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Water soluble polysaccharides extracted from *Pterocladia capillacea* and *Dictyopteris membranacea* and their biological activities

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ABSTRACT

Cold and hot water extracts (CWE, HWE) of both the red alga *Pterocladia capillacea* (*P. capillacea*) and the brown alga *Dictyopteris membranacea* (*D. membranacea*) were studied for their polysaccharide contents. In both (CWE) and (HWE) extracts. Relatively higher yields were obtained in case of *P. capillacea pillacea* (2.87 and 6.46%, respectively). The polysaccharide contents of the CWE hydrolyzate of both studied algae analyzed by HPLC were found to be enriched with glucuronic acid, arabinose and glucose, whereas, HWE hydrolyzate were found to be rich in glucuronic acid and fructose. The polysaccharide contents of the CWE and HWE extracts of (*D. membranacea*) showed appreciable antimicrobial activity in addition to a moderate antitumor activity against HELA (Cervix carcinoma cell line) at IC50 = 9.83 μg/dl, respectively. Whereas the polysaccharide contents of the CWE and HWE extracts of (*P. capillacea*) exhibited a promising anticoagulant activity.

1. Introduction

Recently, macroalgae have been used as a novel food with potential nutritional benefits in industry and medicine for various purposes (Agili & Mohamed, 2012; Khair & El-Shafay, 2013). Furthermore, macroalgae have shown to provide a rich source of natural bioactive compounds with antiviral, antifungal, antibacterial, antioxidant, anti-inflammatory, hypercholesterolemic, hypolipidemic and antineoplasteic properties (El-Baroty et al., 2007). Thus, there is a growing interest in the area of research on the positive effect of macroalgae on human health and other benefits.

In particular, marine algae contain large amounts of polysaccharides, which are polymers of simple sugars (monosaccharides) linked together by glycosidic bonds, and they have numerous commercial applications in products such as stabilizers, thickeners, emulsifiers, food, feed, beverages *etc.* (Bixler & Porse, 2010; Chandini, Ganesan, & Bhaskar, 2008; Zhou, Hu, Wu, Pan, & Sun, 2008). Over the last decade, bioactive polysaccharides isolated from brown and red algae have attracted much attention in the fields of pharmacology and biochemistry (Agili & Mohamed, 2012; Huang, Zhou, & Zhang, 2006; Tiago et al., 2012). Functional polysaccharides such as fucans, alginic acid and carrageenans derivatives produced by brown and red algae are known to exhibit different biological properties including anticoagulant, anti-inflammatory, antiviral, antitumoral activities and tissue engineering and drug delivery approaches (Sahera & Fatimah 2013; Silva et al., 2012). Algal polysaccharides have been described to possess anticoagulant activity similar to heparin. Various polysaccharides have been extracted from species of *Dictyotales* and *Fucales* (Phaeophyta) (Medeiros et al., 2008). The proposed mechanisms of action of these compounds are predominantly related to the "*in vitro*" inhibition of factors Xa and IIa mediated by antithrombin and heparin cofactor II (Li, Lu, Wei, & Zhao, 2008). Algal substances are also said to possess bacteriostatic and bactericidal activity and have been extensively studied by several researchers (Freile-Pelegrin & Morales, 2004; Salvador, Gomez-Garreta, Lavelli, & Ribera, 2007).

A role of polysaccharides from algae as antineoplastic agents has also been suggested. Several investigations have reported that polysaccharides have antiproliferative activity in cancer cell lines *in vitro*, as well as inhibitive activity in tumors growing in mice (Queiroz et al., 2006). Moreover, these polymers have been reported to induce apoptosis in several cancer lines and stimulate immune system cells to induce tumor cell death (Maruyama, Tamauchi, Hashimoto, & Nakano, 2003). In addition, they have antimetastatic activity blocking the interactions between cancer cells and the basement membrane (Rocha et al., 2005). On the basis of these considerations, the purpose of the present study to study polysaccharide contents of the two algal species and to evaluate their

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anticoagulant, antimicrobial and antitumor activity against cancer cell lines

2. Experimental

2.1. Materials

2.1.1. Algal material

The red alga *P. capillacae* and the brown alga *Dictyopteris membranacea* were collected from along the coast of Abo-Qire, Alexandria, Cairo, Egypt, in February, March and May, respectively in two successive years 2003 and 2004. The algal samples were brought to the laboratory in an ice box, cleaned thoroughly with fresh water, the epiphytes were removed. The cleaned algae were dried in shade at room temperature and well grinned.

