CHAPTER 18

Anticoagulant Effect of Marine Algae

Se-Kwon Kim[*,†,1] and **Isuru Wijesekara**[*]

Contents

Abstract

Recently, a great deal of interest has been developed in the nutraceutical and pharmaceutical industries to isolate natural anticoagulant compounds from marine resources. Among marine resources, marine algae are valuable sources of novel bioactive compounds with anticoagulant effect. Phlorotannins and sulfated polysaccharides such as fucoidans in brown algae, carrageenans in red algae, and ulvans in green algae have been recognized as potential anticoagulant agents. Therefore, marine algae-derived phlorotannins and SPs have great potential for developing as anticoagulant drugs in nutraceutical and pharmaceutical areas. This chapter focuses on the potential anticoagulant agents in marine algae and presents an overview of their anticoagulant effect.

* Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan, Republic of Korea
† Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea
[1] Corresponding author: Se-Kwon Kim, *E-mail address:* sknkim@pknu.ac.kr

Advances in Food and Nutrition Research, Volume 64
ISSN 1043-4526, DOI: 10.1016/B978-0-12-387669-0.00018-1

I. INTRODUCTION

As more than 70% of the world's surface is covered by oceans, the wide diversity of marine organisms offers a rich source of natural products, and the importance of marine organisms as a source of novel bioactive substances is growing rapidly. With marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds (Aneiros and Garateix, 2004; Barrow and Shahidi, 2008). Moreover, a very different kind of substances have been obtained from marine organisms among other reasons because they are living in a very exigent, competitive, and aggressive surrounding, very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules. Marine environment contains a source of functional materials, including polyunsaturated fatty acids (PUFA), polysaccharides, essential minerals and vitamins, antioxidants, enzymes, and bioactive peptides (Kim and Wijesekara, 2010; Pomponi, 1999).

The blood coagulation system consists of intrinsic and extrinsic pathways, where a series of factors involve in the mechanism. Blood coagulation is proceeded by coagulation factors in order to stop the flow of blood through the injured vessel wall whenever an abnormal vascular condition and exposure to nonendothelial surfaces at sites of vascular injury occurred. As endogenous or exogenous anticoagulants interfered with the coagulation factors by inactivate or restrict, the blood coagulation can be prolonged or stopped (Jung *et al.*, 2001). These anticoagulants are used in therapeutic purposes, for example, to cure hemophilia.

Heparin has been identified and used for more than 50 years as a commercial anticoagulant, and it is widely used for the prevention of venous thromboembolic disorders. However, several side effects of heparin have been reported such as development of thrombocytopenia, hemorrhagic effect, and ineffectiveness in congenital or acquired antithrombin deficiencies, and incapacity to inhibit thrombin bound to fibrin (Pereira *et al.*, 2002). Moreover, heparin is available in very low concentrations in pig intestine or bovine lungs from where it is primarily extracted. Therefore, the necessity of discovering alternative sources of anticoagulants has been arisen with interesting demand for safer anticoagulant therapy. Therefore, marine organisms have gained much attention to find natural and safe anticoagulant agents.

Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly, and researchers have revealed that marine algal originated compounds exhibit various biological activities with potential anticoagulant effect (Wijesekara and Kim, 2010; Wijesekara *et al.*, 2010, 2011).

Sulfated polysaccharides (SPs) and phlorotannins from marine algae have been shown potent anticoagulant effect, and according to most of the studies, SPs are the main bioactive for this anticoagulant effect. The anticoagulant activity of the SPs and phlorotannins from marine algae has been determined by prolongation of activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) assays. Most of the studies reported that the anticoagulant activity of marine SPs is based on APTT and TT pathways. The prolongation of APTT suggests inhibition of the intrinsic factors and is the measure of the intrinsic pathway-dependent clotting time. The TT revealed the inhibition of thrombin activity or fibrin polymerization as thrombin inhibition-dependent clotting time. PT is the extrinsic pathway-dependent clotting time. This chapter focuses on anticoagulant agents derived from marine algae and presents an overview of their anticoagulant effect.

