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Molecular characteristics of partially hydrolyzed fucoidans from sporophyll of *Undaria Pinnatifida* and their *in vitro* anticancer activity

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ABSTRACT

The effects of molecular characteristics on the anticancer activity of fucoidans were investigated after hydrolysis by copper acetate and then fractionation with 30 and 5 kDa membranes, which produced three fucoidan fractions: $F_{>30\ K}$, $F_{5-30\ K}$ and $F_{<5\ K}$. The $F_{>30\ K}$ and $F_{5-30\ K}$ consisted of mostly carbohydrate (58.2–61.3%) and sulphate (31.7–35.5%) with small amounts of proteins (1.2–6.4%). However, the major constituents of $F_{<5\ K}$ were sulphate (31.8%) and ash (37.5%) with smaller amounts of carbohydrate (15.5%) and protein (1.2%). The molecular weights (M_w) of $F_{>30\ K}$, $F_{5-30\ K}$ and $F_{<5\ K}$, obtained by a light scattering technique, were 262, 5.6 and 1.6 kDa, respectively. The observed anticancer activities were 18.0–28.5% for $F_{>30\ K}$, 19.2–57.5% for $F_{5-30\ K}$ and 26.5–36.5% for $F_{<5\ K}$, respectively, in the concentration range of 0.2–0.8 mg/mL. The results suggest that the anticancer activity of fucoidans could be considerably improved by lowering their M_w and by improving the binding properties of sulphate groups possibly through changing the molecular conformation.

1. Introduction

Fucoidans are complex and heterogeneous sulphated polysaccharides commonly found in brown seaweeds. Their structural and compositional properties vary with the species of brown seaweeds, but they mainly consist of fucose and sulphate with small amounts of galactose, xylose, mannose and uronic acids (Bilan et al., 2002; Chizhov, Dell, Morris, Haslam, & McDowell, 1999; Nagaoka et al., 1999; Partankar, Oehninger, Barnett, Williams, & Clark, 1993; Percival & Ross, 1950). Fucoidans, because of their diverse biological activities (anticoagulant, antiviral and anticancer), have been extensively studied. It was reported by Soeda, Sakaguchi, Shimeno, and Nagamatsu (1992) that the fibrinolytic and anticoagulant activities of fucoidans were due to the stimulation of the tissue plasminogen activator (t-PA), inducing plasma clot lysis and decreasing the rate of fibrin polymer formation. Recent report indicated that galacto-fucans, a major component of the room temperature extract from *Adenocystis utricularis*, had a high inhibitory activity against herpes simplex virus 1 and 2 with no cytotoxicity (Ponce et al., 2003). A number of studies have also been reported on the anticancer activity of fucoidan polymers. It has been suggested that fucoidan polymers activate the host immune system against tumours (Maruyama, Tamauchi, Hashimoto, & Nakano, 2003; Noda, Amano, Arashima, & Nisizawa, 1990; Usui, Asari, & Mizuno, 1980). On the other hand, Koyanagi, Tanigawa, Nakagawa, Soeda, and Shimeno (2003) suggested that fucoidan polymers could inhibit the growth of tumour cells by suppressing angiogenesis, which is the formation of new micro-blood vessel, of tumour cells.

The biological activities of fucoidans have been reported to be closely related to both sulphate content and molecular weight. Recently, it was reported by Qiu, Amarasekara, and Doctor (2006) that oversulphated fucoidans induced higher stimulation of glutamic-plasminogen (Glu-Plg) activation by t-PA and urokinase (UK) showing four times higher anticoagulant activity than native fucoidans. It was also shown that oversulphated fucoidans effectively suppressed the angiogenesis of Sarcoma 180 cells implanted in mice and exhibited more potent anticancer activity than native fucoidans (Koyanagi et al., 2003). However, partially desulphated fucoidans with sulphate contents less than 20% showed drastic decreases in both anticoagulant and anticancer activities (Haroun-Bouhedja, Ellouali, Sinquin, & Boisson-Vidal, 2000). There have been a limited number of studies investigating the effect of the molecular weight of the fucoidan polymers on biological activity. It was reported by Nishino and Nagumo (1991) that the most potent anticoagulant activities were found in the range of the molecular weights of fucoidans from 10 to 300 kDa. However, to the best of our knowledge, the molecular weight effects of fucoidan polymers on the anticancer activity have not yet been reported. In the study of other polysaccharides, Lin et al. (2004) reported that

