ISOLATION, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF THE CRUDE POLYSACCHARIDE FROM PHYLLOPHORA PSEUDOCERANOIDES

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ISOLATION, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF THE CRUDE POLYSACCHARIDE FROM PHYLLOPHORA PSEUDOCERANOIDES (Abstract): The red seaweed Phyllophora pseudoceranoides (Gmelin) New. et Tayl, commonly found in the Romanian Black Sea coastal waters, has not been studied regarding its chemical composition and biological activities. Aims: The aim of the present study was to isolate, characterize and assess the in vitro antioxidant activity of the crude polysaccharide from P. pseudoceranoides. Materials and Methods: The water soluble polysaccharide was characterized by Fourier transform infrared spectroscopy (FT-IR). Total phenolic content was determined by Folin-Ciocalteu method. Antioxidant activity was evaluated by ABTS radical cation scavenging and reducing power assays. Commercial-grade carrageenan was used as positive control. Results and Discussion: FT-IR analysis of the crude polysaccharide showed characteristic bands of carrageenan-type structure. A total phenolic content of 25.08 ± 1.00 mg GAE/g was determined in the crude polysaccharide. At 1.5 mg/mL, the polysaccharide exhibited important ABTS scavenging activity (49.59 ± 0.03%) and showed a good reducing power (0.4060 ± 0.002), when compared with the positive control (ABTS scavenging activity: 30.30 ± 0.03% and reducing power: 0.2494 ± 0.002, at a concentration of 1.5 mg/mL). Conclusions: The antioxidant activity of the sulfated polysaccharide suggests its possible use as an ingredient and antioxidant agent in the food and pharmaceutical industries. Keywords: PHYLLOPHORA PSEUDOCERANOIDES, POLYSACCHARIDES, ALGAE, ANTIOXIDANT ACTIVITY.

Over the past decades, marine macroalgae have attracted an emerging interest due mainly to their bioactive constituents with a broad spectrum of activities (1). Carra-
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3,6-anhydro-D-galactose and the conformation of the pyranose ring, resulting six groups: iota (ι)-, kappa (κ)-, lambda (λ)-, mu (µ)-, nu (ν)- and theta (θ)- carrageenans, the first three carrageenans being of commercial interest (fig. 1). Carrageenans are used in the food industry as thickening and gelling agents, but also in the non-food industries such as the pharmaceutical, cosmetics, printing and textile industries. Furthermore, carrageenans might be used in the development of new therapeutic agents, as they are endowed with anticoagulant, antiviral, antioxidant, antitumor, immunomodulatory and cholesterol-lowering properties (2).

Fig. 1. Chemical structures of carrageenans

Research in the field of antioxidants from macroalgae has gained considerable attention, as these metabolites can delay or inhibit the effects of reactive oxygen species (ROS) in biological systems (3). It is known that increased ROS generation leads to DNA damage, lipid peroxidation and protein denaturation, playing a key role in the etiology and pathogenesis of a wide range of diseases and age-related disorders (4). Furthermore, as the extensive use of synthetic antioxidants (butylhydroxytoluene, butylhydroxyanisole, propyl gallate and tert-butylhydroquinone) has been associated with potential health risks (5), seaweed polysaccharides could offer a natural alternative to synthetic antioxidants by enhancing the safety and preserving the quality of foods (3).

*Phyllophora pseudoceranoides* (Phyllophoraceae) is a red seaweed, commonly found in the coastal areas of the Romanian Black Sea. The aim of this study was to isolate and characterize the crude polysaccharide from *P. pseudoceranoides*; in addition, the total phenolic content and *in vitro* antioxidant activity were investigated in order to assess its potential use in the field of food and pharmaceutical industries.

**MATERIAL AND METHODS**

*Chemicals*

Carrageenan (commercial grade, type I), gallic acid, potassium ferricyanide, iron (III) chloride and sodium carbonate were purchased from Sigma-Aldrich (Steinheim, Germany). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), disodium phosphate dodecahydrate and Folin-Ciocalteu’s phenol reagent were supplied by Fluka (Steinheim, Germany). Monopotassium phosphate was purchased from Merck (Darmstadt, Germany). Trichloroacetic acid and potassium persulfate were purchased from Riedel-de Haën (Seelze, Germany). All other solvents and reagents were of analytical grade.

