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Properties of Polysaccharides in Several Seaweeds from Atlantic Canada and their Potential Anti-influenza Viral Activities

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Abstract

Objective: To explore the polysaccharides from selected seaweeds of Atlantic Canada and to evaluate their potential anti-influenza virus activities. **Methods**: Polysaccharides were isolated from several Atlantic Canadian seaweeds, including three red algae (*Polysiphonia lanosa, Furcellaria lumbricalis,* and *Palmaria palmata*), two brown algae (*Ascophyllum nodosum* and *Fucus vesiculosus*), and one green alga (*Ulva lactuca*) by sequential extraction with cold water, hot water, and different alkali solutions. These polysaccharides were analyzed for monosaccharide composition and other general

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chemical properties, and they were evaluated for anti-influenza virus activities. **Results**: Total sugar contents in these polysaccharides ranged from 15.4 % (in *U. lactuca*) to 91.4 % (in *F. lumbricalis*); sulfation level was as high as 17.6 % in a polysaccharide from *U. lactuca* whereas it couldn't be detected in an alikali-extract from *P. palmaria*. For polysaccharides extracted from red seaweeds, the main polysaccharides were sulfated agar and carrageenan for *P. lanosa, F. lumbricalis*, and xylans for *P. palmata.* In brown seaweeds, the polysaccharides mainly contained sulfated fucans, whereas the polysaccharides in green seaweeds were composed of heteroglycuronans. Screening for antiviral activity against influenza A/PR/8/34 (H1N1) virus revealed that brown algal polysaccharides were particularly effective. **Conclusion**: Seaweeds from Atlantic Canada are a good source of marine polysaccharides with potential antiviral properties.

Key words: Polysaccharides; Anti-influenza viral activity; Monosaccharide composition; H1N1

1 Introduction

Many seaweed species have been used traditionally as food, as well as herbal drugs in Asian countries. Investigations have been reported that the seaweeds have not only nutritional effects but also beneficial properties that may be applied in the prevention or treatment of various diseases (Redgwell and Fischer 2005; Costa, Fidelis et al. 2010; Pomin 2010). Algal polysaccharides exhibit structural features such as sulfate and uronic acid groups, which distinguish them from polysaccharides of terrestrial plants, but are similar to mammalian glycosaminoglycans, such as heparin and chondroitin sulfate. Increasing research on algal polysaccharides, in native form or their derivatives, have revealed a variety of biological activities including anti-virus (Ghosh, Chattopadhyay *et al*. 2009; Harden, Falshaw *et al*. 2009), antioxidant (Josephine, Amudha *et al*. 2007; Rocha de Souza, Marques *et al*. 2007), anti-tumor (Zhou, Sun et al. 2004; Yamasaki-Miyamoto, Yamasaki et al. 2009), anticoagulant/anti-thrombotic (Pereira, Mulloy *et al*. 1999; Pomin and Mourco 2008), and immuno-inflammatory activities (Groth, Grunewald *et al*. 2009; Yang, Yu *et al*. 2011).

The anti-virus properties of algal polysaccharides have generated great interest with activities reported against a wide range of viruses including human immunodeficiency virus (HIV) (Baba, Snoeck *et al.* 1988), herpes simplex virus (HSV) (Hasui, Matsuda *et al.* 1995), human cytomegalovirus (HCMV) (Damonte, Matulewicz *et al.* 2004), vesicular stomatitis virus (Baba, Snoeck *et al.* 1988), papilloma virus (Pérez-Andino, Buck *et al.* 2009) and influenza A virus (Leibbrandt, Meier *et al.* 2010). The influenza pandemic in 2009 (A/H1N1; 2009 H1N1) underscored the need for new anti-virus agents, particularly for use in elderly or immuno-compromised subjects (Hayden 2009). Adamantane-based anti-virus compounds such as amantadine and rimantadine have serious side-effects on gastrointestinal and nervous system (Jefferson, Demicheli *et al.* 2006). Using oseltamivir, a viral neuraminidase inhibitor, to treat influenza (von Itzstein 2007) is being confounded by the rise of resistant H1N1 influenza strains and emerging resistant influenza A seasonal strains (Bantia, Parker *et al.* 2001; Matheson, Harnden *et al.* 2008; Shun-Shin, Thompson *et al.* 2009).

We prepared polysaccharides from several Atlantic Canad seaweeds, *Polysiphonia lanosa, Furcellaria lumbricalis, Palmaria palmata, Ascophyllum nodosum, Fucus vesiculosus* and *Ulva lactuca*, and studied their chemical properties. Their antiviral activity against influenza A/PR/8/34 (H1N1) virus was screened, and the results suggested that some of these seaweeds are good sources for new anti-influenza virus agents.