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sulphated α-glucans, extracted from *Poria cocos* mycelia, having a moderate range of molecular weight from 20 to 400 kDa were beneficial to the enhancement of the antitumor activities, though the effect of the molecular weight within the range on the antitumor activity was negligible.

In this study, fucoidan was extracted from the sporophyll of *Undaria pinnatifida*, hydrolyzed by copper acetate and subsequently fractionated using an ultrafiltration system equipped with the membranes having the molecular weight cut-offs of 30 and 5 kDa, which produced three fucoidan fractions with different molecular weights. The objectives of this study were to determine the physicochemical properties of these fucoidan fractions and to investigate the relationship between their molecular characteristics and *in vitro* anticancer activity.

2. Materials and methods

2.1. Materials

The sporophyll of brown seaweed (*U. pinnatifida*), originated from the coast of Wando, Chunnam province, Korea, was purchased, washed with distilled water and air-dried at 60 °C. The dried sample was milled using a blender, sieved (<0.5 mm) and then stored at −20 °C before analyses. The average particle diameter of the milled sample was 476.9 μm, which was determined by a particle size analyzer (Mastersizer, 2000, Malvern Instruments Ltd., Malvern, UK). All chemicals and reagents used were of analytical grade.

2.2. Extraction, hydrolysis and fractionation of fucoidan

The milled sample (20 g) was refluxed with 200 mL of 80% ethanol at 75 °C for 1 h to remove lipophilic pigments (chlorophylls, fucoxanthin and carotenes etc.) and low molecular weight proteins, and centrifuged at 1800*g* for 10 min, and then dried at 25 °C. The dried biomass (10 g) was extracted with 0.2 M HCl (100 mL) at 60 °C with constant mechanical stirring for 1 h. The extract was centrifuged at 5000*g* for 10 min, and the supernatant was collected. Ethanol (99%, 400 mL) was added into the supernatant to obtain the final ethanol concentration of 75%, and the solution was placed at 4 °C overnight. After centrifugation at 5000*g* for 10 min, the precipitate (crude fucoidan) was collected, washed with ethanol and acetone, dried at room temperature and stored for further experiments.

The extracted crude fucoidan was hydrolyzed by the method of Nardella et al. (1996) to produce the low molecular weight fucoidans. The hydrolysis of crude fucoidan was carried out by dissolving the crude fucoidan (1.0 g) and copper acetate monohydrate mixture (0.08 g, 0.04 mM) in water at 60 °C with maintaining the pH at 7.5 by the addition of 2 M NaOH. Hydrogen peroxide solution (90%, v/v) was added into the fucoidan mixture at a flow rate of 12 mL/h and the hydrolysis by copper acetate monohydrate was performed for 5 h with a constant mechanical stirring. After the hydrolysis of fucoidan, a chelating resin (Chelex-100, Biorad, Hercules, CA, USA) was added into the fucoidan solution to remove copper. The fucoidan solution was subsequently neutralized with 2 M NaOH, dialyzed with a 1000 g/mol cut-off membrane (Millipore Corp., Bedford, MA. USA) and lyophilized.

The hydrolyzed fucoidan was fractionated using a Millipore Ultrafiltration System with two membranes having nominal molecular weight cut-offs of 30 and 5 kDa with the area of 0.1 m^2 (Millipore, USA). After dissolving the hydrolyzed fucoidan in distilled water, the solution was pumped to the membrane surface and the filtrate was collected while the retentate was directed back to the recycle tank. The circulation process was performed until the solution was concentrated to about one sixth of its original volume, and then it was concentrated by rotary evaporation, precipitated by ethanol and lyophilized. The filtrate after each ultrafiltration process was the feed solution of the next ultrafiltration. Finally, the hydrolyzed fucoidan was fractionated into the three fractions, $F_{>30\ K}$ (M_w > 30 kDa), $F_{5-30\ K}$ (30 kDa < M_w < 5 kDa) and $F_{<5\ K}$ (M_w < 5 kDa).

