Towards optimizing the conventional and ultrasonic-assisted extraction of sulfated polysaccharides from marine algae

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Abstract. Recently, there has been a growing interest in sulfated polysaccharides extracted from various natural sources due to their versatile biological activities that find great use in biomedical and industrial applications. In the present study, sulfated polysaccharides were extracted from green algae, Ulva lactuca and red algae, Jania rubens collected from Alexandria city coast, Egypt. Conventional and ultrasonic-assisted extraction methods were investigated under different operating parameters and the optimum conditions for extraction were obtained based on the yield. Algal extracts with optimum SPs yields were then tested for their phenolic and sugar contents, as well as their 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) antioxidant activities. Optimum yields of both green and red algae were obtained via extraction under ultrasonic conditions for 4h. Higher yields were produced from the green algae as compared to the red ones. However, red algal extracts showed relatively higher antioxidant activities, possibly due to their higher mannose content.

Keywords: Ulva lactuca, Jania rubens, marine algae, antioxidant activity, phenolic content

1. Introduction

Marine algae are rich in various potentially bioactive compounds, a major group of which are sulfated polysaccharides (SPs) which are anionic carbohydrate biopolymers consisting of repeated monosaccharide units linked by glycosidic bonds. Algal SPs have drawn great attention in the biomedical as well as food and pharmaceutical industrial areas because of their broad spectrum of biological activities including anticoagulation, antiviral, anti-tumor, anti-inflammation and antioxidation. The potential of marine algae, being green (Chlorophyceae), red (Rhodophyceae) or brown (Phaeophyceae), as a source of bioactive compounds has not yet been fully explored especially with the increase in the health awareness and the advancement in functional foods and pharmaceutical industries [1–6]. Antioxidant activity, in particular, has become a subject of intensive investigations due to the growing demand to develop natural anti-aging and anti-carcinogenic compounds with health
benefits. However, the search for natural antioxidants as alternatives to synthetic ones is of great interest due to safety and cytotoxicity issues associated with the latter [7–10].

Aqueous extraction of the green algae *Ulva lactuca* produced SPs that were mainly constituting L-rhamnose, D-xylose and D-glucose in the molar proportion of 4.2: 1.3: 1.0, in addition to traces of D-mannose, 24% of D-glucuronic acid and 19% of ester sulfate [11]. Furthermore, acid-hydrolysis revealed the presence of the following monosaccharide components: arabinose, xylose, rhamnose, galactose, mannose and glucose in the ratio of 1: 1: 9: 5: 2.5: 16, respectively. As for the red sea weeds, sulfated galactans are its most abundant constituents. The main acidic polysaccharides from the red seaweed *Jania rubens* share the general characteristics of corallinans (agar-like xylogalactans) [12].

The yields and biological activities of SPs produced from algae depend not only on the type of algae, but on the underlying extraction method and the relevant operating conditions. In this regard, a number of conventional techniques for extraction have been reported. The most commonly used ones were employing water [13–19] and other solvents [20–32]. This technique, however, suffers from being time-consuming and hence energy intensive and sometimes not eco-friendly especially when extraction involves the excessive use of organic solvents. Therefore, several modern techniques were proposed in an attempt to overcome these drawbacks. One example of these techniques is the enzyme-assisted extraction (EAE) which has been reported to increase yields and reduce solvent consumption [33–35]. However, the method limitations include high cost of enzymes and unavailability of substrate-specific enzymes. The applications of other relatively recent techniques such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) were also very limited due to their high energy requirements and subsequently high cost [36]. Furthermore, the high temperature involved in microwave-assisted extraction (MAE) and SFE could be an issue for algal species that are thermolabile. Thus with the increasing energy costs and the drive to reduce greenhouse gas emissions, food and plant-based chemical industries are thriving to come up with new technologies that provide shorter extraction times and reduced organic solvent consumption which, in turn, would save energy and cost while increasing quality and safety. This has led to the consideration of the use of enhanced and efficient extraction techniques such as ultrasound-assisted extraction (UAE).

