CHAPTER 12

Functional Properties of Brown Algal Sulfated Polysaccharides, Fucoidans

You-Jin Jeon,[*,1] **W. A. J. P. Wijesinghe,**[*] and **Se-Kwon Kim**[†]

Contents

Abstract

Marine algae are potentially prolific sources of highly bioactive components that might represent useful leads in the development of new pharmaceutical agents and functional foods. This chapter discusses the current literature on biological activities of sulfated polysaccharides, fucoidans, from brown seaweeds. The profound functional properties of fucoidans could be employed in pharmaceutical, nutraceutical functional food, and cosmeceutical

* School of Marine Biomedical Sciences, Jeju National University, Jeju, Republic of Korea
† Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea
[1] Corresponding author: You-Jin Jeon, *E-mail address:* youjinj@cheju.ackr

Advances in Food and Nutrition Research, Volume 64
ISSN 1043-4526, DOI: 10.1016/B978-0-12-387669-0.00012-0

applications. Therefore, the chapter deals with the functional properties of the sulfated polysaccharides, fucoidans, with reference to its industrial applications as a functional ingredient.

I. INTRODUCTION

At present, the field of marine natural products becomes more sophisticated. Seaweeds have drawn worldwide attention due to their involvement in many industrial applications. Seaweeds produce a variety of active components with different structures and interesting biological activities (Amarowicz *et al.*, 2004; Choi *et al.*, 2002; Kim and Bae, 2010; Kong *et al.*, 2009; Shibata *et al.*, 2008). The bioactive components isolated from seaweeds could be divided into polyphenols, peptides, polysaccharides, etc. Many of these active compounds have been found to be useful functional ingredients in many industrial applications such as pharmaceutical, cosmeceutical, and functional food.

Brown seaweeds belong to a very large group (Davis *et al.*, 2003; Mestechkina and Shcherbukhin, 2010; Reddy and Urban, 2009). Most brown seaweeds contain the pigment fucoxanthin, which is responsible for the distinctive greenish-brown color that gives them their name. Brown seaweeds also produce a range of active components including unique secondary metabolites such as phlorotannins and many of which have specific biological activities that give possibilities for their economic utilization. In addition, over the past decade, bioactive sulfated polysaccharides isolated from brown seaweeds have attracted much attention in the fields of pharmacology and biochemistry. Functional polysaccharides such as fucans and alginic acid derivatives produced by brown seaweeds are known to exhibit different biological properties including anticoagulant, anti-inflammatory, antiviral, and antitumoral activities (Boisson-Vidal *et al.*, 1995; Costa *et al.*, 2010; Lee *et al.*, 2008a). In the recent years, sulfated polysaccharides, fucoidans, have been isolated from different brown algal species such as *Ecklonia cava* and *Ascophylum nodusum* (Athukorala *et al.*, 2006; Matou *et al.*, 2002). In this chapter, the biological activities and possible industrial applications of fucoidans have been discussed and summarized.

II. SULFATED POLYSACCHARIDES, FUCOIDANS

Polysaccharides widely exist in animals, plants, microorganisms, and algae (Yang and Zhang, 2009). They are polymeric carbohydrate structures, usually composed of various monosaccharides linked with different glucosidic bonds. Depending on the structure, polysaccharides can have distinct

functional properties from their building blocks. Sulfated polysaccharides are among the most abundant and broadly studied polysaccharides from nonanimal origin (Pereira *et al.*, 2002). They are widespread in nature. Seaweeds are abundant source of sulfated polysaccharides with various biological activities. Therefore, sulfated polysaccharides are of special interest. Most naturally occurring sulfated polysaccharides are complex mixtures of molecules showing wide variations in their structure as well as their activities (Alban *et al.*, 2002).

Fucoidan (Fig. 12.1) is a sulfated polysaccharide mainly found in the cell-wall matrix of various brown seaweed species (Kim *et al.*, 2010a; Teruya *et al.*, 2007). It contains substantial percentages of L-fucose and sulfate ester groups (Jiang *et al.*, 2010; Li *et al.*, 2008; Matou *et al.*, 2002). Fucose is a hexose deoxy sugar with the chemical formula $C_6H_{12}O_5$ and is the fundamental subunit of the fucoidan polysaccharide. For the past decade, fucoidan has been extensively studied due to its numerous biological activities. Recently, researches for new drugs have raised interest in fucoidans. In the past few years, several fucoidans' structures have been isolated and many aspects of their biological activity have been elucidated (Li *et al.*, 2008).

III. PURIFICATION OF FUCOIDAN FROM BROWN SEAWEEDS

Over the years, isolation and chemical characterization of active components from seaweeds have gained much attention. Marine algae appear to be good sources of active polysaccharides presenting great chemical, physicochemical, and rheological diversities (Lahaye, 1991). Naturally occurring sulfated polysaccharides are today among the most talked about classes of bioactive natural products. Extraction is the first step in

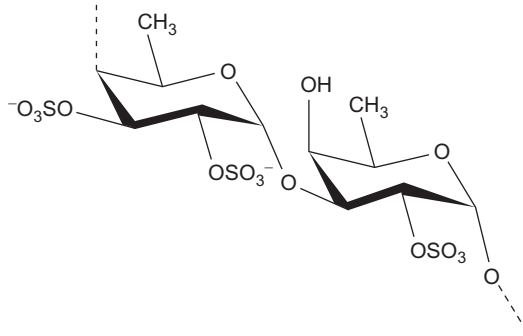

FIGURE 12.1 Chemical structure of fucoidans.

the isolation of active components from plant materials. In addition, extraction is influenced by the chemical nature of the components, the extraction method employed, and the presence of interfering substances (Chirinos *et al.*, 2007).

