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Components and Antimicrobial Activity of Polysaccharides Extracted from Thai Brown Seaweeds

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ABSTRACT

Seven species of brown seaweed, *Colpomenia sinuosa* (CS), *Hydroclathrus clathratus* (HC), *Dictyota dichotoma* (DD), *Padina australis* (PA), *P. minor* (PM), *Sargassum polycystum* (SP) and *Turbinaria conoides* (TC) were collected from Chon Buri (01), Chumphon (02) and Rayong (03) provinces. Polysaccharides were extracted by boiling the dried seaweed samples in water at 100°C (E1) for 2 h and at 75°C, adjusted to pH 3 by addition of 2 mol/l HCl (E2), for 5 h. The total polysaccharide yield, total carbohydrate, sulfate content and sugar components of the E1- and E2-extracts were determined. The E1-extract of SP03 gave a maximum yield of 1.97±1.15% (w/w). The greatest percentage of carbohydrate (55.95±0.72%) was obtained from the E1-extract of PM02. The maximum sulfate content (18.10±0.25%, w/w) was obtained from HC01. For the E2 extraction, the maximum yield (19.69±0.23%, w/w) was obtained from HC01, while the greatest percentage of total carbohydrate obtained was 44.41±0.94% (w/w) in PM02. The sulfate content was high in the E2-extract from CS01 (14.22±0.69%). Fucose was identified as a main component in all extracts, with the highest percentage in HC01 (12.35%). Mannose was observed in samples from CS01, PA03, PM02, SP01 and SP03, but not from DD02, HC01, PA01 PA02 or TC02. Furthermore, regarding the antimicrobial activity determined in both extracts at 2 mg/ml, only the E1- and E2-extracts of SP01 and the E2-extract of CS01 demonstrated activity against *Candida albicans*. In contrast, most of the E1- and E2-extracts were not only inactive against the microorganisms that were tested, but actually promoted microbial growth, particularly in the case of extracts that contained mannose.

Abbreviation: CS = *Colpomenia sinuosa,* HC = *Hydroclathrus clathratus,* DD = *Dictyota dichotoma,* PA = *Padina australis,* PM = *Padina minor,* SP = *Sargassum polycystum* and TC = *Turbinaria conoides*
Code: 01 = Chon Buri, 02 = Chumphon and 03 = Rayong
Key words: antimicrobial activity, acid extraction, hot water extraction, polysaccharide, Thai brown seaweed

INTRODUCTION

Species of brown seaweed are well known to contain large amounts of cell-wall polysaccharides, most of which are the sulfated polysaccharide, fucoidan (Asker *et al*., 2007), which is not found in terrestrial plants. Fucoidan has a substantial component of L-fucose and sulfate ester groups (Bilan *et al*., 2002) and has a wide range of pharmacological and biomedicinal

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properties (Güven *et al*., 1999). There have been several studies on the diverse bioactivities, molecular weights, structural parameters and physiological characteristics of seaweed polysaccharides (Asker *et al*., 2007; Wang *et al*., 2007). Recently, aqueous crude extract of the brown seaweed, *Hydroclathrus clathratus*, from Hong Kong, has been reported to have high antiviral activity against the herpes simplex virus (HSV) and low cytotoxicity to Vero and HEp-2 cells (Wang *et al*., 2007). The antiviral activities of the sulfated polysaccharides extracted from *Sargassum latifolium* have also been reported by Asker *et al*. (2007), who found that the activity was dependent on the degree of sulfation and the molecular weight. Most of the seaweed polysaccharides isolated using the acid extraction method are crude fucoidans (Becker and Lowe, 2003). The polysaccharide yields extracted from brown seaweed species depended on the algal species and the extraction method (Asker *et al*., 2007; Wang *et al*., 2008), as well as on environmental factors (Zvyagintseva *et al*., 2003).

There have been reports on the antimicrobial activity of marine algae in Thailand (Chotigeat *et al*., 2004; Kaewsrithong *et al*., 2007). The crude fucoidan extracted from *Sargassum polycystum* has been observed to reduce the impact of white spot syndrome virus (WSSV) infection in *Penaeus monodon* (Chotigeat *et al*., 2004). However, there is little published information on the polysaccharide content of Thai seaweeds and in particular, on the yield and chemical components of brown seaweeds. Therefore, this study was conducted to determine the yield and analyze the polysaccharide components extracted from a selection of brown seaweeds in Thailand. In addition, the antimicrobial activity of each extract was tested.

MATERIALS AND METHODS

Preparation of the algal sample

Seven species of brown seaweed, *Colpomenia sinuosa* (CS), *Hydroclathrus clathratus* (HC), *Dictyota dichotoma* (DD), *Padina australis* (PA), *P. minor* (PM), *Sargassum polycystum* (SP) and *Turbinaria conoides* (TC) were collected from Samaesan district in Chon Buri province (01), Haad Thung Wua Laen in Chumphon province (02), and Ban Phe in Rayong province (03). The samples were washed in seawater to remove sand, mud and all epiphytes, rinsed with tap water and dried at room temperature. The dried samples were ground separately into powder using a miller before extraction of the crude polysaccharides.

