# Influence of Extraction Methods on the Chemical Composition and Antioxidant Activity of Polysaccharide Extracts from Discarded Sea Grape (*Caulerpa lentillifera*)

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## ABSTRACT

Sea grape (*Caulerpa lentillifera*) is a green marine macroalga which contains polysaccharides as major components of the cell wall. After harvesting and grading for sale, approximately 60-70 % of sea grapes are considered to have defects and are unsaleable. However, their nutritional values are not affected and thus they should be utilized for other purposes such as a source of natural products. The aims of this study were to investigate the impacts of pH of the extraction solvents and extraction time on the polysaccharide yield and chemical composition of *C. lentillifera* extracts, and to investigate their antioxidant potentials. Hot water extraction (pH 6) at 90 °C for 20 min was practical and the most cost-effective method compared to acidic or alkaline conditions and compared to longer extraction times. The polysaccharide extracts contained 47 % total carbohydrate, 31 % minerals, and 19 % sulfate by dry weight (dw). The major monosaccharides (% mol) were galactose (37 %), mannose (32 %), xylose (19 %) and glucose (11 %). The high-molecular weight (Mw) polysaccharide extract was determined to be approximately 3,830 kDa relative to dextran standard. Acid hydrolysis with 1 M hydrochloric acid (HCl) at 70 °C for 120 min was performed on the extract to obtain the low-Mw polysaccharide extract (~21 kDa). The antioxidant activities of the low-Mw polysaccharide extract were ten-fold higher than the high-Mw polysaccharide extract. Low-Mw polysaccharide extract had the half-maximal inhibition concentration ($IC_{50}$) values of 0.08 mg·mL$^{-1}$ for DPPH and 1.07 mg·mL$^{-1}$ for ABTS, which was lower than the high-Mw polysaccharide extract (0.9 and 10.8 mg·mL$^{-1}$). Therefore, the extracted polysaccharide with low-Mw has high potential for use as an antioxidant ingredient in the food industry.

**Keywords:** Components, Defect, Extraction, Fibers, Green seaweed, Scavenging activities

## INTRODUCTION

*Caulerpa lentillifera* (sea grape) is an edible green seaweed in the phylum Chlorophyta that has potential use as a healthy food item or as a source of highly nutritional ingredients in other food products (Sun *et al*., 2018). They contain high amounts of carbohydrates (38.7 % dw), total dietary fiber (33.0 % dw) including 17.2 % dw of soluble fiber and ash (37.2 % dw) including 1.1 % dw of potassium, 1.9 % dw of calcium and 1.0 % dw of magnesium, followed by protein (10.4 % dw) and some other bioactive components such as phenolic compounds and pigments (Matanjun *et al*., 2008). This species grows well when cultivated in open ponds or lagoons, and the domestic Thai market demands approximately 1 tonne per month (Chaiklahan *et al*., 2020). The value of *C. lentillifera*

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produced annually in Thailand is approximately 522,000 USD·ha$^{-1}$ (Phetchaburi Coastal Aquaculture Research and Development Center, 2018). Commonly, 30-40 % of *C. lentillifera* biomass after harvesting and grading is considered food grade, with a selling price of 9-21 USD·kg$^{-1}$ (in 2021). The remaining portion (60-70 %) is discarded and processed into animal feed due to undesirable external characteristics, such as yellow-brown color or short and withered branches. However, nutritional values of these discarded sea grapes are similar to the food-grade types (Chaiklahan *et al*., 2020).

A growing interest in finding phytochemicals like polysaccharides and polyphenols, which have been proven to be effective antioxidants and an alternative to synthetic substances, has fostered research on screening of new antioxidants from natural sources, including seaweed. Green seaweeds are a rich source of antioxidants; for example, sulfated polysaccharides from *Ulva intestinalis* (3 mg·mL$^{-1}$) conferred 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of 56-60 % (Peasura *et al*., 2015). The aqueous extract from green seaweed *Enteromorpha prolifera* (1.0 mg·mL$^{-1}$) showed the highest DPPH activity at 46 % (Cho *et al*., 2011). Apart from variation due to species and extraction process, antioxidant activity also varies with molecular weight (Mw) of the polysaccharide, whereby Zhong *et al*. (2019) reported the low-Mw polysaccharides (54.7 kDa) from green seaweed *Ulva fasciata* had better antioxidant activity than high-Mw polysaccharide (262.7 kDa). In recent years, there have been a few reports on biological potentials of *C. lentillifera*, including antioxidant, anticoagulant, immunostimulatory, anticancer, anti-diabetic, hypoglycemic, and lipogenesis inhibition activities (Chen *et al*., 2019; Osotprasit *et al*., 2021); however, no one has studied these potentials in discarded *C. lentillifera* specifically.