2.3. Determination of chemical composition

The sulphate content of fucoidan was determined by the $BaCl_2$ gelatin method using K_2SO_4 as a standard after hydrolyzing the polysaccharide in 0.5 M HCl at 105 °C for 5 h (Dodgson & Price 1962). The contents of total carbohydrate and protein were determined by the phenol–sulphuric acid method using fucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith 1956) and by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) using DC Protein assay kit (Bio-Rad, Hercules, CA, USA), respectively. Moisture content of fucoidan was determined by drying the samples at the drying oven for 2 h at 131 °C. The content of ash was determined by heating the dried sample in a furnace at 600 °C for 8 h.

2.4. Determination of ash composition

A Perkin–Elmer Model A Analyst 700 atomic absorption spectrometer (Perkin–Elmer, Waltham, MA) equipped with flame and graphite furnace atomizers and Zeeman background correction was used to determine the ash composition of fucoidan fractions after dissolution in 6 M nitric acid. Sodium and potassium were determined by atomic emission at 589.0 and 766.5 nm, using an air/acetylene flame with an oxidant fuel flow of 17.0 and 2.0 mL/min, respectively. Calcium, zinc, iron, copper and manganese measurements were performed by atomic absorption at 422.7, 213.9, 248.3, 324.8 and 279.5 nm, using hollow cathode lamps operated at 10, 15, 30, 15 and 20 mA, and bandwidths of 0.7, 0.7, 0.2, 0.2 and 0.2 nm, respectively.

2.5. Determination of monosaccharide composition

Quantitative determination of the monosaccharide composition of fucoidan was performed using a HPLC system, consisting of a pump (Waters 510, Waters, Milford, MA, USA), an injection valve (Model 7010, Rheodyne, Rohnert Park, CA, USA) with a 20 μL sample loop, a column (Carbohydrate analysis column, 4.6 × 250 mm, Waters, Milford, MA, USA) and a RI detector (Waters 410), after 90 min-hydrolysis of the polysaccharide (6 mg) in 2 M trifluoroacetic acid (TFA, 0.3 mL) at 120 °C. After removing TFA in the sample solution with a dried stream of nitrogen, the hydrolyzed polysaccharide sample was injected into the HPLC system. The mixture of acetonitrile and water (90:10, v/v) was used as a mobile phase at a flow rate of 2 mL/min.

2.6. Determination of weight average molecular weight

To measure the weight average molecular weight (M_w) as well as radius of gyration (R_g) of fucoidan polymers, fucoidan (2 mg) was dissolved in distilled water (1 mL). The fucoidan solution was heated in a microwave oven (RE-552 W, SamSung, Seoul, Korea) using a microwave bomb (#4872, Parr Instrument Co., Moline, IL, USA) for 30 s. The heated fucoidan solution was centrifuged for 10 s at 12,000*g* before injected to a high performance size exclusion chromatography coupled to multi-angle laser light scattering and refractive index detection (HPSEC-MALLS-RI) system.

The HPSEC-MALLS-RI system consisted of a pump (model 321, Gilson, Middleton, WI, USA), an injector valve with a 100 μL sample