*Algal material*

*Phyllophora pseudoceranoides* was collected along the Black Sea coastal areas of Romania in June 2014 (location: Constanța - 44°11′44″N, 28°39′24″E). A voucher specimen was deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy
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**Grigore T. Popa** - Iasi. Fresh seaweed material was washed thoroughly with distilled water to remove salt and sand; the air dried sample (40 ºC) was powdered and kept at -4 ºC until analysis.

**Isolation of the crude polysaccharide**

Algal material was first extracted with ethanol to remove pigments and polyphenols; then, the residue was dried and extracted with ultrapure water (1.5% w/v) for 2 h at 100 ºC. After filtration and concentration of the aqueous extract, the polysaccharide was isolated by precipitation with ethanol (1:3 v/v); the precipitate was washed with acetone and air dried. Then, the polysaccharide was re-dissolved in ultrapure water, followed by the same protocol of precipitation, washing and drying (6). The final precipitate was lyophilized, giving a white-brownish solid (21% of the seaweed dry weight) which was used for further analysis.

**Chemical characterization**

**FT-IR spectroscopic analysis.** FT-IR spectroscopic analysis is a simple, fast, accurate and non-destructive technique that requires small amounts of sample; over the last decades, it has been extensively used to identify polysaccharide structures from various seaweeds, based on the analysis of their characteristic absorption bands (10).

Fig. 2 presents the FT-IR spectrum of the polysaccharide isolated from *P. pseudoceranoides*. The spectrum showed characteristic bands belonging to the stretching vibration of O-H (3741–3363 cm⁻¹) (I, II) and saturated hydrocarbon groups (2958 cm⁻¹, 2891 cm⁻¹) (III, IV). The polysaccharide fraction showed a strong absorption band at 1224 cm⁻¹ (V) that could be assigned to the vibration of S=O group of the sulfate ester. Besides, the spectrum had a characteristic band at 1026.05 cm⁻¹ (VII) which could be assigned to the glycosidic linkage (C-O and C-C stretching vibrations of the pyranose ring). The spectrum presented characteristic bands at 1068 cm⁻¹ (VI) and 929 cm⁻¹ (VIII) that belong to the stretching vibration of C-O group of 3,6-anhydro-D-galactose. The two strong absorption bands at 844.76 cm⁻¹ (IX) and

**RESULTS AND DISCUSSION**

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802.33 cm\(^{-1}\) (X) could be attributed to C-O-SO\(_3\) bond of galactose-4-sulfate and to C-O-SO\(_3\) bond of 3,6-anhydro-D-galactose-2-sulfate, respectively (10-12).

![FT-IR spectrum of the P. pseudoceranoides crude polysaccharide](image)

**Fig. 2.** FT-IR spectrum of the *P. pseudoceranoides* crude polysaccharide

The FT-IR spectrum of *P. pseudoceranoides* polysaccharide showed characteristic absorption bands of carrageenan type structure; FT-IR band assignment of carrageenans are presented in table I. Generally, the repeating disaccharide unit of carrageenans consists of alternating units of β-D-galactopyranose and α-D-galactopyranose or 3,6-anhydro-α-D-galactopyranose. As shown in table I, the FT-IR spectra of ι-carrageenan presents characteristic bands at 1210-1260 cm\(^{-1}\), 840-850 cm\(^{-1}\) and 800-805 cm\(^{-1}\), thus enabling us to tentatively identify the polysaccharide isolated from *P. pseudoceranoides* as ι-carrageenan (10-12).