Despite the potential use of seaweeds as a source of natural products, seaweed cell walls are major obstacles to successfully extracting the intracellular bioactive compounds due to their structural complexity and rigidity (Charoensiddhi *et al*., 2014). Previous studies reported that hot water extraction is commonly used for polysaccharide extraction from green algae and that the extraction time is generally considered to greatly affect the yield of polysaccharides. The polysaccharide yield of green seaweed *C. lentillifera* derived from hot water extraction at 90 °C significantly increased ($p<0.05$) with longer extraction time (e.g., ~3.5 % dw for 30 min extraction time vs. ~5 % dw for 120 min extraction time) (Chaiklahan *et al*., 2020). However, the main disadvantages of hot water extraction are the high extraction temperature and long extraction time (Chi *et al*., 2018). Therefore, different extraction methods, particularly using different pH of the extract solvents, have been developed to enhance the extraction efficiency of the polysaccharides. A few studies have demonstrated that pH influences certain components and properties of extracted polysaccharides from seaweeds (Yaich *et al*., 2017). In particular, Chi *et al*. (2018) demonstrated that acid extraction of polysaccharides from *E. prolifera* resulted in the highest yield (24.7 %) and greater biological activity compared to water and alkali extraction. However, there are no reports suggesting the influence of extraction time and pH of extract solvents on the polysaccharides of discarded *C. lentillifera*. Therefore, the aims of this study were to investigate the impact of the extraction processes on yield and chemical composition of polysaccharide extracts from discarded *C. lentillifera* and determine their antioxidant scavenging activities between high- and low-Mw polysaccharides. The findings can be used to increase the value of the *C. lentillifera* that is unsaleable or used for very low-value products, and instead expand its application as a source of natural antioxidant polysaccharides.

## MATERIALS AND METHODS

### *Material*

*Caulerpa lentillifera* was harvested from a sea grape farm in Phetchaburi Province, Thailand. Sea grapes with yellow-brown color, or short and withered branches were collected as defect grade and were used as the raw material in this study. The sea grapes were rinsed with tap water and dried at 60 °C for 8 h. Dried *C. lentillifera* was ground with a hammer mill (Hosokawa Micron, Japan) and sieved through 0.2 mm mesh. The ground powder was kept in vacuum bags and placed in a desiccator

until further use. All chemical reagents used in this study were of analytical grade (Sigma-Aldrich, USA).

*Composition analysis of Caulerpa lentillifera*

Moisture, protein (N-Kjeldahl ×6.25), fat (Soxhlet extraction), ash (ignition at 550 °C), crude fiber and carbohydrate contents of the dried and ground seaweed were analyzed according to the AOAC (2012). Total dietary fiber including soluble and insoluble fiber was also determined based on the AOAC (2010).

*Polysaccharide extraction from Caulerpa lentillifera*

To determine the effects of pH of the extraction solvents and extraction time on polysaccharide yield and chemical composition, the extraction processes were carried out at three different levels of pH: 0.007 M HCl (pH 4), distilled water (pH 6), and 0.01 M sodium hydroxide (pH 8); and three extraction times: 20, 60 and 120 min. The dried *C. lentillifera* powder and solvent at a ratio of 1:12 (w/v) were used with the extraction temperature of 90±2 °C. After the extraction, the solution was centrifuged at 13,000×g, 4 °C for 20 min to collect supernatant, then neutralized with 1 M HCl or 1 M NaOH. The supernatant was precipitated in absolute ethanol to a final concentration of 70% ethanol, and then centrifuged at 10,000×g 4 °C for 20 min to obtain the high-Mw polysaccharide-rich extracts from *C. lentillifera*. The precipitants were dried in a hot air oven at 65 °C for 6 h, ground to powder and kept at room temperature for further analysis.

