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ASSESSMENT OF NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY OF POLYSACCHARIDE EXTRACTS FROM BROWN SEAWEED *Sargassum flavicans* FOR AQUACULTURE USES

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ABSTRACT

In this study, crude polysaccharides were extracted from brown seaweed Sargassum flavicans by three extraction solvents including hot-water (SF1), 0.1N HCl (SF2), and 90% aqueous ethanol (SF3). The extracts were then analyzed for chemical composition and antioxidant activity. The results showed that among three extraction solvents, 0.1N HCl extract exhibited higher yield (41.1%), followed by hot-water (24.5%) and 90% aqueous ethanol solvent (12.8%). The color of these freeze-dried extracts changed from brown (SF1 and SF2) to brown green (SF3). The crude protein concentrations were 3.6; 6.1; and 5.2% for SF1, SF2, and SF3, respectively. Extract SF2 had higher concentration of total phosphorus (0.34%) than that of SF1 (0.17%). Total phlorotannin concentration of extracts was in the range of 0.27-0.47%. Also, extract SF2 had the highest percentage of sulfate content (3.5%). The free radical scavenging activity, ferrous ion chelating activity, and ferric reducing power of extracts were increased with increasing of concentrations of polysaccharides. Polysaccharide was extracted by 0.1N HCl (SF2) showed the highest antioxidant activity. These results indicated that the polysaccharide extracts of brown seaweed S. flavicans possessed a good antioxidant activity.

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1 INTRODUCTION

The brown seaweed, *Sargassum* sp. (Phaeophyceae), is a common plant distributing around the coastal area and over 400 species worldwide have been described (Tseng and Lu, 2004). In Vietnam, there are 143 brown seaweed species have been identified, of which 22 and 13 *Sargassum* species were found in the North and the South Vietnam, respectively (Pham, 1969; Nguyen *et al*., 1993). Among bioactive natural products, polysaccharides from brown seaweed are considered as the main sources of antioxidants from the ocean. It has been noted that seaweed extracts are important sources of glycolipids, sulfate fucans, and phenolic compounds that serve as antioxidants and immunostimulants (Blondin *et al*., 1994; Franz *et al*., 2000; Hossain *et al*., 2003). In fact, polysaccharides extracted from *Sargassum polycystum*, *S. fusiforme*, and *S. duplicatum* had capable of improvement of the immune response and resistance of black tiger shrimp, *Penaeus monodon* (Chotigeat *et al*., 2004), fleshy prawn *Fenneropenaeus chinensis* (Huang *et al*., 2006), and white shrimp,

Litopenaeus vannamei (Giang *et al*., 2011). Therefore, it is assumed polysaccharides from brown seaweed (Phaeophyta) are nutrient sources and funtional food promising candidates as an alternative source of nutrients for aquafeeds (Niu *et al*., 2015). *S. flavicans* is a brown seaweed distributing in the coast of Kien Giang province, Mekong Delta, Vietnam. Information on bioactivity of polysaccharide extracted from this species is limited. Therefore, this work was carried out to evaluate the effect of different sovent extractions on chemical composition of polysaccharides as well as their antioxidant activity in order to postulate for aquaculture use.

2 METHODOLOGY

2.1 Preparation of polysaccharide extracts

A brown seaweed *S. flavicans* was collected from the coast of Kien Giang Province, Vietnam. *S. flavicans* was washed and kept in plastic bag at 4°C and shipped to laboratory. The species taxonomy classification, extraction, sample analysis were done at the Laboratory of Marine Biology, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University. Polysaccharide extracts of brown seaweed *S. flavicans* were prepared following the method described by Giang *et al*. (2011). Sample was washed with distilled water to separate potential contaminants and then dried in oven at 37°C. After being dried, the sample was ground to powder by the high speed blender, then sieved through the 125 µm mesh size. *S. flavicans* powder was subsequently stored in the refrigerator (4°C) for further extraction. Three solvent extractions were used in this study. Each extract was done 5 replicates and denoted as SF1: hot-water at 100°C for 3 h; SF2: 0.1N HCl at 100°C for 3 h; and SF3: 90% aqueous ethanol at room temperature for 12 h. 10 g of dry *S. flavicans* powder was added to 300 mL of various solvents, and the suspension was boiled following the time as described above. The suspension was filtered through a glass filter paper 0.45 µm GF47 (Whatman, Germany), and the filtrate was centrifuged at 4000 rpm for 10 min at 4°C then lyophilized under reduced pressure. The harvest weight of the polysaccharides samples obtained from extraction of 10 g of *S. flavicans* in powder form was recorded and stored in the refrigerator for later analysis.