The polysaccharide contents of seaweeds vary according to the species. Generally, these polysaccharides have been extracted using water or aqueous organic solvents (Albuquerque *et al.*, 2004). However, as the cell wall consists of complex polymers, it is not easy to extract active polysaccharides using solvent extraction process. The production of different bioactive polysaccharides with lyases is required in order to increase the extraction efficiency of more functional ingredients from seaweeds. Therefore, enzyme-assisted extraction technique can be employed as an alternative method to improve the extraction efficiency of bioactive polysaccharides for industrial use (Athukorala *et al.*, 2009; Kang *et al.*, 2011).

The isolation and purification of sulfated polysaccharides, fucoidans, from seaweeds could be done as previously described method (Athukorala *et al.*, 2006; Matsubara *et al.*, 2000). Briefly, the dried algal sample grinds and sieves through a 50 standard testing sieve. A 100 g of the sample homogenizes with water (2 L), and then 1 mL of enzyme (AMG 300 L) mix. The enzymatic digestion can be performed for 12 h to achieve an optimum degree of the digestion. Before the digestion, pH of the homogenate should be adjusted to its optimal pH value, and after the digestion, it boils for 10 min at 100 °C to inactivate the enzyme. The reactant clarifies by centrifugation (3000 rpm, for 20 min at 4 °C) to remove the residue. The enzymatic digest (240 mL) well mix with 480 mL of 99.5% ethanol. The mixture allows standing for 30 min at the room temperature, and then the crude polysaccharides can be collected by centrifugation at 10,000 $\times$ *g* for 20 min at 4 °C. After that, freeze-dried crude polysaccharide from the digest introduces to diethylaminoethyl cellulose (DEAE cellulose) ion exchange chromatography. And then the sample further purifies on a new DEAE cellulose column to improve the purity of the sample. Thereafter, the sample applies into a gel permeation chromatography on Sepharose-4B to purify the sample according to its molecular weight. The purity of the sample can be confirmed by agarose gel electrophoresis, and the molecular weight of the sample can be determined by gel filtration chromatography system.

IV. BIOLOGICAL ACTIVITIES OF FUCOIDANS

Fucose-containing sulfated polysaccharides from brown seaweeds might exhibit interesting biological properties (Matsuhiro *et al.*, 1996). The profound functional properties of the sulfated polysaccharides are probably due to the presence of sulfate groups in varying amounts. In addition,

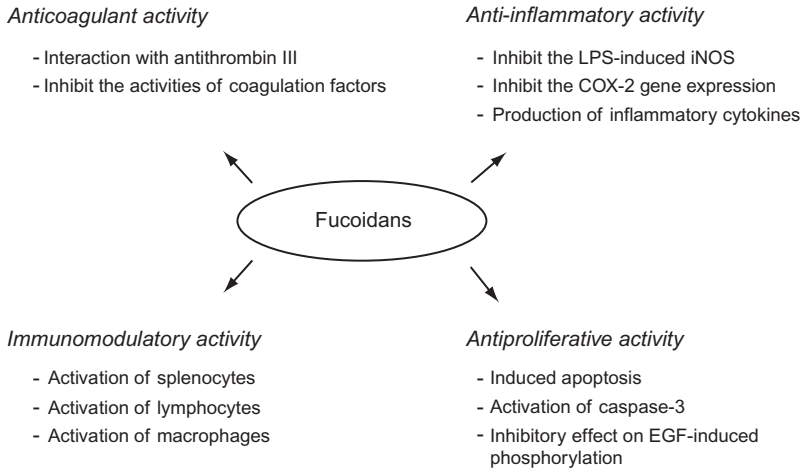

FIGURE 12.2 Schematic showing biological properties of brown algal fucoidans.

positions of the sulfated groups along the macromolecular backbone also play a vital role in their biological activities. Among the sulfated polysaccharides, fucoidans found in seaweeds are well known to have numerous biological activities (Fig. 12.2) and the potent biological properties of fucoidans seem to be determined by their high degree of sulfation, fine structure, and molecular weight (Jiang *et al.*, 2010; Zvyagintseva *et al.*, 2003). However, the composition of algal fucans varies according to several factors such as species, extraction procedure, season of harvest, and climatic conditions (Dietrich *et al.*, 1995; Grauffel *et al.*, 1989). Thus, each newly isolated and described fucans are unique compounds with unique structural features, consequently having the potential of being used as novel pharmaceuticals (Silva *et al.*, 2005). Table 12.1 provides a summary of biological activities of fucose-rich sulfated polysaccharides and fucoidan isolated from various brown seaweeds.

A. Anticoagulant and antithrombotic activity

Anticoagulants are substances that prevent coagulation; that is, they stop blood from clotting (Desai, 2004). Therefore, they are a group of pharmaceuticals that can be used *in vivo* as a medication for thrombotic disorders. Heparin, a highly sulfated polysaccharide present in mammalian tissues, is one of the commonly used drugs of the choice in prevention of thromboembolic disorders (Lee *et al.*, 2008b). However, there are some well-documented problems related to its clinical application (Alban *et al.*, 2002). Therefore recently, alternative drugs for heparin are in high demand due to its bad and long-term side effects (Athukorala *et al.*,

TABLE 12.1 Biological activities of fucose-rich sulfated polysaccharides and fucoidan isolated from various brown seaweeds