*Composition analyses of polysaccharides extracted from Caulerpa lentillifera*

Total carbohydrate (% dw) was analyzed using a phenol-sulfuric acid method (Dubois *et al*., 1956) with glucose as a standard and the absorbance at 490 nm was measured. Total protein content (% dw) was analyzed by the Lowry method using bovine serum albumin (BSA) as a standard and the absorbance at 750 nm was measured (Lowry *et al*., 1951). The ash content (% dw) was determined using ignition at 550 °C (AOAC, 2012). The sulfate content (% dw) was analyzed by a barium chloride method using sodium sulfate as a standard and the absorbance at 360 nm was measured (Yu *et al*., 2017).

For the analysis of monosaccharide composition, the polysaccharides (50 mg) were hydrolyzed with 90% (w/v) formic acid (5 mL) in a hot air oven at 100 °C for 2 h. After removal of the formic acid by parallel evaporator (Buchi, Switzerland), the sample was incubated with 0.125 M sulfuric acid (12.5 mL) at 100 °C for 10 h, neutralized with barium carbonate and centrifuged at 11,000×g, 4 °C for 30 min (Horisberger, 1968). The monosaccharide profile of hydrolyzed samples was analyzed using high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD), following a modified method of Buathongjan *et al*. (2020). The system consisted of Dionex ICS-5000+chromatography system (Sunnyvale, USA) with electrochemical detector (Dionex, USA), Dionex AXP post column and CarboPac PA1 column size 250 mm×4 mm ID connected with guard column size 50 mm×4 mm ID. The sample (25 mL) was injected after equilibrating the column with 100 mM NaOH for 13 min, followed by 100 mM NaOH/17 mM NaOAc for 12 min, then type I water for 3 min at a flow rate of 1.0 mL·min$^{-1}$. An isocratic elution with type I water for 20 min was applied to separate the neutral sugars (fucose, arabinose, galactose, glucose, xylose and mannose). Throughout the column equilibration and neutral sugars analysis, the post column was continuously run with 300 mM NaOH at a flow rate of 0.5 mL ·min$^{-1}$ before being decreased to 0.01 mL·min$^{-1}$ until the end of injection. The measurement was then run for another 15 min using a 100 mM NaOH/ 100 mM acetate buffer pH 5.0 to determine the galacturonic acid content. The standards consisted of fucose, arabinose, galactose, glucose, xylose, mannose and D-galacturonic acid, and the Chromeleon software (Dionex, USA) was used to analyze the data.

In addition, the Mw profiles were carried out by high-performance gel-permeation chromatography (Waters 600E, USA) using refractive index detector with an ultrahydrogel linear column (Mw resolving range 1-20,000 kDa). The injection volume was 20 µL with flow rate of 0.6 mL·min$^{-1}$ using 0.05 M sodium bicarbonate buffer (pH 11) as a mobile phase. Dextrans from Sigma (Mw 4,400-401,000 Da) were used as standards.

*Development of low-Mw polysaccharide*

The low-Mw polysaccharide was prepared by dissolving dried *C. lentillifera* powder in DI water at a ratio of 1:12 (w/v) and extracting at 90 °C, 20 min to achieve high-Mw polysaccharides. Then, the low-Mw polysaccharide was developed by adjusting pH of the high-Mw polysaccharide extract with HCl to a final concentration of 1 M HCl, incubating in water bath at 70 °C for 120 min (modified method from Li *et al*., 2013) and adjusting the pH to 7 with 1 M NaOH. After degradation, absolute ethanol was added to the supernatant in order to obtain a final concentration of 70% ethanol (v/v), and centrifuged at 10,000×g, 4 °C for 20 min. The supernatant was discarded and the precipitant was collected, dried in a hot air oven at 65 °C for 6 h and ground to powder to obtain the low-Mw polysaccharide.

*Antioxidant activities*

*1,1-Diphenyl-2-pycrilhydrazyl (DPPH) radical scavenging activity assay*

The DPPH radical scavenging activity was measured following the method of Pachaiappan *et al*. (2018). Briefly, 100 µM of fresh DPPH solution (190 µL) in 95% ethanol was added to 0.02-10 $mg{\cdot}mL^{-1}$ of polysaccharide solution (20 µL); 0.1-0.6 $mg{\cdot}mL^{-1}$ of trolox was used as a positive control. After incubation in a dark room for 30 min, the absorbance was measured at 517 nm using microplate reader (Tecan-Infinite M200 pro, Switzerland). The radical scavenging activity was calculated as a percentage of the control using the following equation:

$$(\%)\ \text{scavenging activity} = (Abs_{control}-Abs_{sample})/Abs_{control}\times 100$$

where $Abs_{control}$ is the absorbance of the control experiment (without polysaccharide) and $Abs_{sample}$ is the absorbance of the polysaccharide.