2.1.2. Material for antimicrobial study

2.1.2.1. Tested microorganisms. Four pure strains of bacteria were tested *viz*., Gram-positive bacteria; *Bacillus cereus* and *Staphylococcus aureus*, Gram-negative bacteria; *Escherechia coli* and *Pseudomonas fluoresens*, yeast strain; *Saccharomyces cervesia*, fungal strains; *Fusarium oxysporium* and *Aspergillus niger* were obtained from the Microbial Chemistry Department, National Research Centre, Egypt.

2.1.2.2. Nutrient media. Nutrient media used were Lauri-Bertani medium [tryptone 10 g, yeast extract 5 g, NaCl 10 g, 15 g agar and water to 1 l, the pH was adjusted to 7.5 with sodium hydroxide (Moniantis, Fritssh, & Sambrook, 1980), Yeast extract peptone medium [dextrose 20 g, peptone 20 g, yeast extract 10 g, water to 1 l] (Dillon, Nasm, & Nestmann, 1985) and potato dextrose agar medium [peeled and sliced potato 200 g, D-glucose 20 g, agar 15 g, water to 1 l]. These media were used for bacteria, yeast and fungi, respectively. The media were sterilized by autoclaving for 20 min at 121 °C.

2.1.3. Material for in vitro antitumor activity

The used cancer cell lines were U251 (Brain tumor cell line), MCF7 (Breast adino carcinoma cell line), HELA (Cervix carcinoma cell line), HEPG2 (Liver carcinoma cell line), H460 (Lung carcinoma cell line), HCT 116 (Colon carcinoma cell line). They were obtained from the American Type Culture Collection, University Boulevard, Manassas, USA.

2.1.4. Experimental animals for anticoagulant activity

Anticoagulant activity was conducted on male albino rats (120–160 g). Animals were obtained from the animal house colony, N.R.C., Cairo, Egypt. They were housed six animals/cage and fed on standard laboratory diet. Thromboplastine with calcium Kit (Biomerieux Company) was used.

2.2. Methods

2.2.1. Extraction of water soluble polysaccharides

Five grams of the dry algal powder were extracted in distilled water (D.W) at room temperature. (CWE) of each alga was filtered and concentrated under reduced pressure at temperature not exceeding 40 °C and precipitated with ethanol (4 volume, v/v) the precipitated polysaccharides were filtered, washed twice with absolute ethanol and dried at 40 °C to obtain the crude cold water-soluble polysaccharides. The algal residue after cold water was extracted with (D.W) at 80 °C for 3 h. From the filtrate hot water soluble polysaccharides were obtained following the same procedure used for cold water soluble polysaccharides.

2.2.2. Acid hydrolysis of both cold and hot water soluble polysaccharides

The precipitated polysaccharides (0.1 g) were added to 10 ml 1 N H_2SO_4 and heated in a boiling water bath for 5 h to hydrolyze the polysaccharides. Barium carbonate was added then centrifuged and the precipitate was washed twice by water, then the solution was evaporated under reduced pressure at temperature not exceed exceeding 40 °C. The residue, in each case was extracted with 2 ml of hot pyridine. The pyridine extract was evaporated to dryness under reduced pressure and kept for chromatographic analysis.

2.2.3. Paper chromatographic investigation

Part of the CWE and HWE hydrolyzates as well as authentic sugars were dissolved in 10% isopropanol/water then spotted on Whatman No. 1 paper sheets. The chromatograms were developed adopting the descending technique for 18 h using S1 [(n-butanol–acetic acid–water (4:1:5, v/v/v)] and S2 [isopropanol–water (4:1, v/v)]. The spots were visualized by spraying with aniline phthalate reagent and heating in an oven for 5 min at 110 °C.

2.2.4. HPLC chromatographic investigation

Ten mg of the CWE and HWE hydrolyzates as well as authentic reference were homogenized with acetonitrile/water (76/24, v/v). The extract was filtered through a through a Whatman No. 1 filter paper and microfilter (0.45 μm), partitioned three times with ethyl acetate and injected in HPLC apparatus model HP1050 equipped with UV detector on APS column (4.6 mm × 200 mm), the mobile phase was the same used in the extraction and the UV detector was adjusted at 192 nm, flow rate 2 ml/min.