2.2 Chemical composition analysis

Polysaccharide samples were analyzed for protein, phosphate, L-fucose, sulfate content, and phlorotannin. Crude protein and phosphate was determined by AOAC (2001); L-fucose was measured by the phenol-sulfuric acid method using L-fucose as the standard (Dubois *et al*., 1956); sulfate content was determined following the described by Terho and Hartiala (1971); and for phlorotannin concentration, Folin-Ciocalteu method was used with gallic acid as the standard (Koivikko *et al*., 2005).

2.3 Determination of antioxidant activity

The scavenging activity for 2,2-diphenyl-picylhydrazyl ($C_{18}H_{12}N_5O_6^+$) (DPPH$^\bullet$) free radicals was measured according to the method of Shimada *et al*. (1992). Briefly, DPPH$^\bullet$ solution was prepared at the concentration of 0.1 mM in ethanol 100%. Polysaccharide samples were made at the various concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 mg mL^{-1} with deionized distilled water, then 1 mL of test solution was mixed with 1 mL of DPPH$^\bullet$ solution. The mixture was incubated in dark place for 30 min at 25°C. After standing for 30 min, absorbance was recorded at 517 nm by UV-Vis UNICAM spectrophotometer (England). The percentage of DPPH$^\bullet$ free radicals scavenging activity was calculated using the equation given by Duan *et al*. (2006): Scavenging activity =[1-(A1-A2)/A0]×100 where A0, A1 and A2 are the absorbance of the control (without test solution), the presence of the test solution, and without DPPH$^\bullet$, respectively.

The ferric chelating activity of the polysaccharide samples was determined by the method described by Dinis *et al*. (1994). Briefly, one milliliter of the test solution (concentration of 0.1-4.0 mg mL^{-1}) was mixed with 3.8 mL of deionized distilled water and 100 µL of 2 mM $FeCl_2$. After 30 seconds, 0.2 mL of 5 mM ferrozine was added and reacted for 10 min at room temperature. The absorbance of the Fe-ferrozine complex was measured at 562 nm. The chelating activity was calculated as following equation: Chelating activity = [(A0-A1)/A0]×100. A0 and A1 are the absorbance of the control (without test solution) and the presence of the test solution, respectively.

The ferrous reducing power of the polysaccharide extract was determined following the method described by Oyaizu (1988). One milliliter of aliquot of the test sample (concentration of 0.5-4.0 mg mL^{-1}) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$, then incubated at 50°C in a water bath for 20 min. The reaction was stopped by adding 1 mL of 10% CCl_3COOH solution and then centrifuged at 5500 rpm for 10 min. The supernatant (1.5 mL) was

mixed with 1.5 mL of deionized distilled water and 100 µL of 0.1% $FeCl_3$ solution for 10 min. Any rise of the reaction mixture, read at 700 nm using a spectrophotometer (UNICAM, England), was indicative of an increase in reducing activiy.

2.4 Data analysis

The data is presented in mean of five determinations. Polysaccharide concentrations (mg mL^{-1}) and antioxidant activity (%) was graphically estimated using a linear regression algorithm. The values of median inhibit concentration (IC_{50}) for polysacchride extracts were recognized for inhibiting free radicals concentration or increasing chelating activity by 50%; and absorbance up to 0.5 for reducing power.

3 RESULTS

3.1 Yield of crude polysaccharide extracts

Polysaccharide was extracted by 0.1N HCl exhibited the highest yield of 41.1±3.9% followed by hot-water (24.5±0.5%) and 90% aqueous ethanol solvent (12.8±0.1%) (Fig. 1A).