2 Materials and Methods

2.1 Seaweeds and reagents

The seaweed *P. lanosa* (Ceramiales, Rhodomelaceae) was collected at Sandy Cove, NS, Canada during the autumn of 2008. *F. lumbricalis* (Gigartinales, Furcellariaceae) was supplied by Oceanside Seaweeds Ltd. (Miminegash, PEI, Canada); *P. palmata* (Palmariales, Palmariaceae) and *U. lactuca* (Ulvales, Ulvaceae) were from Atlantic Mariculture Ltd. (Grand Manan, NB, Canada); *A. nodosum* and *F. vesiculosus* were from Acadian Seaplants Ltd. (Dartmouth, NS, Canada).

Monosaccharide standards, including glucuronic acid, galactouronic acid, glucosamine, galactosamine, glucose, mannose, xylose, galactose, fucose, rhamnose and arabinose, were purchased from Sigma-Aldrich (MO, USA). Dialysis tubing (6-8 kDa MWCO) was from Thermo Fisher Scientific (Pittsburgh, PA). All reagents and solvents were of analytical grade.

2.2 Polysaccharides preparation

Polysaccharides were extracted following the method of Yu (2007) with some modification and the process was outlined in Fig. 1. Briefly, freeze-dried and grounded seaweed powder was treated with methanol at 80°C for 2 hours to remove lipids and pigments. The air-dried residue was then extracted with 20 volumes of cold water for 3 hours and with 3 times, followed by extracting with 20 volumes of distilled water at 80°C for 3 hours and with 3 times. Then the residue was extracted with 2 % Na_2CO_3 solution at 80°C two times and every time for 2 hours, then with 0.5 M NaOH at 60°C two times and every time for 2 hours. The different extracting solutions were neutralized (if necessary), concentrated, and precipitated with 4 volumes of ethanol. All precipitates were dissolved in distilled water and dialyzed (6-8 kDa MWCO) until the conductivity was similar to that of distilled water. The dialysates were freeze-dried to obtain the polysaccharide extracts.

2.3 Chemical analysis of seaweed polysaccharides

Total sugar content of the polysaccharides was determined by the phenol-sulfuric acid method (DuBois, Gilles *et al*. 1956) with various monosaccharides as the standards. Sulfation level was determined by using the $BaCl_2$-gelatin turbidimetry method (Dodgson and Price 1962). The content of crude protein was determined by Lowry method (Lowry, Rosebrough *et al*. 1951).

2.4 Determination of the molecular weight

The molecular weights (M_w) of algal polysaccharides were determined by high performance liquid chromatography (HPLC, Agilent 1200) with a PL aquagel-OH

MIXED column (7.8 mm×300 mm, Agilent, USA), eluted with 0.2 M $NaNO_3$ (with 10 mM NaH_2PO_4) at 0.5 mL/min at 30°C and detected with a Multi-Angle Laser Light Scattering system (MALLS, Wyatt Technologies, USA) and Refractive Index (RI) detector. Their molecular weights were calculated by using Astra 5.3.4.20 software.

2.5 Monosaccharides composition analysis

Algal polysaccharides were hydrolyzed by 2 M TFA at 105°C for 6 hours to release the monosaccharides. The monosaccharide composition was analyzed by pre-column derivation with 1-Phenyl-3-methyl-5-pyrazolone (PMP) (Honda, Akao *et al*. 1989; Fu, Zhao *et al*. 2008). Briefly, 100 μg of a hydrolysate was combined with 100 μL of 0.3 M NaOH solution and 120 μL of 0.5 M PMP solution and react at 70°C for one hour. After cooling to room temperature, the extra PMP was extracted with chloroform (200 μL three times). The aqueous phase was filtered (0.22 μm filter) prior to loading onto an Agilent XDB C18 column (150 mm×4.6 mm, 5 μm). Elution was conducted with phosphate buffer (pH 6.7)/Acetonitrile (83:17, v/v) for 35 min with UV detection (DAD 245 nm).

2.6 Fourier transform infrared spectrometry (FTIR) analysis

Samples (1-2 mg) were dried in a P_2O_5 desiccator for 48 hours, mixed with 100 mg KBr and pressed under 7 kg/cm^2 to make transparent films that were scanned from 400 to 4,000 cm^{-1} in an FTIR instrument (Nicolet Nexus 470, Thermo Electron, USA).