Advances in UAE have resulted in a number of innovative techniques such as ultrasound-assisted Soxhlet apparatus, ultrasound-assisted Clevenger distillation, continuous ultrasound-assisted extraction and combination of ultrasound with other techniques such as microwave extrusion and supercritical fluid extraction [37]. In recent years, UAE of antioxidants has been widely applied due to its high efficiency, reproducibility and ease of operation. The force of ultrasound can produce heat and disrupt cell walls, thus facilitating the release of bioactive compounds. However, it was claimed that although this promising technique has presented numerous advantages over conventional techniques, yet evidences revealed that it caused degradation of some compounds and modifications in the physicochemical properties of products [38]. Very few studies investigated the use of modern techniques such as UAE [39] and MAE [40] in order to increase the yield and reduce the extraction time.

In the current study, conventional and ultrasonic-assisted extraction methods will be investigated and comparatively assessed under different operating conditions and using red and green algae in order to select a viable efficient extraction method that produces optimum SPs yields with potent antioxidant activities. This comparative screening approach for scouting the optimum extraction conditions should be of great benefit in the design and development of efficient algal extraction processes for food and pharmaceutical industries.

### 2. Materials and Methods

#### 2.1 Collection of Seaweed Samples

*Ulva lactuca* and *Jania rubens* were collected from Alexandria city coast (N 31° 19’ E030° 03’) and were immediately brought to the laboratory in sterile plastic bags containing seawater in order to
prevent evaporation. The algal samples were first cleaned well with the seawater until unwanted impurities, adhering sand particles and epiphytes, pebbles, and shells were removed. They were then washed thoroughly with tap water followed by deionized water to remove the surface salty materials. Afterwards, algae were air dried for one week then finely ground in an electric mixer. The powdered samples were subsequently stored in the refrigerator for future use.

2.2 Extraction of Seaweeds

2.2.1 Conventional extraction. Ten grams of each of the prepared powdered seaweed samples were suspended in 200 mL of distilled water. The suspension was agitated on a magnetic stirrer and its pH was adjusted to 5.5 using 1N HCl. It was then shaken either for 24 h at room temperature (RT) or for 2 h at 80 °C, and subsequently filtered through cheese cloth. The filtrate was dialyzed overnight in a dialysis bag, after which it was centrifuged (Heraeus-Christ, GMBH336 Ostode Ma Harz No.39189) for 10 min at -10 °C and 5500-6000 rpm. The supernatant was collected and added to ethanol in the ratio of 1:3.5 (v/v), respectively. After further centrifugation under the aforementioned conditions, the supernatant (SP fraction) was collected.

2.2.2 Ultrasonic-assisted extraction. Ultrasonic-assisted experiments were conducted for different periods of time in the ultrasonicator (VWR® sonicator model 150HT) at 50 and 60°C for Ulva lactuca and at RT for Jania rubens. Samples were prepared and SP fractions were extracted in the same manner as in the conventional extraction.

2.3 Characterization and chemical analysis of the algal extracts

2.3.1 Chemical analysis. Total carbohydrate content of the polysaccharide extracts was determined by the phenol-sulfuric method [41] using galactose as standard, while the protein content was determined adopting the method of Lowery [42] and using bovine albumin as standard. Total phenolic content of polysaccharide fractions was determined using the Folin-Ciocalteu reagent according to the method described by Singleton & Rossi [43], with minor modifications.

2.3.2 IR Spectroscopy analysis. The IR spectra of the extracts were determined using a Fourier transform IR spectrophotometer (FT-IR) (TGA/FT-IR Nicolet 380). Samples were mixed with KBr powder, ground and then pressed into 1-mm pellets for measurement in the frequency range of 4000-500 cm⁻¹.

2.3.3 Testing antioxidant activity of the algal extracts. The free radical scavenging activity of the investigated algal extracts was measured using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay following the method of Blois [44] with minor modifications.

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\text{Scavenging rate (\%)} = \frac{A_0 - (A_2 - A_1)}{A_0} \times 100 \tag{1}
\]

2.3.4 HPLC analysis of sugar content. Before analysis, samples were filtered through a 0.45 µm membrane. Sugar content in the filtrate was analyzed using HPLC, Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A, Shimadzu detector, LC-16ADVP binary pump, DCou-14 A degasser and ShodexPL Hi-PlexPb column (Sc 1011 No. H706081), Guard column Sc-LcShodex, and heater set at 80 °C. Separation and quantitation were carried out on an amino-bonded column with a mobile phase of (80:20) H₂O to acetonitrile at a flow rate of 1mL/min.