The half-maximal inhibitory concentration ($IC_{50}$) is the concentration of the antioxidant required to scavenge 50 % of the free radical.

*2,2'Azino-bis 3-ethyl benzothiazoline-6-sulfuric acid (ABTS) radical scavenging assay*

The ABTS decolorization scavenging activity was measured according to the method of Karamac *et al*. (2018). The ABTS stock solution was prepared by dissolving 7 mM ABTS (5 mL) in distilled water and 140 mM potassium persulfate (0.3 mL) and incubating in the dark at room temperature for 12 h before use. Directly, the stock solution was diluted in phosphate-buffered saline (PBS) that contained 137 mM sodium chloride, 2.7 mM potassium chloride and 11.9 mM phosphate buffer at a ratio of 1:50 (v/v) to achieve the absorbance of 0.7±0.02 at 734 nm. Ten milligrams per milliliter (10 $mg{\cdot}mL^{-1}$) of polysaccharide was prepared and 0.1-1 $mg{\cdot}mL^{-1}$ of trolox was used as a positive control. Ten microliters (10 µL) of sample were mixed with 200 µL of ABTS reagent and incubated at 30 °C for 6 min. The absorbance at 734 nm was measured using microplate reader (Tecan-Infinite M200 pro, Switzerland). The radical scavenging activity and $IC_{50}$ of polysaccharides were calculated the same way as in the DPPH assay described above.

*Statistical analysis*

Results were expressed as the mean±SD of triplicate analyses. Statistical analysis was performed through an analysis of variance (one-way ANOVA) and differences between treatments were tested using Tukey's test with a significance level of 95 % ($p<0.05$) using IBM SPSS Statistics Version 28.0 (Thaisoftup Co., Ltd., Thailand).

## RESULTS AND DISCUSSION

*Chemical composition of dried Caulerpa lentillifera*

Discarded *Caulerpa lentillifera* used in this investigation contained approximately 34.5 % carbohydrate, including 22.3 % total dietary fiber (6.2 % soluble fiber and 16.1 % insoluble fiber) and 12.7 % crude fiber; 50.5 % ash; 14.5 % protein; and 0.5 % fat dw. This suggests that the major components of discarded seaweed powder are dietary fiber and ash, the latter of which represents the

content of minerals such as sodium, magnesium and calcium (Matanjun *et al*., 2008). These values correspond to those of Matanjun *et al*. (2008), who reported *C. lentillifera* (food-grade type) was rich in ash (37.2-46.2 % dw) and dietary fiber (25.1-39.7 % dw). Therefore, discarded *C. lentillifera* has potential for use as a source of dietary fiber.

*Extraction process of polysaccharides from Caulerpa lentillifera*

Figure 1 shows that the extraction yield of polysaccharides from *C. lentillifera* was not significantly different ($p>0.05$) among pH levels (4, 6 and 8) of the extraction solvents with 20 min extraction time (5.2-5.5 % dw). On the other hand, the extraction yield of polysaccharide at pH 4 (5.8 % dw) was significantly higher than at pH 6 (5.2 % dw) and pH 8 (5.3 % dw) with 60 min of extraction time. Also, the extraction yield of polysaccharide at pH 4 (6.1 % dw) was significantly higher than at pH 8 (5.2 % dw), but was not significantly higher than pH 6 (5.7 % dw) with 120 min of extraction time. From an economic feasibility and industrial perspective, using extraction solvent at pH 4 or pH 8 did not have much impact on the extraction efficiency compared to the hot water extraction (pH 6), as the extraction yield was improved less than 1 %. The use of very strong acid for extraction may not further improve the extraction yield of polysaccharides. In fact, Yaich *et al*. (2017) demonstrated that the use of very strong acid (pH 1.5) extraction of ulvan sulfated polysaccharide from the green seaweed *Ulva lactuca* at 90 °C likely led to the decrease in the extraction yield due to the hydrolysis of polysaccharide linkages into monosaccharides. In contrast, alkali extraction can swell the seaweed cell walls (Chi *et al*., 2018) similar to the hot water extraction. Alkali extraction has shown to be more effective in the release of insoluble polysaccharides (Peasura *et al*., 2015). Therefore, the extraction yield of soluble polysaccharide from alkali extraction may not be different from hot water extraction.