3.2 Chemical composition of polysaccharide extracts

The color of freeze-dried extracts were brown (SF1 and SF2) and brown green (SF3). Crude protein contents were 3.6, 6.1, and 5.2% for SF1, SF2, and SF3 fraction, respectively (Fig. 1B). Polysaccharide in SF1 showed higher L-fucose content (8.0%) than that of fraction SF2 and SF3 (Fig. 2A). However, extract SF2 contained the highest percentage of sulfate content compared to other extracts. The sulfate contents were 2.3, 3.5, and 2.3% for the SF1, SF2, and SF3, respectively (Fig. 2B). Phlorotannin concentrations respectively accounted for 0.35, 0.47, and 0.27% of the extract SF1, SF2, and SF3 (Fig. 3).

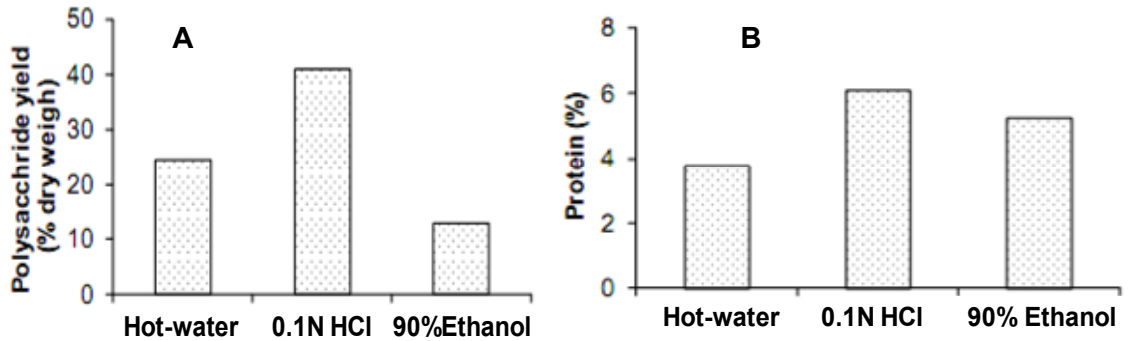

Fig. 1: Yields of polysaccharide (A) and crude protein (B) in polysaccharide extracts

