L^{-1} sodium acetate buffer (pH 5.0), containing 1 g of papain, 5×10^{-3} mol.L^{-1} EDTA, and 5×10^{-3} mol.L^{-1} cysteine, and incubated at 60 °C for 30 min. The incubation solution was then filtered through a nylon membrane, and the homogenate was retained. The polysaccharides in solution were precipitated with 16 mL of 10% cetylpyridinium chloride (CPC) solution. After 24 h at room temperature (25 °C), the mixture was centrifuged at 2,560 × g for 20 min at 20 °C. The polysaccharides were washed with 500 mL of 0.05% CPC solution, dissolved with 100 mL of a 2 mol.L^{-1} NaCl-ethanol (100:15, v/v) mixture, and the excess of salts was removed by precipitation and wash with 200 mL of absolute ethanol. After 24 h at 4 °C, the precipitate was collected by centrifugation (2,560 × g for 20 min at 20 °C), washed extensively with ethanol-80%, then absolute ethanol. After this, the polysaccharides (PLS) were washed with acetone, which was followed by hot air drying (60 ºC) until all the acetone was removed.

2.3 Chemical analysis

Sulfate content of PLS was determined by the barium-gelatin method[34] after hydrolysis of the sample in 1 mol.L^{-1} HCl (5 h, 105 °C) using sodium sulfate (Na_2SO_4) as standard. To measure the amount of total neutral carbohydrates the phenol-sulfuric method was performed[35] with a standard curve prepared with D-galactose. The protein content was measured by the Bradford method[36] using bovine serum albumin (BSA) as standard.

2.4 Monosaccharide composition

Samples of the polysaccharides extracted from *G. intermedia* (5 mg) were hydrolyzed with 5 mol.L^{-1} trifluoroacetic acid for 4 h at 100 °C, reduced with borohydride, and the alditols were acetylated with acetic anhydride:pyridine (1:1, v/v). The alditols acetates were dissolved in chloroform and analyzed in a gas–liquid chromatograph/mass spectrometrer (GCMS-QP2010 Shimadzu, Japan) with a DB-5ms column (Agilent)[37].

2.5 Infrared spectroscopy

The Fourier transform infrared spectra (FTIR) were obtained with a Shimadzu IR spectrophotomer (FTLA 2000, ABB-BOMEM, Canada) with measurements in the wavenumber range of 400-4000 cm^{-1} using 20 scans. The samples were analyzed as KBr pellets.

2.6 Nuclear magnetic resonance (NMR) spectroscopy

The spectroscopic technique of nuclear magnetic resonance (NMR) is a method commonly used for structural characterization of seaweed polysaccharides[38-41].

^{1}H, ^{13}C and 2D ^{1}H COSY NMR spectra in D_2O were recorded at 353 K on a Fourier transform spectrometer (Bruker Avance DRX 500, USA) with an inverse multinuclear gradient probe-head equipped with z-shielded gradient coils.

2.7 Determination of antioxidant activity

2.7.1 Total antioxidant capacity

The total antioxidant capacity test, based on the reduction of Mo^{6+} to Mo^{5+}, was performed by the methodology proposed by Prieto, Pineda & Aguilar[42]. Aliquots of the polysaccharide solution (0.1 mL) of different concentrations (0.1% to 1.5%) were mixed with 1 mL of the reagent solution (0.6 mol.L^{-1} sulfuric acid, 28×10^{-3} mol.L^{-1} sodium phosphate,

and 4×10^{-3} mol.L^{-1} ammonium molybdate). This step was followed by incubation at 95 °C for 90 min. Subsequently, absorbance was read at 695 nm. A standard curve under the same conditions was prepared with solutions of ascorbic acid. Thus, the results are presented as equivalence of ascorbic acid (mg/g EAAsc).