Extraction of the crude polysaccharides

Hot water extraction (E1)

The algal powder was boiled in distilled water (1:5 w/v) at 100°C for 2 h using a reflux condenser under reduced pressure. The hot extract was filtered with a nylon mesh bag (pore size 24 μm) and sequentially filtered with 11, 8 and 0.45 μm Millipore filters. The filtrate was condensed using a rotary evaporator (BÜCHI Rotavapor R 200) to a minimal volume and then freeze-dried. The extract obtained was referred to as E1 crude extract polysaccharide (E1-extract). The polysaccharide yield of the E1-extract was calculated as percent dry weight of the seaweed material (w/w).

Hot acid extraction (E2)

This extraction method was modified from that described by Ozawa *et al*. (2006). The algal powder was boiled in distilled water, at 75°C adjusted to pH 3 by addition of 2.0 mol/l HCl, for 5 h using a reflux condenser under reduced pressure. The hot extract solution was filtered with a nylon mesh bag (pore size 24 μm) and sequentially filtered with 11, 8 and 0.45 μm

Millipore filters. The filtrate was condensed using a rotary evaporator (BÜCHI Rotavapor R 200) to a minimal volume, and then freeze-dried. The extract obtained was referred to as E2 crude polysaccharide (E2-extract). The yield of the E2 crude polysaccharide was calculated as percent dry weight of the seaweed material (w/w).

Purification

The crude extract polymers were dissolved in distilled water (1:20 w/v) at 40°C and centrifuged at 3,000 rpm. The supernatant was dialyzed using dialysis tubing (6-8,000 dt molecular weight cut off) against two volumes of distilled water for 24 h at room temperature and then freeze-dried.

Analysis of the chemical components

Total carbohydrate and sulfate contents

The total carbohydrate content was analyzed by the phenol sulfuric acid method (Dubois *et al*., 1956), using L-fucose as the standard. The sulfate content was determined after acid hydrolysis (2 N HCl at 100°C for 2 h) of the polysaccharides, according to the gelatin-barium method, using potassium sulfate as the standard (Craigie *et al*., 1984).

The sugar component

The sugar component of the crude extract was analyzed using a high performance liquid chromatography (HPLC) system equipped with a pump (Attech 626), detector (Attech ELSD 2000 ES), and column (Lichrocart NH_2 250 × 4 mm); the mobile phase was 85% acetonitrile: 15% H_2O and a flow rate of 2 ml/min was used.

The purified crude polysaccharide (2 mg) was hydrolyzed with 0.5 ml of 2 N HCl at 100°C for 2 h. The hydrolysate was washed with distilled water to remove the acid, then evaporated using the rotary evaporator, then washed again three times or until all the acid was removed. The hydrolysate was then kept in a brown screw-capped vial before monosugar analysis using glucose, fructose, fucose, galactose and mannose as standards.

Assay of the antimicrobial activity of the crude polysaccharide

The antimicrobial activity of the crude polysaccharide was assayed using the paper disc diffusion method. Test microorganisms were obtained from the Aquatic Animal Health Institute, Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangkok and comprised: one species of gram-positive bacteria (*Bacillus subtillis* TISTR 008), six species of gram-negative bacteria (*Aeromonas hydrophilla* TISTR 1321, *Escherichia coli* TISTR 780, *Pseudomonas aeruginosa* TISTR 781, *Vibrio alginolyticus* TISTR 1572, *V. parahaemolyticus* and *V. harveyi*), one yeast (*Candida albicans* TISTR 5779) and one fungus (*Aspergillus niger* TISTR 3254).

The test microorganisms were first cultured on 100 ml of Mueller Hinton broth before inoculation. The gram-positive bacteria were cultured at 30°C for 24 h. Gram-negative bacteria were cultured for 24 h at varying temperatures depending on the species, i.e., 30°C for *A. hydrophilla* TISTR 1321, 32°C for *V. parahaemolyticus* and *V. harveyi* and 37°C for *V. alginolyticus* TISTR 1572. The yeast, *C. albicans* TISTR 5779, and the fungi, *A. niger* TISTR 3254, were cultured at 30°C for 48 h. All test microorganisms were washed in 0.85% NaCl solution and centrifuged at 4,500 rpm, once for 30 min and twice for 10 min. The microorganisms were kept in NaCl solution before measuring their optical density (OD) at 540 nm, after which their OD was adjusted to 0.14. The suspension contained 1×10^8 cells/ml. The microorganism suspension was then diluted 1:10 to obtain a cell concentration of 3×10^6 cells/ml. The microorganism suspension was smeared thoroughly on the test media before assaying the

activity of the polysaccharide.

The crude extracts were dissolved and diluted in distilled water to concentrations of 2, 1, 0.5, 0.25 and 0.125 mg/ml. The crude extracts were then dropped (50 µl/piece) onto sterile filter paper discs (5 mm diameter) and dried for 30 min, prior to the antimicrobial activity assay. These discs were then placed onto the surface of the solidified culture media containing the microorganisms in Petri dishes (6 pieces/dish). The dishes were then incubated under the same conditions as mentioned above. The activity assays were replicated in triplicate. After incubation, the clearance zones around the discs were measured in millimeters. Two antiseptics, gentamicin and nystain, were used as controls. Gentamicin was used against gram-positive and gram-negative bacteria, and nystain was used against yeast and fungi.