loop (model 7072, Rheodyne), a guard column (TSK PWxl, TosoBiosep, Mongomeryville, PA, USA), three SEC columns (TSK G5000 PW, 7.5 × 600 mm; TSK G3000 PWxl, 7.8 × 300 mm; TSK G2500 PWxl, 7.8 × 300 mm; TosoBiosep, Mongomeryville, PA, USA), a multi-angle laser light scattering detector (HELEOS, Wyatt Technology Corp, Santa Barbara, CA, USA) and a refractive index detector (RI-150, Thermo Electron Corp., Yokohama, Japan). The aqueous solution of 0.15 M $NaNO_3$ and 0.02% NaN_3 was used as a mobile phase at a flow rate of 0.4 mL/min. The normalization of MALLS detector and the determination of volume delay between MALLS and RI detectors were carried out with bovine serum albumin (BSA). The d*n*/d*c* value was set to 0.129 for fucoidan polymers (Rioux, Turgeon, & Beaulieu, 2007). The calculations of M_w and R_g were carried out using ASTRA 5.3 software (Wyatt Technology Corp.). Molecular conformation of fucoidan polymers was examined using the following relationship: $R_g = KM_w^{\alpha}$, where K is an optical constant and α is the slope of the plot between *log* R_g versus *log* M_w (sphere if $\alpha < 0.3$; random coil if $0.3 \leqslant \alpha < 0.5$; rod if $\alpha \geqslant 0.5$) according to Roger, Bello-Perez, and Colonna (1999).

2.7. Anticancer activity assay

The anticancer activity of low molecular weight fucoidans was determined using sulforhodamine B (SRB) assay (Doll & Peto, 1981), which was based on the measurement of cellular protein content. Human gastric carcinoma cell line (AGS, ATCC, Rockville, MD, USA) was used in this study. The cell line (100 μL) with concentration of 4–5 × 10^4 cells/mL was placed in a 96-well plate and cultured for 24 h at 37 °C in the presence of 5% CO_2. The three fucoidan fractions (100 μL) having various concentrations from 0.2 to 0.8 mg/mL were added to the cultured cell line in the plate and again cultured for 48 h. The supernatant was removed from the well and subsequently cold TCA (10%, 100 μL, 4 °C) was added into the well. The solution was left at 4 °C for 1 h. After removing TCA by washing with distilled water, the well was dried at room temperature. The SRB staining of the cell line was carried out with the addition of 100 μL of 0.4% SRB dissolved in 1% acetic acid into the dried well. The unstained SRB was removed by washing with 1% acetic acid and the well was dried again at room temperature. The dried well was filled with 100 μL of 10 mM Tris buffer and the absorbance of sample solution (A_s) was measured at 540 nm using a microplate reader (Molecular Devices, THERMOmax, Hayward, CA, USA). The percentage of the cancer cell growth inhibition was calculated using the following equation: Growth inhibition (%) = $100(1 - (A_s/A_c))$, where A_s is the absorbance of sample solution and A_c is the absorbance of control for which 100 μL of H_2O was used instead of 100 μL sample solution.

3. Results and discussion

3.1. Proximate composition of fucoidans

The yield of fucoidan fractions ($F_{>30\ K}$, $F_{5-30\ K}$ and $F_{<5\ K}$), obtained by the ultrafiltration system from the hydrolyzed fucoidan, is presented in Table 1. The largest amount of $F_{>30\ K}$ fraction (43.3%) was collected from the hydrolysate while the least amount of $F_{5-30\ K}$ fraction (16.6%) was obtained. The hydrolysate also contained a substantial amount of $F_{<5\ K}$ fraction (39.9%). It is shown in Table 1 that the fraction of $F_{>30\ K}$ mostly consisted of carbohydrate (61.3%) and sulphate (31.7%) with a small amount of proteins (6.4%). $F_{5-30\ K}$ fraction was also composed of mostly carbohydrate (58.2%) but contained slightly higher sulphate (35.5%) and less protein (1.2%) than $F_{>30\ K}$ fraction. The proximate compositions of $F_{>30\ K}$ and $F_{5-30\ K}$ fractions showed a good agreement with the literature, which indicated that fucoidan polymers from other brown seaweeds had comparable amounts (42–66%) of carbohydrates with smaller amounts of sulphate (11.5–30.0%), and their protein contents varied from 0% to 12% (Bilan, Grachev, Shashkov, Nifantiev, & Usov, 2006; Ponce et al., 2003; Qiu et al., 2006; Rioux et al., 2007). In case of $F_{<5\ K}$ fraction, on the other hand, the major constituents were sulphate and ash with considerably smaller amounts of carbohydrate (15.5%) than $F_{>30\ K}$ and $F_{5-30\ K}$ fractions (Table 1). Among the ash components, sodium (Na) was the major constituent as shown in Table 2. It was not clear why this fraction contained such high amount of ash. This was probably because the low molecular inorganic compounds were accumulated in this fraction during the ultrafiltration process using the membrane (M_w cut-off, 5 kDa). In addition, they were not completely removed during the desalination process. It was, therefore, assumed that these inorganic components would be chemically bound to the backbone of fucoidan polymers, possibly with the anionic sulphate groups. Monosaccharide composition analysis showed that fucose (51.7–72.5%) was the major sugar in these fractions with significant amount of galactose (27.5–34.3%) and little mannose (3.6–8.9%), as shown in Table 1. Other monosaccharides such as glucose and xylose were not detected in these fractions. Fucoidan polymers in the literature showed considerable variations in their monosaccharide compositions. In general, the chemical composition of fucoidan polymers is significantly different depending on species, anatomical regions, growing conditions, extraction procedures