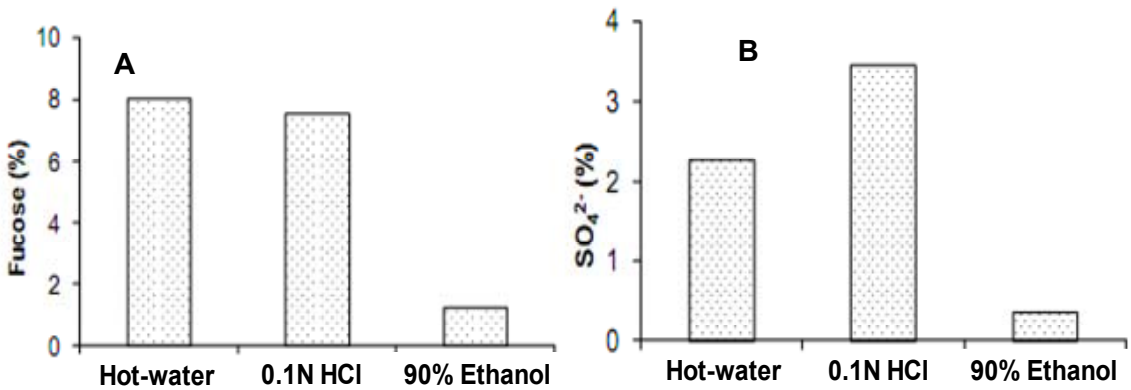

Fig. 2:-fucose (A) and sulfate contents (B) in polysaccharide extracts

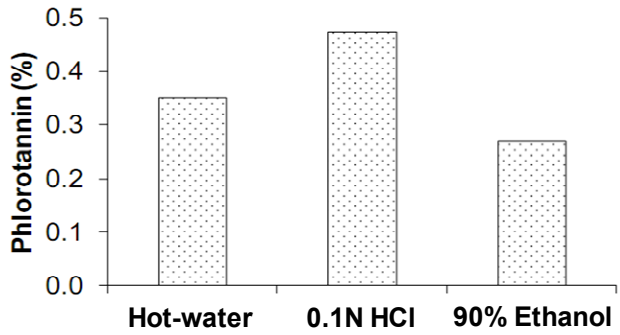

Fig. 3: Phlorotannin in polysaccharide extracts

3.3 Antioxidant activity of polysaccharide extracts

Free radical scavenging activities were increasing with increase of concentration of aqueous polysaccharides and showed a linear relationship by determining with DPPH• free radicals scavenging activity assay. Figure 4 demonstrates SF2 the highest scavenging activity followed by SF1. The values of IC_{50} were 0.73 mg mL^{-1} (Y = 7.6099X + 44.474; R^2 = 0.8384), 2.65 mg mL^{-1} (Y = 13.731 + 13.559; R^2 = 0.9589), and 7.67 mg mL^{-1} (5.2032X + 10.109; R^2 = 0.8975) for SF2, SF1, and SF3, respectively. Among extracts, SF1 showed higher Fe^{2+} chelating activity than that of SF2 and SF3. At the concentration of 4.0 mg mL^{-1}, the chelating activity reached 63.4, 50.6, and 30.7% for SF1, SF2, and SF3, respectively. The IC_{50} values of chelating activity were 4.06, 5.33, and 7.28% for SF1, SF2, and SF3, respectively (Fig. 5). However, Figure 6 indicates that SF2 had the highest reducing power increased (Y = 0.1857 + 0.0718; R^2 = 0.9378) followed by SF2 (Y = 0.1032X + 0.011; R^2 = 0.954).