2.7 Nuclear magnetic resonance (NMR) analysis

Samples (~30 mg) were dissolved in 1 mL D_2O and freeze-dried twice to replace all exchangeable protons with deuterium. ^{1}H-NMR was acquired at 20 ℃ using JEOL 600 MHz equipment. Chemical shift values were calibrated using acetone-d6 as an internal standard.

2.8 Antiviral activity

Algal polysaccharides (250 μg/mL) were screened for their antiviral activities against influenza A/PR/8/34 (H1N1) virus using a cytopathic effect (CPE) reduction method in the Madin Darby canine kidney (MDCK) cell line as described previously (Hung, Tseng et al. 2009; Wang, Zhang et al. 2011). Confluent MDCK cells, grown in 96-well plates were infected with influenza virus (MOI = 1.0) for 60 min. The medium containing virus was then removed and replaced with medium containing different concentrations of polysaccharides, which were tested in triplicate. MDCK cells inoculated with influenza A viruses were incubated under 100 % humidity and 5 % CO_2 at 37℃ for 48 hours. Cells were then fixed with 4 % formaldehyde for 20 min at room temperature and stained with 0.005 % crystal violet solution for 30 min. Cells were then washed and dried and the intensity of crystal violet staining for each well was read at 570 nm. Anti-viral inhibition was calculated by $(OD_{sample}-OD_{virus})/(OD_{blank}-OD_{virus})\times100\%$. The 50% inhibitory concentration (IC_{50}) was determined as the concentration of test sample required to inhibit the influenza virus yield by 50%. Ribavirin (Sigma-Aldrich, MO, USA) was evaluated in parallel as a positive control.

3 Results and Discussion

3.1 Extraction and general analysis of algal polysaccharides

Chemical structures of polysaccharides from marine algae have been extensively studied and the results showed that their chemical and structural properties differ from species to species (Percival, Venegas Jara *et al.* 1983; Sun, Yu *et al.* 2011). Even in the same species, the polysaccharides show structural differences depending on life cycle stage, anatomy or geographical location (Black, Blakemore *et al.* 1965; Chen, McLachlan *et al.* 1973; Reis, Yoneshigue-Valentin *et al.* 2008). In consideration of existing extraction methods, we used a simplified method as shown in Fig. 1 to obtain native algal polysaccharides without degradation or structure modification. All algae were extracted with methanol first to remove the methanol soluble non-polysaccharide components, followed by cold water, hot water and alkali solution to obtain four crude polysaccharide preparations, E1, E2, E3 and E4, respectively. These extracts were soluble in water and

the total polysaccharides yielded from dry algae varied from 19.71 % (*F. lumbricalis*) to 49.20 % (*P. lanosa*) which indicated that these seaweeds were rich in polysaccharides.

General composition analysis was carried out using methods described by other research groups (Lowry, Rosebrough *et al.* 1951; DuBois, Gilles *et al.* 1956; Dodgson and Price 1962) and the results were in Table 1. The reported values have a relatively widespread range from 15.42-91.36 % sugar content, 1.69-35.70 % crude protein content and 0-17.55 % sulfate content. Some of the variation can be attributed to specie differences and environment condition differences.

Depending on their major monosaccharide composition, different sugars were used as standards when running total sugar content determination to reduce the individual error. For example, galactose was used as standard for polysaccharides from *P. lanosa* and *F. lumbricalis*, xylose was used for those from *P. palmata* and *U. lactuca*, and fucose was applied as sugar standard for those from brown seaweeds, *A. nodosum* and *F. vesiculosus*.

The molecular weights of these polysaccharides were determined by using HPLC with MALLS and RI detectors to be within the range from about 26 kDa to 2,482 kDa. The molecular weights of most of the alkali-extracted polysaccharides from red algae and green algae were higher than those of water-extracted polysaccharides. However, the molecular weights of alkali-extracts from brown alge *A. nodosum* and *F. vesiculosus* were lower than those of water-extracted polysaccharides. These data offered the basis for obtaining polysaccharides of different M_w by different extraction methods. The relatively high level of polysaccharides and protein in these seaweeds also suggests that they could be consumed for their nutritional value.