2.7.2 Iron (Fe^{2+}) chelating activity

The iron ion is associated with lipid peroxidation due to the Fenton reaction. This process is related to a series of diseases[43-45]. The antioxidants form a complex with this ion, thus preventing cell damage[46]. The ferrous ion chelation activity of PLS was investigated according to the methodology used by Zhang et al.[18]. Aliquots of 1 mL of the polysaccharide solution at different concentrations (0.1% to 1.5%) were mixed with 0.05 mL of $FeCl_2$ (2×10^{-3} mol.L^{-1}), 0.2 mL of ferrozine (5×10^{-3} mol.L^{-1}), and 2.75 mL of water. The solution was agitated and incubated at room temperature for 10 min. Absorbance of the solution at 562 nm was measured. The chelating activity was calculated using the following Equation 1:

$$Chelating\ activity(\%) = \left[1 - \frac{A_1 - A_2}{A_0}\right] \times 100 \quad (1)$$

where A_0 is the absorbance of the blank, A_1 is the absorbance of the test solution, and A_2 is the absorbance of a solution identical to A_1 with the substitution of $FeCl_2$ for the same aliquot of water. Ascorbic acid was used as the positive control.

2.7.3 Scavenging of DPPH radicals

The 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) scavenging activity of PLS was measured using the method used by Wu et al.[16]. Ascorbic acid was used as a positive control. The inhibition (%) was calculated using the following Equation 2:

$$Scavenging\ of\ DPPH\ radicals(\%) = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (2)$$

where A_0 and A_1 are the absorbance of the blank and sample, respectively.

2.8 Rheological behavior

Rheological measurements were carried out in a rotational rheometer (Ares, TA Instruments, New Castle, DE, USA), using cone-plate geometry (50 mm diameter, cone angle of 0.0399 rad, gap of 0.0553 mm). All measurements were run at 25 ± 1 °C.

Dynamic tests were performed to evaluate the behavior of storage (G') and loss (G") moduli as a function of frequency. The frequency sweep tests were performed in the linear viscoelastic region (LVR), determined through strain sweep tests. Three samples containing the extracted sulfated PLS (1.0%, 1.25%, and 1.5%) were analyzed.

Flow curves were obtained by recording shear stress values when shearing the samples at an increasing shear rate from 0.1 to 100 s^{-1} with increment of 4 s^{-1} and then reducing it through the same path. This test was performed for samples containing PLS in different concentrations (1.0%, 1.25%, and 1.5%). Additionally, for testing the potential of PLS as thickening agent, solutions containing carboxymethylcellulose (CMC), a well-known thickening agent, and blends of CMC and PLS were also analyzed. The concentrations of the referred CMC solutions were of 0.75% of CMC and 1.5%. In the case of the blends, two formulations were prepared, one containing 1.0% of PLS and 0.5% of CMC and another containing 0.75% of PLS and 0.75% of CMC.

The data of the flow curves were fitted using the Ostwald-de Waele model (power-law), Equation 3:

$$\eta = K\dot{\gamma}^{n-1} \quad (3)$$

where: is the shear viscosity (Pa·s), K is the consistency index (Pa.s); is the shear rate (s^{-1}), n is the power-law index (dimensionless) and K is the consistency index (Pa.s). The estimation of the parameters K and n was performed using the least-square method, in the software Microsoft Excel.

3. Results and Discussions

3.1 Yield and chemical analysis

The yield of polysaccharides by mass of seaweed *Gracilaria intermedia* was 17.0 ± 1.18%. Studies which extraction took place by enzymatic digestion of seaweed *Gracilaria cornea* (Brazil) achieved yields of 18.0%[28] and 11.0 to 21.4%[47]. Souza et al.[12] in Brazil obtained a polysaccharide yield of 27.2% for the seaweed *Gracilaria birdeae* while Freile-Pelegrin and Robledo[48], on a seasonal study in Mexico, obtained yield from 25.0 to 39.3% for *Gracilaria cervicornis*. In extraction performed at room temperature, in Brazil, Maciel et al.[23] were able to yield 6.5% of polysaccharides from seaweed *Gracilaria birdeae*. Making the extraction of polysaccharides with autoclave in India, yields of 14.8% for *Gracilaria debilis* and 15.2% for *Gracilaria Salicornia*[49] were achieved.