Table 1
Yield and chemical compositions of fucoidan fractions ($F_{>30\ K}$, $F_{5-30\ K}$ and $F_{<5\ K}$) extracted from the sporophyll of *Undaria pinnatifida*.

Sample	Yield (%)	Total carbohydrate (%)	Sulfate content (%)	Protein (%)	Ash (%)	Monosaccharide composition (%)		
						Fuc	Gal	Man
$F_{>30\ K}$	43.4	61.3 ± 1.8	31.7 ± 2.2	6.4 ± 0.07	nd[a]	64.4 ± 6.0	31.9 ± 4.7	3.6 ± 1.3
$F_{5-30\ K}$	16.6	58.2 ± 0.7	35.5 ± 1.9	1.2 ± 0.04	nd[a]	72.5 ± 0.3	27.5 ± 0.3	nda
$F_{<5\ K}$	39.9	15.5 ± 1.1	31.8 ± 5.4	1.2 ± 0.04	37.4 ± 0.29	52.1 ± 2.12	39.0 ± 3.1	8.9 ± 0.9

[a] Not determined.

Table 2
The ash components of $F_{<5\ K}$ fraction analyzed by atomic absorption spectrometer.

Sample	Ash components (%)							
	Na	K	Ca	Mg	Cu	Fe	Zn	Unknown
$F_{<5\ K}$	55.0 ± 1.4	13.2 ± 2.1	1.4 ± 0.4	1.4 ± 0.0	0.3 ± 0.0	nd[a]	nd[a]	28.7

[a] Not determined.

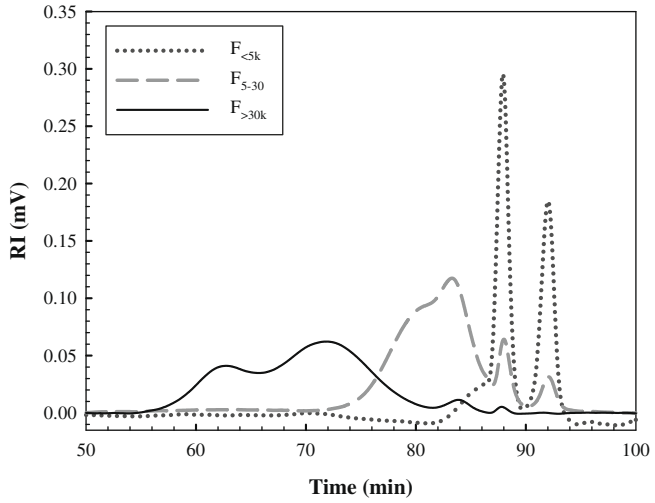

Fig. 1. The HPSEC chromatograms of three fucoidan fractions ($F_{>30\ K}$, $F_{5-30\ K}$ and $F_{<5\ K}$) dissolved in distilled water by heating in a microwave for 30 s.