Statistical analysis

The data are presented as means ± standard deviation (SD) and the differences in the results obtained between species were analyzed utilizing analysis of variance (ANOVA).

RESULTS

Yield of crude polysaccharide

The yields of the crude polysaccharides obtained from the hot water (E1) and acid (E2) extractions are shown in Figure 1. The E1 extraction process gave varying yields of crude polysaccharide, which depended on the species of seaweed used. The E1-extract of SP03 had the highest yield, at 1.97±1.15%, which was higher than those of the same species collected from Chon Buri province (SP01). In contrast, the yields of the E1-extracts were not significantly different among specimens of PA collected from different habitats, while between species there was a significant difference ($p<0.05$).

In this study, yields of the E2-crude extracts of the brown seaweed samples from CS01, HC01, PA01 and SP01, were 4.33±0.18, 19.69±0.23, 11.79±0.19 and 5.06±0.27% (w/w), while DD02, PA02, PM02 and TC02 yielded

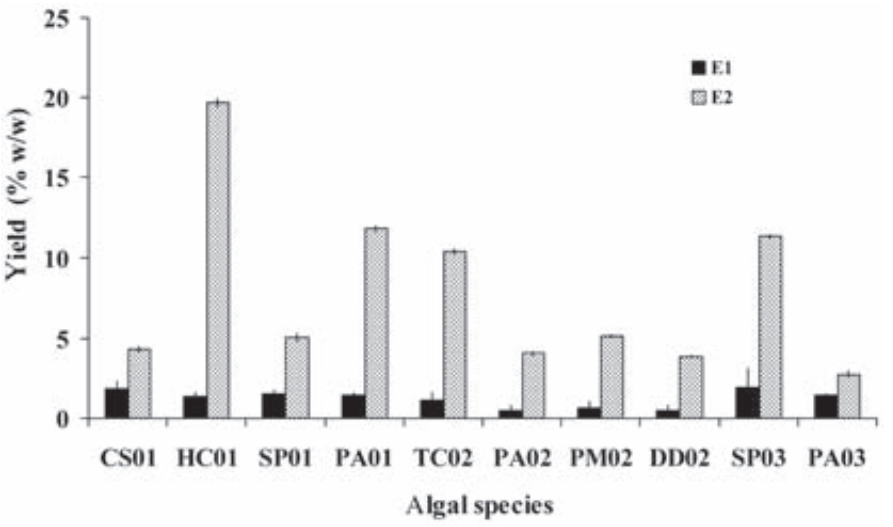

Figure 1 Yield of crude polysaccharides extracted in water at 100°C (E1) and at 75°C adjusted to pH 3 by addition of 2 mol/l HCl (E2) from brown seaweeds. CS = *Colpomenia sinuosa,* DD = *Dictyota dichotoma*, HC = *Hydroclathus clathratus,* SP = *Sargassum polycystum*, PA = *Padina australis*, PM = *Padina minor*, TC = *Turbinaria conoides*, collected from Chon Buri (01), Chumphon (02), and Rayong (03) provinces. The vertical bar represents the standard deviation.

3.87±0.04, 4.06±0.10, 5.15±0.08 and 10.37±0.15% (w/w), respectively. The extract from SP03 yielded 11.32±0.12% (w/w), which was higher than that for the SP01 sample from Chon Buri. The yield from the extract of PA03 (collected from Rayong province) was lower (2.77±0.19% w/w) than that of PA01. The yields of crude polysaccharide from the E2 samples of each species were significantly different ($p<0.05$).

Chemical components

Total carbohydrate content

The crude polysaccharide extracted using the E1 method from the CS01, HC01, PA01 and SP01 samples had total carbohydrate contents of 34.55±0.21, 38.04±0.10, 40.07±0.18 and 40.10±0.23% (w/w), respectively. The extracts from the DD02, PA02, PM02 and TC02 samples, had values of 43.06±0.09, 49.40±0.50, 55.95±0.72 and 32.15±0.18% (w/w), respectively. The extracts from the SP03 samples had a total carbohydrate content of 40.25±0.24%, which was not significantly different from that of SP01. In contrast, the crude polysaccharide extracted from the PA03 samples had a total carbohydrate content of 51.94±0.08% (w/w), higher than that of PA01. The highest carbohydrate content was 55.95±0.72% (w/w), obtained from the crude polysaccharide in the PM02 samples, while the lowest value was 32.15±0.18% (w/w), obtained from the extracts of the TC02 samples (Figure 2). The carbohydrate contents of each species were significantly different ($p<0.05$).

