

Chemical characterization and antioxidant activity of sulfated polysaccharide from the red seaweed *Gracilaria birdiae*

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ABSTRACT

Hydrocolloids from seaweeds have interesting functional properties, such as antioxidant activity and gelling ability. A polysaccharide was isolated by aqueous extraction at 90 °C from the red seaweed *Gracilaria birdiae* (*Gb*), with a yield of 27.2% of the seaweed dry weight. The sulfate content of the polysaccharide was 8.4% and the main sugars present were galactose (65.4 mol%), 3,6-anhydrogalactose (25.1 mol%) and 6-O-methylgalactose (9.2 mol%). Gel permeation chromatography showed that *Gb* polysaccharide is a heterogeneous system, with molar mass at the main peak of $3.7 \times 10^5 \text{ g mol}^{-1}$ and a shoulder of $2.6 \times 10^6 \text{ g mol}^{-1}$. The sulfated polysaccharide of *Gb* characterized by FTIR exhibits the characteristic bands of agarocolloids (at 1375 and 770 cm^{-1}).

The rheological behavior of *Gb* sulfated polysaccharide exhibits a gel-like behavior close to the one observed in commercial agar.

The antioxidant properties of *Gb* sulfated polysaccharide were evaluated by measuring DPPH free-radical scavenging effect, showing that this polysaccharide has a moderate effect in inhibiting the formation of those radicals.

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1. Introduction

In recent years, marine resources have attracted attention in the search for bioactive compounds to develop new drugs and healthy foods (Qi, Zhao, Zhang, Li, & Zhao, 2005). In particular, seaweeds are a very important and commercially valuable resource for food, fodder, soil conditioners and pharmaceuticals (Yang et al., 2006). Moreover, sulfated polysaccharides from marine algae are known to exhibit many biological and physiological activities including anticoagulant, antiviral, antitumor, anti-inflammatory and antioxidant (Becker, Guimarães, Mourão, & Verli, 2007; F-Tischer, Talarico, Nosedá, Guimarães, & Duarte, 2006; Souza et al., 2007; Ye, Wang, Zhou, Liu, & Zeng, 2008).

Red seaweeds synthesize a great variety of sulfated galactans, which are the major components of the extracellular matrix. Polysaccharides from the *Gracilaria* genus are composed mainly of the alternating 3-linked- β -D-galactopyranose unit (Gal) and the 4-linked-3,6-anhydro- α -L-galactopyranose unit (AnGal). The Gal unit can be substituted by either a methyl or a sulfate ester groups (Rees, 1961).

Marine red algae of the genus *Gracilaria* are a major agarophyte resource in the world and are cultivated for the phyto colloid industry or for integrated marine culture (Troell et al., 2003). The red marine alga *Gracilaria birdiae* has a great economic impact in Brazil due to agar production (Plastino, Ursi, & Fujii, 2004). Marinho-Soriano, Moreira, and Carneiro (2006) studied the cultivation of this species in the sea and Maciel et al. (2008) studied the isolation and structural characterization of the cold water-soluble fraction of polysaccharide taken from *G. birdiae* cultivated on the Atlantic coast of Brazil (Fleixiras Beach, in the State of Ceará). No studies have yet been performed on the chemical characterization of its polysaccharides extracted with hot water.

When the natural defenses of an organism (of enzymatic, non-enzymatic, or dietary origin) are overwhelmed by an excessive generation of reactive oxygen species (ROS), a situation of 'oxidative stress' occurs. Consequently, cellular and extracellular macromolecules (proteins, lipids, and nucleic acids) can suffer oxidative damage, causing tissue injury (Halliwell & Auroma, 1991; Halliwell & Gutteridge, 1989). Most organisms are able to defend themselves from oxidations and repair oxidative damages. However, the innate defense in the human body may not be enough for severe oxidative stress. Antioxidants are substances that can

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delay or prevent oxidation of cellular oxidizable substrates (Wang, Zhang, Zhang, Zhang, & Li, 2009). Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the amount of ROS (Li, 2007).

In order to reduce damage to the human body and prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) (Qi et al., 2005).

In recent years, algal polysaccharides have been demonstrated to play an important role as free-radical scavengers and antioxidants for the prevention of oxidative damage in living organisms (Kim et al., 2007; Souza et al., 2007; Wang et al., 2009). More recently Cerqueira et al. (2009) studied the applicability of sulfated polysaccharide from *G. birdiae* as cheese coatings.