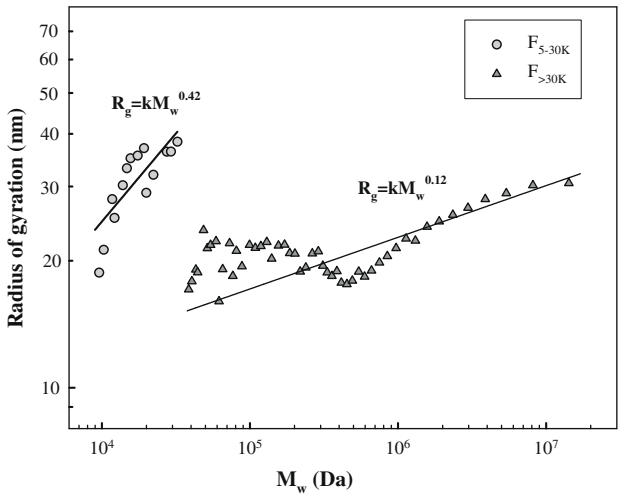

Fig. 2. The plot of weight average molecular weight (M_w) versus radius of gyration (R_g) of fucoidan fractions ($F_{>30\ K}$ and $F_{5-30\ K}$) treated by microwave heating (30 s).

and analytical methods (Bilan et al., 2002; Bilan et al., 2006; Chizhov et al., 1999; Ponce et al., 2003).

3.2. Molecular characteristics of fucoidans

The HPSEC chromatograms of three fucoidan fractions, which were dissolved in distilled water and heated by a microwave for 30 s, are shown in Fig. 1. The majority of $F_{>30\ K}$ fraction was eluted from the SEC column between the elution times of 56 and 82 min and showed two peaks, indicating that they were composed of two types of polymers with different molecular weights. $F_{5-30\ K}$ fraction exhibited a significant shift of the peak and was mostly eluted from 72 to 87 min with additional two small peaks at 88 and 92 min, respectively. $F_{<5\ K}$ fraction was eluted over 82 min with two large and distinct peaks at 88 and 92 min, respectively, indicating significantly lower molecular weights than $F_{>30\ K}$ and $F_{5-30\ K}$ fractions. It is shown in Table 3 that the weight average molecular weights (M_w) of three fucoidan fractions, obtained by a multiangle laser light scattering (MALLS) technique that can provide the absolute molecular weight of polymers, were 262, 5.6 and 1.6 kDa, respectively. These M_w results suggested that the fractionation of hydrolyzed fucoidans using the ultrafiltration technique was successfully performed, leading to the production of fucoidan polymers with the expected M_w. The M_w of $F_{>30\ K}$ fraction found in this study falls in the range of the reported M_w values (21–1600 kDa) of fucoidan polymers (Li, Xue, Xue, Li, & Fu, 2006; Nagaoka et al., 1999; Rioux et al., 2007), on the other hand, $F_{5-30\ K}$ and $F_{<5\ K}$ fractions had significantly lower M_w than the reported values.

Radius of gyration (R_g) was also calculated from the HPSEC peaks of the fucoidan fractions in order to estimate the approximate size of fucoidan polymers (Table 3). The R_g of $F_{>30\ K}$ fraction was found to be 23.5 nm. However, despite the significantly lower M_w, $F_{5-30\ K}$ fraction had similar R_g value (21.2 nm) to $F_{>30\ K}$ fraction. This was probably because of the differences in the molecular conformation of fucoidan polymers, suggesting that $F_{5-30\ K}$ fraction might exist in an extended conformation while $F_{>30\ K}$ fraction in a more compact conformation, probably through the intra-molecular interactions. This was also assumed in the plot of M_w and R_g from Fig. 2 that $F_{>30\ K}$ fraction had conformation of compact sphere ($\alpha = 0.15$), on the other hand, $F_{5-30\ K}$ fraction was in the loosed and random coil conformation ($\alpha = 0.42$). In the study of other polysaccharide such as debranched amylopectin chains using the HPSEC-MALLS-RI system, it was also shown that the R_g value of the lowest M_w chains (A chains) was greater than that of higher M_w chains ($B_{>2}$ chains), implying that $B_{>2}$ chains existed in a more compact molecular conformation than A chains probably due to intramolecular interactions (You, Fiedorowicz, & Lim, 1999). In case of $F_{<5\ K}$ fraction, it was almost impossible to obtain reliable signals to calculate the R_g value because scattering error became large for the small mass.

