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Research Article

PARTIAL CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITY FROM FRACTIONATED POLYSACCHARIDES OBTAINED OF BROWN ALGAE

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ABSTRACT

The polysaccharides that have sulfates in its composition have been widely studied due to their pharmacological potential. One of the main sources are marine algae because they are rich in sulfated polysaccharides and also because they have the advantage of being abundant in nature and are renewable natural resources. The objective of this work was to partially characterize sulfated polysaccharide fractions obtained from the algae species *Dictyota cerviconi*, *Sargassum vulgare C*. and *Padina boergesenii* and evaluate their antioxidants activities. The macroalgae were dried, powdered and treated with acetone to remove lipids and pigments. The extraction of polysaccharide fractions were obtained with increasing amounts of acetone and all the fractions have been assessed for antioxidant activity against DPPH (1,1-dyphenyl-2-picrylhydrazyil) radical. The results show that the fractional polysaccharides of all species of algae showed antioxidant activity, and the brown seaweed *Sargassum vulgare C.* and *Padina boergesenii* had the highest and lowest antioxidant activity, respectively. The algae *Padina boergesenii* showed the greatest percentage of total carbohydrates in its composition as well as more percentage of fucose.

Keywords: Macroalgae, Sulfated polysaccharides, Antioxidant activity.

INTRODUCTION

Brazil has a huge biological diversity in both aquatic and terrestrial environments. Macroalgae in the marine environment is one of the groups of greatest diversity among photosynthetic organisms, representing a strategic natural resource to the biotechnology development in the country [1]. Seaweeds serve as an important source of bioactive natural substances, which provide a rich source of structurally diverse secondary metabolites [2,3].

The presence of macroalgae is common throughout the Brazilian coast, being, however, more abundant and diverse in areas with rocky soil and more transparent water, such as the northeast coast of the country. The main factors that reduce the biodiversity of macroalgae are related to the presence of large inflows of fresh water, sediments and areas subject to strong organic pollution [1].

The seaweeds are distributed among three divisions: *Chlorophyta*, *Phaeophyta* and *Rhodophyta*. Algae of the phylum *Chlorophyta* are characterized by their green color due to a higher concentration of chlorophyll. They also has carotenes and xanthophylls pigments. The phylum *Rhodophyta* is composed of algae that have chloroplasts with predominance of phycobilins on chlorophyll A, and especially phycoerythrin and carotenoids that give the color red, pink or red vinacea. And in the division *Phaeophyta*, object of study in this work, are included species known as brown algae due to the predominance of xanthophylls chloroplasts (mainly fucoxanthin) on chlorophyll A, C and carotenoids [4].

Algae are renewable natural sources potentially rich in sulfated polysaccharides, with a predominance of one or another polysaccharide depending on the group. In red algae, there are mainly sulfated galactans, green algae have more heterogeneous sulfated polysaccharides that are rich in galactose, mannose, xylose, arabinose, glucose and / or uronic acids and brown seaweed are extract fucans, laminarin and alginic acid [5]. Studies show that the structure of these polysaccharides varies not only between different species of algae as well as with the age of the algal tissue, the place where they are growing and in different parts of the seaweed.

Fucans is a term used to characterize a family of polysaccharides rich in sulfated L-fucose with varying molecular weights [6]. In addition to the brown algae, the fucans can also be found in echinoderms. The physiological properties of sulfated fucans are not well known on marine organisms, however, there is a belief that they have a role in organizing the cell wall and may be involved in the binding of alginate and cellulose [7]. The fucans has a broad spectrum of activities in biological systems, such as antiviral activity, anti-adhesive, anticoagulant, anti-inflammatory, antiproliferative, antioxidant, antitumor and interference with mechanisms involved in fertilization [6, 7, 8, 9]. The sulfated fucans are among the most widely sulfated polysaccharides studied.

Studies in the literature regarding the characterization of fucans show specific characteristics to these polysaccharides depending on the selected species of algae. Abdel-Fathah *et al.* [10] showed one fucan of *Sargassum linifolium* consists of a core structure formed by β-D-glucuronide and β-D-mannose and branches composed of galactose-6-sulfate and 3, 6-disulfate, as well of fucose. For a fucan extracted from seaweed *Sargassum stenophyllum* was verified a central structure of (1 → 6)-β-D-galactose and / or (1 → 2)-β-D-mannose, with branches of oligosaccharides from α-L-fucose (1 → 3 and / or 1 → 4)-α-D-glucuronide with β-D-xylose in non-reducing terminal [11]. Studies with fucans extracted from algae of different order are also presented in the literature and confirm the heterogeneity of polysaccharides present in the algae [12, 13, 14, 15]. As mentioned, the sulfated polysaccharides can have antioxidant activity. An antioxidant is any substance which, when present in low concentrations compared to the oxidizable substrate, delays or inhibits oxidation of this substrate effectively [16]. These substances may act through various mechanisms, such as trapping or inhibiting the formation of free radicals, repair of injuries caused by these molecules, or even increased synthesis of antioxidant enzymes due to adaptation of the body in response to generation of these radicals [17].