This study also focused on the effect of extraction time on yield of polysaccharides (Figure 1). Using hot water extraction (pH 6), the yield of polysaccharides was not significantly different ($p>0.05$) between the extraction times of 20 min (5.2 % dw) and 60 min (5.2 % dw) or 120 min (5.7 % dw). Therefore, extraction for 20 min is likely sufficient for complete extraction with hot water at pH 6. Chi *et al*. (2018) reported that the hot water extraction is a conventional method used to prepare soluble polysaccharides. The seaweed cell walls are swollen and broken down as a result

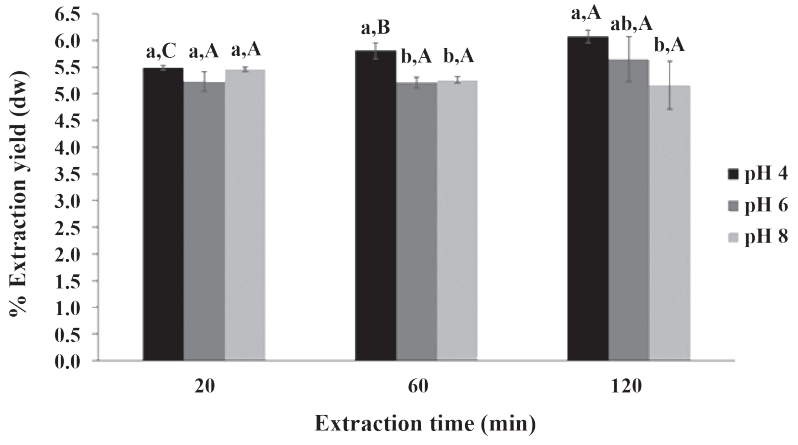


Figure 1. Effect of pH and extraction time on the extraction yield (% dw) of polysaccharide extracts from discarded *Caulerpa lentillifera*. The bar graph represents mean±SD from triplicate analyses. Different lowercase (a, b) and uppercase (A, B, C) letters denote significant ($p<0.05$) differences among treatments with different pH of extraction solvents for the same extraction time, and among different extraction times at the same pH, respectively.

of the continuous heating, leading to the release of soluble polysaccharides. In this study, the increase in extraction time from 20 to 60 and 120 min did not increase the extraction yield, as the diffusion of extraction solvent might be at equilibrium inside and outside the seaweed cell walls. With the pH 4 extraction solvent, the yield of polysaccharide at 120 min (6.1 % dw) was significantly higher ($p<0.05$) than at 20 min (5.5 % dw) and 60 min (5.8 % dw). In the acid solvent, hydrogen ions from HCl could continue to break the glycosidic bonds of hemicellulose and cellulose in the seaweed cell walls, resulting in the diffusion and dissolution of polysaccharides (Chi *et al*., 2018). On the other hand, in the extraction at pH 8, the extraction yields were not significantly different ($p>0.05$) among extraction times of 20 min (5.5 % dw), 60 min (5.3 % dw) and 120 min (5.2 % dw), for the same reason as given for the hot water extraction (i.e., diffusion equilibrium). Therefore, the increase in extraction time of *C. lentillifera* did not impact the extraction yield. The extraction time doubled (60 to 120 min), but the extraction yield was only slightly different.

*Chemical composition of polysaccharides from Caulerpa lentillifera*

Chemical characteristics of extracted polysaccharides prepared by the most practical and cost-effective method, i.e., hot water extraction (pH 6) at 90 °C for 20 min, are presented in Table 1. Results showed that polysaccharide extracts contained carbohydrate (44-46 % dw) and minerals (28-33 % dw) as major components, followed by sulfate (18-19 % dw). There was no difference in total carbohydrate, protein or sulfate when using extraction solvents with different pH. The highest mineral content was found in the acidic extraction, as the acid solution could hydrolyze the polysaccharide structures in cell walls including cellulose, hemicellulose and lignin (Chi *et al*., 2018). Therefore, minerals which are tightly associated with polysaccharides were also extracted (Peasura *et al*., 2015). The sulfate content in the polysaccharide from discarded *C. lentillifera* was rather high, and similar to the results of Rodrigues *et al*. (2011) and Sun *et al*. (2018), who reported that almost all polysaccharides from *Caulerpa* contained approximately 10-23% sulfate. These sulfated polysaccharides commonly exhibit antioxidant activity. Generally, the chemical composition of sulfated polysaccharides influences the desirable bioactivity, particularly the sulfate content that can increase the hydrogen atom donation capability (Peasura *et al*., 2015).