2.2.5. Antimicrobial activity

The antimicrobial test was carried out according to the antibiotic assay disk method (Gnanamanickam & Mansfield, 1981). Nutrient agar media for the bacteria, fungi, and yeast were prepared and sterilized, then distributed in sterile Petri dishes of 12 cm diameter. Each suspension of the tested organism was, separately, inoculated onto the surface of a number of Petri dishes. Each antibiotic assay disk (6 mm diameter) was loaded with 100 μg/disk) of the tested extracts. The air-dried discs were firmly applied to the surface of the inoculated agar plates. Ampicillin as antibacterial (100 μg/disk) and Clotrimazole (100 μg/disk) as antifungal were used as reference drugs. This assay was replicated three times. All these steps were carried out under aseptic conditions, bacterial plates incubated at 37 °C for 24 h, while those containing yeast and fungi were incubated at 28 °C for 48–72 h, diameter of the inhibition zones were recorded for each replicate and the average diameters were calculated.

2.2.6. Antitumor activity

Antitumor activity against the human tumor cell lines previously mentioned was done Skehan et al. (1977). Cells were plated in 96 multi-well plate (104 cells/well) for 24 h before treatment with the extracts to allow attachment of the cell to the wall of the plate. Different concentrations of the extracts (0, 1, 2.5, 5 and10 μg/dl) were prepared and added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with each extract for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulphorhodamine B (SRB) stain. Excess stain was washed with acetic acid and attached stain was recovered with tri-EDTA buffer. Color intensity was measured in an ELIAS reader and the relation between surviving fraction and the algal extracts

Table 1
Yields of the cold and hot water-soluble polysaccharides obtained from *P. capillacea* and *D. membranacea*.

Extract	% (g/100 g)	
	P. capillacea	*D. membranacea*
Cold water extract	2.87	2.14
Hot water extract	6.46	3.89

concentration is plotted to get the survival curve of each tumor cell line.

2.2.7. Anticoagulant activity

The anticoagulant activities were evaluated using the whole blood clotting time and prothrombin time (6-h and 24-h), blood samples were obtained from the retro-orbital venous plexus of each animal using 0.11 mol/litter trisodium citrate as anticoagulant for prothrombin time test. Thromboplastine with calcium kit (Biomerieux Company) was used. The treated groups of animals received the compounds orally (100 mg/kg body weight), the blood samples were taken at six and twenty four hours after administration and the clotting and prothrombine time were recorded (Young, Kisker, & Doty, 1978).

3. Results and discussion

Several methods can be used to extract polysaccharides from marine algae. However, the yield can vary depending on the technique and the algae used (Rodrigo, José, Márjory, Paulo, & Norma, 2011; Rodrigues et al., 2011). Our results revealed that the total yield of the water-soluble polysaccharide extracted from the red alga *Pterocladia capillacea* and the brown alga *D. membranacea* at room temperature (25 °C) were 2.87, 2.14% and at 80 °C were 6.46, 3.89, respectively (Table 1). Paper chromatography and HPLC analysis of the polysaccharide hydrolyzates of cold and hot water of *P. capillacea* illustrated in Tables 2 and 3, showed mainly the presence of six sugars of the cold hydrolyzate constituting 80.87% and 59.10%, respectively. The major sugars were galactose (25.36%), glucose (20.59%), arabinose (12.61%) and fructose (9.17%), while galactos (17.81%), glucose (16.99%), fructose (9.38%) and mannose (7.37%) were the major sugars of the hot hydrolyzate. Meanwhile the HPLC analysis of cold and hot water hydrolyzates of *D. membranacea* revealed the identification of seven and nine sugars constituting 87.29% and 67.87%, respectively. The major sugars of the cold hydrolyzate were glacturonic acid (32.53%), glucuronic acid (30.54%) and fructose (7.56%), while glacturonic acid (27.32%), glucuronic acid (22.02%) and mannose (6.01%) were the major sugars of the hot hydrolyzate. The chemical and structural characteristics of the marine algal polysaccharides are considered a prerequisite for understanding their biological activity (Errea & Matulewicz, 1996; Rodrigo et al., 2011; Zhang et al., 2008).

Table 3
HPLC analysis of the soluble polysaccharide hydrolyzate of *P. capillacea* and *D. membranacea*.

Authentic sugars	R_t	Relative%			
		P. capillacea		*D. membranacea*	
		Cold	Hot	Cold	Hot
Fucose	1:08	–		5.32	1.73
Rhamnose	1:41	–		–	–
Maltose	1:76	–		–	–
Lactose	1:80	–		–	–
Sorbose	1:93	–		–	–
Galactose	2:21	25.36	17.81	–	1.87
Galacturonic acid	2:40	–	–	32.53	27.32
Ribose	2:62	–		–	–
Xylose	2:89	–		–	0.61
Sorbitol	3:19	5.92	2.02	–	–
Arabinose	3:39	12.61	6.53	3.79	2.71
Fructose	3:88	9.17	9.38	7.56	4.07
Mannose	4:29	7.22	7.37	5.13	6.01
Glucose	4:46	20.59	16.99	2.42	1.53
Glucuronic acid	5:50	–	–	30.54	22.02
Total identified sugar	–	80.87	59.10	87.29	67.87