For the polysaccharides extracted using the E2 method from the CS01, HC01, PA01 and SP01 samples (collected from Chon Buri province), the total carbohydrate contents were 36.72±0.55, 10.46±0.52, 33.64±0.67 and 23.91±0.29% (w/w), respectively. The crude extracts from the Chumphon samples, DD02, PA02, PM02 and TC02, had total carbohydrate contents of 33.95±0.78, 35.10±0.86, 44.41±0.94 and 21.46±0.71% (w/w), respectively. The crude polysaccharide extracts of the brown seaweed

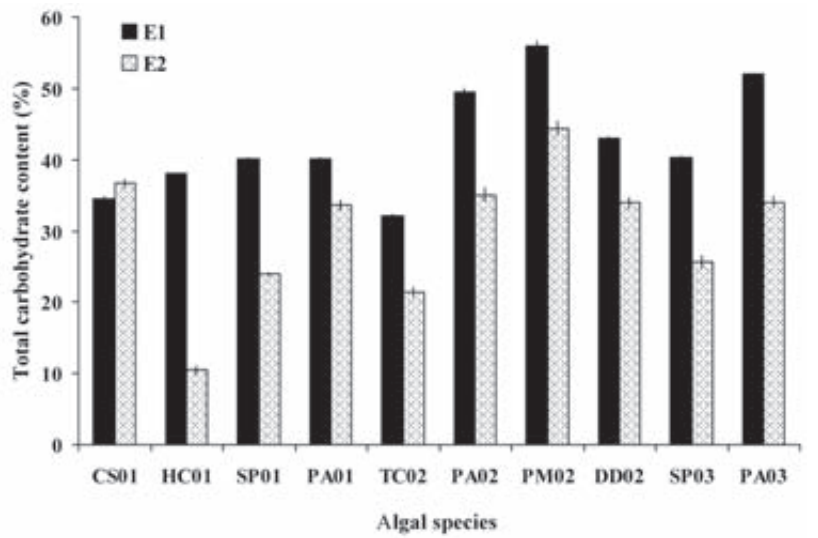

Figure 2 Total carbohydrate content of crude polysaccharides extracted in water at 100°C (E1) and at 75°C adjusted to pH 3 by addition of 2 mol/l HCl (E2) from brown seaweeds. CS = *Colpomenia sinuosa,* DD = *Dictyota dichotoma*, HC = *Hydroclathus clathratus,* SP = *Sargassum polycystum*, PA = *Padina australis*, PM = *Padina minor*, TC = *Turbinaria conoides*, collected from Chon Buri (01), Chumphon (02), and Rayong (03) provinces. The vertical bar represents the standard deviation.

samples collected from Rayong province had total carbohydrate contents of 25.68±0.91% w/w (SP03) and 34.07±0.70% w/w (PA03), which were slightly higher than those of the Chon Buri seaweeds. The total carbohydrate content was highest in the polymers of PM02 from Chumphon and lowest in HC01 from Chon Buri province (Figure 2). The carbohydrate contents obtained from each species were significantly different ($p<0.05$).

Sulfate content

The E1-crude polysaccharide extracts had varying sulfate contents that were dependent on the species of the brown seaweed sampled. The sulfate content of the Chon Buri seaweed samples was 5.83±0.28, 18.10±0.25, 8.97±0.09 and 11.09±0.17% (w/w), respectively, in CS01, HC01, PA01 and SP01. The sulfate content of samples of DD02, PA02, PM02 and TC02 from Chumphon province was 7.37±0.54, 7.95±0.43, 9.16±0.35 and 10.19±0.20% (w/w), respectively. For samples collected from Rayong, the sulfate content was 10.33±0.42 and 9.10±0.10% (w/w) in SP03 and PA03, respectively. The sulfate content was highest at 18.10±0.25% (w/w) in HC01 and lowest in CS01 (Figure 3). The sulfate contents obtained from each species showed significant differences ($p<0.05$).

The E2-crude polysaccharide extracts showed sulfate contents of 14.22±0.69, 13.82±0.18, 9.88±0.20 and 10.90±0.85% (w/w), respectively, in CS01, HC01, PA01 and SP01, while that from DD02, PA02, PM02 and TC02 contained 9.74±1.49, 10.10±0.65, 12.75±0.10 and 10.25±0.13% (w/w), respectively. For the samples of SP03 and PA03, the crude extracts had sulfate contents of 11.13±0.22% and 11.00±0.13% (w/w), respectively. The highest sulfate content was obtained from the crude extract of CS01 (14.22±0.69% w/w) and the lowest two readings were obtained from DD02 (9.74 ± 1.49% w/w)