Seaweed species	Biological activity	Reference
E. cava	Anticoagulant (*in vitro*)	Athukorala *et al.* (2006)
E. cava	Antithrombotic	Jung *et al.* (2007)
F. evanescens	Anticoagulant	Kuznetsova *et al.* (2003)
E. cava	Anticoagulant (*in vivo*)	Wijesinghe *et al.* (2011)
P. gymnospora	Anticoagulant	Silva *et al.* (2005)
A. nodosum	Anticoagulant	Chevolot *et al.* (1999)
—	Anticoagulant	Soeda *et al.* (1992)
S. fulvellum	Anticoagulant	De Zoisa *et al.* (2008)
Hizikia fusiforme	Anticoagulant	Dobashi *et al.* (1989)
Laminaria cichorioides	Anticoagulant	Yoon *et al.* (2007)
U. pinnatifida	Antitumor	Synytsya *et al.* (2010)
E. cava	Antiproliferation	Athukorala *et al.* (2009)
—	Antiproliferation	Aisa *et al.* (2004)
F. evanescens	Antitumor and antimetastatic	Alekseyenko *et al.* (2007)
L. guryanovae	Anticancer	Lee *et al.* (2008a)
F. vesiculosus	Immunomodulation	Kim and Joo (2008)
—	Immunomodulation	Choi *et al.* (2005)
F. vesiculosus	Immunomodulation	Do *et al.* (2010)
F. vesiculosus	Immunomodulation	Jintang *et al.* (2010)
U. pinnatifida	Immunomodulation	Yoo *et al.* (2007)
F. vesiculosus	Immunomodulation	Yang *et al.* (2008)
L. japonica	Anti-inflammation	Li *et al.* (2011)
E. cava	Anti-inflammation	Kang *et al.* (2011)
A. nodosum	Angiogenesis	Matou *et al.* (2002)
U. pinnatifida	Antivirus	Hemmingson *et al.* (2006)

2007). Over the years, isolation and purification of natural sulfated polysaccharides responsible for anticoagulant activity from different seaweed species had been reported (De Zoisa *et al.*, 2008). The ability of sulfated polysaccharides to interfere with biological systems has a longstanding record, as illustrated with heparin (Huynh *et al.*, 2001). In addition, anticoagulant and antithrombotic activities are among the most widely studied properties of sulfated polysaccharides.

Athukorala *et al.* (2006) tested anticoagulant activity of fucose-containing sulfated polysaccharide isolated from brown seaweed *E. cava*

including activated partial thromboplastin time, thrombin time, and prothrombin time. According to their results, the pure compound showed almost similar anticoagulant activity to that of heparin. Further study demonstrated that fucose-containing sulfated polysaccharide isolated from *E. cava* strongly inhibits the activities of coagulation factors via interaction with antithrombin III in both the extrinsic and the common coagulation pathways (Jung *et al.*, 2007). Possible anticoagulation mechanism and molecular interaction of fucoidan isolated from the brown seaweed *E. cava* with blood coagulation factors are shown in Fig. 12.3. Fucoidans enhance ATIII-mediated coagulation factor inhibition in coagulation pathways. This contributes to its high anticoagulant activity. Wijesinghe *et al.* (2011) demonstrated *in vivo* anticoagulant activity of isolated fucose-rich sulfated polysaccharide obtained from *E. cava*. Anticoagulant and antithrombin activities of over sulfated fucans having different sulfate contents were reported (Nishino and Nagumo, 1992). There results showed that heparin cofactor II-mediated antithrombin activity of the over sulfated fucans also increased significantly with increase in sulfate content. In addition, it was reported that the major antithrombin activity by fucoidan was mediated by heparin cofactor II (Qui *et al.*, 2006). Another previous study reported the partial characterization and anticoagulant activity of a heterofucan from the brown seaweed, *Padina gymnospora* (Silva *et al.*, 2005). Further, they have reported that 3-*O*-sulfation at C-3 of 4-α-L-fucose-1→ units was responsible for the anticoagulant activity of fucoidan from the particular seaweed species.

De Zoisa *et al.* (2008) reported the isolation and characterization of fucose-containing sulfated polysaccharide as an anticoagulant agent from the edible brown seaweed *Sargassum fulvellum* by means of a simple fermentation process and chromatography technique. According to their

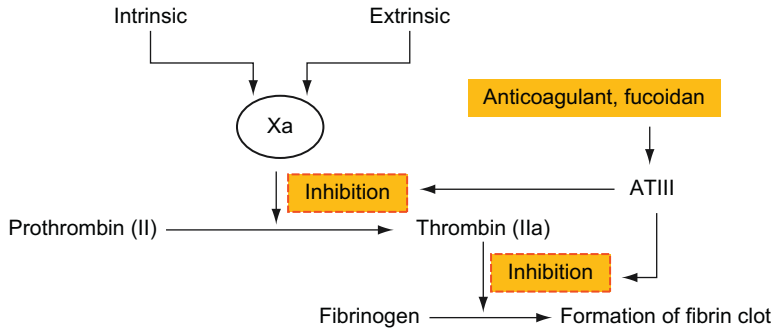

FIGURE 12.3 Possible anticoagulation mechanism of fucoidan from brown seaweed *Ecklonia cava*.

report, fermentation could offer a tool to increase the bioactive potentials. Therefore, the report facilitates further screening and large-scale production of the bioactive molecules from fermented marine seaweed in the future.

With the evidence from previous studies, brown algal sulfated polysaccharide, fucoidan attracted extensive interest in anticoagulative drug discovery.

B. Antiproliferative/antitumor/anticancer activity

In recent years, it has been reported that fucose-rich sulfated polysaccharides isolated from brown seaweeds exhibited antitumor activity which is one of the most important biological activities of seaweeds.