II. ANTICOAGULANT AGENTS IN MARINE ALGAE

A. Sulfated polysaccharides

Edible marine algae, sometimes referred as seaweeds, have attracted a special interest as good sources of nutrients, and one particular interesting feature is their richness in SPs, the uses of which span from food, cosmetic, and pharmaceutical industries to microbiology and biotechnology (Ren, 1997). Marine algae are the most important source of nonanimal SPs, and the chemical structure of these polymers varies according to the algal species (Costa *et al.*, 2010). The amount of SPs present is found to differ according to the three major divisions of marine algae, Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae). The major SPs (Fig. 18.1) found in marine algae include fucoidan and laminarans of brown algae, carrageenan of red algae, and ulvan of green algae. Recently, various SPs from marine algae have attracted much attention in the fields of food, cosmetic, and pharmacology. For examples, carrageenans from marine red algae are widely used as food additives, such as emulsifiers, stabilizers, or thickeners (Campo *et al.*, 2009; Chen *et al.*, 2007). Ulvan displays several physiochemical and biological features with potential interest for food, pharmaceutical, agricultural, and chemical applications (Lahaye and Robic, 2007).

B. Phlorotannins

Marine brown algae accumulate a variety of phloroglucinol-based polyphenols, as phlorotannins of low, intermediate, and high molecular weight containing both phenyl and phenoxy units. Based on the means

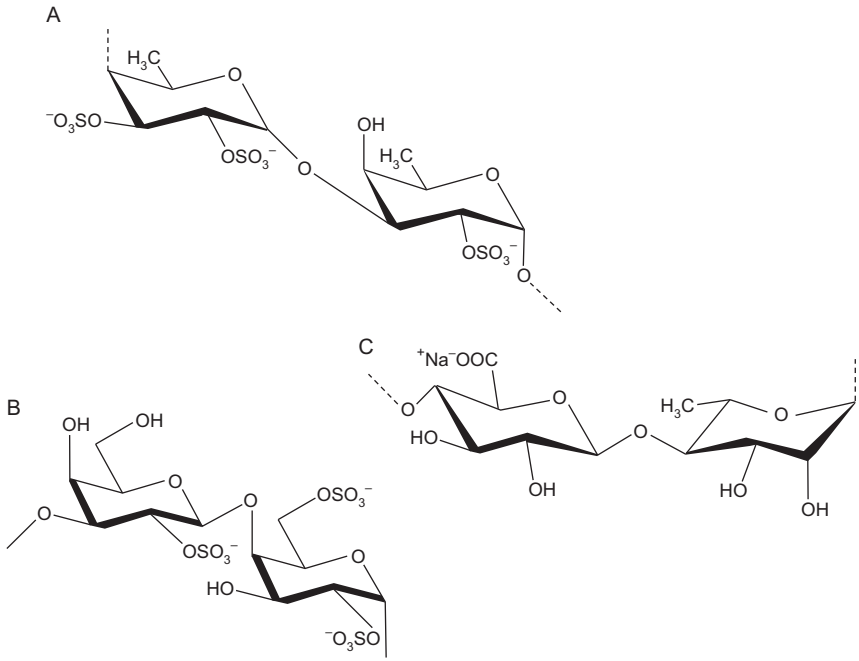

FIGURE 18.1 Anticoagulant SPs from marine algae (A) fucoidan, (B) carrageenan, and (C) ulvan.