Polysaccharides from *Gracilaria intermedia* (PLS) exhibited 6.60 ± 0.13% of sulfate, which is within the range of sulfate polysaccharides content from the other Gracilaria species (2.30-8.90%)[23]. More recent studies indicate that this interval is actually wider, since percentages of sulphate of 0.76 ± 0.08% (*Gracilaria debilis*, India)[49], 1.00 ± 0.05% (*Gracilaria caudata*, Brazil)[26], and 15.66% (*Gracilaria cornea*, Brazil)[28] have already been reported.

The carbohydrate content was 54.64 ± 1.19%, consistent with the percentage value of D - galactose found in other Gracilaria species. Amorin et al.[50] performed the test sulfuric phenol in different fractions of *Gracilaria ornata*, Brazil, and found levels of sugars ranging between 33.14 and 62.20%. In polysaccharide fractions of seaweed *Gracilaria birdeae*, Brazil, sugar content between 30.8 and 68.2%[51] were found.

Proteins were not detected in the polysaccharide fraction obtained from seaweed *Gracilaria intermedia*.

3.2 Monosaccharide composition

The monosaccharide composition of the polysaccharide extracted from *G.intermedia* was determined based on gas chromatography/mass spectrometry analysis of the alditol acetates formed after acid hydrolysis. The major monosaccharide detected was galactose. This finding is in agreement with results presented by Pomin & Mourão[52]

who describe the presence of sulfated galactans in red algae. No other sugar was detected up to a limit of < 2% as % of dry weight, ensuring the purity of the material.

3.3 Infrared spectroscopy

Figure 2 shows the FTIR spectrum of the extracted polysaccharide fraction, expanded in the region between 1400 and 700 cm^{-1} to better identify the sulfate groups bands. The bands found at 1375 and 1258 cm^{-1} may be attributed to sulfate ester groups[51,52], the band at 1075 cm^{-1} corresponds to galactan[53,54], and the band at 892 cm^{-1} corresponds to the agar-specific band[55]. The band at 931 cm^{-1} corresponds to the C-O-O group present in the 3,6 -anhydrogalactose, and the bands between 820 and 860 cm^{-1} indicate the presence of sulfate groups[53-57].

3.4 NMR spectroscopy

Figures 3 and 4 show, respectively, the 1D (^{1}H and ^{13}C) and 2D NMR spectra of the polysaccharide fraction extracted from *G.intermedia*. The ^{1}H NMR spectrum is somewhat complex due to overlap and enlargement of the signs (Figure 3a). It shows the signals from the α-anomeric proton at δ 5.62, 5.14 and 4.69 (assigned, respectively, to 3,6-α-L-anhydrogalactose linked to β-D-galactose, α-L-galactose-6-sulfate linked to β-D-galactose and β-D-galactose linked to 3,6-α-L-anhydrogalactose), and from the H-1 of β-D-galactose linked to 3,6-α-L-anhydrogalactose with a signal at δ 4.69[23,26,38-41,58]. However, the H-1 β-D-galactose linked to α-L-galactose-6-sulfate was not detected, probably due to the signal overlap. Others studies state that when this unit is linked with α-3,6-anhydrogalactose and L-α-L-galactose-6-sulfate, there are minor chemical variations in the region of 4.65-3.90 ppm. Therefore, its identification is difficult[23,26,58,59].

The anomeric region of ^{13}C NMR (90-110 ppm) shows four main signals (Figure 3b), which were assigned based on the literature data[23,26,38-41,58,60,61], just like the other carbons observed in the region of 59 – 85 ppm (Table 1). The C-1 of β-D-galactose linked to α-L-galactose-6-sulfato at δ 103.7; C-1 of β-D-galactose linked to 3,6-α-L-anhydrogalactose at δ 102.5; C-1 of α-L-galactose-6-sulfato linked to β-D-galactose at δ 101.25 and C-1 of 3,6-α-L-anhydrogalactose linked to β-D-galactose at δ 98.4.

