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Evaluation of the antioxidant activity of enzymatically-hydrolyzed sulfated polysaccharides extracted from red algae; *Pterocladia capillacea*

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ABSTRACT

Sulfated polysaccharides (SPs) were extracted from red algae, *Pterocladia capillacea* and the produced fractions were hydrolyzed using different enzymes. 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) and peroxide scavenging activities as well as phenolic content of the extracted fractions and their hydrolysates were determined. Viscozyme hydrolysate exhibited the highest phenolic content and a DPPH scavenging activity of about 92% with more than 50% increase over its mother fraction. It also showed a high antibacterial activity relative to commercial antibiotics. Phenolic content determination and HPLC analysis suggested that the high antioxidant activity of viscozyme hydrolysate could be owed to its short-length chain and its high content of phenolic compounds as well as the predominance of mannose and galactose sugars in the polysaccharide chain.

1. Introduction

In recent decades, sulfated polysaccharides (SPs) isolated from various sources (animals, plants and microorganisms) have found a wide range of applications in food, cosmetic and pharmaceutical industries because of their broad spectrum of biological activities and relatively low toxicity. In marine algae, SPs are found in varying amounts in three major divisions namely, *Rhodophyta* (red algae), *Phaeophyta* (brown algae) and *Chlorophyta* (green algae). They exhibit various beneficial biological activities such as anti-coagulation, antiviral, anti-tumor, anti-inflammation and antioxidation.

There has been recently a growing interest in natural antioxidants as alternatives to synthetic ones due to safety and cytotoxicity issues associated with the latter (Duan et al., 2006, Wang et al., 2010 & Wijesekara et al., 2010). Antioxidants are vital in protecting the body against cell and tissue damage caused by reactive oxygen species such as superoxide radical ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), peroxyl radical ($ROO^{\cdot}$) and nitric oxide radical ($NO^{\cdot}$). Several antioxidants have been extracted from different terrestrial and marine sources such as vegetables, leaves, herbs, seeds, crops and seaweeds or algae (Lim et al., 2002, Shon et al., 2003, Takamatsu et al., 2003, Huang & Wang, 2004, & Wijesekara et al., 2010).

SPs in marine algae are potential rich sources of antioxidants. SPs were extracted from three different algal samples (*Ulva lactuca*, *Sargassum crassifolium* and *Digenea simplex*) that were collected from along Jeddah Corniche. Polysaccharides from brown algae showed higher antioxidant activity relative to those from red and green algae (Al-Amoudi, Mutawie, Patel, & Blunden, 2006). In addition, SPs extracted from five different algal species revealed strong scavenging effects for superoxide and hydroxyl radicals (Zhang et al., 2010). Furthermore, water soluble crude polysaccharides extracted from the brown algae *Turbinaria ornata* exhibited high antioxidant and anti-inflammatory activity (Ananthi et al., 2010).

SPs extracted from red algae have manifested good antioxidant activities. Polysaccharides containing mainly galactose and xylose

Abbreviations: SPs, Sulfated polysaccharides; S1(M), High molecular weight polysaccharide mother fraction; S2(M), Low molecular weight polysaccharide mother fraction; S1(Visco), Viscozyme hydrolysate of S1; S2(Visco), Viscozyme hydrolysate of S2; $S1(Visco)_{col}$, Viscozyme hydrolysate of S1 after ion exchange purification; S1(Glu), Gluconase hydrolysate of S1; S2(Glu), Gluconase hydrolysate of S2; $S1(Glu)_{col}$, Gluconase hydrolysate of S1 after ion exchange purification; S1(Gal), Galactosidase hydrolysate of S1; S2(Gal), Galactosidase hydrolysate of S2.

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were isolated from the Chinese *Corallina officinalis* seaweeds through anion exchange column chromatography. It was found that SPs fractions have considerable antioxidant properties that are superior to those of the de-sulfated fractions (Yang, Liu, Wu, Chen, & Wang, 2011). Potent antioxidant activities were also demonstrated by the crude and fraction extracts derived from the Chinese red algae *Polysiphonia urceolata*, and significant association between antioxidant potency, total phenolic content and reducing power was found (Duan et al., 2006). In addition, the complex polysaccharides sulfated galactans and carrageenans, were isolated from the Lebanese red algae *Pterocladia* and a more pronounced antioxidant effect was shown for the sulfated galactans (Sebaaly et al., 2012). SPs composed of 65.4% galactose were extracted from the red seaweed *Gracilaria birdie* and moderate inhibitory effect was observed. In general, reported studies suggest that marine food-derived SPs promise to be effective, non-toxic potential antioxidants (Ngo, Wijesekara, Vo, Ta, & Kim, 2011).

New methods for extraction of SPs have been investigated for the purpose of enhancing their antioxidant activity. Supercritical CO_2 and ultrasonic-aided extraction were employed to obtain SP fractions from the brown algae *Sargassum pallidum*. These fractions were further purified onto a DEAE Cellulose-52 anion-exchange column and were tested for their antioxidant activity using DPPH method. The purified fractions showed lower antioxidant activities as compared to the crude ones and their activities increased at high concentrations (Ye, Wang, Zhou, Liu, & Zeng, 2008). Enzymatic hydrolysis of seven species of brown seaweeds was performed using five carbohydrate degrading enzymes (Viscozyme, Celluclast, AMG, Termamyl and Ultraflo). The antioxidant activities of the enzymatic hydrolysates were evaluated using two assays; DPPH and lipid peroxidation. Even though different results were obtained with different assays, all hydrolysates showed potential antioxidation (Heo, Park, Lee, & Jeon, 2003). Enzyme-assisted extraction was also effective in enhancing the recovery of polyphenols and other lipophilic antioxidant compounds from the red algae *Palmaria palmata* (Wang et al., 2010).