3.2 Structural feature analysis

The absorption peaks of algal polysaccharides were summarized in Table 2. Based on FTIR analysis, the samples revealed polysaccharides features at about 3430 cm^{-1} (ν_{O-H} of -OH group), 2928 cm^{-1} (ν_{C-H} of pyranose) and 1166 cm^{-1} (ν_{C-O-C} of glycoside). The strong absorption bands at about 1257 cm^{-1} ($\nu_{O=S=O}$ of sulfate) and a sharp band at 850 cm^{-1} (ν_{C-O-S} of C4-S), 820 cm^{-1} (ν_{C-O-S} of C6-S) or 805 cm^{-1} (ν_{C-O-S} of C2-S) suggested the

presence of sulfate groups and substitution positions. The strength of the S-O absorption bands corresponded well with the sulfate content determined by chemical analysis.

Structural analysis of a polysaccharide requires knowledge of its monosaccharide composition. In our previous work, we compared four methods to determine the monosaccharide composition of polysaccharides. The results showed that PMP precolumn-derivatized HPLC worked with a higher sensitivity and better resolution, and could analyze neutral, acidic and alkaline monosaccharides simultaneously (Fu, Zhao *et al*. 2008). Because of the instability of 3,6-anhydro-galactose (AnG) in an acid environment, it could not be derived with PMP. Thus our data did not include the AnG composition of polysaccharides from red seaweeds.

As shown in Table 1, four kinds of polysaccharides from *P. lanosa* were mainly composed of sulfated galactose. Water-extracted polysaccharides, FL1 and FL2, from *F. lumbricalis* were also composed of galactose but its alkali-extracts, FL3 and FL4, mainly contained glucose. These two polysaccharides were proved to be floridean starch by reacting with iodide solution. Four polysaccharides extracted from *P. palmata* were xylans according to the monosaccharide composition analysis. Fucose was the dominant component in extracts from brown algae *A. nodosum* and *F. vesiculosus*, which also contained other monosaccharides, such as glucouronic acid, mannose, xylose, galactose and glucose. Thus, these brown algal polysaccharides could be regarded as heterofucans. In the green alga *U. lactuca*, the polysaccharides were determined to be heteroglycuronans containing rhamnose, xylose and glucose. Although some of the polysaccharides from these seaweeds have already been reported before, they were extracted with different conditions but not in a sequential fashion. For example, Batey and Turvey reported that hot water extract of *P. lanosa* contained sulfated agar (Batey and Turvey 1975). Yu *et al.* and Yang *et al.* found that *F. lumbricalis* mainly contained sulfated carrageenan and a complex floridean (Yu 2007; Yang, Yu et al. 2011). Xylans and ulvans were investigated from *P. palmata* (Turvey and Williams 1970; Deniaud, Quemener et al. 2003) and *U. lactuca* (Lahaye, Jegou et al. 1994; Siddhanta 2001), respectively. A number of studies have reported on fucoidans and other sulfated fucans from *A. nodosum* and *F. vesiculosus* (Chevolot, Mulloy et al. 2001; Usov and Bilan 2009).

However, most of these algal polysaccharides were extracted only using water or alkali condition. Here we used a sequential extraction procedure to obtain the polysaccharides and compared the structural features of polysaccharides in the different extraction products. The compositions of these polysaccharides were consistent with those published results.

3.3 Anti-influenza virus activities

Reports have revealed recently that algal polysaccharides exhibited antiviral activities both *in vivo* and *in vitro* (Witvrouw and De Clercq 1997; Schaeffer and Krylov 2000; Hidari *et al.* 2008) with an effectiveness that depends on the sugar composition, main chain length, sulfation level and sulfate pattern (Ghosh *et al.* 2009; Jiao *et al.* 2011). As shown in Table 1, the inhibition of influenza A/PR/8/34 virus by algal polysaccharides varied depending on the algae species. Generally, PL3 from *P. lanosa,* AN1, AN2, AN3, AN4 from *A. nodosum*, and FV1, FV3, FV4 from *F. vesiculosus* showed high or medium antiviral activities against influenza A/PR/8/34 virus (Table 1). Overall, polysaccharides extracted from the brown seaweeds exhibited higher anti-H1N1 activities than those from red or green algae. In particular, FV1, the cold water extract from *F. vesiculosus* inhibited the influenza A/PR/8/34 virus by 77.9 % (Table 3). ^{1}H-NMR spectrum of FV1 (Fig. 2) showed an intense band at 1.8 ppm due to 6-deoxi sugar protons, corresponding to the main neutral sugar, fucose, in this polysaccharide. Collectively, the absorption on FTIR spectrum of FV1 (Fig. 3) at about 1255.43 cm^{-1} ($\nu_{O=S=O}$), 1620.67 cm^{-1} ($\nu_{C=O}$) and 824.16 cm^{-1} (ν_{C-O-S} of C6-S) suggested that FV1 was a sulfated fucan substituted with uronic acid groups.