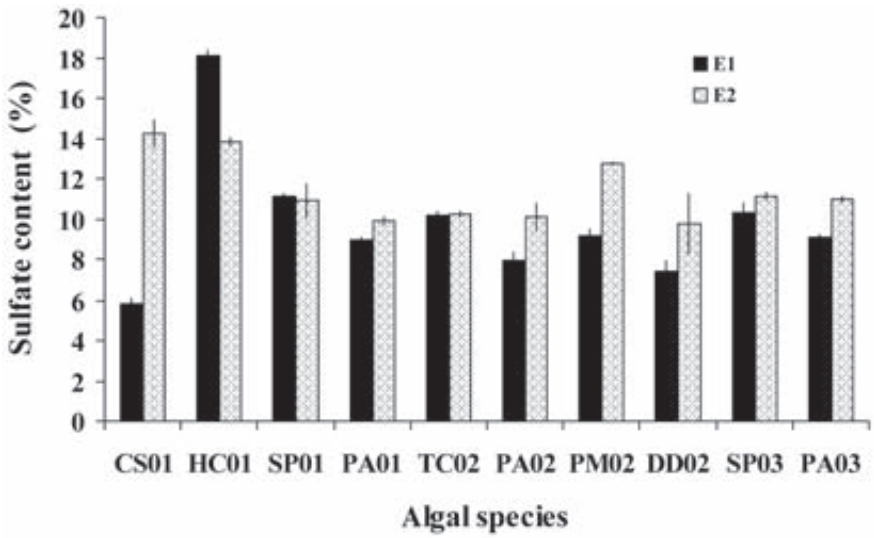

Figure 3 Sulfate content of crude polysaccharides extracted in water at 100°C (E1) and at 75°C adjusted to pH 3 by addition of 2 mol/l HCl (E2) from the brown seaweeds. CS = *Colpomenia sinuosa,* DD = *Dictyota dichotoma*, HC = *Hydroclathus clathratus,* SP = *Sargassum polycystum*, PA = *Padina australis*, PM = *Padina minor*, TC = *Turbinaria conoides*, collected from Chon Buri (01), Chumphon (02), and Rayong (03) provinces. The vertical bar represents the standard deviation.

and PA01 (9.88±0.20% w/w), as shown in Figure 3. The sulfate contents obtained from each species were significantly different ($p<0.05$).

Sugar components

The HPLC analysis of the sugar component in the E1- and E2-crude polysaccharide extracts showed that all species had fucose as the main sugar component (Tables 1 and 2). The highest value of fucose was obtained from the E1-extract of HC01 (12.35%). The lowest sugar component was mannose, which was found in the E1-extracts of CS01, PA03, PM02, SP01 and SP03, but not in the polysaccharide extracts of DD02, HC01, PA01, PA02 and TC02. In addition, very low values of galactose were detected in PM02 and SP03, while traces of glucose were observed in the crude polysaccharides of DD02 and SP01 (Table 1). For the E2-extracts, the highest level of fucose (9.095%) was obtained from the extract of CS01 (Table 2). The lower sugar component was glucose, which was found in the extracts of DD02, TC02, PA01 and SP01. Mannose and galactose were only detected in the extract from HC01. In this study, fructose was not detected in any of the algal species.

Table 1 Sugar components (%) in the E1 crude polysaccharide extracts analyzed using HPLC.

Algal species	Sugar component (%)				
	glucose	mannose	fucose	galactose	fructose
CS01	-	0.035	3.900	-	-
DD02	0.095	-	0.800	-	-
HC01	-	-	12.350	-	-
PA01	-	-	3.450	-	-
PA02	-	-	6.350	-	-
PA03	-	0.250	6.650	-	-
PM02	-	0.050	7.100	0.210	-
SP01	0.090	0.450	2.100	-	-
SP03	-	0.050	4.250	0.170	-
TC02	-	-	6.150	-	-

Table 2 Sugar components (%) in the E2 crude polysaccharide extracts analyzed using HPLC.

Algal species	Sugar component (%)				
	glucose	mannose	fucose	galactose	fructose
CS01	-	-	9.095	-	-
DD02	0.320	-	5.750	-	-
HC01	-	0.020	7.950	0.110	-
PA01	0.450	-	3.760	-	-
PA02	-	-	8.950	-	-
PA03	-	-	4.900	-	-
PM02	-	-	6.800	-	-
SP01	0.010	-	3.800	-	-
SP03	-	-	5.750	-	-
TC02	0.146	-	4.000	-	-

Antimicrobial activity

Antimicrobial activity was determined in crude extracts using both procedures (E1 and E2) at 2, 1, 0.5, 0.25 and 0.125 mg/ml. At 2 mg/ml, activity was observed only against *Candida albicans* in the crude extracts of SP01, with a clear zone of 0.122±0.004 mm in the E1-extracts and a clear zone of 0.123±0.003 mm in the E2-extracts. The E2-extracts of CS01 also inhibited the growth of *C. albicans* with a clear zone of 0.156±0.035 mm (Figure 4). Most of the microorganisms tested grew in the presence of the E1-extracts, except for *A. hydrophila* and *A. niger* (Table 3).