Synytsya *et al*. (2010) demonstrated the antitumor activity of fucoidan from *Undaria pinnatifida* in PC-3, HeLa, A549, and HepG2 cancer cells in similar pattern to that of commercial fucoidan. In addition, fucose-rich sulfated polysaccharide of *E. cava* has antiproliferative effects on murine colon carcinoma (CT-26), human leukemic monocyte lymphoma (U-937), human promyelocytic leukemia (HL-60), and mouse melanoma (B-16) cell lines (Athukorala *et al*., 2009). Fucoidan was found to inhibit proliferation and induce apoptosis in human lymphoma HS-Sultan cell lines (Aisa *et al*., 2004). Further, they have reported the fucoidan-induced apoptosis was accompanied by the activation of caspase-3. In another recent study, antitumor and antimetastatic activities of fucoidan, isolated from brown seaweed *Fucus evanescens*, were studied in C57Bl/6 mice with transplanted Lewis lung adenocarcinoma (Alekseyenko *et al*., 2007). Another *in vitro* study demonstrated the inhibitory effects of fucoidan on activation of epidermal growth factor receptor (EGFR) and cell transformation in JB6 C141 cells (Lee *et al*., 2008a). Their results provided the first evidence that fucoidan from *Laminaria guryanovae* exerted a potent inhibitory effect on EGF-induced phosphorylation of EGFR. The EGFR, one of the receptor tyrosine kinases, plays an important role in regulating cell proliferation, differentiation, and transformation (Chen *et al*., 1987). Therefore, it is an important target for cancer therapy (Yarden and Sliwkowski, 2001).

Antiproliferative and antitumor properties of fucoidan were reported for several studies (Itoh *et al*., 1995; Maruyama *et al*., 2003, 2006). Fucoidans inhibit tumor growth and metastatic process both by direct action on tumor cells and by the enhancement of immune response (Khotimchenko, 2010). Identification of novel effective cancer chemopreventive agents has become an essential worldwide strategy in cancer prevention (Eldeen *et al*., 2009). Therefore, finding of anticancer properties of brown algal fucoidans could elevate the value of brown seaweeds as functional ingredients in pharmaceuticals or functional foods.

C. Immunomodulatory activity

Immunomodulation refers to the action undertaken by the medication on auto-regulating processes that steer the immunological defense system. Many polysaccharides obtained from natural sources are considered to be biological response modifiers and have been shown to enhance various immune responses (Li *et al.*, 2008). Previous studies have shown that brown algal fucoidans have immunological effects both *in vitro* and *in vivo*. Figure 12.4 shows the possible immunomodulatory effects of brown algal fucoidan by activated splenocytes via JNK, NF-κB, and NFAT signal pathways.

Kim and Joo (2008) reported the immunomodulatory effects of fucoidan purified from brown seaweed *Fucus vesiculosus* on dendritic cells. Further, they suggested that the fucoidan has immunostimulating and maturing effects on bone marrow-derived dendritic cells, via a pathway involving at least NF-κB. In another recent study, Choi *et al.* (2005) investigated the immunomodulating effects of arabinogalactan and

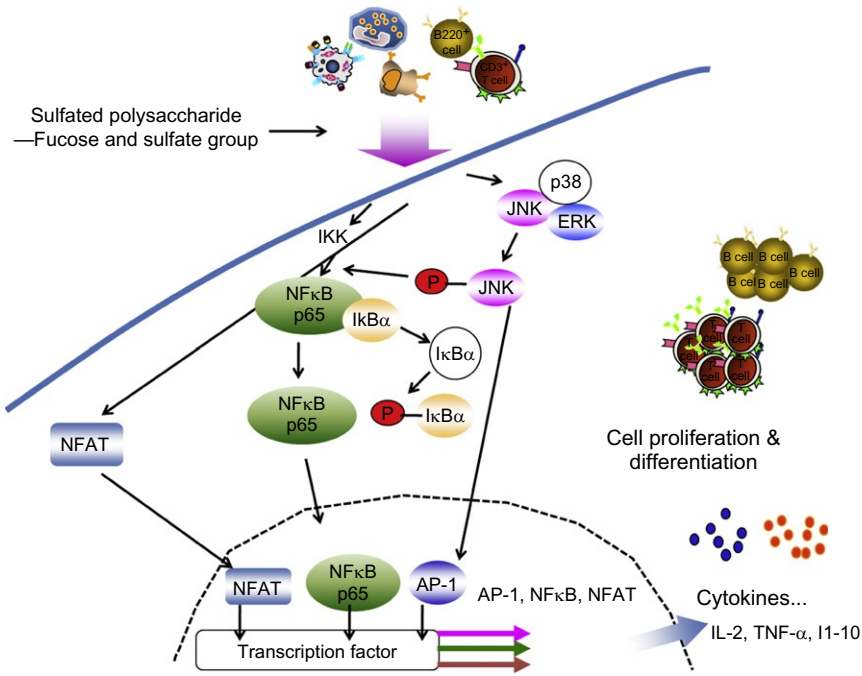

FIGURE 12.4 Possible immunomodulatory effects of brown algal fucoidan by activated splenocytes via JNK, NF-κB, and NFAT signal pathways.

fucoidan *in vitro*. Their data suggest that arabinogalactan and fucoidan are activators of lymphocytes and macrophages. This property may contribute to their effectiveness in the immunoprevention of cancer. Yang *et al*. (2008) reported the effects of fucoidan on maturation process and activation of human monocyte-derived dendritic cells. Their results suggest that dendritic cells appear to be a potential target for the immunomodulatory capacity of fucoidan. Therefore, fucoidan may be used on dendritic cells-based vaccines for cancer immunotherapy.