3. Results and discussion

3.1 Yields of algal extracts

Extraction of SPs from the two employed algal species was performed using both ultrasonic-assisted and conventional methods under different operating conditions. Obtained yields and antioxidant activities were then compared in order to obtain the optimum conditions of extraction pertaining to each algal species. The UAE of SPs was first investigated for the green algae at 50 and 60 °C and for the red one at RT. These temperatures were chosen because the algae were sensitive and susceptible to degradation at higher temperatures [45]. The effect of extraction time on the percentage yields of SPs
(by weight) extracted from *Ulva lactuca* using the UAE method at 50 and 60 °C and different incubation periods is depicted in Figure 1.

![Figure 1](image1.png)

**Figure 1.** Time courses for the % yield of *Ulva lactuca* SPs at different temperatures.

As can be seen from the figure, the yield at both temperatures increases with prolonging the incubation time until it reaches a plateau where it remains constant. However at the higher temperature, the yield takes longer time to reach the plateau. Furthermore, yields obtained at 60 °C dramatically exceed those obtained at 50 °C which indicates that temperature is favorable for the extraction of SPs from *Ulva lactuca*. The maximum % yield obtained at 60 °C is more than ten times higher than its counterpart obtained at the lower temperature. In view of these results, the yields of *Ulva lactuca* at 60 °C and different extraction times were compared to those of *Jania rubens* at RT as shown in Figure 2. Clearly, the yields obtained from the green algae are more than 12 fold higher than those of the red algae. Maximum yields for *Ulva lactuca* and *Jania rubens* were obtained at 4 and 2 h, respectively. From the above, it can thus be deduced that the optimum yields for UAE of SPs from green algae were obtained at 60 °C and 4h extraction time, while those from red algae were obtained at RT and 2h.

Yields of SPs from ultrasonic-assisted extraction were then compared to their counterparts obtained under conventional conditions as depicted in Figure 3. For the red algae, conventional extraction was conducted at RT for 24 h; while for the green algae, two sets of operating conditions were employed. Extraction conditions for the first set are RT and 24 h, while those for the second set are 80 °C and 2h.

The figure shows that the yields of *Ulva lactuca* are much higher than those of *Jania rubens*. For the green algae, the highest yield is obtained via conventional extraction at RT and 24 h. This yield exceeds those obtained from the other adopted conventional and ultrasonic-assisted extractions by about 1.4 and 1.5 fold, respectively. In addition, the yields obtained via these other adopted methods are comparable. However, the difference between the highest yield obtained conventionally at room temperature and 24 h and the lowest yield obtained ultrasonically at 60 °C and 4 h is slightly above 2%. This finding therefore does not justify performing the extraction conventionally for 24 h to gain only a 2% increase in yield. Thus among the adopted operating conditions, UAE at 60 °C and 4 h would offer a good compromise for saving both time and energy. As for *Jania rubens*, conventional and ultrasonic-assisted extraction gave comparable yields. Again for the purpose of saving time, UAE would be more favorable than the conventional extraction.

### 3.2 DPPH Antioxidant activities of the SP fractions

The DPPH antioxidant activities were then measured for the algal extracts obtained under conditions that gave maximum yields. Figures 4A and B show the % DPPH scavenging activity as a function of concentration for the polysaccharide fractions extracted respectively from *Ulva lactuca* and *Jania rubens*. The DPPH scavenging activity is depicted in Figure 4A and B.
rubens at different extraction conditions. The inset of the figure shows the % DPPH scavenging activity for ascorbic acid which was used as a standard reference. As clear from the figures, the scavenging activity increases linearly with time. It increases by about 5-10% for Ulva lactuca (Figure 4A) and by about 10-20% for Jania rubens (Figure 4B) as the concentration increases by ten folds from 100 to 1000 μg/mL.

As shown earlier, maximum scavenging activities for all SPs extracts under the different employed extraction conditions were obtained at a concentration of 1000 μg/mL. Values thereof are presented in Figure 5.