2D COSY was used to determine the proton resonance sequence (Figure 4). Regarding the dimer formed by 3,6-α-L-anhydrogalactose linked to β-D-galactose five couplings relating the 3,6-α-L-anhydrogalactose u and two couplings assigned β-D-galactose are observed. The protons that are coupled are H-1 (3,6-α-L-anhydrogalactose)/H-2 (3,6-α-L-anhydrogalactose) at δ 5.62/4.60; H-2 (3,6-α-L-anhydrogalactose)/H-3 (3,6-α-L-anhydrogalactose) at δ 4.60/5.04; H-3 (3,6-α-L-anhydrogalactose)/H-4 (3,6-α-L-anhydrogalactose) at δ 5.04/4.11; H-4 (3,6-α-L-anhydrogalactose)/H-5 (3,6-α-L-anhydrogalactose) at δ 4.11/4.26; H-1 (β-D-galactose)/H-2 (β-D-galactose) at δ 4.69/4.44 and H-2 (β-D-galactose)/H-3 (β-D-galactose) at δ 4.44/3.91. For the α-L-galactose-6-sulfate dimer linked to β-D-galactose a coupling is observed for the H-1 (α-L-galactose-6-sulfato)/H-2 (α-L-galactose-6-sulfato) at δ 5.14/5.05. Although other engagements can be observed,

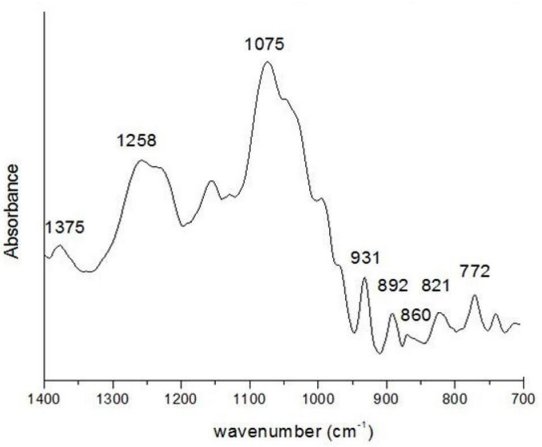

Figure 2. The IR spectra of the PLSs were determined using a Fourier transform infrared spectrometer (FTIR).

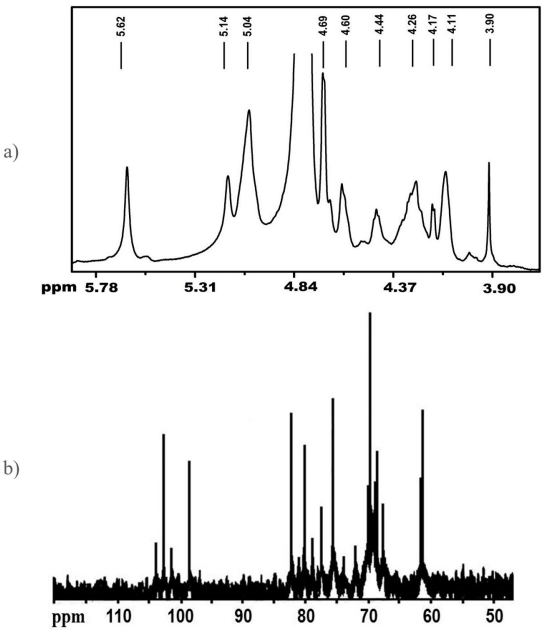

Figure 3. ^{1}H NMR (a) and ^{13}C NMR (b) spectra of the PLSs extracted from *Gracilaria intermedia* in D_2O solution.

it is not possible to identify the sequence of hydrogens because the one-dimensional ^{1}H spectrum overlap them.

3.5 Determination of antioxidant activity

The total antioxidant capacity was determined by forming a phosphomolybdenum complex when Mo^{6+} is reduced to Mo^{5+}; PLS showed activity with 28.98 ± 1.86 mg/ g EAAsc. In studies performed by Costa et al.[62] the amount of ascorbic acid in the seaweeds *Codium isthmocladum* and S*patoglossum schroederi* were (9.2 mg/g EAAsc) and (14.4 mg/g EAAsc) respectively. These values are inferior amount to the ones exhibited by the polysaccharides extracted from *G. intermedia.*

The ability of the PLS to chelate iron (II) ions was dose-dependent. Even though PLS led to higher chelation percentage than the ones obtained from ascorbic acid

Table 1. ^{1}H and ^{13}C NMR chemical shifts for residues of G. intermedia polysaccharide.

