Can Tho University Journal of Science
website: sj.ctu.edu.vn

DOI: 10.22144/ctu.jen.2016.045

CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY OF CRUDE POLYSACCHARIDE EXTRACTED FROM BROWN SEAWEED *Sargassum microcystum* AND ITS EFFECT ON GROWTH PERFORMANCE AND SURVIVAL OF WHITELEG SHRIMP *Litopenaeus vannamei* VIA DIETARY ADMINISTRATION

Huynh Truong Giang, Tran Trung Giang, Duong Thi Hoang Oanh, Tran Suong Ngoc and Vu Ngoc Ut

College of Aquaculture and Fisheries, Can Tho University, Vietnam

Article info.

Received date: 31/08/2015
Accepted date: 30/11/2016

Keywords

Antioxidant activity, growth performance, polysaccharide, Sargassum microcystum, Litopenaeus vananmei

ABSTRACT

The aims of this study were to evaluate antioxidant activity of polysaccharide extracted from S. microcystum and examine its positive effect on growth performance and survival of whiteleg shrimp (Litopenaeus vannamei). Polysaccharides from brown seaweed Sargassum microcystum were extracted by four methods (1) hot-water within 3 h, (2) hot-water within 6 h, (3) 0.1N HCl (100°C) within 3 h, and (4) 0.1N HCl (100°C) within 6 h. The extracts were then analyzed for chemical composition and antioxidant activities. The extract showed the highest antioxidant activity could be used to examine its effectiveness on whiteleg shrimp culture via dietary administration. For trial on shrimp, whiteleg shrimp were reared in a recirculating seawater system with 500-L tanks and fed the pellet diets containing 0.5, 1.0, and 2.0% of hot-water extract. Shrimp were fed the diet without extract supplemented served as control group (0%). After 60 days of feeding, survival rate and growth parameters of shrimp were evaluated. The results showed that protein concentrations of polysaccharide extracts were low and varied from 1.3 to 6.8%. The polysaccharide extracted with hot-water within 6h was higher phlorotannin, glucose and L-fucose concentrations than those in other extracts. However, higher SO_4^{2-} concentration was found in 0.1N HCl extract within 3 h extract. The significant interactions between solvent and extraction time were observed that affecting yield of polysaccharide and chemical composition as phlorotannin, glucose and SO_4^{2-} concentrations. Hot-water within 6 h extract showed the highest antioxidant activity indicating by high DPPH$^{\bullet}$ free radical scavenging (IC_{50} = 0.434 mg mL^{-1}) and ferric reducing power activity ($OD_{0.5}$ = 2.55 mg mL^{-1}). Whiteleg shrimp that being fed the diets incorporating with hot-water extracts at 1.0% had significantly higher growth performance than those in the control diet. However, there was no significant differences in daily length gain and survival rates among treatments. Therefore, it is concluded that polysaccharide extracted from S. microcystum could be growth promoter in whiteleg shrimp culture.

Cited as: Giang, H.T., Giang, T.T., Oanh, D.T.H., Ngoc, T.S. and Ut, V.N., 2016. Chemical composition, antioxidant activity of crude polysaccharide extracted from brown seaweed *Sargassum microcystum* and its effect on growth performance and survival of whiteleg shrimp *Litopenaeus vannamei* via dietary administration. Can Tho University Journal of Science. Vol 4: 71-80.

1 INTRODUCTION

The quantities of polysaccharide produced by marine algae quite likely exceed those of fresh water plants (Wood, 1974). The first polysaccharide to be described having an interesting biological activity was probably the β-glucan (Paulsen, 2002). Cell walls from brown seaweed characteristically contain sulfated polysaccharides, which are not found in land plants and which may have specific functions in ionic regulation (Kloareg and Quatrano, 1988). Fucoidan, laminaran, and alginate are the main polysaccharides of brown seaweed (Zvyagintseva *et al.*, 1999; Giang *et al.*, 2011a; Immanuel *et al.*, 2012). There are additional therapeutic areas that are under extensive investigations by anticoagulant, anti-inflammatory, antiparasitic, antiviral and antibacterial activities (Giang *et al.*, 2011a). Previous studies have been intensively demonstrated that some of brown seaweed *Sargassum* are now being considered as a rich source of antioxidants and nutrients from the ocean (Ye *et al.*, 2008; Hwang *et al.*, 2010; Giang *et al.*, 2013a). Recently, phlorotannin, fucoxanthin and fucoidan are known as bioactive compounds present in brown seaweed showing chemopreventive and chemotherapeutic effects against cancer (Padua *et al.*, 2015). In shrimp, polysaccharide extracts from brown seaweed have been showed promoting immune response and disease resistance (Huang *et al.*, 2006; Yeh *et al.*, 2006; Giang *et al.*, 2011b; Immanuel *et al.*, 2012; Yudiati *et al.*, 2016). Previous studies have also evaluated the uses of seaweed extracts for improvement of growth performance of Indian white prawn (*Penaeus indicus*) (Immanuel *et al.*, 2004), Kuruma shrimp (*Marsupenaeus japonicas*) (Traifalgar *et al.*, 2010); tiger shrimp (*P. monodon*) (Traifalgar *et al.*, 2009; Immanuel *et al.*, 2010; Sivagnanavelmurugan *et al.*, 2014; 2015). In whiteleg shrimp (*Litopenaeus vannamei*), positive effects of brown seaweed meals on growth performance and survival were also evaluated (Cruz-Suarez *et al.*, 2009; Cardenas *et al.*, 2015). Among brown seaweed *Sargassum* distributing around the coast of Mekong Delta, Vietnam, previous studies have been reported that polysaccharides extracted from *Sargassum* species had potential antioxidant activities and capable of improving feed value (Giang *et al.*, 2013a; 2013b; 2016). However, studies on applications of polysaccharides extracted from *Sargassum* on whiteleg shrimp culture are limited. Therefore, the aims of this study were to evaluate antioxidant activity of polysaccharide extracted from *Sargassum microcystum* and examine its positive effect as a growth-promoting factor on whiteleg shrimp to postulate for use in intensive shrimp farming.