of linkage, phlorotannins can be classified into four subclasses such as fuhalols and phlorethols (phlorotannins with an ether linkage), fucols (with a phenyl linkage), fucophloroethols (with an ether and phenyl linkage), and eckols (with a dibenzodioxin linkage). The isolated and characterized phlorotannins from marine brown algae are compounds **1**–**7** (Fig. 18.2), such as phloroglucinol (**1**), eckol (**2**), fucodiphloroethol G (**3**), phlorofucofuroeckol A (**4**), 7-phloroeckol (**5**), dieckol (**6**), and 6,6′-bieckol (7). In addition, triphloroethol A, 8,8′-bieckol, and 8,4‴-dieckol have been isolated. Among marine brown algae, *Ecklonia cava* is a rich source of phenolic compounds as phlorotannins than other brown algae (Wijesekara *et al.*, 2010). However, other brown seaweeds also have been reported for various types of phlorotannins. These phlorotannins help to protect algae from stress conditions and herbivores. Due to the health beneficial various biological activities of phlorotannins, marine brown algae are known to be a rich source of healthy food. Among marine brown algae, *E. cava*, *Ecklonia stolonifera*, *Ecklonia kurome*, *Eisenia bicyclis*, *Ishige okamurae*, *Sargassum thunbergii*, *Hizikia fusiformis*, *Undaria pinnatifida*, and *Laminaria japonica* have been reported for phlorotannins with health beneficial biological activities.