Effect of fucoidan on NO production induced by IFN-γ and the molecular mechanisms underlying these effects in two types of cells including glia (C6, BV-2) and macrophages (RAW264.7, peritoneal primary cells) were reported (Do *et al*., 2010). According to the results, they have reported that the effects of fucoidan on iNOS expression through IFN-g-mediated signaling between two cell types can suggest the possibility not only as a promising candidate for treating inflammatory-related neuronal injuries but also as a immune modulating nutrient for altering sensitivity of cells.

Matrix metalloproteinase-9 (MMP-9) is a secreted multidomain enzyme, which plays an important role in the migration of immune cells. In a recent study, it is reported that fucoidan posttranslationally regulated MMP-9 secretion from U937 (Jintang *et al*., 2010). Fucoidan isolated from *U. pinnatifida* possesses immunomodulating activity to produce cytokines and chemokines from macrophages and splenocytes (Yoo *et al*., 2007).

Besides having direct anticancer or antiproliferative properties, fucoidans can also suppress the development of tumor cells through enhancing body's immunomodulatory activity.

D. Anti-inflammatory activity

The inflammatory process involves a series of events that can be elicited by numerous internal or external stimuli. Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Macrophages are key players in inflammation (Kazlowska *et al*., 2010).

Potent effect of the fucose-containing sulfated polysaccharide from *E.* cava on anti-inflammatory activity in LPS-stimulated RAW 264.7 cells was successfully investigated (Kang *et al*., 2011). According to their results, isolated sulfated polysaccharide-containing fucose, dose-dependently inhibited the LPS-induced iNOS and COX-2 gene expression, as well as the subsequent production of NO and PGE_2 by LPS in RAW 264.7 macrophages. Resent *in vivo* study revealed that the administration of fucoidan, isolated from brown seaweed *Laminaria japonica*, could regulate the inflammation response via HMGB1 and NF-κB inactivation in I/R-induced myocardial damage on rats (Li *et al*., 2011).

Connective tissue destruction during inflammatory diseases, such as chronic wound, chronic leg ulcers, or rheumatoid arthritis, is the result of continuous supply of inflammatory cells and exacerbated production of inflammatory cytokines and matrix proteinases (Senni *et al.*, 2006). According to the Senni *et al.* (2006), fucoidan from *Ascophyllum nodosum* is a potent modulator of connective tissue proteolysis. Further, the authors suggested that fucoidan could be used for treating some inflammatory pathology in which uncontrolled extracellular matrix degradation takes place.

E. Other biological activities of fucoidan

The effect of fucoidan from *A. nodosum* on fibroblast growth factor (FGF)-2-induced proliferation and differentiation of human umbilical vein endothelial cells (Matou *et al.*, 2002) was reported. Their results showed that fucoidan can enhance vascular tube formation induced by FGF-2 with a modulation of the expression of surface proteins involved in angiogenesis. In another study, however, smooth muscle cell proliferation was inhibited by fucans, suggesting an antiproliferative effect (Logeart *et al.*, 1997). Together with these results, Matou *et al.* (2002) suggested a potential preventive effect of fucoidan on restenosis.

Hemmingson *et al.* (2006) demonstrated the potential antiviral activity of galactofucan sulfates extracted from *U. pinnatifida* against herpes viruses HSV-1, HSV-2, and HCMV. In recent years, few other antivirus activities of sulfated polysaccharides-containing fucose have been demonstrated (Hayashi *et al.*, 2008; Mandal *et al.*, 2007).

Despite these biological activities, detailed study on the toxicology of brown algal fucoidan has been performed (Kim *et al.*, 2010b). They have tested the toxicity of a 4-week oral trial of fucoidan extracted from the *U. pinnatifida* in Sprague–Dawley rats.

The study showed that fucoidan from *U. pinnatifida* is not toxic when orally administered at 150, 450, and 1350 mg/kg bw/day for 4 weeks and does not have anticoagulant activity, reducing concern about adverse effects related to excess bleeding.

V. POSSIBLE INDUSTRIAL APPLICATIONS OF FUCOIDANS

Over the years, there are significant developments in the fields of pharmaceutical, nutraceutical, cosmeceutical, and functional food. There is a growing interest among producers and the public in those areas that may provide health benefits beyond basic nutrition. This fact has brought great interest for searching new functional ingredients that can contribute to develop new opportunities in the relevant applications. Therefore, today,

in the modern market, there is an increasing number of novel products are available with functional ingredients from different natural sources. Discussed biological properties of sulfated polysaccharides fucoidans might give a clear evidence of its potential uses in medical and food industry.

VI. CONCLUDING REMARKS

Apart from being a source of food, brown seaweeds are also produce a range of unique active components, many of which have specific biological activities that give them possibilities for their economic utilization. Brown seaweeds have been identified as easily accessible producers of sulfated polysaccharides. This chapter discussed the isolation and purification of the sulfated polysaccharides, fucoidans together with its numerous biological properties. The potent biological activities of brown algal fucoidans may represent an interesting advance in the search for novel functional applications in many industrial uses such as functional foods and pharmaceuticals.

REFERENCES

Aisa, Y., Miyakawa, Y., Nakazato, T., Shibata, H., Saito, K., Ikeda, Y., and Kizaki, M. (2004). Fucoidan induces apoptosis of human HS-Sultan cells accompanied by activation of caspase-3 and down-regulation of ERK pathways. *Am. J. Hematol.* **78**, 7–14.

Alban, S., Schauerte, A., and Franz, G. (2002). Anticoagulant sulfated polysaccharides: Part I. Synthesis and structure-activity relationships of new pullulan sulfates. *Carbohydr. Polym.* **47**, 267–276.

Albuquerque, I. R. L., Queiroz, K. C. S., Alves, L. G., Santos, E. A., Leite, E. L., and Rocha, H. A. O. (2004). Hetarofucans from Dictyota menstrualis have anticoagulant activity. *Braz. J. Med. Biol. Res.* **37**, 167–171.