2 MATERIALS AND METHODS

2.1 Experiment 1: Evaluating effect of different extraction methods on yields, chemical composition and antioxidant activities of extracts from *S. microcystum*

2.1.1 Preparation of polysaccharide extract

A fresh *S. microcystum* was collected from the coast of Kien Giang province, Vietnam. *S. microcystum* meal powder was prepared following method described by Giang *et al.* (2011b). Samples was washed with distilled water to separate potential contaminants and then dried under the sunshine until the weight of seaweed was immutable. After being dried, the sample was exhaustively ground to powder form by high speed crusher (Grinder-RT), and then sieved with the mesh 125 µm. *S. microcystum* powder was subsequently stored in the refrigerator (4°C) until extraction. The *S. microcystum* was extracted by four methods including (1) hot-water within 3 h, (2) hot-water within 6 h, (3) 0.1N HCl (100°C) within 3 h, and (4) 0.1N HCl (100°C) within 6 h. The polysaccharide fraction of *S. microcystum* was done following the method described by Giang *et al.* (2011b). Briefly, 10 g of dry *S. microcystum* powder was added to 300 mL of various solvents. The suspension was filtered through a glass filter paper 0.45 µm GF (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 obtained from 10 g of *S. microcystum* in powder form was recorded.

2.1.2 Chemical composition analysis

Polysaccharide extracts were analyzed for crude protein, glucose, L-fucose, SO_4^{2-}, phlorotannin. Crude protein was analyzed following the method of AOAC (2001). L-fucose determined by the phenol-sulfuric acid method using L-fucose as the standard (Dubois *et al.*, 1956); glucose was quantified by GC/MS; SO_4^{2-} was measured following the described by Terho and Hartiala (1971); phlorotannin was determined by the Folin-Ciocalteu method using Gallic acid as the standard (Koivikko *et al.*, 2005). The chemical composition data is presented in mean of five determinations.

2.1.3 Screening for antioxidant activity

The scavenging activity for DPPH$^{\bullet}$ free radical was measured according to the method of Shimada *et al.* (1992). DPPH$^{\bullet}$ solution was prepared at the concentration of 0.1 mM in ethanol 100%. Polysacchride extract was made at the various concen-

trations of 0.5, 1.0, 2.0, 3.0 and 4.0 mg mL^{-1} with deionized distilled water, and then 1 mL of test solution was mixed with 1 mL of diluted DPPH• solution. The mixture was incubated in dark place for 30 min at 25°C. Absorbance was recorded at wavelength of 517 nm using UV-VIS UNICAM spectrophotometer (England). The percentage of DPPH• 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 were the absorbance of the control without test solution, the present of the test solution, and without DPPH•, respectively. Reducing power activity of the polysaccharide extracts were determined by the method described by Oyaizu (1998). 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 0.1 mL of 0.1% $FeCl_3$ solution for 10 min. Absorbance of mixture was recorded at wavelength of 700 nm using UV-VIS UNICAM spectrophotometer (England). Increased optical density (OD) of the reaction was indicative of an increase in reducing activity. Polysaccharide concentrations (mg mL^{-1}) and antioxidant activities (%) was graphically estimated using a linear regression algorithm then the values of median inhibit concentration (IC_{50}) for polysacchride extracts were recognized for inhibiting free radicals concentration by 50% and OD up to 0.5 and 1.0 for reducing power.

2.2 Experiment 2: Effects of polysaccharide extracted from *Sargassum microcystum* on growth performance and survival of whiteleg shrimp via dietary administration

2.2.1 Experimental design

One hundred shrimp postlarvae (mean body weight of 0.08 g) were randomly allocated to each circular composite tank (capacity of 0.5 m^3) containing 0.4 m^3 of 15‰ brackish water. The experiment was carried out with four treatments according to the concentrations of polysaccharide extracted from *S. microcystum* incorporating with commercially available artificial pellet at 0.5, 1.0 and 2.0%. The shrimp fed the diet with no polysaccharide supplemented served as control group (0%). Each treatment was done in three replicates. The experiment was lasted 60 days.

2.2.2 Preparation of test diets

The selective hot-water extract was incorporated with commercial pellet feed (Grobest, 40% crude protein) at the concentrations of 0, 0.5, 1.0 and 2.0%. The required concentration of polysaccharide extracted was dissolved in the water and coated onto pellets. For coating, the polysaccharide extract was mixed with the food pellets and incubated at room temperature for 15 min to allow the absorption of the polysaccharide extracted. The feed was then dried and stored at room temperature until given to shrimp. After feeding, the remaining feed was stored in refrigerator 4°C. Polysaccharide-containing diets were prepared every two days.

2.2.3 Experimental system and management

Shrimp were reared in recirculation water system at the College of Aquaculture and Fisheries, Can Tho University. Aeration was supplied via a single airstone to maintain the dissolved oxygen at ≥ 5 mg L^{-1}. Shrimp were fed at 3% body weight dividing into three times daily at 07:00, 14:00 and 20:00. The amount of all diets fed was calculated by subtracting the uneaten portion, and data were recorded daily. Any dead shrimp were removed to prevent cannibalism. To provide a suitable environment for whiteleg shrimp, recirculating system was maintained uniform flow rates, fixed water levels, and uninterrupted operation following described by Masser *et al.* (1999) (Fig. 2). During the period of feeding experiment, some crucial water quality parameters as temperature, pH, dissolved oxygen (DO), alkalinity, ammonia and nitrite-nitrogen were also monitored. The methods were as described in Standard Methods for Waste Water and Water Examination (APHA, 1999).