Natural compounds produced by red and brown marine algae specially their polysaccharides, are able to prevent QS-regulated virulence factor production (quorum sensing systems which are involved in the adaptation of populations of bacteria to environmental cues.) in several human pathogens, including the bacteria associated with cholera and cystic fibrosis-associated lung infections (Aoun, Said, & Farhat, 2010; Diane, Rice, & Kjelleberg, 2006; Laio, Lin, Shich, Jeng, & Huang, 2003). This reflects the necessity of testing the antimicrobial activity of the algal extracts under investigation. Results depicted in Table 4 revealed that cold and hot water extracts of *P. capillacea* showed wide range of antimicrobial activity against (G−ve and G+ve) bacteria and fungi. Besides their higher antifungal activity (125% activity) than clotrimazol (100% activity) against *Fusarium oxysporium*. For *D. membranacea* as shown in Table 4, both cold and hot water extracts has no effect on G+ve bacteria and fungi but have a good effect on G−ve bacteria. Our results were in agreement with the results obtained by Salvador et al. (2007).

They reported that the extracts that were prepared from fresh and lyophilized samples of algae showed differences in their antimicrobial activities depending on the season that samples were collected and the type of samples which the extracts have been prepared. Also Taskin, Caki, Ozturk, and Taskin (2010) proved that the difference observed in the antimicrobial activities are contributed

Table 2
Paper chromatographic investigation of polysaccharide hydrolyzates of *P. capillacea* and *D. membranacea*.

Spot no.	R_f value		*P. capillacea*		*D. membranacea*		Color of spot	
	S1	S2	I	II	I	II		
1	0.59	0.69	–	–	–	–	Yellowish brown	Rhamnose
2	0.54	0.63	–	–	–	–	Brown	Ribose
3	0.38	0.56	–	–	+	+	Brown	Fucose
4	0.32	0.55	–	–	–	+	Reddish brown	Xylose
5	0.27	0.52	+	+	+	+	Brown	Arabinose
6	0.25	0.48	+	+	+	+	Yellowish brown	Fructose
7	0.18	0.46	+	+	+	+	Brown	Glucose
8	0.16	0.45	+	+	–	+	Brown	Galactose
9	0.16	0.38	–	–	+	+	Pale brown	Glucuronic acid
10	0.20	0.30	–	–	+	+	Pale brown	Galacturonic acid
11	0.29	0.61	+	+	+	+	Brown	Mannose

S1, n-butanol–acetic acid–water (4:1:5, v/v/v); S2, isopropanol–water (4:1); I, Hydrolyzate of cold water extract; II, hydrolyzate of hot water extract.

Table 4
Anti-microbial activity of cold and hot water extracts of *P. capillacea* and *D. membranacea*.

Micro-organism	Diameter of inhibition zone in mm (% activity)					
	I	II	III	IV	A	B
Bacillus cereus (G+)	6 (33.3)	6 (33.3)	–	–	18 (100)	–
Staphylococcus aureus (G+)	12.5 (56.8)	9.5 (43.2)	–	–	22 (100)	–
Streptococcus pyogen (G+)	15 (39.5)	14.5 (38.1)	–	–	38 (100)	–
Escherichia coli (G−)	–	–	2 (20)	11 (110)	20 (100)	–
Pseudomonas flouresens (G−)	10 (40)	9.5 (38)	18 (72)	–	25 (100)	–
Candida albicans	–	–	7.2 (25.7)	7 (25)	–	20 (100)
Saccharomyces cerevisea	–	–	–	–	–	28 (100)
Asperagillus niger	–	–	–	–	–	22 (100)
Macrophomina phaseli	–	–	8.2 (41)	8.4 (42)	–	25 (100)
Fusarium oxysporium	25 (125)	–	–	–	–	20 (100)

I, CWE of *P. capillacea*; II, HWE of *P. capillacea*; III, CWE of *D. membranacea*; IV, HWE of *D. membranacea*; A, Ampicillin; B, Clotrimazol.

Table 5
The percentage of surviving fraction of different cell lines against different concentrations of the cold and hot water extracts of *P. capillacea*.