Alekseyenko, T. V., Zhanayeva, S. Y., Venediktova, A. A., Zvyagintseva, T. N., Kuznetsova, T. A., Besednova, N. N., and Korolenko, T. A. (2007). Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk sea *Fucus evanescens* brown alga. *Bull. Exp. Biol. Med.* **143**, 730–732.

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., and Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.* **84**, 551–562.

Athukorala, Y., Jung, W. K., Vasanthan, T., and Jeon, Y. J. (2006). An anticoagulative polysaccharide from an enzymatic hydrolysate of *Ecklonia cava*. *Carbohydr. Polym.* **66**, 184–191.

Athukorala, Y., Lee, K. W., Kim, S. K., and Jeon, Y. J. (2007). Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. *Bioresour. Technol.* **98**, 1711–1716.

Athukorala, Y., Ahn, G. N., Jee, Y. H., Kim, G. Y., Kim, S. H., Ha, J. H., Kan, J. S., Lee, K. W., and Jeon, Y. J. (2009). Antiproliferative activity of sulfated polysaccharide isolated from an enzymatic digest of *Ecklonia cava* on the U-937 cell line. *J. Appl. Phycol.* **21**, 307–314.

Boisson-Vidal, C., Haroun, F., Ellouali, M., Blondin, C., Fischer, A. M., de Agostini, A., and Josefonvicz, J. (1995). Biological activities of polysaccharides from marine algae. *Drugs Future* **20**, 1237–1249.

Chen, W. S., Lazar, C. S., Poenie, M., Tsien, R. Y., Gill, G. N., and Rosenfeld, M. G. (1987). Requirement for intrinsic protein tyrosine kinase in the immediate and late actions of the EGF receptor. *Nature* **328**, 820–823.

Chevolot, L., Foucault, A., and Chauber, F. (1999). Further data on the structure of brown seaweed fucans: Relationships with anticoagulant activity. *Carbohydr. Res.* **319**, 154–165.

Chirinos, R., Rogetz, H., Campos, D., Pedreschi, R., and Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruiz & Pavon) tubers. *Sep. Purif. Technol.* **55**, 217–225.

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., Park, S. H., and Kim, S. K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Sci.* **163**, 1161–1168.

Choi, E. M., Kim, A. J., Kim, Y., and Hwang, J. K. (2005). Immunomodulating activity of arabinogalactan and fucoidan *in vitro*. *J. Med. Food* **8**, 446–453.

Costa, L. S., Fidelis, G. P., Cordeiro, S. L., Oliveira, R. M., Sabry, D. A., Câmara, R. B., Nobre, L. T., Costa, M. S., Almeida-Lima, J., Farias, E. H., Leite, E. L., and Rocha, H. A. (2010). Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed. Pharmacother.* **64**, 21–28.

Davis, T. A., Volesky, B., and Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.* **37**, 4311–4330.

De Zoisa, M., Nikapitiya, C., Jeon, Y. J., Jee, Y., and Lee, J. (2008). Anticoagulant activity of sulfated polysaccharide isolated from brown seaweed *Sargassum fulvellum*. *J. Appl. Phycol.* **20**, 67–74.

Desai, U. R. (2004). New antithrombin-based anticoagulants. *Med. Res. Rev.* **24**, 151–181.

Dietrich, C. P., Farias, G. G. M., Abreu, L. R. D., Leite, E. L., Silva, L. F., and Nader, H. B. (1995). A new approach for characterization of polysaccharides from algae: Presence of four main acidic polysaccharides in three species of the class Phaeophyceae. *Plant Sci.* **108**, 143–153.

Do, H., Kang, N. S., Pyo, S., Billiar, T. R., and Sohn, E. H. (2010). Differential regulation by fucoidan of IFN-γ-induced NO production in glial cells and macrophages. *Journal of Cell Biochemistry* **111**, 1337–1345.

Dobashi, K., Nishino, T., and Fujihara, M. (1989). Isolation and preliminary characterization of fucose-containing sulfated polysaccharides with blood-anticoagulant activity from seaweed *Hizikia fusiforme*. *Carbohydr. Res.* **194**, 315–320.

Eldeen, A. M. G., Ahmed, E. F., and Zeid, M. A. A. (2009). *In vitro* cancer chemopreventive properties of polysaccharide extract from the brown alga, *Sagassum latifolium*. *Food Chem. Toxicol.* **47**, 1378–1384.

Grauffel, V., Kloareg, B., Mabeau, S., Durand, P., and Josefonficz, J. (1989). New natural polysaccharides with potent antithrombotic activity: Fucans from brown algae. *Biomaterials* **10**, 363–369.

Hayashi, K., Nakano, T., Hashimoto, M., Kanekiyo, K., and Hayashi, T. (2008). Defensive effects of a fucoidan from brown alga *Undaria pinnatifida* against herpes simplex virus infection. *Int. Immunopharmacol.* **8**, 109–116.

Hemmingson, J. A., Falshaw, R., Furneaux, R. H., and Thompson, K. (2006). Structure and antiviral activity of the galactofucan sulfates extracted from *Undaria pinnatifida* (Phaeophyta). *J. Appl. Phycol.* **18**, 185–193.

Huynh, R., Chaubet, F., and Jozefonvicz, J. (2001). Anticoagulant properties of dextran-methylcarboxylate benzylamide sulfate (DMCBSu); a new generation of bioactive functionalized dextran. *Carbohydr. Res.* **332**, 75–83.