Fig. 1: Fresh *S. microcystum* and its extract (Photo: Giang *et al.*, 2012)

Fig. 2: Experimental recirculation water system

2.2.4 Data collection

At the end of the experimental period of 60 days, the survival rate (SR) was assessed; experimental shrimp from each tank was individually measured length (cm) and weight (g). Growth measured as daily lenght gain (DLG), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR) were calculated as described previously (Niu *et al.*, 2015).

2.3 Statistical analysis

All data were analyzed by the SAS computer software version 9.1 (SAS Institute, Cary, NC, USA). A multiple-comparison test (DUNCAN's) was used to examine if significant differences among treatments. Two-way ANOVA was used for analyzing the effects between solvent and extraction time on yield of polysaccharide and chemical composition of polysaccharides. For growth performance trial, all values in percentage (survival rate) were arcsine-transformed to satisfy the requirement for the normal distribution. Significance of differences were considered at $p<0.05$.

3 RESULTS

3.1 Yield of crude polysaccharide

Yields of polysaccharides extracted by hot-water were lower than that of 0.1N HCl. *S. microcystum* was extracted by 0.1N HCl within 3 h exhibited higher yield of polysaccharide (18.9±0.5%) followed by HCl 0.1N within 6 h (18.7±0.4%). However, there was no significant difference in yield of polysaccharide among methods extracted with 0.1N HCl ($p>0.05$). Polysaccharide extracted by hot-water within 6 h was 8.0±0.5% and significant lower than that by hot-water within 3 h (9.9±0.6). A two-way ANOVA test showed that yield of polysaccharide was significantly affected by both solvent and extraction time ($p<0.05$). Yield of polysaccharide was significantly affected by interaction between solvent and extraction time ($p<0.05$) (Table 1).

Table 1: Yield of crude polysaccharide extracted from brown seaweed with different methods

Method		**Yield of crude polysaccharide (%)**
Solvent	**Extraction time (h)**	%
Hot-water	3	9.9±0.6[a]
Hot-water	6	8.0±0.5[b]
0.1N HCl	3	18.9±0.5[a]
0.1N HCl	6	18.7±0.4[a]
Two-way ANOVA		
Solvent		*
Extraction time		*
Interaction *Solvent × Extraction time*		*

Data are means of five replicates. Data (mean±SE) in the same solvent with different letters differ significantly ($p<0.05$). $^{ns}p>0.05$, $^{*}p<0.05$

3.2 Chemical composition

Among polysaccharide extracts, protein concentrations of polysaccharide extracted from *Sargassum microcystum* were low and varied from 1.3±0.59 to 6.8±0.84%. Highest protein concentration was found in the hot-water extract within 6 h while the lowest one was found in 0.1N HCl extract within 3 h. Phlorotannin concentrations slightly changed among methods and varied from 0.48±0.01 to

0.84±0.03%. Interestingly, the polysaccharide extracted with hot-water within 6h showed higher phlorotannin, glucose and L-fucose levels than those in other extracts (Table 2). Conversely, significantly higher SO_4^{2-} concentration was found in 0.1N HCl extract within 3 h while the lowest one was in hot-water extract within 6 h. Protein was significantly affected by extraction time ($p<0.05$), but not by solvent ($p>0.05$; Table 2). Interestingly, phlorotannin containing in polysaccharide was significantly affected by solvent ($p<0.05$), but not by solvent ($p>0.05$). Glucose, fucose and SO_4^{2-} were significantly affected by both solvent and extraction time ($p<0.05$; Table 2). No significant interaction between solvent and extraction time was observed that affecting protein and L-fucose concentrations. However, phlorotannin, glucose and SO_4^{2-} were significantly affected by interaction of solvent and extraction time. The two-way ANOVA analysis is shown in Table 2.

Table 2: Chemical composition of polysaccharide

Method		Protein	Phlorotannin	Glucose	Fucose	SO_4^{2-}
Solvent	**Extraction time (h)**	%	%	%	%	%
Hot-water	3	5.7±1.5a	0.48±0.01b	14.3±0.2b	8.5±0.2b	4.9±0.11a
Hot-water	6	6.8±0.8a	0.84±0.03a	18.4±0.3a	9.9±0.3a	4.4±0.16b
0.1N HCl	3	1.3±0.2a	0.59±0.11a	2.8±0.5b	3.6±0.6b	5.8±0.11b
0.1N HCl	6	2.0±0.7a	0.63±0.07a	3.4±0.5a	4.4±0.6a	7.9±0.18a
Two-way ANOVA						
Solvent		ns	*	*	*	*
Extracttion time		*	ns	*	*	*
Interaction *Solvent × Extraction time*		ns	*	*	ns	*

Data are means of five replicates. Data (mean±SD) in the same row and the same solvent with different letters differ significantly ($p<0.05$). $^{ns}p>0.05$, $^{*}p<0.05$

3.3 Antioxidant activity

DPPH• free radical scavenging activity assay is directly proportional to concentration of polysaccharides with relatively high correlation coefficient. Polysaccharides extracted by hot-water in 6 h had the highest free radical scavenging activity followed by that of hot-water within 3 h. Polysaccharide extracted by 0.1N HCl in 6 h had the lowest free radical scavenging activity. The values of IC_{50} were 0.434, 0.565, and 1.678 mg mL^{-1} for hot-water extract 6 h, hot-water extract 3 h and HCl 0.1N extract in 6 h, respectively (Table 3). Similarly, polysaccharide extracted by hot-water in 6 h also showed the highest a ferric reducing power activity with $OD_{1.0}$ of 6.59 mg mL^{-1} followed by hot-water extract in 3 h ($OD_{1.0}$ = 7.44 mg mL^{-1}). The values of $OD_{1.0}$ were 19.2 and 20.2 mg mL^{-1} for methods of 0.1N HCl within 3 and 6 h, respectively (Table 4).