Polysaccharides from seaweeds have also interesting thickening and gelling properties. Since the rheological behavior is directly linked to the structure of the polysaccharide, the rheological characterization of sulfated polysaccharide from *G. birdiae* is required.

The aim of this work was to obtain sulfated polysaccharides from *G. birdiae* isolated by aqueous extraction at 90 °C and evaluate their chemical and rheological properties and to test their antioxidant potential.

2. Materials and methods

2.1. Chemical analyses

Total sugar content of each fraction was determined according to the method of Dubois, Gillis, Hamilton, Rebers, and Smith (1956). Protein content was measured by Bradford's method (Bradford, 1976).

2.2. Extraction of polysaccharides

Specimens of the red seaweed *G. birdiae* were collected in the Atlantic coast of Brazil (Fleixeiros Beach, Trairi, Ceará). This species grows attached to rocks or dead coral. The diploid phase that develops directly on the female thallus, the carposporophyte, is evident all year in the area, and was selected as seed material. The seedlings were cleaned and then tied in a structure made of string, which was placed in the sea (03° 13' 25" S and 039° 16' 65" W), where it was anchored and submerged for two months. After that period algae were collected, cleaned of epiphytes, washed with distilled water and stored at –20 °C.

The samples were air dried and then milled. The powder was extracted with water (1.5% w/v) at 25 °C with mechanical stirring for 15 h. The algal residue was removed by filtration and supernatant discarded. The algal residue obtained from the first extraction was extracted with water at 90 °C with mechanical stirring for 45 min. The residue was removed by centrifugation, and the supernatant was precipitated with ethanol (1:3 v/v), lyophilized and stored.

2.3. Determination of sulfate content in polysaccharides

Sulfate content in polysaccharides was determined by the barium chloride–gelatin method (Lloyd, Dodgson, Price, & Rose, 1961). A standard curve was made as follows: 0.02; 0.04; 0.06; 0.08; 0.10; 0.12; 0.16; 0.18 and 0.20 mL K₂SO₄ standard solution (0.6 mg mL⁻¹) were accurately put into test tubes; hydrochloric acid (1 M) was compensated to 0.2 mL solution. Then 3.8 mL of

trichloroacetic acid (3% v/v) and 1.0 mL of barium chloride–gelatin solution (5 g L⁻¹) were added, vortexed, and absorbances were measured at 360 nm after incubation for 15 min at room temperature; 0.2 mL hydrochloric acid solution was used as a blank.

2.4. Polysaccharide analyses

The monosaccharide composition of red seaweed galactans was obtained by reductive hydrolysis (Stevenson & Furneaux, 1991). This technique combines hydrolysis (with CF₃CO₂H) and reduction (with 4-methylmorpholine-Borane) followed by acetylation to produce alditol acetates. After acetylation, these alditol acetate derivatives were analyzed by gas chromatography (GC) (Carlo Erba 6000, Carlo Erba, Milan, Italy) with a split injector (split ratio 1:60) and a flame ionization detector. The column was a DB-225 (J & W, USA) with 30 m × 0.25 mm and film thickness of 0.25 μm; the oven temperature program was: 220 °C during 5 min, being then the temperature raised at a rate of 20 °C min⁻¹ to 230 °C and maintained at this temperature for further 6 min. The flow rate of the carrier gas (H₂) was set at 1 mL min⁻¹ at 220 °C. The injector temperature was 220 °C and the flame ionization detector temperature was 230 °C. The monosaccharide composition was quantified by Gas chromatography–mass spectrometry (GC–MS), the analysis was performed in a HP (USA) series 2 gas chromatograph and Trio-1S VG mass-lab with scans between 400 and 35 m/e/s with a 70 eV ionization energy. All mass spectra were compared with the library data system of the GC–MS equipment (Wiley 275). The hydrolysis of all samples was performed in duplicate and each one was injected twice.