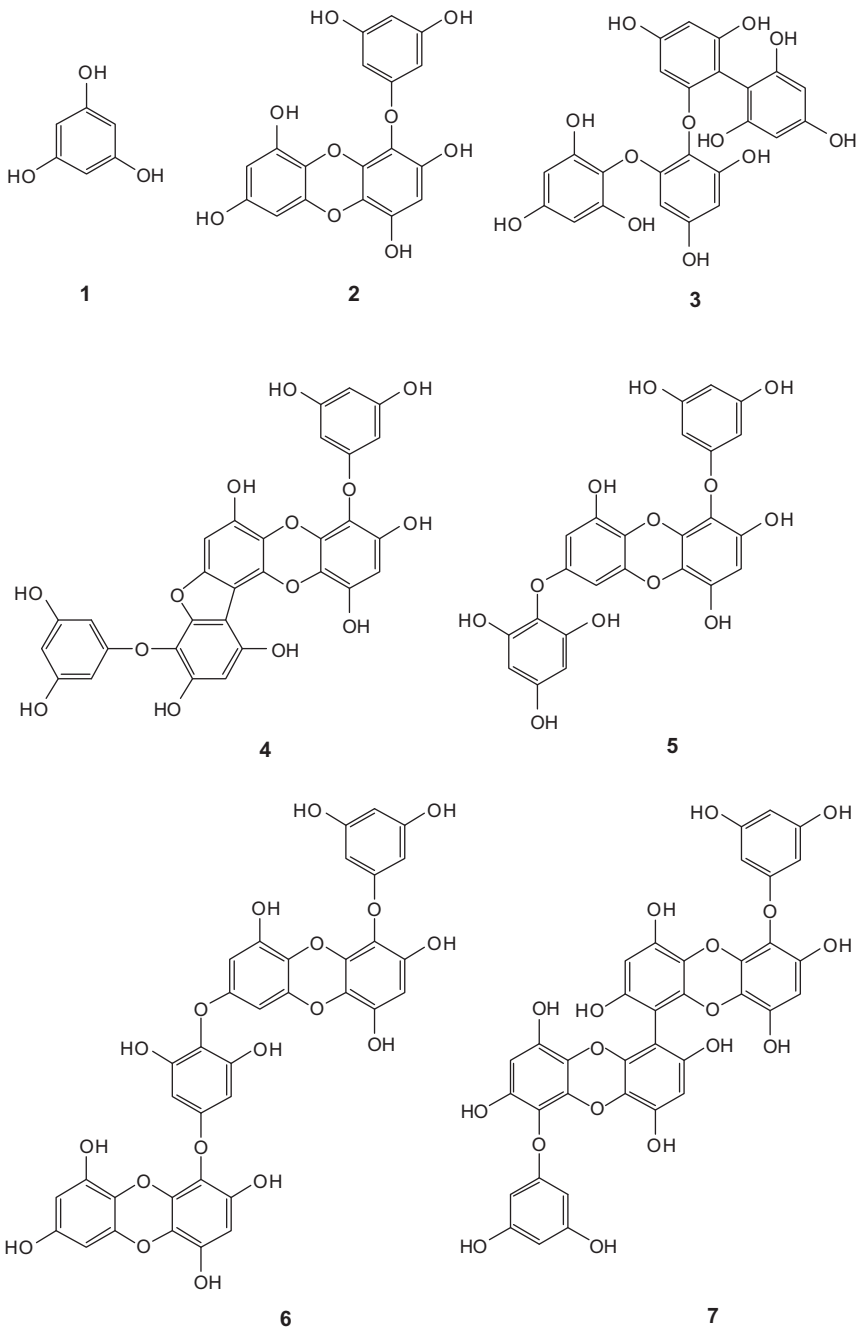

FIGURE 18.2 Phlorotannin derivatives from marine brown algae.

III. ANTICOAGULANT ACTIVITY OF MARINE ALGAE

After the investigation of blood anticoagulant properties from marine brown algae, it has been reported that SPs derived from marine algae are alternative sources for manufacture of novel anticoagulant drugs (Church *et al.*, 1989; Matsubara, 2004; Nishino *et al.*, 2000). Anticoagulant activity is among the most widely studied properties of SPs and anticoagulants from marine algae have previously been reviewed (McLellan and Jurd, 1992; Mestechkina and Shcherbukhin, 2010). Various anticoagulant SPs from marine algae have been isolated and characterized (Table 18.1). Two types of SPs are identified with high anticoagulant activity including sulfated galactans, also known as carrageenan, from marine red algae (Carlucci *et al.*, 1997; Kolender *et al.*, 1997; Sen *et al.*, 1994) and sulfated fucoidans from marine brown algae (Chevolot *et al.*, 1999; Colliec *et al.*, 1991; Dobashi *et al.*, 1989). However, there are fewer reports of anticoagulant SPs reported from marine green algae compared to brown and red algae (Mao *et al.*, 2009). Jurd *et al.* (1995) found that

TABLE 18.1 Some marine algae with anticoagulant sulfated polysaccharides (SPs)

Marine algae with anticoagulant SPs	Ref.
Chlorophyta	
Monostroma latissimum	Mao *et al.* (2009)
Monostroma nitidum	Maeda *et al.* (1991)
Ulva conglobata	Mao *et al.* (2006)
Codium fragile	Hayakawa *et al.* (2000)
Codium pugniformis	Matsubara *et al.* (2000)
Codium cylindricum	Matsubara *et al.* (2001)
Phaeophyta	
Ecklonia cava	Athukorala *et al.* (2006)
Ecklonia kurom	Nishino *et al.* (1991)
Laminaria japonica	Wang *et al.* (2010)
Ascophyllum nodosum	Nardella *et al.* (1996)
Lessonia vadosa	Chandia and Matsuhiro (2008)
Rhodophyta	
Lomentaria catenata	Pushpamali *et al.* (2008)
Gigartina skottsbergii	Carlucci *et al.* (1997)
Schizymenia binderi	Zuniga *et al.* (2006)
Grateloupia indica	Sen *et al.* (1994)
Porphyra haitanensis	Zhang *et al.* (2010)
Nothogenia fastigiata	Kolender *et al.* (1997)

the anticoagulant-active SPs from *Codium fragile* subspecies *atlanticum* (Chlorophyta) contain xyloarabinogalactans. A sulfated galactan with anticoagulant activity has also been reported from *Codium cylindricum* (Matsubara *et al.*, 2001). In addition, Maeda *et al.* (1991) have revealed that the anticoagulant SPs from *Monostroma nitidum* (Chlorophyceae) yielded a sixfold higher activity than that of heparin. In comparison, marine brown algae extracts exhibit higher anticoagulant activity than red and green algae extracts (Chevolot *et al.*, 1999; Patankar *et al.*, 1993).