Table 3: DPPH• free radical scavenging activity

Method	IC_{50} (mg mL^{-1})	IC_{100} (mg mL^{-1})	Equation	R^2
Hot-water, 3 h	0.565	1.309	y = 67.222x + 12.03	0.9653
Hot-water, 6 h	0.434	1.084	y = 76.867x + 16.67	0.9690
0.1N HCl, 3 h	1.643	3.939	y = 21.778x + 14.21	0.9785
0.1N HCl, 6 h	1.678	4.074	y = 20.865x + 14.98	0.9887

Table 4: Ferric reducing power activity

Method	$OD_{0.5}$ (mg mL^{-1})	$OD_{1.0}$ (mg mL^{-1})	Equation	R^2
Hot-water, 3 h	2.94	7.44	y = 0.1111x + 0.1731	0.918
Hot-water, 6 h	2.55	6.59	y = 0.1237x + 0.1852	0.917
0.1N HCl, 3 h	8.52	19.2	y = 0.0468x + 0.1013	0.763
0.1N HCl, 6 h	8.01	20.2	y = 0.0412x + 0.1701	0.546

3.4 Shrimp growth performance trial

During experimental period water quality parameters were within suitable ranges for shrimp growth. In fact, temperature maintained in a range of 25.7-27.7°C; pH 8.33-8.59; DO 5.52-7.41 mg L^{-1}; alkalinity 121-169 mg $CaCO_3$ L^{-1}; ammonia 0.001-0.033 mg L^{-1}; and nitrite-nitrogen 0.003-0.138 mg L^{-1}. Over 60 days of feeding trial, whiteleg shrimp that being fed the commercial feed supplemented at 1% of hot-water extract from *S. microcystum* had significant higher final weight, WG, DWG and SGR compared to those of control group ($p<0.05$). No significant difference in growth rate among

shrimp fed hot-water extract was observed. Interestingly, there was no significant difference in growth performance among 0.5%, 2% and control treatments. Shrimp fed the diets containing 0.5 and 1.0% of polysaccharide had the highest survival rates with the mean values of 56.0±6.2% and 56.7±11.9%, respectively. However, insignificance in DLG and SR was found among treatments ($p>0.05$). For the feed utilization, whiteleg shrimp fed 1% polysaccharide showed the lowest FCR while the highest one was found in control treatment ($p<0.05$) (Table 5).

Table 5: Data of whiteleg shrimp cultured with the polysaccharide extract-containing diets

Growth parameters	*S. microcystum* polysaccharide extract supplemented in diet			
	0%	0.5%	1%	2%
Initial length (cm)	1.3±0.1	1.3±0.1	1.3±0.1	1.3±0.1
Final length (cm)	4.23±0.39^{b}	4.86±0.46^{ab}	5.10±0.14^{a}	4.61±0.32^{ab}
DLG (cm shrimp^{-1} day^{-1})	0.05±0.01^{a}	0.06±0.01^{a}	0.06±0.0^{a}	0.06±0.01^{a}
Initial weight (g shrimp^{-1})	0.08±0.006	0.08±±0.006	0.08±±0.006	0.08±±0.006
Final weight (g shrimp^{-1})	1.08±0.25^{b}	1.37±0.24^{ab}	1.59±0.18^{a}	1.39±0.05^{ab}
WG (g shrimp^{-1})	1.00±0.25^{b}	1.29±0.24^{ab}	1.51±0.18^{a}	1.21±0.05^{ab}
DWG (g shrimp^{-1} day^{-1})	0.017±0.004^{b}	0.022±0.004^{ab}	0.025±0.003^{a}	0.020±0.001^{ab}
SGR (% shrimp^{-1} day^{-1})	4.31±0.42^{b}	4.72±0.31^{ab}	4.98±0.19^{a}	4.63±0.07^{ab}
Survival rate (%)	44.7±20.3^{a}	56.0±6.2^{a}	56.7±11.9^{a}	47.5±15.9^{a}
FCR	1.94±0.91^{a}	1.07±0.18^{ab}	0.86±0.02^{b}	1.36±0.29^{ab}

Data (mean ± SE) in the same row with different letters differ significantly ($p<0.05$)

4 DISCUSSION

Eluvakkal *et al*. (2010) had extracted two Sargassum species as *S. ilicfolium* and *S. marginatum* by 0.1N HCl and obtained yields of more than 20%. In addition, by using the hot-water extract in 3 h, Giang *et al*. (2011b) reported the yield of polysaccharide in *S. hemiphyllum* var. *chinense* was 31%. Moreover, Giang *et al*. (2012; 2013b) also revealed brown seaweed *S. microcystum* had the highest yield of polysaccharide by 0.1N HCl in 3 h (with a mean of 40.2±1.8%). In this study, *S. microcystum* extracted by 0.1N HCl had much lower yield of polysaccharide than that reported by Giang *et al*. (2013b) with the same extraction method. It may be explained that yield of polysaccharide is not depending only on the methods of extraction, but also season of sampling and stage of development of brown seaweed (Jormalainen and Honkanen, 2004). Of interest, it was found that yield of polysaccharide extracted was not only affected by solvent and extraction time but also their interaction. Almost brown seaweed species has low protein concentration. In fact, study of Ruperez *et al.* (2002) on brown seaweed *Fucus vesiculosus* revealed that protein level depended on ecological regions and protein containing at low level (1.0-6.0%). Several papers reported that protein containing in *S. microcystum* varied from 3.6 to 9.3% (Badrinathan *et al*., 2011; Giang *et al*., 2012). The present results are consistent with those reported in previous studies. These results were also demonstrated that fact that protein was significantly affected by extraction time, but not by solvent. Researchers have demonstrated phlorotannins are one of the most effective antioxidants in brown seaweed (Padua *et al*., 2015). Giang *et al*. (2013a) reported phlorotannin concentrations in polysaccharides extracted from brown seaweed *S. mcclurei* ranged from 0.40 to 0.53%. Chowdhury *et al*. (2011) also stated that early stage of seaweeds contains higher level than that of the senescent stage. In the present work, phlorotannin concentrations containing in polysaccharide extracts were relatively higher and varied from 0.48 to 0.84%. Interestingly, the polysaccharide extracted with hot-water within 6 h had significantly higher phlorotannin that caused higher antioxidant activity. It is known that L-Fucose is a rare sugar, one of the eight known bioactive sugars essential for proper cell to cell communication. Seaweed is purported to be one of the best sources of L-fucose. In brown seaweed *Undaria pinnatifida*, Kim *et al*. (2007) reported that concentration of L-fucose was very high and reached 72%. For *S. wightii*, glucose and L-Fucose concentrations were 28.3 and 23%, respectively (Eluvakkal *et al*., 2010). However, Giang *et al*. (2011b) reported that concentration of L-fucose in polysaccharide extracted from *S. hemiphyllum* var. *chinense* were 31.8%. The concentrations of glucose and L-fucose in the present study were relatively lower as compared with previous studies. The results of present study also indicated that the phlorotannin, glucose and SO_4^{2-} concentrations were affected by interaction of solvent and extraction time.