Table 3
The weight average molecular weight (M_w) and radius of gyration (R_g) of fucoidan fractions ($F_{>30\ K}$, $F_{5-30\ K}$ and $F_{<5\ K}$) dissolved in distilled water by heating in a microwave for 30 s.

Samples	M_w (kDa)	R_g (nm)
$F_{>30\ K}$	262 + 13	23.5 + 1.2
$F_{5-30\ K}$	5.6 + 0.3	21.2 + 1.1
$F_{<5\ K}$	1.6 + 0.1	nd[a]

[a] Not determined.

3.3. Anticancer activity of fucoidans

The anticancer activity of three fucoidan fractions, expressed as a percentage of the growth inhibition of cancer cell line AGS, is shown in Fig. 3. Among the fucoidan fractions, $F_{5-30\ K}$ fraction exhibited a strong dose-dependent manner in the inhibition potency of cancer cell growth while the other two fractions, $F_{>30\ K}$ and $F_{<5\ K}$ fractions, had a relatively weak concentration dependence. It was shown that the anticancer activities of fucoidan fractions were 18.0–28.5% for $F_{>30\ K}$, 19.2–57.5% for $F_{5-30\ K}$ and 26.5–36.5% for $F_{<5\ K}$ in the concentrations from 0.2 to 0.8 mg/mL. The anticancer activities of these fucoidans were lower than those of fucoidans from *Fucus vesiculosus* and *Laminaria japonica*, which showed about 89% of inhibition activity on the growth of HS-Sultan and MCF-7 cells, respectively (Aisa et al., 2005; Park et al., 2002). These considerable variations in the anticancer activity between fucoidans are probably because of the various chemical compositions of fucoidans originated from differences in species, anatomical regions, growing conditions of brown seaweeds and extraction and purification procedures as well as the use of different cancer cell lines.

Among the three fucoidan fractions, it was found that $F_{5-30\ K}$ fraction had more potent inhibition activity for the growth of AGS cells than $F_{>30\ K}$ fraction. This is likely to be the differences in the M_w of fucoidan fractions. It is assumed that low molecular

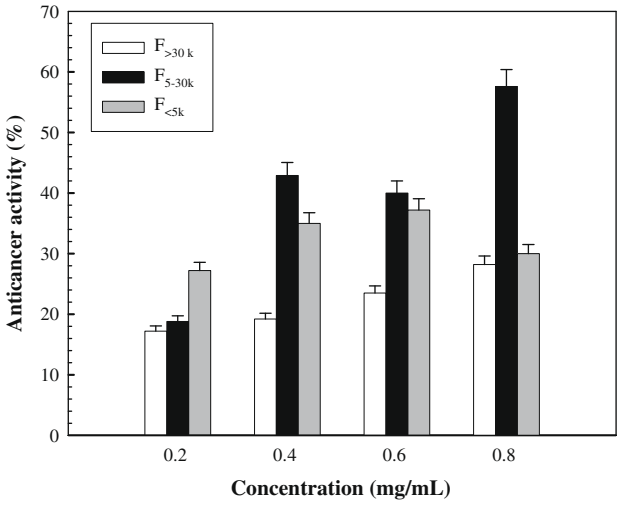

Fig. 3. The growth inhibition of cancer cell lines (AGS) by three fucoidan fractions ($F_{>30\ K}$, $F_{5-30\ K}$ and $F_{<5\ K}$) in the various concentrations (0.2–0.8 mg/mL).