2.5. Molar mass distribution

The peak molar masses (M_{pk}) were estimated by gel permeation chromatography (GPC) with a Shimadzu equipment at room temperature using an Ultrahydrogel linear column (7.8 × 300 mm, exclusion limits 10⁶ g mol⁻¹), flow 0.5 mL min⁻¹, 0.5% polysaccharide concentration and 0.1 M NaNO₃ as solvent. A differential refractometer and an ultraviolet photometer (at 280 nm) were used as detectors and the elution volume corrected to the internal marker of ethylene glycol at 10.45 mL. Pullulan samples (Shodex Denko) of M_w 5.9 × 10³, 1.18 × 10⁴, 4.73 × 10⁴, 2.12 × 10⁵ and 7.88 × 10⁵ g mol⁻¹ were used as standards.

2.6. FTIR analysis

The IR spectra of the polysaccharides were determined using a Fourier transform infrared spectrometer (FTIR) (Perkin–Elmer 16 PC spectrometer, Boston, USA). The polysaccharide was ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into 1 mm pellets for FTIR measurement in the wavenumber range of 600 and 4000 cm⁻¹ using 16 scans.

2.7. Rheological measurements

The measurements under frequency sweep of *G. birdiae* sulfated polysaccharide and commercial-grade agar (Fluka, Switzerland) have been performed using a controlled – strain rheometer (AR-G2) from TA Instruments (USA), using a plate and plate geometry (diameter: 25 mm) with a gap between plates of 1 mm. Frequency sweeps were performed, at 25 °C, in the 0.1–10 Hz range and the strain was fixed at 0.7% in order to assure that the working conditions lied in the linear viscoelastic region. In fact, before performing frequency spectra, the linear viscoelastic region was determined and the appropriate strain was selected, by means of

strain sweeps conducted at different frequencies (0.1, 1 and 10 Hz) and variable strain ranging from 0.01 to 10%. This type of test determines the maximum deformation attainable for a system without structural failure. Measurements were performed in triplicate.

2.8. Determination of antioxidant activity

2.8.1. Effect of scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The free-radical scavenging capacity of the sulfated polysaccharides (*Gb*) was analyzed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test according to the method of Blois (1958), with some modifications. BHT was used as reference material. Briefly, 0.2 mL of MeOH and 0.3 mL of various sample concentrations (0.1–2.0 mg mL⁻¹) dissolved in MeOH were mixed in a 10-mL test tube. DPPH (2.5 mL of 75 μM in MeOH) was then added to achieve a final volume of 3.0 mL. The solution was kept at room temperature for 30 min, and the absorbance at 517 nm (A_{517}) was measured.

The DPPH scavenging effect was calculated as follows:

$$\text{Scavenging effect (\%)} = [A_0 - (A - A_b)/A_0] \times 100 \quad (1)$$

where $A_0 = A_{517}$ of DPPH without sample; $A = A_{517}$ of sample and DPPH; and $A_b = A_{517}$ of sample without DPPH.

2.8.2. Hydroxyl radical scavenging activity

The scavenging activity of seaweed polysaccharides against the hydroxyl radical was investigated using Fenton's reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}$). The results were expressed as an inhibition rate. Hydroxyl radicals exhibit a small diffusion capacity and are most reactive in the induction of injuries to cellular molecules and, accordingly, deserve special attention. Hydroxyl radicals were generated using a modified Smirnoff and Cumbes' (1989) method: in 3 mL sodium phosphate buffer (150 mM, pH 7.4), which contained 10 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mM EDTA, 2 mM sodium salicylate, 30% H_2O_2 (200 μL) and varying concentrations of polysaccharides (0.1–2.0 mg mL⁻¹). In the control sample, sodium phosphate buffer replaced H_2O_2 . The solutions were incubated at 37 °C for 1 h, and the presence of hydroxyl radical was detected by monitoring absorbance at 510 nm.

2.9. Statistical analyses

Statistical analyses were performed using Analysis of Variance (ANOVA) and linear regression analysis. The Tukey test ($\alpha = 0.05$) was used to determine any significance of differences between specific means (SigmaStat, trial version, 2003, USA).