As mentioned above, due to higher phlorotannin concentration, the hot-water extract within 6 h showed higher DPPH$^{\bullet}$ free radical scavenging ac-

tivity and reducing power activity. In the present work, IC_{50} values of hot-water extracts ranged from 0.434 to 0.565 mg mL^{-1} while 0.1N HCl extracts varied from 1.643 to 1.678 mg mL^{-1}. However, it is interesting that the yields of hot-water extract were significant lower than those of 0.1N HCl extracts. Therefore, it is proposed for the solvent extract exhibiting more yield of polysaccharide may lead low antioxidant activity. Previous studies found that DPPH$^{\bullet}$ free radical scavenging activity of polysaccharide extracted from *S. hemiphyllum* by hot-water had IC_{50} of 1.58 mg mL^{-1} (Ye *et al.*, 2008; Hwang *et al.*, 2010). In addition, Giang *et al.* (2012; 2013b) reported IC_{50} values of extracts from *S. microcystum* by hot-water and 0.1N HCl in 3 h were 2.0 and 4.45 mg mL^{-1}. This is surprising that with the same methods of extraction as hot-water and 0.1N HCl in 3 h, the antioxidant activity of extracts from *S. microcystum* in present work were much higher (IC_{50} values of 0.57 and 1.64 mg mL^{-1}, respectively). This refers that seasons and nutrient level in environment might affect bioactivity of polysaccharide of seaweed. Hence, further research is imperatively needed to evaluate variation of antioxidant activity with fluctuation of salinity, light intensity, or settlement density of the brown seaweed.

In aquatic animals, a variety of brown seaweed have often been regarded as one of the most promising candidates as an alternative source of nutrients for aquafeeds in earlier studies (Immanuel *et al.*, 2004; Cruz-Suarez *et al.*, 2009; Immanuel *et al.*, 2010; Qi *et al.*, 2010; Sivagnanavelmurugan *et al.*, 2014; Niu *et al.*, 2015 Sivagnanavelmurugan *et al.*, 2015; Peixoto *et al.*, 2016). However, most of the previous studies focused on uses of seaweed meals in aquafeeds to replace animal ingredients, although the average protein content of seaweeds is low. In Penaeid shrimp, polysaccharide extracted from seaweed have been widely used in promoting immune response and disease resistance (Yeh *et al.*, 2006; Giang *et al.*, 2011b; Immanuel *et al.*, 2012; Yudiati *et al.*, 2016), whereas some studies evaluated their effects on growth performance. For instance, India whiteleg shrimp cultured in the environment inoculating with *Vibrio parahaemolyticus* at 10^7 CFU mL^{-1} and then fed with butanolic extracts from brown seaweed *S. wightii* enriched *Artemia* for 30 days showed higher SR, WG and SGR than those of control shrimp (Immanuel *et al.*, 2004). In addition, in Kuruma shrimp (1.17±0.08 g), Traifalgar *et al.* (2010) reported that significantly higher WG and SGR were observed in treatment groups fed the 0.05 and 0.1% dietary polysaccharide extracted from brown seaweed *U. pinnatifida* (70% fucoidan having 40% of fucose and 23.5% of sulfate content) supplementation when compared with control group after 56 days of feeding. Moreover, Immanuel *et al.* (2010) reported that tiger shrimp (PL_{15}) fed the *Artemia* nauplii enriched with seaweed polysaccharide extracts of *S. duplicatum* and *S. wightii* at various concentrations (250, 500 and 750 mg L^{-1}) for 20 days had significantly higher WG and SGR than those of control group. Also, growth of tiger shrimp (PL_{15}) that being fed *S. wightii* fucoidan enriched *Artemia* nauplli at 400 mg L^{-1} was significantly higher than that of control shrimp (Sivagnanavelmurugan *et al.*, 2015). By incorporating with the pellet feed, Traifalgar *et al.* (2009) reported that tiger shrimp postlavae fed diets supplemented with graded levels of *U. pinnatifida* fucoidan at concentration of 0.05-0.2% significantly improved growth performance after 30 days of feeding. However, there was no significant difference in survival rate. A study of Sivagnanavelmurugan *et al.* (2014) also revealed that tiger shrimp (PL_{20}) were fed the diet containing ethanolic polysaccharide extract from *S. wightii* at three concentrations of 0.1, 0.2 and 0.3% for 60 days had higher WG and SGR as compared with those of control group. The previous studies concluded that the growth performance of experimental groups of shrimp was depending on the concentrations of polysaccharide extracts in the diets. In the present study, the concentrations of polysaccharide incorporated with pellet feed are relatively higher (0.5, 1.0 and 2.0%), and growth performance of experimental shrimp was slightly decreased after being fed the diet containing 2% of polysaccharide.