Residue	^{1}H chemical shift (ppm)					
	H-1	H-2	H-3	H-4	H-5	H-6
3,6-α-L-anhydrogalactose linked to β-D-galactose	5.62	4.60	5.04	4.11	4.26	nd*
β-D-galactose linked to 3,6-α-L-anydrogalactose	4.69	4.44	3.91	nd*	nd*	nd*
α-L-galactose-6-sulfato linked to β-D-galactose	5.14	5.05	nd*	nd*	nd*	nd*
	^{13}C chemical shift (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
3,6-α-L-anhydrogalactose linked to β-D-galactose	98.4	nd*	81.2	77.5	nd*	69.5
β-D-galactose linked to 3,6-α-L-anhydrogalactose	102.5	nd*	82.1	68.7	73.8	61.5
α-L-galactose linked to β-D-galactose	101.25	nd*	nd*	78.75	71.9	67.5
β-D-galactose linked to α-L-galactose-6-sulfato	103.7	70.5	80.2	69.3	75.6	61.8

nd, not detected; *Signal which can be overlapped with the signal of similar units.

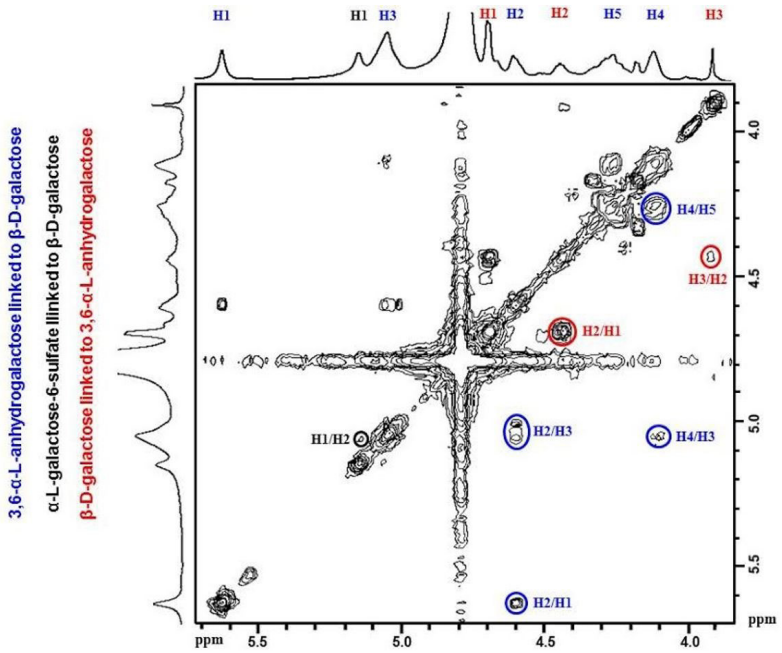

Figure 4. 2D COSY spectrum of PLSs from *Gracilaria intermedia* in D_2O.

(Figure 5a), its activity is low if compared to other polysaccharides extracted from seaweeds. The values for PLS were 11.64 ± 0.83% (PLS 0.1%) and 2819 ± 0.97% (PLS 1.5%). Costa et al.[62] found a value of 40.2% of iron (III) chelation in solutions of 1.5 mg.mL^{-1} of polysaccharides from *Gracilaria caudata*. Wang et al.[63] studied different polysaccharide fractions from *Laminaria japonica* and found a value of 29.48% for quelation in a 1.17 mg.mL^{-1} solution.

The DPPH radical scavenging capacity of PLS was also dose-dependent, with a blockage varying from 9.89 ± 1.32% (at a concentration of 0.1%) to 41.83 ± 0.97% (at a concentration of 1.5%). Although significant, the DPPH radical scavenging capacity of PLS was lower than that ascorbic acid over the concentration range tested (Figure 5b) and also lower than the activity of other polysaccharides from seaweeds. Dore et al.[14], with polysaccharides from *Sargassum vulgare,* found values of 10.0 ± 0.7% a 22.0 ± 0.6% in solutions that varied from 0.15 to 3.0 mg.mL^{-1}