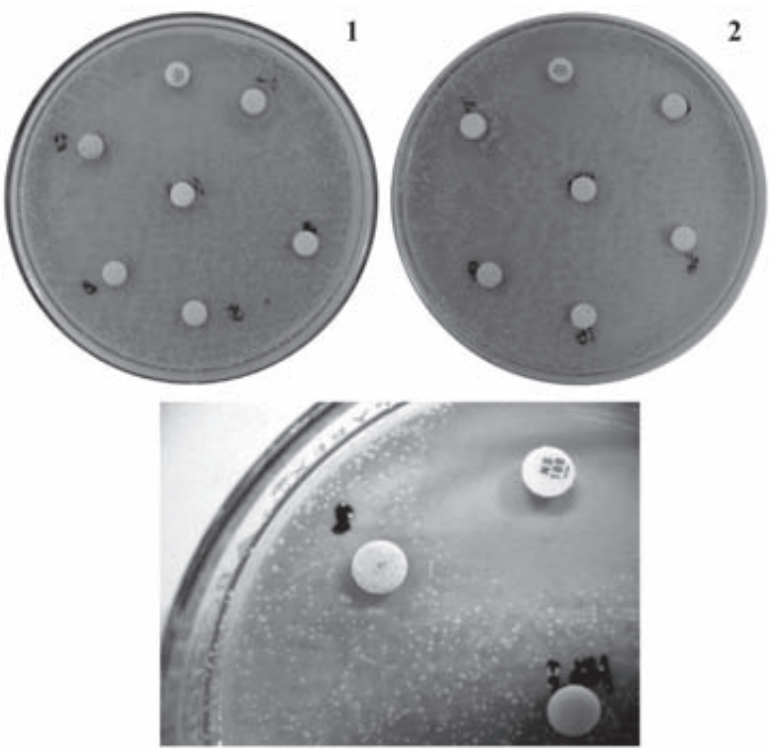

Figure 4 E1 and E2 crude polysaccharide extracted from SP01 showing inhibition of the growth of the yeast, *Candida albicans,* at 2 mg/ml (1 = E1-SP01, 2 = E2-SP01, 3 = the clear zone of the inhibition).

Table 3 Antimicrobial activity assays of the E1 crude polysaccharides.

Algal species	Microorganism test								
	B. subtillis	*E. coli*	*P. aeruginosa*	*V. parahaemolyticus*	*V. alginolyticus*	*V. harveyi*	*A. hydrophilla*	*C. albicans*	*A. niger*
CS01	×	×	×	×	×	×	-	×	-
DD02	×	×	×	×	×	×	-	×	-
HC01	×	×	×	×	×	×	-	×	-
PA01	×	×	×	×	×	×	-	×	-
PA02	×	×	×	×	×	×	-	×	-
PA03	×	×	×	×	×	×	-	×	-
PM02	×	×	×	×	×	×	-	×	-
SP01	×	×	×	×	×	×	-	+	-
SP03	×	×	×	×	×	×	-	×	-
TC02	×	×	×	×	×	×	-	×	-

Note: Antimicrobial activity: + = trace, - = inactive, × = stimulate growth.

Table 4 shows that some of the microorganisms tested, for example, *B. subtilis,* could grow in the presence of the E2-extracts of CS01, HC01, PA02, PM02, SP03 and TC02. *E. coli* growth was observed when tested in the presence of the E2-extracts of CS01, HC01, PM02, SP03 and TC02. *P. aeruginosa* could grow in the presence of the extracts of HC01 and PA01, while *V. parahaemolyticus* and *A. hydrophilla* grow in the presence of the extracts of PM02 and PA02, respectively. The extracts of DD02, PA02 and SP01 were inactive against *V. alginolyticus* and *V. harveyi*. As a result, most of the E1- and E2-extracts were not only inactive against the tested microorganisms, but in fact, promoted microbial growth (Figure 5).

DISCUSSION

In this study, crude polysaccharide was extracted by boiling samples of brown seaweed species in distilled water at 100°C (hot water extraction, E1). The extract mainly consisted of water-soluble sulfated polymers, while the polysaccharide extracted by boiling the samples in HCl at 75°C (hot acid extraction, E2) was mostly crude fucoidan. The results obtained were similar to the studies reported by Becker and Lowe (2003) and Asker *et al*. (2007). The high levels of fucose detected in this study agreed with Berteau and Mulloy (2003), who reported that sulfated L-fucose was the main component of the polysaccharide in the cell wall of brown seaweeds. As has been reported in other research, the E1- and E2-extracts that were obtained in the current

Table 4 Antimicrobial activity assays of the E2 crude polysaccharide.

Algal species	Microorganism test								
	B. subtillis	*E. coli*	*P. aeruginosa*	*V. parahaemolyticus*	*V. alginolyticus*	*V. harveyi*	*A. hydrophilla*	*C. albicans*	*A. niger*
CS01	×	×	-	-	×	×	-	+	-
DD02	-	-	-	-	-	-	-	×	-
HC01	×	×	×	-	×	×	-	×	-
PA01	-	-	×	-	×	×	-	×	-
PA02	×	-	-	-	-	×	×	×	-
PA03	-	-	-	-	×	×	-	×	-
PM02	×	×	-	×	×	×	-	×	-
SP01	-	-	-	-	-	-	-	+	-
SP03	×	×	-	-	×	×	-	×	-
TC02	×	×	-	-	×	×	-	×	-

Antimicrobial activity: + = trace, - = inactive, × = stimulate growth

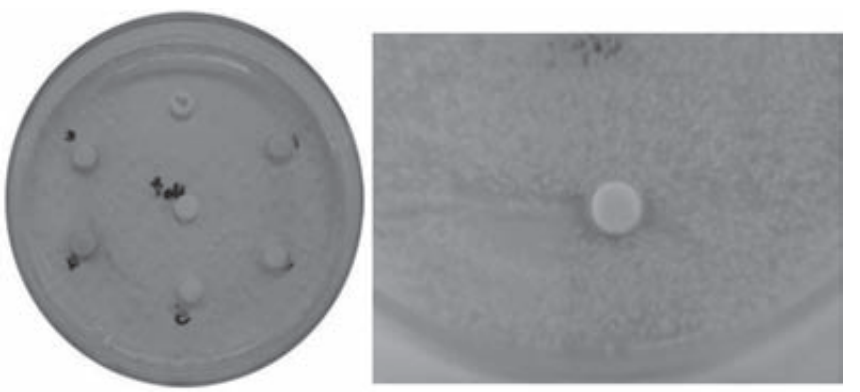

Figure 5 An example of the antimicrobial assay, showing the stimulation of the growth of the microorganism tested by the crude polysaccharide extracts.