Figure 3. Yields for the green and red algal SPs obtained under optimum conventional and ultrasonic conditions. Ultrasonic temperature employed was 60°C for Ulva lactuca and RT for Jania rubens.

Figure 4. % DPPH scavenging activity as a function of concentration for the polysaccharide fractions extracted from Ulva lactuca (A) and Jania rubens (B) at optimum extraction conditions. The inset of the figure represents the % DPPH scavenging activity for ascorbic acid.

As clear from the figure, both the conventional and ultrasonic-assisted extraction methods yielded comparable antioxidant activities for the red algal species. The figure also shows that UAE of SPs from Ulva lactuca provided either slightly higher or comparable % scavenging activities to those obtained via the conventional method.
3.3 Protein, carbohydrate and phenolic contents

Table 1 compiles the values of the protein, carbohydrate and phenolic contents of the algal extracts obtained at the optimum extraction conditions deduced earlier. The table also shows the corresponding percent yields and DPPH scavenging activity. For each algal extract, the higher antioxidant activities correspond to higher phenolic and protein contents probably due to the presence of hydroxyl and amide functional groups, respectively. The direct proportionality between phenolic content and scavenging activity was confirmed in previous literature [46,47]. Furthermore, the carbohydrate contents for both red and algal extracts obtained from the conventional extraction are higher than their counterparts obtained from UAE, probably due to the breaking of the carbohydrate chains by means of the ultrasonic force.

3.4 FTIR analysis

FTIR spectra of SPs extracted from Ulva lactuca under the previously selected optimum conditions are shown in Figure 6A. The main bands appearing in the figure and their corresponding functional groups are compiled in Table 2. These bands are characteristic of SPs functional groups [49, 50].

On the other hand, infrared spectra for the SPs extracted from Jania rubens are illustrated in Figure 6B. Bands at around 3400-3500 cm\(^{-1}\) are ascribed to vibration of O-H, while those at around 2925.8-2927.8 cm\(^{-1}\) correspond to the stretching vibration of the C-H group [6,48,49]. In addition, the band at 875.3 cm\(^{-1}\) could be attributed to galactose sulfate groups [47]. It can be observed that all these bands were present in the SPs extracted from Ulva lactuca. In addition, the ester sulfate band is not shown, however the presence of sulfate is confirmed by the appearance of the bands corresponding to the galactose sulfate groups.

The type of sugars present in the algal extracts and their content could affect their antioxidant activity. The molar composition of some of the sugars present in the different investigated extracts was determined using HPLC analysis and the resulting sugar contents are presented in Table 3. For Ulva lactuca extracts, it is clear that, among the tested sugars, glucose and galactose are the most dominant sugars in the fractions that were conventionally extracted at RT. However, glucuronic acid was the most dominant sugar in the fractions extracted conventionally at 80 °C or ultrasonically at 60 °C. High temperatures could have favored the oxidation of glucose and galactose sugars to glucuronic acid. The
higher antioxidant activities exhibited for the fractions extracted at high temperatures relative to the fraction extracted at RT could be attributed to the high glucuronic acid content. As for *Jania rubens* extracts, glucose and galactose are the dominant sugars for the ultrasonically and conventionally extracted fractions, respectively. In previous work, it was reported that conventionally extracted fractions of *Jania rubens* primarily constitute galactose which is confirmed in this work [48]. The dominant glucose content for the ultrasonically extracted fraction could be a result of the ultrasonic-aided conversion of galactose to glucose. Although glucose and galactose could be partially responsible for the antioxidant activity of *Jania rubens* extracts, the higher antioxidant activity of these extracts relative to those of the green algae could be owed to their relatively higher mannose content. We showed in previous work that mannose was responsible for the high antioxidant activity of the viscozyme hydrolysate of the *Pterocladia capillacea* extract [47].