3.6 Rheological characterization

Based on the strain sweep tests performed to determine the region linear viscoelastic behavior of the samples in the dynamic oscillatory tests, the frequency sweep (FS) tests were carried out at 8% of a deformation. The results of the FS tests are presented in Figure 6, where it can be seen that the values of loss modulus (G") were higher than those of the storage modulus (G') at all concentrations and frequencies tested. These results indicate that the extracted polysaccharide fraction does not lead to gel-like solutions. This behavior can be attributed to the elevated amount of sulfate that was observed in the polysaccharide obtained by papain digestion. The structure of agarans is strongly influenced by the presence of charged groups that participate in intermolecular hydrogen bonds[48]; the strength of this polymer gel is inversely proportional to the amount of sulfate present in its structure[64].

The predominantly viscous response reflected in the dynamic and steady state tests suggest that a potential use

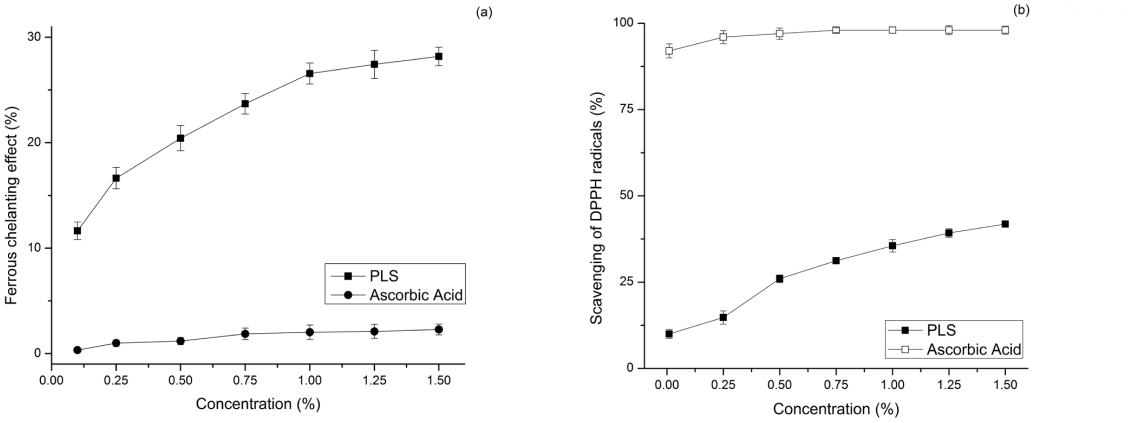

Figure 5. Antioxidant activity of the PLSs extracted from *Gracilaria intermedia*. (a) Chelating activity of Fe^{2+} with ascorbic acid used as a positive control. (b) DPPH radical scavenging activity. Values are means ± SD (n = 3).

for the polysaccharides extracted from the red seaweed *Gracilaria intermedia* is as a thickening agent, especially for applications where antioxidant activity and absence of color and odor are mandatory requirements. To check this hypothesis, the flow curves at 25 ± 1 °C of solutions of the extracted polysaccharides, either pure or in mixture with carboxymethylcellulose (CMC), were compared to those of two solutions containing pure CMC. The concentrations of the pure CMC solutions were of 0.5% and 1.5%, corresponding, respectively, to the lower and upper limit of the range of concentrations typically used in commercial applications of CMC as thickening agent. In the mixtures of CMC with the extracted polysaccharides, the total concentration of thickening agents was 1.5%. The obtained flow curves are presented in Figure 7.

Figure 7 shows that all the tested solutions presented pseudoplastic behavior, since an increase in shear rate led to a decrease in viscosity. Besides, for all samples, the upward and downward (not shown) curves of shear stress vs. shear rate were identical, with no observed hysteresis behavior. Therefore, the extracted polysaccahride fraction solutions, as well as the two solutions with pure CMC, showed no thixotropy in the range of concentrations tested.

In the case of the extracted polysaccharide fraction solution, the pseudoplastic behavior is likely due to the rupture of the double helix structure present in agarans[65]. Additionally, the fact that this rupture only occurs above a certain value of tension (t_{crit})[65] is in agreement with the two-region pattern observed in the flow curves, with constant viscosity at low shear rates (Newtonian plateau) and shear-thinning behavior at higher ones. The higher the polysaccharide concentration, the lower the upper limit of the Newtonian plateau because an increase in viscosity causes t_{crit} be reached at lower values of shear rate.