Information in improvement of growth performance of whiteleg shrimp via dietary polysaccharide extracted from brown seaweed is limited. Several studies have been evaluated for uses of brown seaweed meals in improvement of whiteleg shrimp growth performance (Cruz-Suarez *et al.*, 2009; Cardenas *et al.*, 2015). Whiteleg shrimp juvenile that being fed with diets containing 3.3% seaweed meals, made from brown seaweed *Macrocytis pyrifera*, *Ascophyllum nodosum* or green seaweed *Ulva clathrate*, for 28 days had high survival rate (ranging from 90-100%). WG were 2.7, 2.97 and 3.2 g and FCR were 2.10, 1,92 and 1.71 for the shrimp fed the diets containing *M. pyrifera*, *A. nodosum* and *U. clathrata*, respectively (Cruz-Suarez *et al.*, 2009). Recently, Cardenas *et al.* (2015) reported that whiteleg shrimp fed the diets containing powdered mixtures of the genera *Macrocystis*, *Lessoniaceae* and *Lessonia* at 4 and 8% insignificantly improved growth performance as compared with control group. Moreover, a study of Tien *et al.* (2013) reported that white shrimp cul-

tured in recirculation water system with density of 1,000 shrimp m^{-3} had DWG in a range of 11.9-12.0 g day^{-1} and SGR in range of 3.88-3.90 % day^{-1}. As compared with these results, the survival and WG in the present study were relatively lower. The highest shrimp survival rate in study of Cardenas *et al.* (2015) was 93.3±9.4% (treatment 8%), whereas the highest one in the present study was only 56.7±11.9% (treatment 1%). This can be explained differences in initial weight of shrimp and brown seaweed forms (meal vs extract), resulting in difference in growth performance of experimental shrimp. On the other hand, as observation at the last two weeks of experimentation, mortalities in treatments occurred due to cannibalism immediately after the molting that caused variations and lowering survival rates in treatments.

Polysaccharide extracted brown seaweed *S. microcystum* showed positive effect on improvement of growth performance of whiteleg shrimp. However, understanding on how polysaccharides extracted from brown seaweed participating in improvement of aquatic animals' growth still remain largely unknown. Azad *et al.* (2005) stated that polysaccharide extracts might be attributed to the efficient nutrient digestion and assimilation caused by the activation of fixed phagocytes in the hepatopancreas that secrete hydrolytic enzymes in the digestive gland. However, Immanuel *et al.* (2004) explained that growth and survival may be attributed to the antimicrobial activity of seaweed extracts, whereas Traifalgar *et al.* (2009) have demonstrated as fucose-enriched sulfated polysaccharides supplementation can promote growth. However, high level of seaweed meal or its extract may lead lower growth performance because high polysaccharide contents might affect digestibility of protein and dry matter (Burtin, 2003). In addition, Potty (1996) noted that fiber structures possibly reduce the accessibility of intestinal enzymes to food nutrients, thereby acting as physical hindrances between nutrients and digestive enzymes in the intestine. Similarly, higher supplementation levels of polysaccharide from seaweed have been observed to block the efficacy of beneficial compounds and inhibit digestion (Nakagawa and Montgomery, 2007). In interest, Niu *et al.* (2015) also revealed that shrimp fed higher supplementation levels of seaweed had lower protein efficiency resulted in a progressive decline in growth performance. Hot-water extract of *S. microcystum* showed high antioxidant activity that plays an important role in removing reactive oxygen species (ROS) such as singlet oxygen, peroxy nitrite or hydrogen peroxide; generally produced by endogenous and exogenous factors. An antioxidant compound promotes health benefits through scavenging the free radicals that cause far-reaching oxidative damage to healthy cells by reacting with their nucleic acids, proteins, lipids and enzymes (Vinayak *et al.*, 2011). At optimal concentrations, however, antioxidants may stimulate intestinal beneficial bacteria by dietary. Antioxidants containing tannins and alkaloids may exert an antibacterial influence and should be preceded by careful studies about their influence via oral administration (Duda-Chodak *et al.*, 2008). That may explain why whiteleg shrimp that being fed polysaccharide at 2% slightly declined growth performance in present study.

5 CONCLUSIONS

Polysaccharide extracted from brown seaweed *S. microcystum* by hot-water within 6 h showed the highest antioxidant activity. Whiteleg shrimp that being fed the commercial feed incorporating with hot-water extract 1.0% had significantly higher growth performance than that of control group under indoor culture condition. Therefore, *S. microcystum* extract could be considered as a growth-promoting factor in whiteleg shrimp. However, the actions of mechanism of polysaccharide on growth of shrimp should be elucidated. Further researches are also needed to study the binding of polysaccharide as well as its effect growth and survival of shrimp under various challenged factors.

ACKNOWLEDGEMENTS

This research was supported by grants (B2014-16-36) from Ministry of Education and Training, Vietnam. We appreciate Mr. Ngoc-Son Chau, Ms. Thi-Cam-Tu Phan and Ms. Phuong-Trang Nguyen for their assistances with the experiment.

REFERENCES

AOAC, 2001. Official Methods of Analysis. Association of Official Analytical Chemists Arlington.

APHA, AWWA, WEF, 1999. Standard methods for the examination of water and wastewater, 19th edition. American Public Health Association 1015 Fifteenth Street, NW Washington, DC 20005.

Azad, I.S., Panigrahi, A., Gopal, C., Paulpandi, S., Mahima, C., Ravichandran, P., 2005. Routes of immunostimulation vis-a-vis survival and growth of *Penaeus monodon* postlarvae. Aquaculture. 248: 227-234.