The molecular weight of polysaccharides from all extraction solvents was 3,200-4,000 kDa, which was higher than polysaccharides from other seaweed species in the family *Caulerpa*. Wang *et al*. (2014) reported that polysaccharides derived from *Caulerpa racemosa* and extracted by hot water had Mw of 70-80 kDa. Molecular weight might vary according to species, compositional complexity, structural diversity, extraction procedure, season of harvest and climatic cultivation conditions.

The extraction pH and time did not impact the yield or chemical characteristics of polysaccharides in this study. Therefore, hot water extraction (pH 6) at 90 °C for 20 min has more potential for applications at an industrial scale, as it involves a simple preparation technique, lower cost for extraction solvent and time, as well as being more environmentally friendly.

Table 1. Chemical composition (% dw) and Mw of polysaccharides extracted from discarded *Caulerpa lentillifera* with 20 min of extraction time.

| pH extraction solvent | Total carbohydrate | Total protein | Ash | Sulfate content | Molecular weight (kDa) |
|---|---|---|---|---|---|
| pH 4 | 44.66±0.21 | 2.68±0.39 | 33.24±1.20$^{a}$ | 18.23±0.13 | 3,269.668 |
| pH 6 | 46.83±0.93 | 2.73±0.06 | 31.41±0.42$^{ab}$ | 19.09±1.48 | 3,830.329 |
| pH 8 | 44.79±0.06 | 2.99±0.61 | 28.82±0.63$^{b}$ | 18.58±1.66 | 4,354.173 |

**Note:** Means±SD in the same column superscripted with different lowercase letters are significantly ($p<0.05$) different.

*Monosaccharide profiles of polysaccharides from Caulerpa lentillifera*

Monosaccharide composition of polysaccharide extracts from discarded *C. lentillifera* as analyzed by HPAEC-PAD is shown in Table 2. The polysaccharides contained four major neutral sugars (galactose, glucose, mannose and xylose) and a minor acidic sugar (D-galacturonic acid). Overall, monosaccharide composition of polysaccharides extracted from different pH solvents was similar, although the content of fucose, xylose, mannose and D-galacturonic acid showed significant differences ($p<0.05$) among pH treatments. The monosaccharide profiles of our extracted polysaccharides were in line with previous studies. According to Sun *et al*. (2018), three monosaccharide components (xylose, mannose and galactose) were identified in food-grade *C. lentillifera* polysaccharides, and xylogalactomannans were the main components. Additionally, the β-1, 3-xylan polysaccharide was also identified among polysaccharides found in food-grade *C. lentillifera* (Konishi *et al*., 2012). Also, Chaiklahan *et al*. (2020) reported that discarded *C. lentillifera* polysaccharides mainly contained neutral sugars, including mannose, galactose, glucose and xylose. Although these sugars are usually found in *C. lentillifera* polysaccharides, the content might vary depending on cultivation methods and environmental factors such as salinity, temperature, nutrients and season.

*Composition of low-Mw polysaccharide extract from Caulerpa lentillifera*

Key composition and Mw profile of low-Mw polysaccharide extract degraded by acid hydrolysis using 1 M HCl at 70 °C for 120 min are presented in Table 3. Results showed that total carbohydrate, protein and sulfate content of the low-Mw polysaccharide was similar to the high-Mw polysaccharide extracts, although total carbohydrate and sulfate content was slightly lower in the low-Mw polysaccharide extract. A significantly lower Mw profile was found for low-Mw polysaccharides (~21 kDa) than high-Mw polysaccharides.