Additionally, the data corresponding to the shear thinning region of all flow curves presented in Figure 7 were fitted to power-law model. The obtained values of consistency index (K) and power-law index (n) are presented in Table 2. It is observed that increase of the polysaccharide concentration in the PLS solutions led to increase of the consistency index and reduction of the power-law index. Taking into account

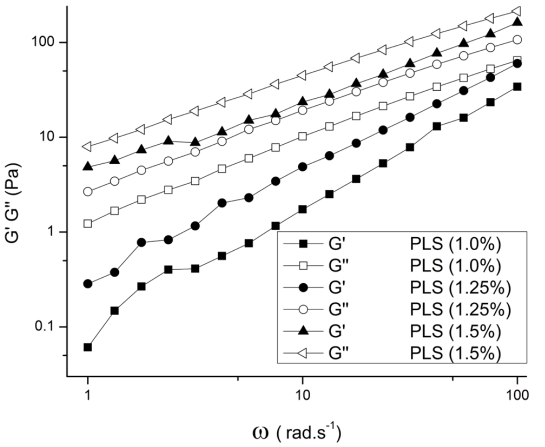

Figure 6. Effect of the concentration of PLS extracted from *Gracilaria intermedia* on the storage and loss moduli measured during frequency sweeps.

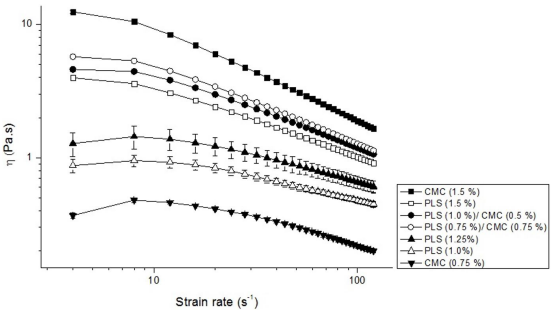

Figure 7. Flow curves of the PLSs extracted from *Gracilaria intermedia* and of CMC at 25 ± 1 °C.

that the consistency index is related to the resistance of a fluid to the flow and power-law index indicates how quickly the viscosity is reduced with an increase in the shear rate, the increase of K and reduction of n with the increase of the concentration of PLS in the solution can be explained

Table 2. Power index (*n*) and consistency index (*K*) values for PLS, CMC solutions, and blends of both samples.

Sample	Concentration (%)	*n*	*K* (Pa.s)
PLS *G. intermedia*	1.0	0.67	2.31
	1.25	0.62	5.76
	1.5	0.47	11.64
CMC	0.75	0.60	1.44
	1.5	0.29	47.23
PLS + CMC	1.0 PLS + 0.5 CMC	0.43	15.83
	0.75 PLS + 0.75 CMC	0.39	10.11

in terms of the increase in the level of interaction among polysaccharide molecules resulting from the increase in the concentration. As observed in Figure 7, although pure CMC led to the highest viscosity, both pure PLS and the PLS/CMC blends were able to produce a significant increase of viscosity. Actually, all over the range of strain rates studied, pure PLS and the PLS/CMC blends provided viscosity values closer to the that obtained with the CMC solution of 1.5% than to those obtained with 0.5%. These results confirm the potential of the extracted sulfated PLS to be used as thickening agent.

4. Conclusions

In this study, a water-soluble polysaccharide was successfully extracted from the red seaweed *Gracilaria intermedia*. The data obtained by spectroscopic methods suggest that the extracted polymeric material is rich in agaran. In vitro studies showed that PLS had antioxidant activity, which may be used for cellular protection against free radicals and to increase the viability of products. The rheological characteristics of PLS indicate that it can be used as a viscosity modifier in industrial processes. The antioxidant characteristics and rheological behavior combined with the organoleptic properties observed (absence of color and odor) make polysaccharides from the seaweed *Gracilaria intermedia* a promising agent to be used in manufacturing processes of food and pharmaceuticals.

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