study varied, depending on the species of algae used and the extraction method (Asker *et al*., 2007; Wang *et al*., 2008), as well as the environment (Zvyagintseva *et al*., 2003). In all algal species, the E1-crude polysaccharide extracts had lower yields than the corresponding E2-extracts. However, the crude extracts from the same algal species collected at different habitats also yielded different levels of polysaccharides. This was the case with the E2-extract of *P. australis* collected from Chon Buri (PA01), which gave a higher yield than samples collected from Rayong (PA03) and Chumphon (PA02). On the other hand, the *S. polycystum* (SP03) sample collected from Rayong gave a higher yield of the crude extract than the sample collected from Chon Buri (SP01). However, the yields of the crude extract obtained in this study were lower than in previous reports for *S. latifolium* (4.75%) extracted in hot water at 100°C by Asker *et al*. (2007) and *S. fulvellum* (8.9%) and *S. thunbergii* (9.6%) by Kang *et al*. (2008).

The E2 polysaccharide yield was highest in *H. clathratus* (HC01) collected from Chon Buri (19.69±0.23%) while it was lowest in *P. australis* (PA03) from Rayong (2.77±0.19% w/w). Similar to the E1 extract, the E2 crude polysaccharide extract from the same species grown at different habitats gave different yields. This can be seen in *S. polycystum* collected from Rayong (SP03), which gave a higher yield of the extract than the samples collected from Chon Buri (SP01). However, the yield of the extract obtained was lower than previously reported in the same species (22.3±4.51%) that was extracted with 0.1 N HCl at 95°C (Chotigeat *et al*., 2004). This difference in yield could have been due to the higher acid concentration (2N HCl) used in the present study than that in Chotigeat *et al*. (2004). Alternatively, the differences in yield of the crude polysaccharide extracted by the E1 and E2 methods may also have been due to the different solubilities of the polysaccharides in the solvent used in the study (Usov *et al*., 2004).

Chemical components of crude extracted polysaccharides

Total carbohydrate content

The total carbohydrate content in the E1 crude polysaccharide extract was higher than that of E2-extract, except for the sample of CS01 collected from Chon Buri. The total carbohydrate content was highest in the E1-extract of PM02 (55.95±0.72%). The lowest value was 32.15±0.18%, and was found in the crude polysaccharide extracted from TC02 collected from Chumphon province. Among different species from the same genus, PM02 had a higher carbohydrate content than that of PA02 collected from Chumphon and PA01 from Chon Buri. Similar to the E1-extracts, the E2 polysaccharide extract that had the highest total carbohydrate content came from the PM02 sample (44.41± 0.94%), while the lowest value was in HC01 (10.46±0.52%). The carbohydrate content varied depending on the algal species used. In previous studies, differences in the carbohydrate contents have been reported for the fractionation of the brown seaweeds, *Laminaria cichorioides*, *L. japonica* and *Fucus evanescens,* which yielded 20-25% from fraction 1 and 26-32% from fraction 2 (Zvyagintseva *et al*., 2003). In addition, the fractionation of the polysaccharide from *Padina gymnospora* gave the highest carbohydrate content of 38.7% (Silva *et al*., 2005). In the current study, there was variation in the carbohydrate contents obtained according to the algal species studied and the habitat, which was similar to Zvyagintseva *et al*. (2003).

Sulfate content

The sulfate content in the crude polysaccharide extracts varied depending on the algal species used. The sulfate content of some species was the same for the same species grown in different habitats, as can be seen for *Sargassum*

polycystum and *Padina australis* (Figure 5). Similar to other reports, the current study found that the structure of the polysaccharides was substituted with different amounts of sulfate ester groups depending on the algal species (Chevolot *et al*., 1999) and that the highly sulfated polysaccharides generally had antimicrobial activity (Chevolot *et al*., 1999; Berteau and Mulloy, 2003). In the present study, the crude polysaccharides that were obtained could be divided into two groups: 1) those with a moderate sulfate content (5-9%), namely, the crude extracts from *Colpomenia sinuosa* (CS), *Dictyota dichotoma* (DD), *Padina australis* (PA) and *P. minor* (PM); and 2) those with a high sulfate content (10-18%), namely, the crude extracts from *Sargassum polycystum* (SP), *Hydroclathrus clathratus* (HC) and *Turbinaria conoides* (TC). The lowest sulfate content in the E1-extracts was in CS01 (5.83±0.28%) and the highest was in HC01 (18.10±0.25%). The lowest sulfate content in the E2-extracts was in DD02 (9.74±1.49%) and the highest was in CS01 (14.22±0.69%). The E1- and E2-extracts of SP01 and SP03 could both be classified in the high sulfate group (~10-11%). This level was similar to that reported in *Steochospermum marginatum* (10%) collected from the Arabian Sea (Adhikari *et al*., 2006), but lower than that in *Sargassum latifolium* (22.10%) collected from the Red Sea in Saudi Arabia (Asker *et al*., 2007). The E1-extracts of PA01, PA02 and PA03 had moderate sulfate levels (~9%), which were not significantly different from the E2 extracts (~10%). However, the sulfate contents obtained in this study were lower than those found in *Padina gymnospora* (18.40-27.57%) by Rocha de Souza *et al*. (2007).