The present paper evaluates the antioxidant capacity and antibacterial effect of sulfated polysaccharide fractions extracted from the red algal species *Pterocladia capillacea* collected from rocky bay of Abu-Qir along the Mediterranean coast, North of Egypt. Fractions of high molecular weight S1(M) and low molecular weight S2(M) were extracted from the algae. They were then subjected to enzymatic hydrolysis using different carbohydrases such as, viscozyme, gluconase, galactosidase enzymes to produce S1(Glu), S2(Glu), S1(Visco), S2(Visco), S1(Gal) and S2(Gal) fractions. Selected S1(Visco) and S1(Glu) fractions were further purified onto an anion-exchange column to obtain $S1(Visco)_{col}$ and $S1(Glu)_{col}$. The extracted fractions and their hyrolysates were tested for their scavenging activity toward DPPH and peroxide radicals. In addition, their total phenolic content and their antibacterial activity were determined. Furthermore, selected fractions were analyzed for their sugar content and their FTIR spectra. It has to be noted that in all reported literature, enzymatic hydrolysis was performed on the algae itself during the extraction process. In this work, however, the crude mother fractions were enzymatically hydrolyzed to reduce their chain length and potentially enhance their antioxidant properties. This approach, to the best of the authors' knowledge, has not been adopted elsewhere.

2. Materials and methods

2.1. Materials

The marine red algae *Pterocladia capillacea* used for this study were freshly collected during the summer season from the rocky bay of Abu-Qir at a depth of 3.5 m along the Mediterranean coast, North of Egypt. It was then washed with fresh water, air dried, ground into powder and stored before extraction.

The three carbohydrate degrading enzymes including viscozyme L (a multi-enzyme complex containing arabanase, cellulase, β-glucanase, hemicellulase and xylanase), β-glucanase from *Trichoderma longibrachiatum* and β-galactosidase from *Kluyveromyces lactis* were purchased from Sigma–Aldrich (St. Louis, Missouri USA).

1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH), gallic acid GA, ascorbic acid AscA, bovine albumin and Folin- Ciocalteu's phenol reagent were all purchased from Sigma–Aldrich (USA), while DEAE (Diethyl amino ethyl cellulose) was purchased from Whatman International Ltd. (England). The total antioxidant capacity kit used for determination of H_2O_2 scavenging activity was purchased from Biodiagnostics for Research Reagents (Egypt).

2.2. Extraction of crude sulfated polysaccharide fractions

SPs fractions S1 and S2 were extracted from the red seaweed *Pterocladia capillacea*. Twenty grams of the dried, ground seaweeds were suspended in 400 mL of distilled water, adjusted to pH 4 using 1N HCl solution and then shaken for 48 h at room temperature using a rotatory shaker (VWR advanced digital shaker, 5000 ADV). The extracts were filtered through cheese cloth and neutralized to pH 7 with a saturated solution of Na_2CO_3, then dialyzed against distilled water in dialysis bags for 48h. The dialyzed solution was then centrifuged (Heraeus-Christ, GMBH336 Osteode Ma Harz No.39189) at 12000 × g for 15 min at −10 °C, and the supernatant containing the extracted polysaccharide was then treated with 4 volumes of absolute ethanol. The precipitated product which is a high molecular weight fraction S1(M), was collected after centrifugation. Both S1(M) and the supernatant which is a low molecular weight fraction S2(M) were then lyophilized, weighed and stored.

2.3. Determination of total carbohydrate, protein and sulfate content

Total carbohydrate content of the polysaccharide fractions S1(M) and S2(M) was determined by the phenol-sulfuric method (Dubois, Gillis, Hamilton, Rebers, & Smith, 1956) using galactose as standard. The protein content was determined adopting the method of Lowery, Rosenbrough, Farr, & Randall, (1952) and using bovine albumin as standard. Estimation of the sulfate content (Larsen, Haug, & Painter, 1966) from the polysaccharide molecule was determined after cleavage of sulfate ester groups using the barium chloride turbidimeteric assay (Garrido, 1964) with minor modifications. The amount of sulfate was calculated from anhydrous sodium sulfate standard curve which relates the optical density to sulfate concentration.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was performed for the algal polysaccharide fractions S1(M) and S2(M), under the optimal conditions pertinent to each employed enzyme. About 0.5–1 g of S1 and S2 substrates were dissolved in 10–15 mL of 0.1M sodium acetate buffer at pH 5.4 for both β-glucanase and β-galactosidase enzymes, and at pH 4.5 for viscozyme L enzyme. To each of the prepared S1(M) and S2(M), either 20 μL (60 units) of β-galactosidase, 225 μL (30 units) of viscozyme L, or 0.016 g (50 units) of β-glucanase were added. All reaction mixtures were then incubated in a water bath rotatory shaker (Precision Scientific, Model 25) at 37 °C and 50 rpm. The optimum time allowed for the enzymatic hydrolysis reaction