weight fucoidan ($F_{5-30\ K}$ fraction) may have greater molecular mobility and diffusivity than high molecular weight fucoidan ($F_{>30\ K}$ fraction), which appears to improve the interaction with cancer cell components, and thus to induce the enhanced anticancer activity. Haroun-Bouhedja et al. (2000) also reported that low molecular weight fucoidans (18.6 kDa) from *Ascophyllum nodosum* at the concentration of 0.1 mg/mL showed more than 70% inhibition activity on CCL39 cell growth. Recent studies, however, indicated that fucoidan polymers could modulate the cell growth in a different manner depending on their molecular weights. Koyanagi et al. (2003) reported that fucoidan polymers obtained from *F. vesiculosus* having the M_w of 100–130 kDa effectively suppressed the growth of Sarcoma 180, Lewis lung carcinoma and B16 melanoma cells by inhibiting the neovascularization, called angiogenesis, due to their anti-angiogenic potency. On the other hand, low molecular weight fucoidans (16 kDa and 4 kDa) obtained from *A. nodosum* enhanced the neovascularization of human umbilical vein endothelial cells (HUVEC) and endothelial progenitor cells (EPC) in the presence of fibroblast growth factor-2, which resulted in the increase of cell proliferation (Matou, Helley, Chabut, Bros, & Fischer, 2002; Zemani et al., 2005).

Recently, several studies have also indicated that sulphate groups of fucoidan play a major role in the suppression of cancer cell growth by binding with cationic proteins on the cell surface (Koyanagi et al., 2003; Soeda, Kozako, Iwata, & Shimeno, 2000; Qiu et al., 2006). This implied that the molecular conformation of fucoidan, which might influence the binding properties of sulphate groups, could be another factor affecting the inhibition potency of the cancer cell growth. This is because that if fucoidan polymers were in the compact spherical conformation through the intramolecular interactions, the anionic sulphate groups available to bind proteins on the cell surface may be hidden inside the chains. Consequently, this would cause the reduction of sulphates available to bind the proteins. However, if the fucoidan polymers were in the loosed and entangled conformation, most sulphate groups existing in the chain are likely to be available to bind the proteins, thus effectively enhancing their biological activity. Therefore, in the case of $F_{5-30\ K}$ fraction as mentioned earlier, it was assumed that the loosed and entangled conformation might enhance the inhibition potency of cancer cell growth. On the other hand, sulphate groups of $F_{>30\ K}$ fraction, due to its compact spherical conformation, were assumed to partially participate in the binding with the cell surface proteins, which appeared to considerably lower the anticancer activity of $F_{>30\ K}$ fraction.

$F_{<5\ K}$ fraction, regardless of its lower molecular weight, exhibited significantly lower growth inhibition activity of AGS cells than $F_{5-30\ K}$ fraction (Fig. 3). This was probably related with the composition of fucoidans because $F_{<5\ K}$ fraction contained excessively high amount of inorganic compounds. Among the inorganic compounds, as mentioned earlier, sodium (Na) was the major component (Table 2) and seemed to be bound to the sulphate groups. This was likely to make the sulphate groups of fucoidan unable to bind and/or interact with cationic cell surface proteins, resulting in the considerable reduction of anticancer activity.

4. Conclusions

The anticancer activities of fucoidan fractions were 18.0–28.5% for $F_{>30\ K}$, 19.2–57.5% for $F_{5-30\ K}$ and 26.5–36.5% for $F_{<5\ K}$, respectively, inhibiting the cancer cell growth in a dose-dependent manner. The different anticancer activities of these fucoidan fractions were attributed to the various factors among which the lower molecular weight and loosed conformation with sulphate groups having the effective binding properties appeared to be the major factors. The current study suggests that the anticancer activity of fucoidans can be considerably improved by lowering their molecular weight and by improving the binding properties of sulphate groups possibly through altering the molecular conformation. It is expected that the more elucidation of the combined effects of molecular weight, conformation and sulphate contents would provide the better understanding of the relationship between the molecular structure and the anticancer activity of fucoidans.

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