As the influenza virus life cycle critically depends on a balance between available receptor sites (neuraminidase) and receptor binding (hemagglutinin) (Wagner *et al.* 2002; Moscona 2009), antiviral activities of algal polysaccharides may be the result of interfering with adsorption of virus to cells or blocking the fusion event, which leads to uncoating of the nucleocapsid (Talarico and Damonte 2007). Algal polysaccharides can interfere the adsorption of virus to host cells through binding to the cellular surface and coating cellular structures that are usually required for viral binding (Leibbrandt, Meier *et*

al. 2010). Brown algal polysaccharides have fucose, sulfate and uronic acid groups along their backbones providing a degree of ionization, which may be involved in an improved ability to inhibit the influenza A/PR/8/34 virus. The strong negative charges of these functional groups could bind to the virus and inhibit viral reverse transcriptase (RT) activity to block its adsorption; alternatively, the functional groups could bind the anionic receptor of the host cellular surface to inhibit virus adsorping to host cells. We further isolated fucoidans, which contained fucose and sulfate groups but no uronic acids, from these brown algal polysaccharides and screened their antiviral activities against influenza A/PR/8/34 virus. Surprisingly, these fucoidans did not show significant antiviral effects (data not shown). This is in agreement with the report by Hidari *et al.* (2008) who prepared an antiviral polysaccharide with glucuronic acid and sulfated fucose residues from *Cladosiphon okamuranus* and found that antiviral properties of this polysaccharide was abolished when the glucuronic acids was carboxyl-reduced (Hidari, Takahashi *et al.* 2008). Both uronic acids and fucose might be important for anti-influenza virus activity of polysaccharides.

Although the other kinds of polysaccharides showed low or no anti-influenza A/PR/8/34 virus activities, many groups reported that sulfated galactans from red seaweeds and ulvan from green seaweeds possessed inhibitory activities against HSV, HCMV, and HIV (Baba, Snoeck *et al.* 1988; Mazumder, Ghosal *et al.* 2002; Damonte, Matulewicz *et al.* 2004). The current observations proved that certain algal polysaccharides could be applied as anti-virus agents.

4 Conclusions

Sequential extraction of six different seaweeds from the Atlantic coast showed them good sources of polysaccharides. The study of their general chemical properties and *in vitro* screening against A/PR/8/34 virus revealed brown algal polysaccharides are potential antiviral agents. Further studies are necessary to determine the structure-function relationship of antiviral polysaccharides and their *in vivo* efficacy.

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Table 1 General chemical properties and anti-H1N1 activities of algal polysaccharides derived from six Atlantic Canadian seaweed species

Seaweeds	PS	Extraction methods	Yield (%)	Total sugar (%)	Sulphation level (%)	Protein (%)	Mw (KDa)	Sugar composition										Anti-HIN1* (%)
								Man	Rha	GlcA	GalA	GalN	Glc	Gal	Xyl	Ara	Fuc	
P. lanosa	PL1	Cold water	10.50	63.39	17.49	1.69	45.89						3.76	74.43	21.81			+
	PL2	Hot water	22.92	66.61	13.27	5.62	26.23						4.18	75.82	20.00			+
	PL3	2 % Na_2CO_3	13.31	17.33	6.78	9.60	112.10						31.01	56.02	12.98			++
	PL4	0.5 M NaOH	2.47	17.80	2.77	26.11	137.20						21.21	41.73	37.06			-
F. lumbricalis	FL1	Cold water	1.30	28.02	9.41	19.86	142.70					4.23	10.02	76.33	9.42			+
	FL2	Hot water	12.56	25.03	9.93	4.09	152.70				0.71	7.26	34.81	57.22				-
	FL3	2 % Na_2CO_3	1.76	64.56	1.50	18.81	2482.00				1.73	1.50	82.57	14.20				-
	FL4	0.5 M NaOH	4.09	91.39	0.37	8.82	121.70				0.69		97.25	2.06				-
P. palmata	PP1	Cold water	12.37	66.09	2.92	10.11	230.70							0.90	99.10			-
	PP2	Hot water	4.18	68.81	3.19	6.63	108.00			1.92			1.31	3.75	93.01			-
	PP3	2 % Na_2CO_3	4.54	23.25	0.18	15.50	368.00						13.77		86.23			+
	PP4	0.5 M NaOH	4.40	61.54	--	4.53	101.40						2.82		97.18			-