Conc. in (μg/dl)	% of surviving fraction											
	Cold water extract						Hot water extract					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
0.0	99.50	99.90	100.2	100.0	100	100	99.50	99.90	100.2	100.0	100	100
1.00	94.00	88.50	90.50	99.40	100.10	98.00	97.00	86.90	101.4	96.10	104.10	102.3
2.50	89.20	86.90	85.00	96.50	98.50	92.30	85.00	86.40	98.60	94.00	100.10	84.40
5.00	74.40	79.70	84.40	87.40	92.30	81.20	74.40	84.90	95.20	87.40	96.80	84.10
10.00	59.10	77.70	81.00	86.00	85.50	81.20	59.10	81.30	84.40	85.80	88.30	80.90

I, (HELA) cell line; II, (MCF7) cell line; III, (HCT116) cell line; VI, (U251) cell line; V, (H460) cell line; VI, (HEPG2) cell line.

Table 6
The percentage of surviving fraction of different cell lines against different concentrations of the cold and hot water extracts of *D. memmbranacea*.

Conc. in (μg/dl)	% of surviving fraction											
	Cold water extract						Hot water extractف					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
0.0	99.50	99.90	100.2	100.0	100	100	99.50	99.90	100.2	100.0	100	100
1.00	61.20	86.90	97.30	82.10	96.20	70.10	63.90	86.90	95.90	89.10	89.50	96.70
2.50	53.30	73.60	92.50	69.70	91.10	63.30	59.10	73.60	94.60	81.20	86.10	77.60
5.00	51.50	73.60	88.40	68.50	86.60	62.30	54.90	73.60	74.80	67.60	82.70	74.80
10.00	49.70	73.60	65.30	62.70	79.90	59.40	53.60	73.60	71.40	64.30	80.90	69.80

I, (HELA) cell line; II, (MCF7) cell line; III, (HCT116) cell line; VI, (U251) cell line; V, (H460) cell line; VI, (HEPG2) cell line.

Table 7
Anticoagulant activity of cold and hot water extracts of *P. capillacea* and *D. membranace*.

Extract no.	Anticoagulant activity			
	6-h		24-h	
	Clotting time (s)	Prothrombin time (s)	Clotting time (s)	Prothrombin time (s)
I	91.3 ± 3.4*	36.1 ± 1.4*	78.2 ± 2.3*	35.6 ± 2.9*
II	82.4 ± 2.9*	47.6 ± 1.8*	63.1 ± 3.7*	48.1 ± 2.5*
III	67.9 ± 2.3*	28.6 ± 1.3*	51.2 ± 1.5	24.4 ± 0.4*
IV	54.6 ± 2.1	19.8 ± 0.9*	56.2 ± 1.7*	21.2 ± 0.3*
Control	46.2 ± 2.7	12.8 ± 0.3	47.3 ± 2.9	13.5 ± 1.2

I, CWE of *P. capillacea*; II, HWE of *P. capillacea*; III, CWE of *D. membranacea*; IV, HWE of *D. membranacea*.
* Significantly different from control at $P < 0.01$.

by several factors, mainly infar-specific variabilities in the production of secondary metabolites.

Antitumor activity of algal polysaccharides has been reported in recent years (Costa et al., 2010; Queiroz et al., 2006; Yubin, Chong, Tao, & Chenfeng, 2007). These reports were in agreement with the results obtained in this study, where, both the cold and hot water polysaccharides extracted from *D. membranacea* represented an effective antitumor activity against HELA (Cervix carcinoma cell line) at IC_{50} = 9.83 μg (Table 6). In contrast, *P. capillacea* showed insignificant antitumor activity upon application on the same cell line (Table 5).

Table 7 shows the anticoagulant activity of polysaccharides extracted from the two algae, in the current work. As is evident, the polysaccharides extracted from *P. capillacea* by the cold and hot method of extraction exhibited the highest anticoagulant activity as compared with those of *D. membrnacae*. This may be due to the differences in the type and ratio of these polysaccharides in the two examined algae (Hayakawa et al., 2000; Shanmugam & Mody, 2000). This outcome is coincident with that reported by other authors (Mao et al., 2008; Mourão, 2004; Mourão, 2008; Zhang et al., 2008).

4. Conclusion

The water soluble extracts (cold and hot) of the red alga *P. capillacae* and the brown alga *D. membranacea* exhibited appreciable antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi in comparison with ampicillin and clotrimazol as the standard antibiotics. In addition, these algal extracts possessed noticeable anticoagulant and antitumor activitry. From the present study it is evident that both the cold and hot extracts of the studded algae could be utilized as a good natural source of antimicrobial, anticoagulant and antitumor against HELA cell line. However, the nature of the specific bioactive components responsible for the observed biological activities from these two algae remains to be evaluated for pharmaceutical and industrial applications.

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