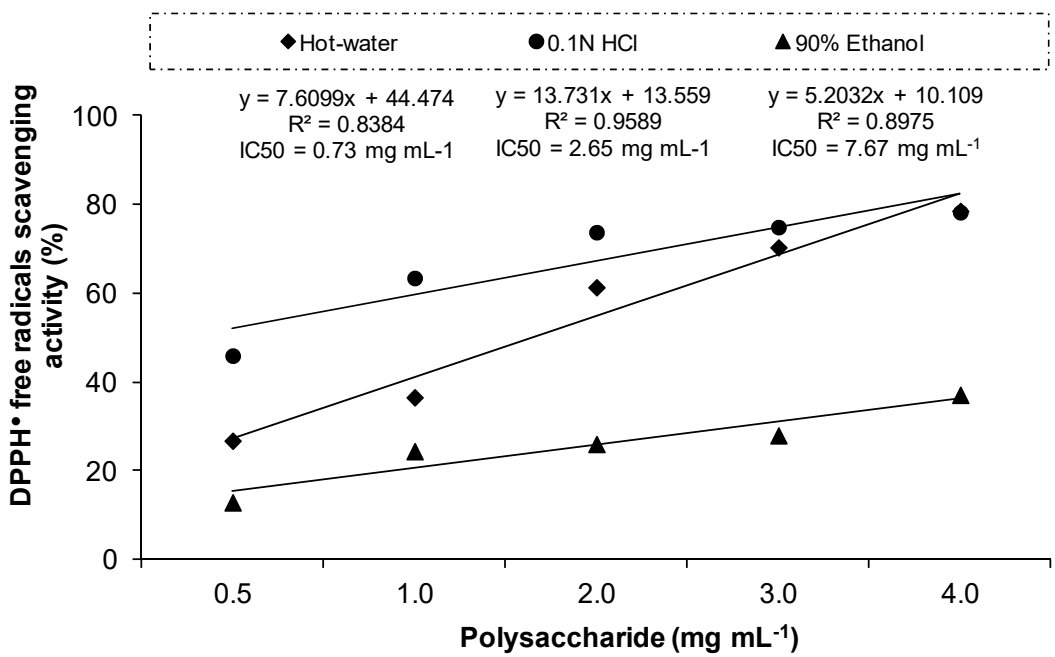

Fig. 4: DPPH• free radicals scavenging activity of polysaccharide extracts

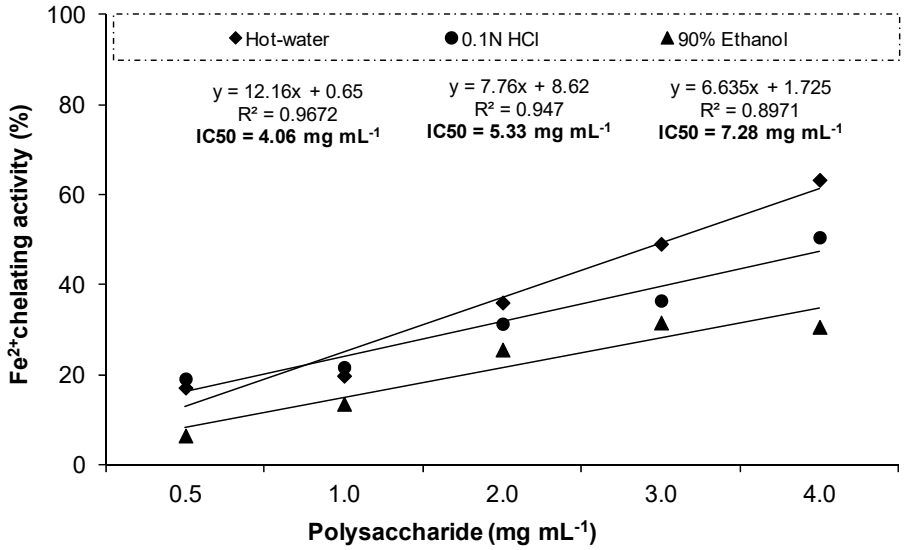

Fig. 5: Ferrous chelating activity of polysaccharide extracts

4 DISCUSSION

Properties of polysaccharides from brown seaweed (Phaeophyta) have been intensively investigated. In fact, yield of polysaccharide from brown seaweed *S. siliquastrum* by aqueous methanol and hot-water presented 6.42 and 2.41%, respectively (Lim *et al*., 2002). Moreover, Ruperez *et al*. (2002) revealed that polysaccharide extracted from *Fucus vesiculosus* by 0.1N HCl was 42.1%. In this study, the yield of polysacchade extracted with 0.1N HCl are consistent with those reported by Ruperez *et al*. (2002), Giang *et al*. (2013a; 2013b). However, as comparison with those reported by Eluvakkal *et al*. (2010), yield of polysaccharide from *S. flavicans* extracted with aqueous ethanol and 0.1N HCl were relatively higher. It is hence assumed that species of seaweeds and different solvents could affect polysaccharide yield. This state is in accordance with (Jormalainen and Honkanen, 2004). The interesting finding of this study is that 0.1N HCl is a good solvent for polysaccharide extraction in brown seaweed. However, it is important to recommend that further study is needed to examine polysaccharide yield by using different solvents with different intervals of extraction; different part of brown seaweed as blade, stipe, and vesicle; or growth stage of this brown seaweed species.