Sugar components in the crude polysaccharide extracts

The crude polysaccharide extracted from all brown seaweed samples in this study had fucose as the main monosaccharide component. Mannose, galactose and glucose were present in small amounts. However, fructose was absent in all of the crude polysaccharide extracts. A similar result has been reported by Dias *et al*. (2008) on the polysaccharides extracted from *Sargassum stenophyllum*, which had fucose, galactose, mannose, xylose, glucose and uronic acid as the main components. The results of the current study were similar to a study on the polysaccharides extracted from *Colpomenia peregrine*, which consisted mainly of fucose, xylose and galactose, although sometimes glucose and mannose were present (Usov *et al*., 2004). The HPLC analysis showed galactose in the E1-extract of PM02 and SP03. The E2-extract of HC01 consisted mainly of fucose, similar to the polysaccharide that was extracted from *Laminaria japonica* (Wang *et al*., 2008). However, in that paper, it was reported that after fractionation, fractions 1 and 3 consisted mainly of galactose. In addition, the HPLC results showed that fucoidan was the main component of the crude polysaccharide extracts, as reported by Chandía and Matsuhiro (2008).

Antimicrobial activity

In the current study, the E1 crude polysaccharides extracted from samples from SP01 and CS01 showed activity against the yeast, *Candida albicans,* at a maximum concentration of 2 mg/ml, but did not affect the other microorganisms tested. The E2 crude polysaccharide extracted from sample SP01 also inhibited yeast in the assay. This contrasts with a paper by Chotigeat *et al*. (2004), which reported that the fucoidan extracted from *Sargassum polycystum* showed antibacterial activity against *Vibrio harveyi*, *Staphylococcus aureus* and *Escherichia coli* at concentrations of 12, 12 and 6 mg/ml, respectively. However, these concentrations were higher than the concentrations used in this study. The polysaccharide extracted from some algal species showed a high content of sulfate, but the sulfate content did not show a clear

relation to the antimicrobial activity. In contrast, Berteau and Mulloy (2003) reported that the antimicrobial activity of the polysaccharides was related to their chemical structure and ester sulfate groups. On the other hand, several species of brown seaweed that have a high sulfate content have been reported to show differences in antimicrobial activities (Adhikari *et al*., 2006; Asker *et al*., 2007), with some sugars showing antimicrobial activity (Zemek *et al*., 1985). However, in the current study, the E1 and E2 crude polysaccharides obtained had rather low sugar contents and a large amount of the crude polysaccharide was needed for the assay. A similar result has been reported in the crude polysaccharide extracted from *Sargassum polycystum*, which inhibited the growth of the bacteria *V. harveyi* at 12 mg/ml (Chotigeat *et al*., 2004). In addition, the crude polysaccharide may have contained pigments, which could have also inhibited the growth of microorganisms, as has been reported by Kaewsrithong *et al*. (2007). They reported that extracts from *Padina australis*, *Sargassum polycystum* and *Turbinaria conoides* using methanol, ethanol, dichloromethane and petroleum ether contained terpenoids, flavonoids and alkaloids. The report also showed that methanolic and ethanolic extracts of the seaweed species inhibited the growth of *Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

In some cases, differences in antimicrobial activity have been thought to be related to the environment in which the algae grew (Martí *et al*., 2004; Salvador *et al*., 2007). Salvadol *et al*. (2007) reported that brown and red seaweeds collected in the spring and autumn inhibited microorganisms, while green seaweed showed inhibition in samples collected in summer. In the current study, the crude polysaccharide extract from most of the brown species did not show antimicrobial activity, possibly due to the samples having been collected during the summer. However, there are no reports to date on the effects of seasonality and environmental factors on the inhibitory activity of the polysaccharides from Thai seaweeds. Moreover, the crude polysaccharide extracted from *Hydroclathrus clathratus* and *Colpomenia sinuosa* had high sulfate contents, but did not inhibit the microorganisms tested. In contrast, a report by Berteau and Mulloy (2003) showed that highly sulfated polysaccharides had the highest antimicrobial activity. The results in the current study showed that most of the E1 and E2 extracts were not only inactive against the microorganisms tested, but in fact promoted microbial growth, particularly in the extracts that contained mannose.

CONCLUSION

The E1 extract of the brown seaweeds mainly consisted of water-soluble sulfated polymers, while the E2 extract was mostly crude fucoidan. The E1- and E2-extracts obtained varied, depending on the species of algae used and the extraction method. The crude extract from the same algal species collected at different habitats yielded different levels of polysaccharides. The total carbohydrate content in the E1 crude polysaccharide extract was mostly higher than that of the E2-extract. The carbohydrate and sulfate contents varied depending on the algal species, with all of the extracts obtained containing fucose as a main component. The study showed that crude polysaccharide, with high sulfate contents, did not inhibit the microorganisms tested, but promoted microbial growth. This may have been explained by the high carbohydrate content in the crude polysaccharide extracted, which could have been used as a source of carbon to promote growth by the microbes tested.

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