Itoh, H., Noda, H., Amano, H., and Ito, H. (1995). Immunological analysis of lung metastasis by fucoidan (GIV-A) prepared from brown seaweed *Sargassum thunbergii*. *Anticancer Res.* **15**, 1937–1947.

Jiang, Z., Okimura, T., Yokose, T., Yamasaki, Y., Yamaguchi, K., and Oda, T. (2010). Effect of sulfated, ascophyllan, from the brown alga *Ascophyllum nodosum* on various cell lines: A comparative study on ascophyllan and fucoidan. *J. Biosci. Bioeng.* **110**, 113–117.

Jintang, S., Alei, F., Yun, Z., Shanzhen, S., Weixu, H., Meixiang, Y., fengcai, W., and Xun, Q. (2010). Fucoidan increases TNF-α-induced MMP-9 secreation monocytic cell line U937. *Inflammation Res.* **59**, 271–276.

Jung, W. K., Athukorala, Y., Lee, Y. J., Cha, S. H., Lee, C. H., Vasanthan, T., Choi, K. S., Yoo, S. H., Kim, S. K., and Jeon, Y. J. (2007). Sulfated polysaccharide purified from *Ecklonia cava* accelerates antithrombin III-mediated plasma proteinase inhibition. *J. Appl. Phycol.* **19**, 425–430.

Kang, S. M., Kim, K. N., Lee, S. H., Ahn, G., Cha, S. H., Kim, A. D., Yang, X. D., Kang, M. C., and Jeon, Y. J. (2011). Anti-inflammatory activity of polysaccharide purified from AMG-assistant extract of *Ecklonia cava* in LPS-stimulated RAW264.7 macrophages. *Carbohydr. Polym* **85**, 80–85.

Kazlowska, K., Hsu, T., Hou, C. C., Yang, W. C., and Tsai, G. J. (2010). Antiinflammatory properties of phenolic compounds and crude extract from *Porphyra dentata*. *J. Ethanopharmacol.* **128**, 123–130.

Khotimchenko, Y. S. (2010). Antitumor properties of nonstarch polysaccharides: Fucoidans and chitosans. *Russ. J. Mar. Biol.* **36**, 321–330.

Kim, T. H. and Bae, J. S. (2010). *Ecklonia cava* extracts inhibit liposaccharide induced inflammatory responses in human endothelial cells. *Food Chem. Toxicol.* **48**, 1682–1687.

Kim, M. H. and Joo, H. G. (2008). Immunomodulatory effects of fucoidan on bone marrow-derived dendritic cells. *Immunol. Lett.* **115**, 138–143.

Kim, K. J., Lee, O. H., and Lee, B. Y. (2010a). Fucoidan, a sulfated polysaccharide, inhibits adipogenesis through the mitogen-activated protein kinase pathway in 3T3-L1 preadipocytes. *Life Sci.* **86**, 791–797.

Kim, K. J., Lee, O. H., Lee, H. H., and Lee, B. Y. (2010b). A 4-week repeated oral dose toxicity study of fucoidan from *Sporophyll* of *Undaria pinnatifida* in Sprague-Dawley rats. *Toxicology* **267**, 154–158.

Kong, C. S., Kim, J. A., Yoon, N. Y., and Kim, S. K. (2009). Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-t human breast cancer cells. *Food Chem. Toxicol.* **47**, 1653–1658.

Kuznetsova, T. A., Besednova, N. N., Mamaev, A. N., Momot, A. P., Shevchenko, N. M., and Zvyagintseva, T. N. (2003). Anticoagulant activity of fucoidan from brown algae *Fucus evanescens* of the Okhotsk sea. *Bull. Exp. Biol. Med.* **136**, 471–473.

Lahaye, M. (1991). Marine algae as sources of fibres: Determination of soluble and insoluble dietary fibre contents in some ''sea vegetables''. *J. Sci. Food Agric.* **54**, 587–594.

Lee, N. Y., Ermakova, S. P., Zvyagintseva, T. N., Kang, K. W., Dong, Z., and Choi, S. (2008a). Inhibitory effects of fucoidan on activation of epidermal growth factor receptor and cell transformation in JB6 C141 cells. *Food Chem. Toxicol.* **46**, 1793–1800.

Lee, S. H., Athukorala, Y., Lee, J. S., and Jeon, Y. J. (2008b). Simple separation of anticoagulant sulfated galactan from red algae. *J. Appl. Phycol.* **20**, 1053–1059.

Li, B., Lu, F., Wei, X., and Zhao, R. (2008). Fucoidan: Structure and bioactivity. *Molecules* **13**, 1671–1695.

Li, C., Gao, Y., Xing, Y., Zhu, H., Shen, J., and Tian, J. (2011). Fucoidan, a sulfated polysaccharide from brown algae, against myocardial ischemia-reperfusion injury in rats via regulating the inflammation response. *Food Chem. Toxicol.* **49**, 2090–2095.

Logeart, D., Prigeant-Richard, S., Jozefonvicz, J., and Letourneur, D. (1997). Fucan, a sulfated polysaccharide extracted from brown seaweeds, inhibits vascular SMC proliferation: Part I.

Comparison with heparin for the antiproliferative activity, binding and internalization. *Eur. J. Cell Biol.* **74**, 385–390.

Mandal, P., Mateu, C. G., Chattopadhyay, K., Pujol, C. A., Damonte, E. B., and Ray, B. (2007). Structural features and antiviral activity of sulphated fucans from the brown seaweed *Cystoseira indica*. *Antivir. Chem. Chemother.* **18**, 153–162.

Maruyama, H., Tamauchi, H., Hashimoto, M., and Nakano, T. (2003). Antitumor activity and immune response of Mekabu fucoidan extracted from Sporophyll of *Undaria pinnatifida*. *In Vivo* **17**, 245–249.