Most of the oxidizing compounds or pro-oxidants have mutagenic and carcinogenic activity and act through the formation of oxygen radicals also called reactive oxygen species (ROS), and usually their formation depends on increased cytoplasmic calcium. The oxygen radicals (hydroxyl and peroxyl radicals) and superoxide anion play an important role in biochemical and physiological reactions of the human body. However, if these are produced in excess during the pathophysiological processes or due to adverse environmental factors, diseases and deep tissue damage can occur, if antioxidants in vivo are not available. These substances retard the rate of oxidation, through one or more mechanisms such as inhibition of free radicals and metal complexation. They can be synthetic or natural, and for use in food, should be safe for health [18].

Xue *et al*. [19] tested the antioxidant activity of a fucan (F-A) and a lower molecular weight polysaccharide (L-A), derived from acid hydrolysis of the original fucan isolated from *Laminaria japonica*. The authors found that L-A had a lower proportion of fucose residues, but a similar proportion of sulfate. The antioxidant properties of the two polysaccharides were using two systems of oxidation of low density lipoproteins, and L-A showed a greater effect against the oxidation of lipoproteins in both systems. The fucan F-A had little effect against oxidation of lipoproteins in a system which was added oxidation inducer of low density lipoprotein (Cu^{+}), a fact explained by the authors due to the fucan high molecular weight. The authors deduced that the molecular mass of F-A interferes with the inhibition of oxidation of low density lipoproteins. These results were further confirmed in more recent work of Zhao *et al*. [20]. When testing fucans fractions (F-A and F-B) and their derivatives from hydrolysis (L-A and L-B) the authors found that F-A and F-B have very little inhibitory effect on lipoprotein oxidation of low molecular weight systems containing Cu^{+}.

Given the importance of sulfated polysaccharides, this work partially characterized the polysaccharide fractions obtained from some species of brown algae from the coast of Alagoas - Brazil, (*Dictyota cervicornis, Padina borgenesii and Sargassum vulgare*) and evaluated their antioxidant activity.

MATERIALS AND METHODS

Collection of seaweed

The algae were collected manually with the aid of spatulas from natural deposits found in São Miguel dos Milagres - AL, packed in styrofoam boxes and transported to the botanic laboratory at ICBS/UFAL. They were initially weighed (wet mass), washed with water in appropriate screens and dried at the sun for a period of four days until they reached a constant weight. We pulverized the dried seaweed in a blender, sieved it through a 710 micrometers aperture sieve, and stored it in labeled containers for use in the next step.

Extraction and fractionation of polysaccharides

The pulverized and dried seaweed (100 g) were treated twice with two volumes of acetone for 24 hours to remove lipids and pigments from the material. After 48 hours the acetone was discarded and the material placed in an aerated oven to dry at 45°C until it reached constant weight. We added 1000 mL of 0.5 M NaCl to the "ketonic powder" obtained from each species, and afterwards adjusted the pH to 7.0 using NaOH. To this material we added the proteolytic enzyme papain from Merck (15 mg per gram of "ketonic powder") and the suspension was maintained at 60°C under constant stirring for 24 hours. The suspension was then filtered through cotton and centrifuged at 4000 rpm for 20 minutes at room temperature. The supernatant, termed "crude polysaccharides" was fractionated with increasing volumes of acetone, which separated the polysaccharides according to their apolar solubility as proposed by Rocha [21]. Initially, we added a volume of acetone equal to 50% of the initial volume of "crude polysaccharides", maintaining this solution to stand at 4°C for 18 hours. The precipitate was collected by centrifugation at 4000 rpm for 20 min at 4°C and dried in an aerated oven at 45°C. To the supernatant was added one volume of acetone needed for a final concentration of 100% acetone with respect to the initial volume of crude polysaccharides, being maintained at 4°C for 18 hours. This procedure was repeated using volumes of acetone needed for the solution to be in the final concentrations of acetone 150% and 200%, with respect to the initial volume of "crude polysaccharide", giving rise, respectively, the fractions of acetone F0,5V; F1,0V; F1,5V and F2,0 V, which correspond to the fractions precipitated with 50, 100, 150 and 200% acetone.

Chemical analysis

The determination of total carbohydrates was done by the method phenol-sulfuric acid [22] and the dosages of fucose by Dische [23] and xylose by the method Wheeler and Tollens,1889, modified by Dische and Boren [24]. The total sulfate was measure by the method gelatin-barium [25].

Antioxidant activity

The evaluation of antioxidant activity was determined by the method DPPH [26] based on the capture of the radical DPPH (2,2-diphenyl-1-picril-hidrazyl) by antioxidants, producing a decrease in absorbance at 517 nm. With this decrease, the percentage of radical DPPH consumption was calculated using the following equation:

$$C(\%) = \frac{(\mathrm{ABS}_{\mathrm{control}} - \mathrm{ABS}_{\mathrm{fraction}}) \times 100}{\mathrm{ABS}_{\mathrm{control}}}$$

Where, C(%) is the radical DPPH consumption percentage, $ABS_{control}$ is the absorbance of the control solution (1,5mL of DPPH 0,45 mg/mL and 1,5 mL of methanol) and $ABS_{fraction}$ is the absorbance of the 1,5 mL of solution containing certain concentration of fractionated polysaccharide (5 - 250 µg/mL) with 1,5mL of DPPH.