**TABLE I**

**FT-IR band assignment of carrageenans** (adapted from Gomez-Ordonez et al. (12))

<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>Assignment</th>
<th>Carrageenan type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1210-1260</td>
<td>sulfate ester</td>
<td>ι, κ, λ, μ, ν, θ</td>
</tr>
<tr>
<td>928-933, 1070 (shoulder)</td>
<td>3,6-anhydro-D-galactose</td>
<td>κ</td>
</tr>
<tr>
<td>840-850</td>
<td>D-galactose-4-sulfate</td>
<td>ι, κ, μ, ν</td>
</tr>
<tr>
<td>830</td>
<td>D-galactose-2-sulfate</td>
<td>λ, θ</td>
</tr>
<tr>
<td>820, 825 (shoulder)</td>
<td>D-galactose-2,6-disulfate</td>
<td>λ, ν</td>
</tr>
<tr>
<td>810-820, 867 (shoulder)</td>
<td>D-galactose-6-sulfate</td>
<td>μ</td>
</tr>
<tr>
<td>800-805, 905 (shoulder)</td>
<td>3,6-anhydro-D-galactose-2-sulfate</td>
<td>ι, θ</td>
</tr>
</tbody>
</table>

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**Total phenolic content.** The crude polysaccharide obtained from *P. pseudoceranoides* showed a higher amount of phenolics (25.08 ± 1.00 mg GAE/g), when compared with commercial-grade carrageenan (11.17 ± 0.73 mg GAE/g). Lim et al. reported lower total phenolic content (0.039 mg GAE/g) for a fucoidan fraction isolated from *Sargassum binderi*, native to Malaysia (13).

**Antioxidant activity**

It has been reported that algae polysaccharides exhibit significant antioxidant effects; the antioxidant activity depends on several chemical features of polysaccharides such as molecular weight, type of major sugar units, sulfate content and solubility (3).

**ABTS radical cation scavenging activity.** The assay is based on the ability of antioxidants to transform the preformed radical cation ABTS, a blue-green chromophore with characteristic absorption at 734 nm, into its reduced form with a consequent decolorization (8). The polysaccharide and the positive control showed good ABTS radical scavenging abilities which were dose-dependent (fig. 3). The scavenging activity of *P. pseudoceranoides* polysaccharide increased from 19.00 ± 0.30% (at 0.15 mg/mL) to 49.59 ± 0.03% (at 1.5 mg/mL). However, at all tested concentrations, the positive control, commercial-grade carrageenan, was less active than the crude polysaccharide, with activities ranging from 15.68 ± 0.45% to 30.30 ± 0.03%. Shao et al. reported higher ABTS radical cation quenching abilities (36.94%) for a sulfated polysaccharide isolated from Chinese green alga *Ulva fasciata*, tested in a concentration of 0.15 mg/mL (14). Additionally, a polysaccharide isolated from *Sargassum horneri* showed higher scavenging activity (94%) at a concentration of 1.5 mg/mL (15). Several studies reported linear concentration dependent reaction kinetics for algae polysaccharides (14, 16).

Reducing power. This method was used to evaluate the ability of the crude polysaccharide to donate an electron, thus reducing the Fe$^{3+}$/ferricyanide complex to the ferrous form (Perl’s Prussian blue), quantified at 700 nm (17). As shown in fig. 4, the
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polysaccharide reduced Fe$^{3+}$ in a concentration-dependent manner. At 1.5 mg/mL, the crude polysaccharide exhibited higher reducing power (0.406 ± 0.002) than the positive control, commercial-grade carrageenan (0.249 ± 0.002). A linearity of the response in the reducing power assay was observed for sodium alginate isolated from Tunisian Cystoseira barbata; however, according to the absorbance values at 700 nm, sodium alginate showed stronger reducing activity (the reducing power was 2 at a concentration of 1.2 mg/mL) (9).

On the contrary, a polysaccharide extracted from Chinese red alga Porphyra haitanensis was reported to possess lower reducing activity than the P. pseudo cera- noides polysaccharide; the reducing power of P. haitanensis polysaccharide was only 0.12 at a concentration of 1.84 mg/mL (18).

CONCLUSIONS

The sulfated polysaccharide isolated from P. pseudoceranoides as characterized by FT-IR showed the characteristic bands of carrageenans. The crude polysaccharide, with a low phenolic content, was more active as free radical scavenger and reducing agent than the commercial-grade carrageenan. The crude polysaccharide isolated from P. pseudoceranoides might be used, in its present form or after purification, as an ingredient and potential antioxidant agent in the food and pharmaceutical industries.

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