3. Results and discussion

3.1. Extraction and chemical analyses

The milled red seaweed was washed with water at room temperature and the residue left was exhaustively extracted with hot water (90 °C). This method revealed to have a higher yield (27.2%) than cold extraction (see Table 1). Maciel et al. (2008) studied the structural characterization of cold extracted fraction of sulfated polysaccharide from *G. birdiae* and showed that the low yield might be due to the low extraction temperature. However the agar yield of *Gracilaria cornea* from Mexico and from Brazil was found to range from 35.6 to 42.1% (Freile-Pelegriñ & Robledo, 1997) and from 29 to 41% (Marinho-Soriano, Silva & Moreira, 2001), respectively, depending on the season. Melo, Feitosa, Freitas, and de Paula (2002) showed that the temperature is one important factor responsible for the yield of extraction. Armis (1995) showed that in general the yield of extraction of agar from *Gracilaria* species is very variable due to several factors, such as environmental conditions, seasonal variation, physiological factors and extraction methods. As can be seen in Table 2, the extraction with hot water originated levels of sulfate of 8.4%, higher than those found in the literature for other tropical species of *Gracilaria* (Cote & Hanisak, 1986; Freile-Pelegriñ & Murano, 2005; Marinho-Soriano & Bourret, 2005), such as *G. cornea* (collected in Mexico), which showed variations from 4.8 to 5.5%. The *G. birdiae* polysaccharide obtained by cold extraction has a sulfate content (6.4%) (Maciel et al., 2008) in the range observed for polysaccharides from other *Gracilaria* species (2.3–8.9%). However, Mazumder et al. (2002), found levels of sulfate ranging from 2.1 to 11.7% for fractions of *Gracilaria corticata* and the higher levels were attributed to fractions obtained from cold extraction. It may thus be concluded that low temperatures are responsible for lower agar extraction yields but they allow higher sulfate concentrations to be attained.

In the present work *Gb* exhibits low levels (2.5%) of protein content (Table 2), which are in agreement with the values observed in the literature (Melo et al., 2002). Analyses of total sugars by the method of phenol/sulfuric acid according to Dubois et al. (1956), showed high percentages of these compounds (85.6%).

3.2. Monosaccharide composition and molar mass distribution

Table 2 shows the monosaccharide composition of galactans obtained from *G. birdiae*. The results show that these galactans have a high content of galactose (65.4%) and 3,6-anhydrogalactose (25.1%) obtained by reductive hydrolysis and quantified by GC–MS. The methyl derivatives: 6-O-methylgalactose (9.2%) and in smaller quantities 3-O- and 4-O-methyl-galactose (0.33%) were also quantified by GC–MS. The presence of galactose and 3,6-anhydrogalactose as major monosaccharides has been observed

Table 1
Yield of extraction of agarans obtained from different species of *Gracilaria*.

Species	Local	Type of extraction	Yield of extraction (%) ^a	Reference
<i>G. birdiae</i>	Brazil	Hot extraction	27.2	Present work
<i>G. birdiae</i>	Brazil	Room temperature	6.5	Maciel et al., 2008
<i>G. bursa-pastoris</i>	France	Autoclaving	34.8	Marinho-Soriano, 2001
<i>G. cervicornis</i>	Brazil	Hot extraction	11.0–20.0	Marinho-Soriano, Silva, & Moreira, 2001
<i>G. cervicornis</i>	Mexico	Hot extraction	25.0–39.3	Freile-Pelegriñ & Murano, 2005
<i>G. cornea</i>	Brazil	Enzymatic digestion	11.0–21.4	Melo et al., 2002
<i>G. dura</i>	France	Autoclaving	33.5	Marinho-Soriano, 2001
<i>G. gracilis</i>	France	Autoclaving	11.1–18.7	Mollet, Rahaoui, & Lemoine, 1998

^a Percentages based on milled seaweed.

Table 2
Chemical analysis and monosaccharide composition of the sulfated polysaccharide obtained from *G. birdiae*.

Sample	Temp. of extraction (°C)	Weight (g)	Carbohydrate (%)	Sulfate ^a (%)	Protein (%)	Monosaccharide composition ^b (mol%)			
						AnGal	6 Gal	3 and 4 Gal	Gal
Gb	90	20.0	85.6	8.4	2.5	25.1	9.2	(tr)	65.4

tr = Traces (<1 mol%).

^a Expressed as SO₃Na.

^b AnGal corresponds to 3,6-anhydrogalactose; 6 Gal to 6-O-methylgalactose, etc.

in several other algae of the order *Gracilariales* (Melo et al., 2002; Mollet et al., 1998), and their quantities are generally variable.

In order to estimate the peak molar mass (M_{pk}) for *Gb* polysaccharide, a calibration was obtained using pullulan fractions. The equation obtained from the calibration plot was:

$$M_w = 13.142 - 0.964V_e \quad (2)$$

where V_e is the elution volume in mL. The linear correlation coefficient was 0.9991.