*Antioxidant activities*

*Effect of extraction pH on antioxidant properties*

The free radicals DPPH and ABTS are commonly used in assays to assess antioxidant activities of natural compounds (Chaiklahan *et al*., 2020). In this study, the DPPH and ABTS scavenging activities of polysaccharide extracts

Table 2. Monosaccharide composition (% mol) of discarded *Caulerpa lentillifera* and their polysaccharides after 20 min of extraction time.

| Sample | Fucose | Arabinose | Galactose | Glucose | Xylose | Mannose | D-Galacturonic acid |
|---|---|---|---|---|---|---|---|
| pH 4 | 0.06±0.00$^{a}$ | 0.10±0.01 | 37.34±0.49 | 11.07±0.30 | 20.84±0.15$^{a}$ | 30.28±0.64$^{b}$ | 0.31±0.04$^{ab}$ |
| pH 6 | 0.05±0.01$^{ab}$ | 0.07±0.02 | 37.31±0.27 | 11.41±0.16 | 18.69±0.03$^{b}$ | 32.11±0.45$^{a}$ | 0.36±0.05$^{a}$ |
| pH 8 | 0.04±0.00$^{b}$ | 0.08±0.01 | 37.02±0.38 | 11.24±0.08 | 18.82±0.11$^{b}$ | 32.55±0.50$^{a}$ | 0.25±0.03$^{b}$ |

**Note:** Means±SD in the same column superscripted with different lowercase letters are significantly ($p<0.05$) different.

Table 3. Chemical composition (mean±SD) of high- and low-Mw polysaccharides extracted from discarded *Caulerpa lentillifera.*

| Polysaccharide | Total carbohydrate (% dw) | Total protein (% dw) | Sulfate (% dw) | Molecular weight (kDa) |
|---|---|---|---|---|
| High Mw | 46.83±0.93 | 2.73±0.06 | 19.09±1.48 | 3,830.329 |
| Low Mw | 39.70±0.59 | 2.68±0.16 | 15.56±0.21 | 20.928 |

from *C. lentillifera* were not significantly different ($p>0.05$) among the three different extraction solvents at pH 4, 6, and 8 (all with 20 min extraction time) (Table 4). This might be because the sulfate content observed did not differ among solvents (18-19 % dw). Yuan and Macquarrie (2015) reported that the sulfate content plays a key role in antioxidant activity, as the sulfate group can activate the hydrogen atom of the anomeric carbon in polysaccharides.

Polysaccharide extracts in this study at the concentration of 0.9 mg·mL$^{-1}$ possessed about 50 % DPPH radical scavenging activity, higher than the sulfated polysaccharides extracted from *Caulerpa taxifolia*, which were reported for their antiproliferative activity against human lung carcinoma. The concentration of *C. taxifolia* at 1 mg·mL$^{-1}$ resulted in DPPH radical scavenging activity of 15.88 % (Bayro *et al*., 2021). However, the DPPH scavenging activity we recorded with *C. lentillifera* extracts was lower than that of extracts (81.6 mg·mL$^{-1}$) from the same species reported by Yap *et al*. (2019). For ABTS, concentrations at 10 mg·mL$^{-1}$ of all polysaccharide extracts in this study showed approximately 50 % scavenging activity, which was higher than the concentration used for polysaccharide extracts from another seaweed species, *C. racemosa* (*var. macrophysa*) (0.2 mg·mL$^{-1}$) (Tanna *et al*., 2018). Generally, the hydroxyl group in natural polysaccharides can provide the hydrogen atom to free radicals to form stable molecules. This happens the same way in polysaccharides from seaweeds, which can provide hydrogen atoms to DPPH free radicals to form DPPH-H. It was indicated that the hydroxyl group of polysaccharide extracts from all pH extractions might reduce the DPPH radical by donating hydrogen (Zhong *et al*., 2019). Additionally, our results showed that the ability of these polysaccharide extracts to scavenge ~50 % of DPPH radicals (0.9 mg·mL$^{-1}$) was higher than for ABTS (10 mg·mL$^{-1}$). This might be due to the different mechanisms of antioxidant activity between DPPH and ABTS free radicals (Song *et al*., 2019).