Maruyama, H., Tamauchi, H., Iizuka, M., and Nakano, T. (2006). The role of NK cells in antitumor activity of dietary fucoidan from *Undaria pinnatifida* sporophylls (Mekabu). *Planta Med.* **72**, 1415–1417.

Matou, S., Helley, D., Chabut, D., Bros, A., and Fischer, A. M. (2002). Effect of fucoidan on fibroblast growth factor-2-induced angiogenesis in vitro. *Thromb. Res.* **106**, 213–221.

Matsubara, K., Matsuura, Y., Hori, K., and Miyazawa, K. (2000). An anticoagulant proteoglycan from the marine green alga, *Codium pugniformis*. *J. Appl. Phycol.* **12**, 9–14.

Matsuhiro, B., Zuniga, E., Jashes, M., and Guacucano, M. (1996). Sulfated polysaccharides from *Durvillaea antarctica*. *Hydrobiologia* **321**, 77–81.

Mestechkina, N. M. and Shcherbukhin, V. D. (2010). Sulfated polysaccharides and their anticoagulant activity: A review. *Appl. Biochem. Microbiol.* **46**, 267–273.

Nishino, T. and Nagumo, T. (1992). Anticoagulant and antithrombin activities of oversulfated fucans. *Carbohydr. Res.* **229**, 355–362.

Pereira, M. S., Silva, A. C. E. S. V., Valente, A. P., and Mourao, P. A. S. (2002). A 2-sulfated, 3-linked α-L-galactan is an anticoagulant polysaccharide. *Carbohydr. Res.* **337**, 2231–2238.

Qui, X., Amarasekara, A., and Doctor, V. (2006). Effect of oversulfation on the chemical and biological properties of fucoidan. *Carbohydr. Polym.* **63**, 224–228.

Reddy, P. and Urban, S. (2009). Meroditerpenoids from the southern Australian marine brown alga *Sargassum fallax*. *Phytochemistry* **70**, 250–255.

Senni, K., Gueniche, F., Bertaud, A. F., Tchen, S. I., Fioretti, F., Jouault, S. C., Durand, P., Guezennec, J., Godeau, G., and Letourneur, D. (2006). Fucoidan a sulfated polysaccharide from brown algae is a potent modulator of connective tissue proteolysis. *Arch. Biochem. Biophys.* **445**, 56–64.

Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H., and Hama, Y. (2008). Antioxidant activities of phlorotannins isolated from Japanese Laminariacea. *J. Appl. Phycol.* **20**, 705–711.

Silva, T. M. A., Alves, L. G., Queiroz, K. C. S., Santos, M. G. L., Marques, C. T., Chavante, S. F., Rocha, H. A. O., and Leite, E. L. (2005). Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. *Braz. J. Med. Biol. Res.* **38**, 523–533.

Soeda, S., Sakaguchi, S., Shimeno, H., and Nagamatsu, A. (1992). Fibrinolytic and anticoagulant activities of highly sulfated fucoidan. *Biochem. Pharmacol.* **43**, 1853–1858.

Synytsya, A., Kim, W. J., Kim, S. M., Pohl, R., Synytsya, A., Kvasnicka, F., Copikova, J., and Park, Y. I. (2010). Structure and antitumor activity of fucoidan isolated from sporophyll of Korean brown seaweed *Undaria pinnatifida*. *Carbohydr. Polym.* **81**, 41–48.

Teruya, T., Konishi, T., Uechi, S., Tamaki, H., and Tako, M. (2007). Anti-proliferative activity of oversulfated fucoidan from commercially cultured *Cladosiphon okamuranus* TOKIDA in U937 cells. *Int. J. Biol. Macromol.* **41**, 221–226.

Wijesinghe, W. A. J. P., Athukorala, Y., and Jeon, Y. J. (2011). Effect of anticoagulative sulfated polysaccharide purified from enzyme-assistant extract of a brown seaweed *Ecklonia cava* on Wistar rats. *Carbohydr. Polym.* **86**, 917–921.

Yang, L. and Zhang, L. M. (2009). Chemical structure and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydr. Polym.* **76**, 349–361.

Yang, M., Ma, C., Sun, J., Shao, Q., Gao, W., Zhang, Y., Li, Z., Xie, Q., Dong, Z., and Qu, X. (2008). Fucoidan stimulation induces a functional maturation of human monocyte-derived dendritic cells. *Int. Immunopharmacol.* **8**, 1754–1760.

Yarden, Y. and Sliwkowski, M. X. (2001). Untangling the ErbB signaling network. *Nat. Rev. Mol. Cell Biol.* **2**, 127–137.

Yoo, Y. C., Kim, W. J., Kim, S. Y., Kim, S. M., Chung, M. K., Park, J. W., Suh, H. H., Lee, K. B., and Park, Y. I. (2007). Immnomodulating activity of a fucoidan isolated from Korean *Undaria pinnatifida* sporophyll. *Algae* **22**, 333–338.

Yoon, S. J., Pyun, Y. R., Hwang, J. K., and Mourão, P. A. S. (2007). A sulfated fucan from the brown alga *Laminaria cichorioides* has mainly heparin cofactor II-dependent anticoagulant activity. *Carbohydr. Res.* **342**, 2326–2330.

Zvyagintseva, T. N., Shevchenko, N. M., Chizhov, A. O., Krupnova, T. N., Sundukova, E. V., and Isakov, V. V. (2003). Water-soluble polysaccharides of some far-eastern brown seaweeds. Distribution, structure and their dependence on the developmental conditions. *J. Exp. Mar. Biol. Ecol.* **294**, 1–13.