Since a few studies reported the prolongation of PT by marine SPs, it suggests that marine SPs interfered a little or may not inhibit the extrinsic pathway of coagulation. The relationship between structure and anticoagulant activity of some SPs has been reported (Colliec *et al.*, 1991; Hayakawa *et al.*, 2000). The presence of sulfate groups in SPs can increase both their specific and nonspecific binding to a wide range of biologically active proteins. Anticoagulant activity of sulfated galactans depends on the nature of the sugar residue, the sulfation position of the structure, and the sulfate content in the SPs (Melo *et al.*, 2004; Silva *et al.*, 2010). Moreover, the *O*-sulfated 3-linked α-galactans enhanced the inhibition of thrombin and factor Xa by antithrombin and/or heparin cofactor II in the intrinsic pathway of blood coagulation (Pereira *et al.*, 2002). Further, high molecular weight carrageenans with high sulfate content have shown higher anticoagulant activity than low molecular weight and low sulfate content SPs (Shanmugam and Mody, 2000).

Unfractionated heparins and low molecular weight heparins are the only sulfated polysaccharides currently used as anticoagulant drugs. Seaweed-derived SPs have been described to possess anticoagulant activity similar to or higher than heparin (Costa *et al.*, 2010). Collectively, these evidences suggest that SPs derived from seaweeds have a promising potential to be used as anticoagulant agents in the pharmaceutical industry.

Phlorotannins from *S. thunbergii* have been analyzed for their potential anticoagulant activity and suggested that phlorotannins are potential anticoagulants *in vitro* and *in vivo*. According to their results, phlorotannins from *S. thunbergii* had a significant effect on the prolongation of APTT, PT, and TT especially at the concentration of 1 mg/ml. In addition, phloroglucinol can be developed as a novel anticoagulant in the pharmaceutical industry (Bae, 2011).

IV. CONCLUSIONS

Recent studies have provided evidence that marine algal derived SPs and phlorotannins play a vital role in human health and nutrition. Further, seaweed processing by-products with bioactive SPs and phlorotannins can be easily utilized for producing functional ingredients. The possibilities

of designing new functional foods and pharmaceuticals to support reducing blood clotting or regulating the coagulant-related chronic malfunctions are promising. Therefore, it can be suggested that due to valuable biological functions with health beneficial effects, marine algae have much potential as active ingredients for preparation of nutraceutical and pharmaceutical products. Until now, most of researches of marine algal anticoagulant effect have been observed *in vitro* or in mouse model systems. Therefore, further research studies are needed in order to investigate their activity in human subjects.

REFERENCES

Aneiros, A. and Garateix, A. (2004). Bioactive peptides from marine sources: Pharmacological properties and isolation procedures. *J. Chromatogr. B* **803**, 41–53.

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.

Bae, J. S. (2011). Antithrombotic and profibrinolytic activities of phloroglucinol. *Food Chem. Toxicol.* **49**(7), 1572–1577. doi: 10.1016/j.fct.2011.04.003.

Barrow, C. and Shahidi, F. (2008). Marine Nutraceuticals and Functional Foods. CRC Press, New York, USA.

Campo, V. L., Kawano, D. F., da Silva, D. B., and Carvalho, I. (2009). Carrageenans: Biological properties, chemical modifications and structural analysis: A review. *Carbohydr. Polym.* **77**, 167–180.

Carlucci, M. J., Pujol, C. A., Ciancia, M., Noseda, M. D., Matulewicz, M. C., Damonte, E. B., and Cerezo, S. A. (1997). Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: Correlation between structure and biological activity. *Int. J. Biol. Macromol.* **20**, 97–105.

Chandia, N. P. and Matsuhiro, B. (2008). Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties. *Int. J. Biol. Macromol.* **42**, 235–240.

Chen, H., Yan, X., Lin, J., Wang, F., and Xu, W. (2007). Depolymerized products of λ-carrageenan as a potent angiogenesis inhibitor. *J. Agric. Food Chem.* **55**, 6910–6917.