Seaweeds	PS	Extraction methods	Yield (%)	Total sugar (%)	Sulphation level (%)	Protein (%)	Mw (KDa)	Sugar composition										Anti-HIN1* (%)
								Man	Rha	GlcA	GalA	GalN	Glc	Gal	Xyl	Ara	Fuc	
A. nodosum	AN1	Cold water	16.97	54.19	12.41	18.71	387.30	7.96		11.49			3.07	12.61	16.83		48.06	++
	AN2	Hot water	2.77	67.05	10.81	12.52	315.10	7.92		14.38			3.61	4.86	17.03		52.22	+++
	AN3	2 % Na_2CO_3	17.56	53.08	5.30	8.66	147.30	7.3		15.05			1.82	4.66	16.8		54.37	++
	AN4	0.5 M NaOH	0.85	58.25	5.02	29.74	116.00	5.85		10.16			3.68	15.09	15.9		49.31	++
F. vesiculousus	FV1	Cold water	7.95	34.20	9.65	35.70	334.20	4.16		7.49			7.78	14.5	6.69		59.38	+++
	FV2	Hot water	2.74	64.26	14.51	15.31	452.10	7.78		7.33			3.49	12.55	14.44		54.42	+
	FV3	2 % Na_2CO_3	14.15	54.47	7.39	5.55	113.60	4.88		11.48	2.82			17.08	17.47		49.09	++
	FV4	0.5 M NaOH	0.14	46.44	2.77	5.75	165.20	3.84		9.3			25.56	10.53	6.35		44.41	++
U. lactuca	UL1	Cold water	6.02	38.26	17.55	8.37	120.10		18.54	49.59					29.72	2.15		+
	UL2	Hot water	6.50	36.14	15.12	6.09	135.70		18.89	37.11			8.63		35.37			+
	UL3	2 % Na_2CO_3	4.85	15.42	9.17	9.08	213.90		9.73	23.51			49.60	1.56	12.66	2.94		+
	UL4	0.5 M NaOH	3.41	29.64	7.04	18.90	699.50		8.15	15.43			44.88		31.54			+

-- Not detected

* Determined base on the average of three independent replications, inhibition value is presented as +++ = 70-100%, ++ = 50-70%, + = 20-50%, - = ＜20%. Concentration of samples was 250 μg/mL.

** Inhibition of positive control, Ribavirin, was 85 % at the same concentration.

Table 2 Absorption peaks of algal polysaccharides on FTIR spectra

Absorption peak (cm^{-1})	**Types of vibration**	**Functional group**
3700-3100	ν_{O-H}	-OH
1060-1030	δ_{O-H}	C-OH
2962, 2928	ν_{C-H}	CH_3, CH_2 in pyranose
1465, 1450	δ_{C-H}	
1640, 1540	δ_{N-H}	CO-NH
1620-1550	$\nu_{C=O}$	-COO
1400-1300	$\nu_{C=O}$	
1166	ν_{C-O-C}	C-O-C in pyranose
1260-1230	$\nu_{O=S=O}$	$-OSO_3^-$
890-840	ν_{C-O-S}	
850	ν_{C-O-S}	^4C- OSO_3^-
820	ν_{C-O-S}	^6C- OSO_3^-
805	ν_{C-O-S}	^2C- OSO_3^-
1070	ν_{C-OH}	C-OH of galactose
930	ν_{C-O-C}	C-O-C of 3,6-anhydro-galactose

Table 3 Antiviral activities of polysaccharides from brown algae and their IC_{50} values.

Samples	**Inhibition (%)**	**IC_{50} value**	
		μg/mL	**μM**
Control	85.0 ± 1.4	24.6	100.73
AN1	67.1 ± 5.6	112.1	0.29
AN2	69.3 ± 7.5	100.7	0.32
AN3	55.2 ± 5.0	151.6	1.02
AN4	52.7 ± 7.8	191.4	1.65
FV1	77.9 ± 2.2	74.8	0.22
FV2	47.6 ± 10.1	180.5	0.40
FV3	50.3 ± 2.5	176.8	1.56
FV4	62.9 ± 6.5	124.5	0.75

Note: Concentration of polysaccharides for inhibition test was 250 μg/mL. Molarities of IC_{50} value of polysaccharides was determined by the molecular weight in Table 1. Ribavirin was used as positive control. Results are the mean ± SD of three independent determinations.