With these data, by plotting graphs of DPPH consumption percentage versus concentration of fractionated polysaccharide (linear regression), it was possible to calculate the IC_{50} (concentration of substrate needed to consume 50% of DPPH).

RESULTS AND DISCUSSION

After obtaining the polysaccharide fractions, we carried out measurements of sugars, sulfate and evaluation of antioxidant activity. The results for sugars and sulfate are shown in Table 1 and for the antioxidant activity in Table 2.

Dosage of total carbohydrates, fucose, xylose and sulfate

It can be observed that the fractions F1,0V for all species of seaweed showed the highest amount of total carbohydrates and fucose. Furthermore, for all the polysaccharide fractions, except for F2,0V, we saw a higher percentage of total carbohydrates and fucose in the seaweed *Padina boergesenii*. Rocha et al. [5] also verified higher total carbohydrates in precipitated fractions by using lower acetone volumes although with different algae. In relation to sulfate, the fractions F0,5V are those with the lowest percentage of sulfate. The fucose and sulfate determination in the fractions indicated the possible presence of fucans in all fractions. Studies have indicated that fucans activities are directly dependent on their sulfation degree [27]. The method of xylose determination showed that the studied seaweed have no xylose in its composition.

Table 1: Percentage (%) of total carbohydrates, fucose and sulfate present in the fractionated of the seaweed

Fractions	*D. cervicornis*			*S. vulgare*			*P. boergesenii*		
	TC	SU	FU	TC	SU	FU	TC	SU	FU
F0,5V	29,5	2,8	7,0	36,2	12,8	7,9	48,3	0,3	27,5
F1,0V	52,7	14,5	26,5	58	19,1	46,4	64,2	13,4	52,0
F1,5V	19,4	36,4	10,5	36,4	15,7	21,7	59,7	31,4	34,9
F2,0V*	-	-	-	23,3	14,2	9,0	21,7	4,8	7,9

* The fraction F2,0V from the seaweed *D. cervicornis* was not obtained.

TC: total carbohydrates; SU: sulfate, FU: Fucose

Evaluation of antioxidant activity

Lower IC_{50} values indicate higher antioxidant activities, because this indicator provides us the fractionated concentration needed to consume 50% of DPPH. Thus, it can be observed that the *Sargassum vulgare C*. has the highest antioxidant activity, since their fractionated have the lowest IC_{50}, with exception of the fractionated F2,0V which did not show antioxidant activity. Similarly, we can claim that *Padina boergesenii* has the lowest antioxidant activity.

According to the results showed in Tables 1 and 2, there was no direct relation between antioxidant activity and fucan percentage. Mulloy *et al.* [14] observed that polysaccharides activities depend on exact patterns of sulfate substitution. These authors verified that a small alteration in dermatan sulfate structure, 4-O-sulfated to 6-O-sulfated galactosamine, leads to an almost complete loss of anticoagulant activity in spite of an overall high level of sulfation. Possibly, the relation between antioxidant activity and fucan percentage depends upon the structure of this sort of polysaccharides.

Table 2: IC_{50}, concentration of fractionated (in µg/mL) from the seaweeds needed to consume 50% of DPPH.

Frações	*D. cervicornis*	*S. vulgare*	*P. boergesenii*
F0,5V	4263	553	No activity
F1,0V	850	812	9381
F1,5V	2694	784	7454
F2,0V	-	No activity	3127

* The fraction F 2,0V from the seaweed *D. cervicornis* was not obtained.

CONCLUSIONS

The brown seaweed *Padina boergesenii* had the highest percentage of carbohydrates in their composition, as well as a higher percentage of fucose. The highest percentage of fucose found shows the presence of fucans, a family of polysaccharides rich in sulfated L-fucose. However, this higher percentage of fucose did not result in better antioxidant activities. The fact that *Padina boergesenii* presented the lowest antioxidant activity does not imply a lesser importance to it, since it showed a high percentage of polysaccharides in their constitution, and most of them fucans, which has a wide spectrum of activities in biological systems such as antiviral activity, anti-adhesive, anticoagulant, anti-inflammatory, antiproliferative, antitumor, and antioxidant activity.

The analysis made in this study indicated that the brown seaweed *Dictyota cervicornis*, *Sargassum vulgare C*. and *Padina boergesenii* obtained in the Brazilian northeastern coast have antioxidant activity (in the range 4-24%), although these percentages are smaller than the ones in the literature. For instance, Souza *et al.* [28] has found percentages of inhibition around 40% for fractionated polysaccharides from brown seaweed. In our study the polysaccharides from *Sargassum vulgare C* showed the best antioxidant activity. It should be noted that the composition of the seaweed, and, consequently, their biological activities, varies among different species of seaweed, with the age of the algal tissue, with the location where they are growing, and sometimes among different parts of the seaweed. Despite the relatively low percentage of antioxidant activity, has been in a natural resource perspective to obtain products with pharmacological activities.

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