Chevolot, L., Foucault, A., Chaubet, F., Kervarec, N., Sinquin, C., Fisher, A. M., and Boisson-Vidal, C. (1999). Further data on the structure of brown seaweed fucans: Relationships with anticoagulant activity. *Carbohydr. Res.* **319**, 154–165.

Church, F. C., Meade, J. B., Treanor, E. R., and Whinna, H. C. (1989). Antithrombin activity of fucoidan. The interaction of fucoidan with heparin cofactor II, antithrombin III, and thrombin. *J. Biol. Chem.* **264**, 3618–3623.

Colliec, S., Fischer, A. M., Tapon-Bretaudiere, J., Boisson, C., Durand, P., and Jozefonvicz, J. (1991). Anticoagulant properties of a fucoidan fraction. *Thromb. Res.* **64**(2), 143–154.

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

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

Hayakawa, Y., Hayashi, T., Lee, J. B., Srisomporn, P., Maeda, M., Ozawa, T., and Sakuragawa, N. (2000). Inhibition of thrombin by sulfated polysaccharides isolated from green algae. *Biochim. Biophys. Acta* **1543**, 86–94.

Jung, W. K., Je, J. Y., and Kim, S. K. (2001). A novel anticoagulant protein from *Scapharca broughtonii*. *J. Biochem. Mol. Biol.* **35**, 199–205.

Jurd, K. M., Rogers, D. J., Blunden, G., and McLellan, D. S. (1995). Anticoagulant properties of sulphated polysaccharides and a proteoglycan from *Codium fragile ssp. atlanticum*. *J. Appl. Phycol.* **7**, 339–345.

Kim, S. K. and Wijesekara, I. (2010). Development and biological activities of marine-derived bioactive peptides: A review. *J. Funct. Foods* **2**, 1–9.

Kolender, A. A., Pujol, C. A., Damonte, E. B., Matulewicz, M. C., and Cerezo, A. S. (1997). The system of sulfated α-(1 → 3)-linked D-mannans from the red seaweed *Nothogenia fastigiata*: Structures, antiherpetic and anticoagulant properties. *Carbohydr. Res.* **304**, 53–60.

Lahaye, M. and Robic, A. (2007). Structure and functional properties of ulvan, a polysaccharide from green seaweeds. *Biomacromolecules* **8**, 1765–1774.

Maeda, M., Uehara, T., Harada, N., Sekiguchi, M., and Hiraoka, A. (1991). Heparinoid-active sulfated polysaccharides from *Monostroma nitidum* and their distribution in the Cholorophyta. *Phytochemistry* **30**, 3611–3614.

Mao, W., Zhang, X., Li, Y., and Zhang, H. (2006). Sulfated polysaccharides from marine green algae *Ulva conglobata* and their anticoagulant activity. *J. Appl. Phycol.* **18**, 9–14.

Mao, W., Li, H., Li, Y., Zhang, H., Qi, X., Sun, H., Chen, Y., and Guo, S. (2009). Chemical characteristics and anticoagulant activity of the sulfated polysaccharide isolated from *Monostroma latissimum* (Chlorophyta). *Int. J. Biol. Macromol.* **44**, 70–74.

Matsubara, K. (2004). Recent advances in marine algal anticoagulants. *Curr. Med. Chem.* **2**, 13–19.

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.

Matsubara, K., Matsuura, Y., Bacic, A., Liao, M. L., Hori, K., and Miyazawa, K. (2001). Anticoagulant properties of a sulfated galactan preparation from a marine green alga, *Codium cylindricum*. *Int. J. Biol. Macromol.* **28**, 395–399.

McLellan, D. S. and Jurd, K. M. (1992). Anticoagulants from marine algae. *Blood Coagul. Fibrinolysis* **3**, 69–80.

Melo, F. R., Pereira, M. S., Foguel, D., and Mourao, P. A. S. (2004). Antithrombin-mediated anticoagulant activity of sulfated polysaccharides. *J. Biol. Chem.* **279**, 20824–20835.