The GPC chromatogram is shown in Fig. 1. A peak at 7.86 mL and a shoulder at 6.98 mL were detected. *Gb* polysaccharide behaves as a heterogeneous system similar to other seaweeds' polysaccharides, such as those from *G. cornea* (Melo et al., 2002) and *Botryocladia occidentalis* (Farias, Valente, Pereira, & Mourão, 2000).

Based on Eq. (2), *Gb* polysaccharide shown M_{pk} values of 2.6×10^6 and 3.7×10^5 g mol⁻¹, corresponding to the identified shoulder and peak. This high molar mass can be justified by grouping of polysaccharide chains, which can also be observed by the highly heterogeneous profile.

Both synthetic polymers and naturally occurring polysaccharides are polydisperse, meaning that in general they do not have sharply defined molecular weights, but rather average molecular weights representing a distribution of molecular species nearly identical in structure but of varying chain length (Stanley, 2006).

3.3. FTIR analysis

Table 3 shows results from FTIR analyses performed on *Gb* polysaccharide and reveals the presence of most characteristic bands of polysaccharides from red seaweeds.

The regions of the spectrum between 1400 and 700 cm⁻¹ were expanded to better identify the positions of sulfate groups present

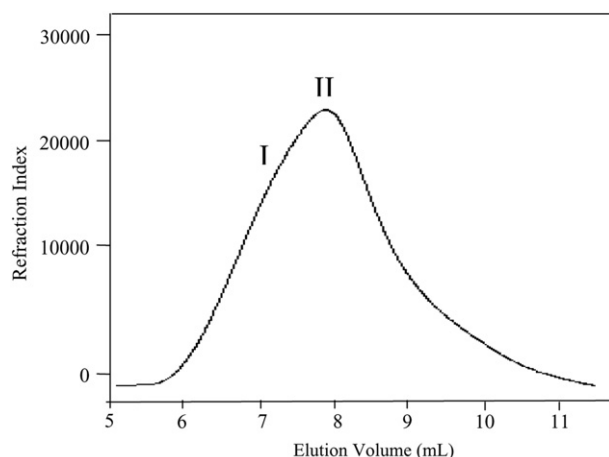


Fig. 1. GPC curve for *G. birdiae* sulfated polysaccharide. I = shoulder; II = peak.

in these polysaccharides (Fig. 2). The most important bands were those found at 1375 cm⁻¹ and 1255 cm⁻¹, corresponding to ester sulfate groups; the region around 1072 cm⁻¹ and 890 cm⁻¹ are equivalent to the skeleton of galactans and agar specific band, respectively. The region around 931 cm⁻¹ can be attributed to the C–O–C group of 3,6-anhydro- α -L-galactopyranose and the absorption around 855 cm⁻¹ indicates the presence of sulfate groups on the C-4 of galactose. The band around 820 cm⁻¹ is attributed to the 6-sulfate group of D-galactose units.

Studies with algae *G. corticata*, *Gracilaria domingensis*, *Gracilaria mammillaris* and *Gracilaria pseudoverrucosa* showed the presence of the band around 820 cm⁻¹ and the absence of the band around 855–840 cm⁻¹. However, after alkaline treatment, the infrared spectrum of the same polysaccharide registered the presence of a band around 850–840 cm⁻¹ and no band around 820 cm⁻¹. It was also observed that the same alkaline treatment resulted in an increase of the bands intensity around 930 cm⁻¹. This means that the band around 820 cm⁻¹ is related to the presence of the sulfate group at C-6 of L-galactose units, which are converted to 3,6-anhydrogalactose after alkaline treatment (Mazumder et al., 2002). The residues of 6-sulfate- α -L-galactose are known to be precursors of 3,6-anhydro- α -L-galactose (Talarico et al., 2004).

Melo et al. (2002) also found bands at 1370 and 770 cm⁻¹ and Maciel et al. (2008), when studying the cold extraction of *G. birdiae* polysaccharide, observed the same bands characteristic of agarocolloids (1375 and 775 cm⁻¹).

3.4. Rheological characterization

The rheological behavior of *G. birdiae* sulfated polysaccharide was compared to that of commercial agar, a gel-forming polysaccharide thoroughly used by the food industry. The mechanical spectra obtained for *Gb* sulfated polysaccharide and for commercial agar at 1.5% (w/w) and at 25 °C are presented in Fig. 3a and b, respectively.