*Effect of high- and low-Mw polysaccharide extracts on antioxidant properties*

Previous research has shown that the antioxidant activity of polysaccharides was relative to their Mw (Zhang *et al*., 2013). It was suggested that low-Mw polysaccharides (18 kDa) extracted from green seaweed *Enteromorpha linza* were ten-fold higher in antioxidant activity than high-Mw (100 kDa) polysaccharides. Therefore, the degradation of polysaccharides from high to low Mw using HCl was performed in order to improve their antioxidant activity. The results in Table 5 show that the $IC_{50}$ of DPPH and ABTS scavenging activities of the low-Mw polysaccharide extract (21 kDa) decreased significantly ($p<0.05$) and by ten-fold compared to the high-Mw (3,830 kDa) polysaccharide extract. This suggests that low-Mw polysaccharide extract exposed more active moieties, which could donate hydrogen ions to scavenge DPPH and ABTS radicals. Our result is in agreement with the study of Li *et al*. (2013), who demonstrated that the degradation of sulfated polysaccharides from *E. prolifera* to lower Mw improves antioxidant activity. Relative to the positive control Trolox, the high Mw-polysaccharide extract from discarded *C. lentillifera* showed antioxidant activities to scavenge 50 % of DPPH and ABTS radicals ($IC_{50}$ DPPH 0.88 mg·mL$^{-1}$; ABTS 10.79 mg·mL$^{-1}$) that were lower than Trolox ($IC_{50}$ DPPH 0.29 mg·mL$^{-1}$; ABTS 0.50 mg·mL$^{-1}$). However, the low-Mw polysaccharide extract showed scavenging activities

Table 4. DPPH and ABTS radical scavenging activities of polysaccharide extracts from discarded *Caulerpa lentillifera* after 20 min of extraction time.

| pH | % DPPH scavenging* | % ABTS scavenging** |
|---|---|---|
| pH 4 | 50.59±2.09 | 49.71±1.57 |
| pH 6 | 50.60±0.73 | 51.39±1.68 |
| pH 8 | 50.32±0.29 | 49.53±1.14 |

**Note:** Means±SD in the same column are not significantly different ($p>0.05$); *% DPPH scavenging at 0.9 mg·mL$^{-1}$ of polysaccharide extract; **% ABTS scavenging at 10 mg·mL$^{-1}$ of polysaccharide extract

Table 5. DPPH and ABTS radical scavenging activities of high- and low-Mw polysaccharides from discarded *Caulerpa lentillifera*.

| Polysaccharide | $IC_{50}$ DPPH ($mg \cdot mL^{-1}$) | $IC_{50}$ ABTS ($mg \cdot mL^{-1}$) |
|---|---|---|
| High-Mw (3,830 kDa) | $0.88 \pm 0.00^{a}$ | $10.79 \pm 0.03^{a}$ |
| Low-Mw (21 kDa) | $0.08 \pm 0.00^{c}$ | $1.07 \pm 0.01^{b}$ |
| Trolox | $0.29 \pm 0.01^{b}$ | $0.50 \pm 0.01^{c}$ |

**Note:** Means±SD in the same column superscripted with different lowercase letters are significantly ($p<0.05$) different.

of both free radicals ($IC_{50}$ of DPPH 0.08 $mg \cdot mL^{-1}$; ABTS 1.07 $mg \cdot mL^{-1}$) that were greater than or comparable to Trolox. Moreover, the low-Mw polysaccharide from *C. lentillifera* in our study demonstrated higher antioxidant activities than other antioxidant-rich plants. Roselle extract, which is commonly known to be rich in anthocyanins and has good antioxidant capacity, showed $IC_{50}$ DPPH of 4.06 $mg \cdot mL^{-1}$ and ABTS of 3.7 $mg \cdot mL^{-1}$ (Wu *et al*., 2018). Also, our results were comparable to the $IC_{50}$ DPPH of polysaccharide extracts from mushrooms (0.05 to >0.2 $mg \cdot mL^{-1}$). Mushrooms are known as an excellent source of bioactive properties, including antioxidants. They are used for the treatment of many diseases in clinical applications such as immunotherapy and cancer treatments (Deveci *et al*., 2019).

## CONCLUSION

The major chemical components of discarded *Caulerpa lentillifera* were carbohydrate and minerals. The extraction yield and chemical composition of extracted polysaccharides did not differ by pH of solvent or by extraction time. Hot water extraction (pH 6) at 90 °C for 20 min is worth applying in industry because it is practical and cost-effective. The polysaccharides extracted by this method had high carbohydrate with neutral monosaccharides (galactose, mannose and xylose) and minerals. The antioxidant activities were not significantly different among polysaccharide extracts from solvents at three pH levels (4, 6, and 8). Producing low-Mw polysaccharide extract increased the antioxidant activity compared to high-Mw polysaccharide extract. Therefore, polysaccharide extract with low-Mw made from discarded green seaweed *C. lentillifera* has high potential to be further developed as a source of natural antioxidants and as a functional food ingredient.

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