Badrinathan, S., Suneeva, S.C., Shiju, T.M., Girish-Kumar, C.P., Pragasam, V., 2011. Exploration of a novel hydroxyl radical scavenger from *Sargassum myriocystum*. Journal of Medicinal Plants Research. 5: 1997- 2005.

Burtin, P., 2003. Nutritional value of seaweeds. Journal of Agriculture and Food Chemistry. 2: 498-503.

Cardenas, J.V., Galvez, A.O., Brito, L.O., Galarza, E.V., Pitta, D.C., Rubin, V.V., 2015. Assessment of differ-

ent levels of green and brown seaweed meal in experimental diets for whiteleg shrimp (*Litopenaeus vannamei*, Boone) in recirculating aquaculture system. Aquaculture International. 23: 1491-1504.

Chowdhury, T.T.H., Bangoura, I., Kang, J.Y., Park, N.G., Ahn, D.H., Hong, Y.K., 2011. Distribution of Phlorotannins in the brown alga *Ecklonia cava* and comparison of pretreatments for extraction. Fisheries and Aquatic Sciences. 14: 198-204.

Cruz-Suarez, L.E., Tapia-Salazar, M., Nieto-Lopez, M.G., Guajardo-Barbosa, C., Ricque-Marie, D., 2009. Comparison of *Ulva clathrata* and the kelps *Macrocystis piryfera* and *Ascophyllum nodosum* as ingredients in shrimp feeds. Aquaculture Nutrition. 15: 421-430.

Duan, X.J., Zhang, W., Li, X.M., Wang, B.G., 2006. Evaluation of antioxidant property of extract and fractions obtained from a red alga *Polysinophia urceolata*. Food Chemistry. 95: 37-43.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28: 350-356.

Duda-Chodak, A., Tarko, T., Statek, M., 2008. The effect of antioxidants on *Lactobacillus casei* cultures. Acta Scientiarum Polonorum Technologia Alimentaria. 7: 39-51.

Eluvakkal, T., Sivakumar, S.R., Arunkumar, K., 2010. Fucoidan in some Indian brown seaweeds found along the Coast Gulf of Mannar. International Journal of Botany. 6: 176-181.

Giang, H.T., Giang, T.T., Oanh, D.T.H., Phu, T.Q., 2016. Assessment of nutritional value and antioxidant activity of polysaccharide extracts from brown seaweed *Sargassum flavicans* for aquaculture use. Can Tho University Journal of Science. In press.

Giang, H.T., Oanh, D.T.H, Tam, N.T., 2012. Characteristic of polysaccharides extracted from some brown algae *Sargassum* (Phaeophyta) and potential use in aquaculture. Can Tho University. 86 pp. (In Vietnamese)

Giang, H.T., Oanh, D.T.H, Ut, V.N., 2013a. Bioactivity of polysaccharide extracted from *Sargassum mcclurei* by different solvents. Collection of Marine Research Works. 19: 124-133. (In Vietnamese)

Giang, H.T., Oanh, D.T.H, Ut, V.N., Phu, T.Q., 2013b. Chemical composition, antioxidant activities of polysacharide extracts from brown seaweed *Sargassum microcystum*. Scientific Journal of Can Tho University. 25: 183-191. (In Vietnamese)

Giang, H.T., Ut, V.N., Phu, T.Q., 2011a. Use of β-glucan extracted from seaweed in shrimp immunity enhancement: a review. Scientific Journal of Can Tho University. 4: 103-113. (In Vietnamese)

Giang, H.T., Yeh, S.T., Lin, Y.C., Shyu, J.F., Chen, L.L., Chen, J.C., 2011b. White shrimp *Litopenaeus vannamei* immersed in seawater containing *Sargassum hemiphylum* var. *chinense* powder and its extract showed increased immunity and resistance against *Vibrio alginolyticus* and white spot syndrome virus. Fish and Shellfish Immunology. 31: 286-293.

Huang, X., Zhou, H., Zhang, H., 2006. The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp, *Fenneropenaeus chinensis*. Fish and Shellfish Immunology. 20: 750-757.

Hwang, P.A., Wu, C.H., Gau, S.Y., Chien, S.Y., Hwang, D.F., 2010. Antioxidant and immune-stimulating activities of hot-water extract from seaweed *Sargassum hemiphyllum*. Journal of Marine Science and Technology. 18: 41-46.

Immanuel, G., Sivagnanavelmurugan, M., Balasubramanian, V., Palavesam, A., 2010. Effect of hot water extracts of brown seaweeds *Sargassum* spp. on growth and resistance to white spot syndrome virus in shrimp *Penaeus monodon* postlarvae. Aquaculture Research. 41: 545-553.

Immanuel, G., Sivagnanavelmurugan, M., Marudhupandi, T., Radhakrishnan, S., Palavesam, A., 2012. The effect of fucoidan from brown seaweed Sargassum wightii on WSSV resistance and immune activity in shrimp *Penaeus monodon* (Fab). Fish and Shellfish Immunology. 32: 551-564.

Immanuel, G., Vincybai, V.C., Sivaram, V., Palavesam, A., Marian, M.P., 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquaculture. 236: 53-65.

Jormalainen, V., Honkanen, T., 2004. Variation in natural selection for growth and phlorotannins in the brown alga *Fucus vesiculosus*. Journal of Evolution Biology. 17: 807-820.

Kim, W.J., Kim, S.M., Kim, H.G., Oh, H.R., Lee, K.B., Lee, Y.K., Park, Y.I., 2007. Purification and anticoagulant activity of a fucoidan from Korean *Undaria pinnatifida* Sporophyll. Algae. 22: 247-252.

Kloareg, B., Quatrano, R.S., 1988. Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharide. Oceanography Marine Biology Annual Review. 26: 259-315.