Both polysaccharides exhibit a gel-like behavior: storage modulus (G') higher than loss modulus (G'') in the whole range of frequencies covered and with both moduli being almost frequency independent. The mechanical spectra of the two sulfated polysaccharides are similar however, for the same concentration, the

Table 3
Assignment of the most important IR bands in agarocolloids.

Wavenumber (cm ⁻¹)	Assignment	Reference
1380–1355	Ester sulfate (–S=O)	[4,5]
1250–1240	ν_{as} S=O (Ester sulfate)	[1,4,5]
1080–1040	Skeleton of galactans	[2,4]
940–930	Vibrations of the C–O–C de 3,6-anhydro-L-galactose	[1,3,4,5]
900–890	Agar specific band	[2]
850–845	Galactose 4-sulfate	[1,4,5]
820	Galactose 6-sulfate	[2]

[1] = Chopin & Whalen, 1993; [2] = Mollet et al., 1998; [3] = Parekh, Doshi, Rao, & Chauhan, 1987; [4] = Prado-Fernández, Rodríguez-Vázquez, Tojo, & Andrade, 2003; [5] = Rochas, Lahaye, & Yaphe, 1986.

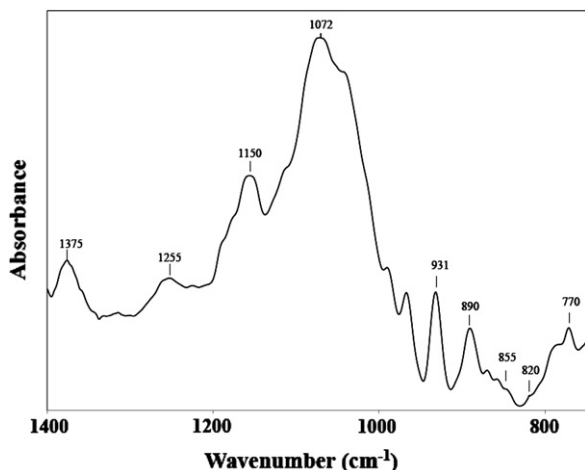


Fig. 2. FTIR spectra of sulfated polysaccharide of *G. birdiae*.

commercial agar solution shows a higher elastic behavior that can be due to the higher purity of the commercial sample (>95%). Also, as expected for gels, the plot of $[\eta^*]$ versus $\log \omega$ was linear with a slope of -1 (Fig. 3).

Several authors reported that the 3,6-anhydro- α -L-galactopyranose (AnGal) content in *Gracilaria* agars is an important component in the control of gel textural quality (Lahaye & Rochas, 1991). Also, Bird, Hanisak, and Ryther (1981) observed that high gel strength of agar sample is associated with high molecular weight and larger polymers and these characteristics reflect a high capability to form 3-dimensional lattices between water and gel helices. The polysaccharide extracted in this work exhibits a solid-like behavior, that can be due to the high content in 3,6 AnGal (25.1 mol%). Rodríguez, Matulewicz, Noseda, Ducatti, and Leonardí (2009) characterized the polysaccharides isolated from *Gracilaria gracilis* at three temperatures and observed that the polysaccharide

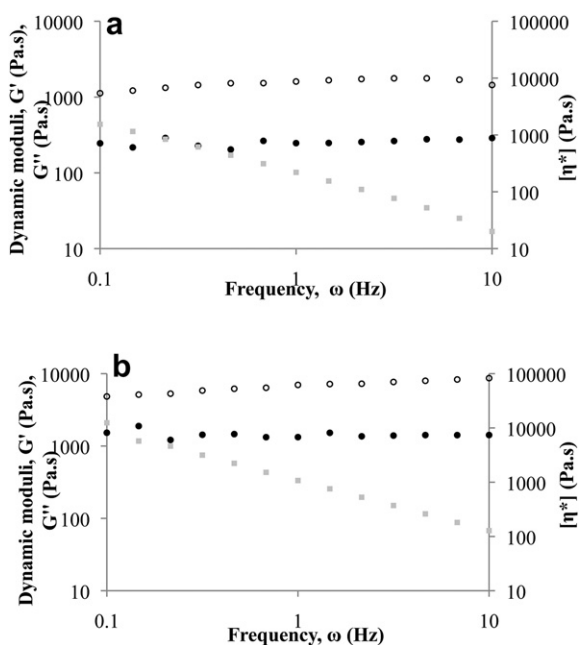


Fig. 3. Mechanical spectra of a) *G. birdiae* sulfated polysaccharide and b) commercial agar at a total polysaccharide concentration of 1.5%, at 25 °C: (G' (○), G'' (●) and $[\eta^*]$ (■)).