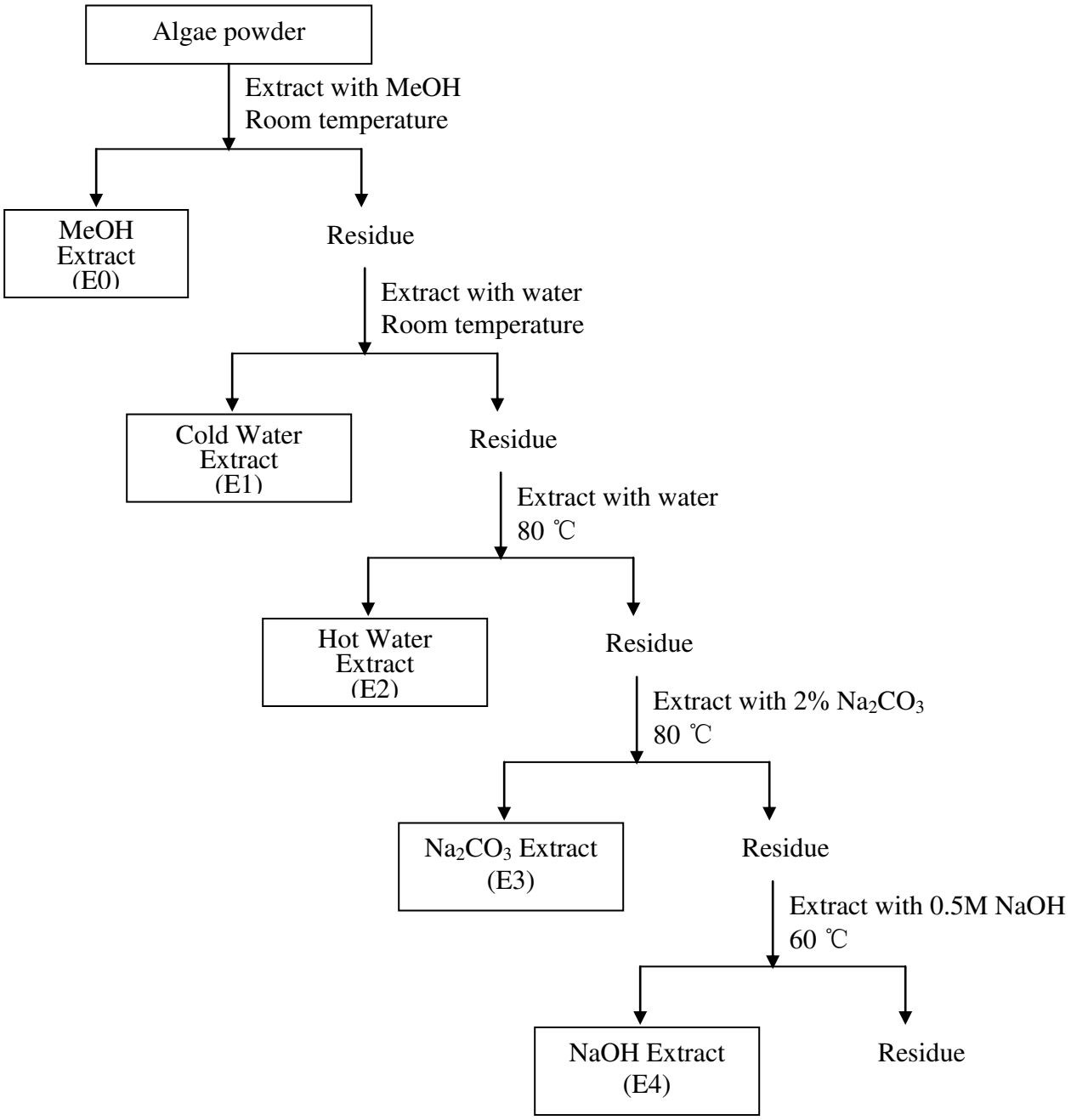

Fig.1 Algal polysaccharides extraction flowchart

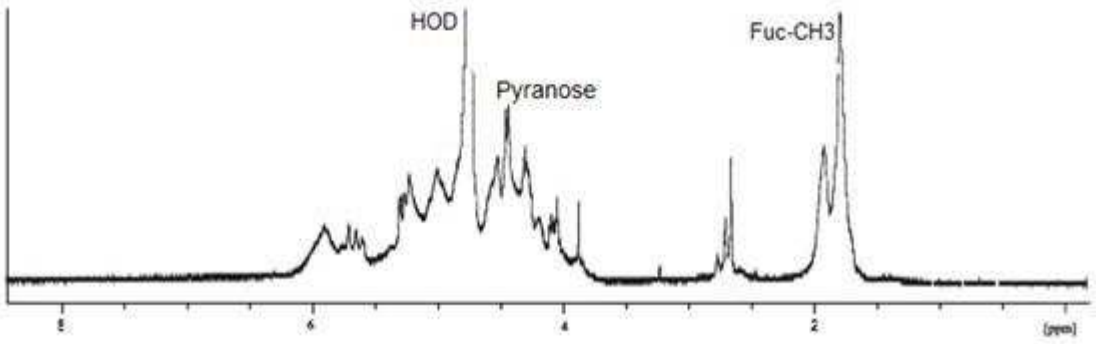

Fig. 2 ^{1}H-NMR spectrum of FV1 from Atlantic Canadian *F. vesiculosus*

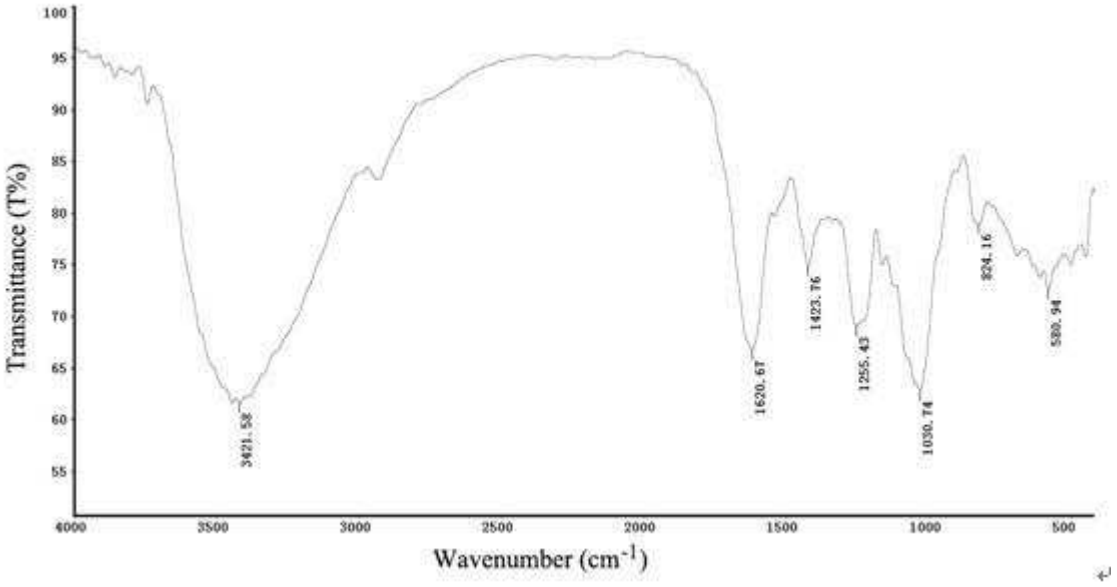

Fig. 3 FTIR spectrum of FV1 from Atlantic Canadian *F. vesiculosus*

加拿大大西洋沿岸海藻多糖的理化性质及其抗流感病毒活性的研究

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摘要：目的：开发加拿大大西洋海域海藻中的多糖资源及寻找具有潜在的抗流感病毒活性的多糖。