Table 1. Protein, carbohydrate and total phenolic contents for the algal extracts obtained under optimum extraction conditions.

| Algal extract          | %Yield | %Protein | %Carbohydrates | Total phenolic content (mgGA/g) | %DPPH activity |
|------------------------|--------|----------|----------------|------------------------------|----------------|
| Ulva.CV (RT, 24h)      | 8.00±0.82 | 4.0±0.9 | 34.5±3.1       | 36.2±2.9                     | 50-52          |
| Ulva.CV (80°C, 2h)     | 5.80±0.59 | 7.7±0.4 | 26.8±2.7       | 71.3±6.2                     | 53-61          |
| Ulva.US (60°C, 4h)     | 5.30±0.25 | 5.8±0.7 | 7.7±0.1        | 60.6±7.9                     | 55-60          |
| Jania.US (RT, 4h)      | 0.36±0.04 | 9.4±1.7 | 2.6±0.1        | 132.6±2.5                    | 55-68          |
| Jania.CV (RT, 24h)     | 0.25±0.02 | 19.6±3.7 | 8.6±0.3       | 136.1±0.7                    | 64-71          |

Values are expressed as mean±SD (n=3)

Figure 6. FTIR spectra of the extracts of (A) *Ulva lactuca* under conventional conditions of RT and 24 h (red), 80°C and 2 h (violet) and ultrasonic conditions of 60°C and 4 h (blue), (B) *Jania rubens* under conventional conditions of RT and 24h (blue), and ultrasonic conditions of RT and 4 h (red).
Table 2. FTIR bands pertaining to Figure 6 and their corresponding functional groups

| Wavelength, cm\(^{-1}\) | functional groups          |
|------------------------|-----------------------------|
| 3500-3400              | OH groups                   |
| 1433.4-1424.2          | Uronic acid and phenolic groups |
| 1260.1-1258.1          | Ester Sulfate group         |
| 1088.3-112.9           | Acidic polysaccharide       |
| 963.6-927.6            | Glycosidic linkage          |
| 850.3-850.2            | Galactose sulfate group     |

Table 3. Sugar content of the algal extracts, expressed as molar composition (% mol)

|                  | Glucuronic acid | Mannose | Galactose | Glucose | Xylose |
|------------------|-----------------|---------|-----------|---------|--------|
| Ulva. CV (RT,24h)| 8.04            | 0.00    | 29.02     | 62.95   | 0.00   |
| Ulva. CV (80C, 2h)| 94.83           | 0.36    | 0.51      | 1.24    | 3.06   |
| Ulva. US (60C, 4h)| 89.92           | 0.00    | 3.53      | 6.55    | 0.00   |
| Jania. US (RT, 4h)| 0.16            | 3.51    | 0.10      | 94.04   | 2.14   |
| Jania. CV (RT, 24h)| 0.27            | 2.19    | 94.02     | 0.80    | 2.72   |

4. Conclusion
This study investigates the conventional and ultrasonic-assisted extraction of sulfated polysaccharides from green *Ulva lactuca* and red *Jania rubens* algal species. For each method, extraction was conducted under different operating conditions of time and temperature in order to select the conditions that produce optimal yields of SPs. The extracts (SPs fractions) obtained under these optimum conditions were analyzed for their antioxidant activities as well as their carbohydrates, proteins and phenolic contents. It was found that the highest yields for *Ulva lactuca* were achieved under conventional extraction conditions of RT and 24h. Under ultrasonic-assisted conditions of 60°C and 4h, slightly lower yields were obtained. However for the sake of saving time and energy, the optimum extraction conditions were selected to be those of ultrasonic-assisted extraction. As for *Jania rubens*, SPs yields from conventional extraction at RT and 24h were comparable to those of ultrasonic-assisted extraction at RT and 4h, hence the latter was the optimum choice. DPPH antioxidant activity results showed that the scavenging activities for different concentrations of *Ulva lactuca* and *Jania rubens* extracts range between 55-60% and 55-68%, respectively. The relatively higher antioxidant activities exhibited by the red algae could be attributed to its high mannose content. Furthermore, the green algae fractions extracted at relatively higher temperatures showed better antioxidant activities owing to their high glucuronic content. Relevant phenolic content measurements indicated direct correlation between phenolic content and antioxidant activity. In addition, FTIR analysis confirmed the presence of OH, uronic acid and sulfate groups as well as glycosidic linkages typical of sulfated polysaccharide chains.

Acknowledgements
Thanks are due to the Chemistry Department at the American University in Cairo for funding this project
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