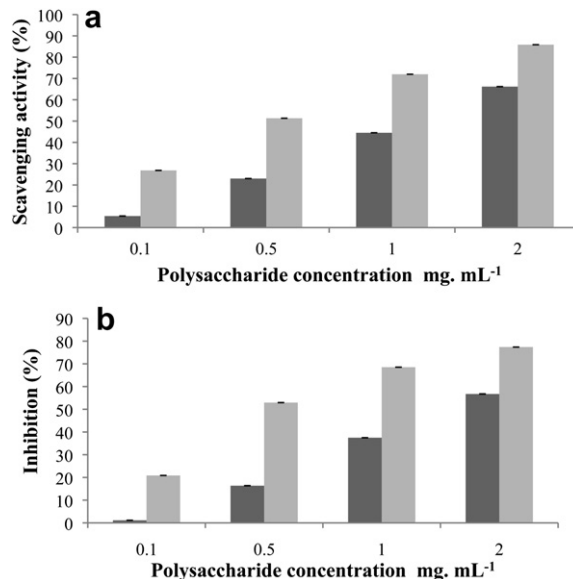


Fig. 4. Antioxidant activity: a) effect of scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and b) Inhibition of hydroxyl radicals effects by sulfated polysaccharide of *G. birdiae*. ■ Gb; ■ BHT as a positive control. Values are means \pm SD ($n = 3$).

extracted at 90 °C exhibited an 3,6 AnGal content of 35–36 mol% and formed a gel with high strength.

3.5. Free-radical scavenging activity

DPPH is a free-radical compound that has been widely used to determine the free-radical scavenging ability of samples (Qi et al., 2005; Wang et al., 2009). This method allows determining the anti-radical activity of an antioxidant by measuring the decrease in absorbance of the DPPH radical caused by the scavenging of the hydroxyl radical through hydrogen donation. In this work, DPPH free-radical scavenging effect of each sample was measured (Fig. 4a). Results demonstrated that the sulfated polysaccharide had a noticeable effect on inhibiting the formation of these radicals ($IC_{50} = 1.62 \text{ mg mL}^{-1}$). However, none of the samples had stronger activity than BHT at the same concentration.

The results obtained for the inhibition of hydroxyl (OH^-) radical formation demonstrated that scavenging activity of *Gb* polysaccharide increased significantly ($p < 0.05$) for higher polysaccharide concentrations. The IC_{50} value of *Gb* eliminating OH^- was about 1.73 mg mL^{-1} , which indicates that the scavenging activity of *Gb* against OH^- was less than that of BHT. Qi et al. (2005) concluded that the OH^- scavenging activity of different polysaccharides was related to the presence of the same structural feature in which all of the polysaccharides had one or more $-\text{OH}$ and $-\text{OSO}_3\text{H}$ groups in the molecule. These results proved that sulfate content had a significant effect on OH^- scavenging activity. Souza et al. (2007) showed that iota-carrageenan had a higher inhibitory effect on OH^- formation in relation to the lambda- and kappa-carrageenans. Several works have demonstrated that the presence of sulfate groups in seaweed polysaccharides is responsible for numerous types of biological activities, such as antioxidant activities (Qi et al., 2005; Wang et al., 2009).

4. Conclusion

The sulfated polysaccharide from marine red algae *G. birdiae* is composed of galactose (65.4%) and methyl derivatives 6-O-methyl-

galactose (9.2%) and in smaller quantities 3-O- and 4-O-methylgalactose (0.33%). This polysaccharide also presents a high content of 3,6-anhydrogalactose (25.1%) and has a sulfate content of 8.4%.

The sulfated polysaccharide of *Gb* characterized by FTIR exhibits the characteristic bands of agarocolloids (at 1375 and 770 cm^{-1}).

It has also been shown that the *G. birdiae* sulfated polysaccharide is a promising agent to be evaluated for the application in the food industry, and that it presents a significant antioxidant activity.

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