**方法：**对来自加拿大大西洋海藻，包括三种红藻（多管藻，蠕虫叉红藻和掌叶树），两种褐藻（泡叶藻和墨角藻），一种绿藻（石莼），通过冷水、热水、弱碱和强碱四种提取方法，制备了一系列海藻多糖，并对其理化性质及其潜在的抗流感病毒 A/PR/8/34 (H1N1) 活性进行了对比。**结果：**所得海藻多糖的总糖含量从 15.4 %（UL3）到 91.4 %（FL4）不等，硫酸根含量从 0（PP4）到 17.6 %（UL1）不等。其中，多管藻和蠕虫叉红藻多糖主要由硫酸化的硫琼胶和卡拉胶组成，掌叶树多糖主要由木聚糖组成；褐藻中的泡叶藻和墨角藻多糖主要包含硫酸化的岩藻聚糖；而绿藻中石莼多糖主要由异聚葡糖糖醛酸及其他单糖聚合而成。通过对其抗流感病毒活性进行对比，发现几种褐藻多糖的抗流感病毒活性高于其他海藻多糖。其中，墨角藻冷水提取的多糖 FV1，一种被糖醛酸取代的硫酸化岩藻聚糖抗流感病毒 A/PR/8/34 (H1N1) 的活性最高。**结论：**加拿大大西洋海域海藻中具有丰富的多糖资源，其多糖具有潜在的抗流感病毒的活性。

关键词：多糖；抗流感病毒活性；单糖组成；H1N1