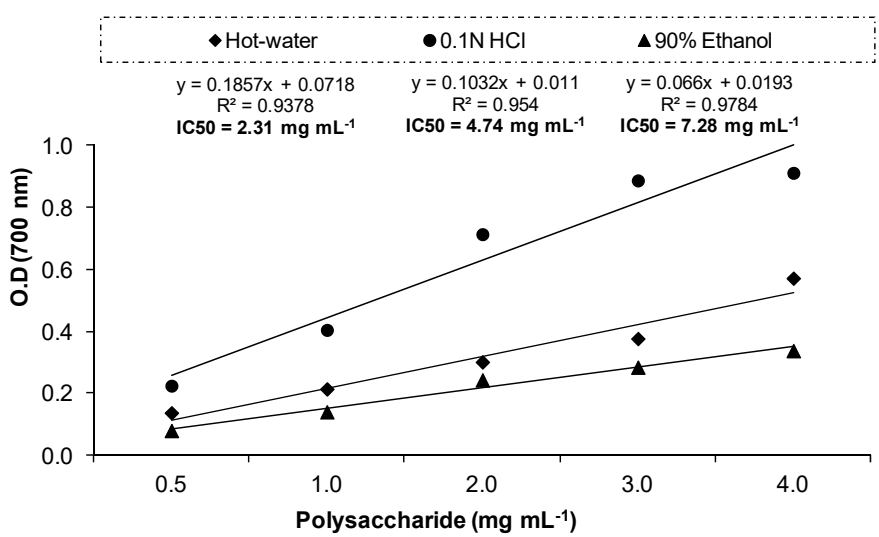

Fig. 6: Ferric reducing power activity of polysaccharide extracts

Earlier studies have been illustrated high protein content in polysaccharide extracts of *S. tenerrimum* (22.1%), *S. congkinhii* (16.0%), *S. mcclurei* (11.4%), and *S. longicruris* (27.7%) (Nguyen, 1997; Rioux *et al*., 2007). A study of Ruperez *et al*. (2002) revealed that the protein content in *F. vesiculosus* varied from 1.0 to 6.0%. Brown seaweed (Phaeophyta) produces families of sulfated fucans among other polysaccharides in which neutral sugars namely L-fucose is the major component. According to Kim *et al*. (2007), the L-fucose content containing in polysaccharide extracts from brown seaweed *Undaria pinnatifida* by using 0.1N HCl solvent reached 70%. In this work, L-fucose content varied from 1.2 to 8.0%. Obviously, the L-fucose contents of *S. flavicans* were lower those reported by Kim *et al*. (2007) and Giang *et al*. (2011). According to recent investigations, tannins are commonly divided into three chemically distinct groups based on their structures. Hydrolysable tannins are commonly present in angiosperms (Waterman and Mole, 1994). Flavonoid-based condensed tannins are found mainly in woody plants as wine, tea, and cocoa beans (Santos-Buelga and Scalbert, 2000). The third is the phlorotannins which consist of polymers of phloroglucinol units and is restricted to the brown seaweed (Ragan and Glombitza, 1986). Phlorotannins are the most effective antioxidants in brown seaweed. Chowdhury *et al*. (2011) reported phlorotannin concentration in brown seaweed *Ecklonia cava* was 0.18% and these authors also revealed that early stage of seaweeds contain higher level than that of the senescent stage. Therefore, phlorotannin concentrations containing in polysaccharide extracted from *S. flavicans* were relatively higher that of *E. cava*.

The DPPH$^{\bullet}$ free radical is a stable free radical that is widely used as a tool for estimating the free radical scavenging activities of antioxidants. The role of an antioxidant is to remove free radicals. One mechanism through which this is achieved involves donating hydrogen to a free radical, and hence, its reduction to an unreactive species. Addition of hydrogen removes the odd electron feature which is responsible for radical reactivity (Shao *et al*., 2014). The results of this study indicated that 0.1N HCl and hot-water extracts showed higher free radical scavenging activity than that of 90% aqueous ethanol. A previous study appeared that hot-water extract of seaweed had higher free radical scavenging activity than that of aqueous ethanol extract (Kuda and Ikemori, 2009). The high radical scavenging activities of SF1 and SF2 may be due to the difference in phlorotannin concentration in crude polysaccharide extraction. At the concentration of 3.8 mg mL^{-1}, polysaccharide extracted from *S. pallidum* showed 19.1% scavenging activity (Ye *et al*., 2008), whereas polysaccharide from *Sargassum* sp. showed the highest antioxidant activity at 0.8 mg mL^{-1}. In addition, for the scavenging activity, Hwang *et al*. (2010) found that the IC_{50} value of hot-water of *S. hemiphyllum* was 1.58 mg mL^{-1}. In *S. horneri*, Shiao *et al*. (2014) found that hot-water extract of *S. horneri* showed radical scavenging activity of 85.0% at concentration of 2.5 mg mL^{-1}. It is known that iron is a transition metal and can accelerate or stimulate lipid peroxidation, while the oxidation of Fe^{2+} was inhibited by adding the polysaccharide extracts from *S. flavicans*, indicating the chelating activities were the good linear dose-depending relationships. Ferrous cheating activity of SF2 and SF3 in the present study showed relatively lower than those reported by Hwang *et al*. (2010). Interestingly, the 0.1N HCl extract with high level of phlorotannin showed the strongest reducing activity.

5 CONCLUSION

Polysaccharide extracted from *S. flavicans* by 0.1N HCl showed good antioxidant activity and high concentrations of L-fucose, SO_4^{2-}, and phlorotannin, which have utilitarian properties of biologically active compounds. Antioxidant activities of extracts from brown seaweed are promising candidates for application of natural bioactive compound in improvement of aquatic animal health. However, molecular weight and monosaccharide composition of polysaccharides extracted from brown seaweed should be elucidated in further research.

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