Koivikko, R., Loponen, J., Honkanen, T., Jormalainen, V., 2005. Contents of soluble, cell wall bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. Journal of Chemistry Ecology. 31: 195-212.

Masser, M.P., Rakocy, J., Losordo, T.M., 1999. Recirculating aquaculture tank production systems: Maenagement of Recriculating Systems. SRAC Publication No. 452. 12 pp.

Nakagawa, H., Montgomery, L.W., 2007. Algae. In: Nakagawa, H., Sato, M., Gatlin, D.M. (Eds.), Dietary Supplements for the Health and Quality of Cultured Fish. CAB International, Oxon, pp. 133-162.

Niu, J., Chen, X., Lu, X., Jiang, S.G., Lin, H.Z., Liu, Y.J., Huang, Z., Wang, J., Wang, Y., Tian, L.X., 2015. Effects of different levels of dietary wakame (*Undaria pinnatifida*) on growth, immunity and in-

testinal structure of juvenile *Penaeus monodon*. Aquaculture. 435: 78-85.

Oyaizu, M., 1988. Antioxidative activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakkaishi. 46: 571-575.

Padua, D., Rocha, E., Gargiulo, D., Ramos, A.A., 2015. Bioactive compounds from brown seaweeds: Phloroglucinol, fucoxanthin and fucoidan as promising therapeutic agents against breast cancer. Phytochemistry Letters. 14: 91-98.

Paulsen, B.S., 2002. Biologically active polysaccharides as possible lead compounds. Phytochemistry Reviews. 1: 379-387.

Peixoto, M.J., Salas-Leiton, E., Pereira, L.F., Queiroz, A., Magalhaes, F., Pereira, R., Abreu, H., Reis, P.A., Goncalves, J.F.M., Ozorio, R.O.A., 2016. Role of dietary seaweed supplementation on growth performance, digestive capacity and immune and stress responsiveness in European seabass (*Dicentrarchus labrax*). Aquaculture Reports. 3: 189-197.

Potty, H.V., 1996. Physico-chemical aspects, physiological functions, nutritional importance and technological significance of dietary fibres - a critical appraisal. Journal of Food Science and Technology. 33: 1-18.

Qi, Z., Liu, H., Li, B., Mao, Y., Jiang, Z., Zhang, J., Fang, J., 2010. Suitability of two seaweeds, *Gracilaria lemaneiformis* and *Sargassum pallidum*, as feed for the abalone *Haliotis discus hannai Ino*. Aquaculture. 300: 189-193.

Ruperez, P., Ahrazem, O., Leal, J.A., 2002. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. Journal of Agricultural Food Chemistry. 50: 840-845.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agriculture and Food Chemistry. 40: 945-948.

Sivagnanavelmurugan, M., Thaddaeus, B.J., Palavesam, A., Immanuel, G., 2014. Dietary effect of *Sargassum wightii* fucoidan to enhance growth, prophenoloxidase gene expression of *Penaeus monodon* and immune resistance to *Vibrio parahaemolyticus*. Fish and Shellfish Immunology. 39: 439-49.

Sivagnanavelmurugan, M., Ramnath, G.K., Thaddaeus, B.J., Palavesam, A., Immanuel, G., 2015. Effect of *Sargassum wightii* fucoidan on growth and disease resistance to *Vibrio parahaemolyticus* in *Penaeus monodon* postlarvae. 21: 960-969.

Terho, T.T., Hartiala, K., 1971. Method for determination of the sulfate content of Glycosaminoglycan. Analytical Biochemistry. 41: 471-476.

Tien, N.V., Chi, N., Phuong, L.H., Truc, V.L.T., Hai, T.N., 2013. Super-intensive culture of white leg shrimp (*Litopenaeus vannamei*) in recirculating system. Journal of Agriculture and Rural Development. 12: 259-265.

Traiflgar, R.F.M., Serrano, A.E., Corre, V.L., Kira, H., Tung, H.T., Michael, F.R., Kader, M.A., Laining, A., Yokoyama, S., Ishikawa, M., Koshio, S., 2009. Evaluation of dietary fucoidan supplementation effects on growth performance and vibriosis resistance of *Penaeus monodon* postlarvae. Aquaculture Science. 57: 167-174.

Traiflgar, R.F.M., Kira, H., Tung, H.T., Michael, F.R., Laining, A., Yokoyama, S., Ishikawa, M., Koshio, S., 2010. Influence of dietary fucoidan supplementation on growth and immunological response of juvenile *Marsupenaeus japonicus*. Journal of World Aquaculture Society. 41: 234-244.

Vinayak, R.C., Sabu, A.S., Chatterji, A., 2011. Bioprospecting of a few brown seaweeds for their cytotoxic and antioxidant activities. Evidence-Based Complementary and Alternative Medicine. 9 pp.

Wood, C.G., 1974. Seaweed extract - a unique ocean resource. Journal of Chemistry Education. 51: 449-452.

Ye, H., Wang, K., Zhou, C., Liu, J., Zeng, X., 2008. Purification, antitumor and antioxidant activities *in vitro* of polysaccharides from the brown seaweed *Sargassum pallidum*. Food Chemistry. 111: 428-432.

Yeh, S.T., Lee, C.S., Chen, J.C., 2006. Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune resistance of white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology. 20: 332-345.

Yudiati, E., Isnansetyo, A., Murwantoko, Ayuningtyas, Triyanto, Retna, C., 2016. Handayani Innate immune-stimulating and immune genes up-regulating activities of three types of alginate from *Sargassum siliquosum* in pacific white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology. 54: 46-53.

Zvyagintseva, T.N., Shevchenko, N.M., Popivnich, I.B., Isakov, V.V., Scobun, A.S., Sundukova, E.V., Elyakova, L.A., 1999. A new procedure for the separation of water-soluble polysaccharides from brown seaweeds. Carbohydrate Research. 322: 32-39.