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

Nardella, A., Chaubet, F., Boisson-Vidal, C., Blondin, C., Durand, P., and Jozefonvicz, J. (1996). Anticoagulant low molecular weight fucans produced by radical process and ion exchange chromatography of high molecular weight fucans extracted from the brown seaweed *Acosphyllum nodosum*. *Carbohydr. Res.* **289**, 201–208.

Nishino, T., Aizu, Y., and Nagumo, T. (1991). The influence of sulfate content and molecular weight of a fucan sulfate from the brown seaweed *Ecklonia kurome* on its antithrombin activity. *Thromb. Res.* **64**, 723–731.

Nishino, T., Yamauchi, T., Horie, M., Nagumo, T., and Suzuki, H. (2000). Effects of fucoidan on the activation of plasminogen by u-PA and t-PA. *Thromb. Res.* **99**, 623–634.

Patankar, M. S., Oehninger, S., Barnett, T., Williams, R. L., and Clerk, G. F. (1993). A revised structure for fucoidan may explain some of its biological activities. *J. Biol. Chem.* **268**, 21770–21776.

Pereira, M. S., Melo, F. R., and Mourao, P. A. S. (2002). Is there a correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans? *Glycobiology* **12**, 573–580.

Pomponi, S. A. (1999). The bioprocess-technological potential of the sea. *J. Biotechnol.* **70**, 5–13.

Pushpamali, W. A., Nikapitiya, C., De Zoysa, M., Whang, I., Kim, S. J., and Lee, J. (2008). Isolation and purification of an anticoagulant from fermented red seaweed *Lomentaria catenata*. *Carbohydr. Polym.* **73**, 274–279.

Ren, D. (1997). Biotechnology and the red seaweed polysaccharide industry: Status, needs and prospects. *Trends Biotechnol.* **15**, 9–14.

Sen, A. K., Sr., Das, A. K., Banerji, N., Siddhanta, A. K., Mody, K. H., Ramavat, B. K., Chauhan, V. D., Vedasiromoni, J. R., and Ganguly, D. K. (1994). A new sulfated polysaccharide with potent blood anti-coagulant activity from the red seaweed *Grateloupia indica*. *Int. J. Biol. Macromol.* **16**, 279–280.

Shanmugam, M. and Mody, K. H. (2000). Heparinoid-active sulfated polysaccharides from marine algae as potential blood anticoagulant agents. *Curr. Sci.* **79**, 1672–1683.

Silva, F. R. F., Dore, C. M. P. G., Marques, C. T., Nascimento, M. S., Benevides, N. M. B., Rocha, H. A. O., Chavante, S. F., and Leite, E. L. (2010). Anticoagulant activity, paw edema and pleurisy induced carrageenan: Action of major types of commercial carrageenans. *Carbohydr. Polym.* **79**, 26–33.

Wang, J., Zhang, Q., Zhang, Z., Song, H., and Li, P. (2010). Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. *Int. J. Biol. Macromol.* **46**, 6–12.

Wijesekara, I. and Kim, S. K. (2010). Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: Prospects in the pharmaceutical industry. *Mar. Drugs* **8**, 1080–1093.

Wijesekara, I., Yoon, N. Y., and Kim, S. K. (2010). Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits. *Biofactors* **36**, 408–414.

Wijesekara, I., Pangestuti, R., and Kim, S. K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydr. Polym.* **84**, 14–21.

Zhang, Z., Zhang, Q., Wang, J., Song, H., Zhang, H., and Niu, X. (2010). Regioselective syntheses of sulfated porpyrans from *Porphyra haitanensis* and their antioxidant and anticoagulant activities *in vitro*. *Carbohydr. Polym.* **79**, 1124–1129.

Zuniga, E. A., Matsuhiro, B., and Mejias, E. (2006). Preparation of a low-molecular weight fraction by free radical depolymerization of the sulfated galactan from *Schizymenia binderi* (Gigartinales, Rhodophyta) and its anticoagulant activity. *Carbohydr. Polym.* **66**, 208–215.