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Review

Chemical Structures and Bioactivities of Sulfated Polysaccharides from Marine Algae

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Abstract: Sulfated polysaccharides and their lower molecular weight oligosaccharide derivatives from marine macroalgae have been shown to possess a variety of biological activities. The present paper will review the recent progress in research on the structural chemistry and the bioactivities of these marine algal biomaterials. In particular, it will provide an update on the structural chemistry of the major sulfated polysaccharides synthesized by seaweeds including the galactans (e.g., agarans and carrageenans), ulvans, and fucans. It will then review the recent findings on the anticoagulant/antithrombotic, antiviral, immuno-inflammatory, antilipidemic and antioxidant activities of sulfated polysaccharides and their potential for therapeutic application.

Keywords: sulfated polysaccharide; marine macroalgae; carbohydrate structure; bioactivity; oligosaccharides

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1. Introduction

Many species of seaweed (marine macroalgae) are used as food and they have also found use in traditional medicine because of their perceived health benefits. Seaweeds are rich sources of sulfated polysaccharides, including some that have become valuable additives in the food industry because of

their rheological properties as gelling and thickening agents (e.g., carrageenan). In addition, sulfated polysaccharides are recognized to possess a number of biological activities including anticoagulant, antiviral, and immuno-inflammatory activities that might find relevance in nutraceutical/functional food, cosmetic/cosmeceutical and pharmaceutical applications.

In this review, we will examine current progress in research on sulfated polysaccharides, relating to their structural diversity, bioactivities and mechanisms of action. The literature contains vast information on the structure and bioactivities of marine sulfated polysaccharides that cannot be completely considered in this short review. There are several excellent reviews where the reader may find additional information on various aspects of this subject [1–9].

2. Structural Diversity of Algal Sulfated Polysaccharides

2.1. Carrageenans and Agarans from Red Algae

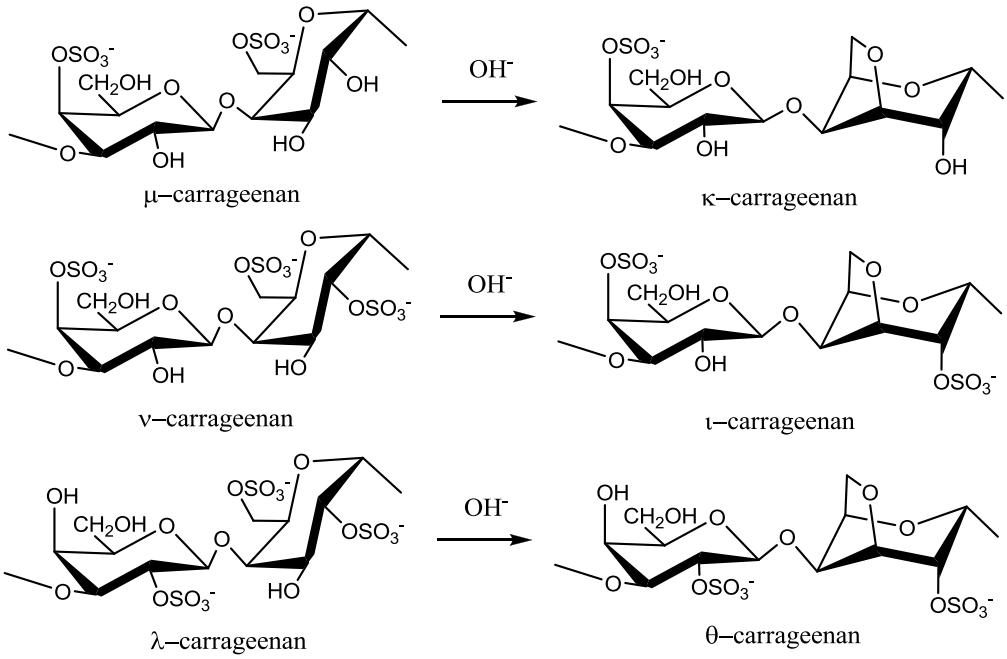
Red seaweed galactans are of great commercial importance as they are used widely in the food industry because of their rheological properties as gelling and thickening agents. These sulfated polysaccharides are primarily classified as agarans and carrageenans based on their stereochemistry, specifically galactans with 4-linked α-galactose residues of the L-series are termed agarans and those of the D-series are termed carrageneens [10]. Thus, carrageenans are high molecular weight sulfated D-galactans composed of repeating disaccharide units with alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-galactopyranose (D-units) or 3,6-anhydro-α-galactopyranose (AnGal-units).

Carrageenans are normally classified according to their structural characteristics, including their sulfation patterns and the presence or absence of AnGal on D-units. There are at least 15 different carrageenan structures [2]. The most industrially relevant carrageenans are κ, ι and λ forms, the structures of which are illustrated in Figure 1. The major source of κ-carrageenan is the red seaweed *Kappaphycus alvarezii* [11]. Its structure was reported as alternating 3-linked β-D-galactose 4-sulfate and 4-linked AnGal units [12]. The ι-carrageenans have an additional sulfate group on C2(O) of the AnGal residue, resulting in two sulfates per disaccharide repeating unit. Funami *et al.* examined ι-carrageenan extracted from *Eucheuma spinosum* using atomic force microscopy and suggested that ι-carrageenans were more homogeneous and flexible than κ-carrageenans [13]. The λ-carrageenans have three sulfate groups per disaccharide unit with the third sulfate group of this form at the C6 position of the 4-linked residue, but there is no 3,6-anhydride bridge on the 4-linked residues. Lambda-carrageenan is obtained from species of the *Gigartina* and *Chondrus* genera [14].

Alternative forms of carrageenan can be obtained by chemical modification. For example, formation of an anhydride bridge in λ-carrageenan can be induced by alkali modification to produce θ-carrageenan (Figure 1). Extraction of λ-carrageenan from hand-sorted tetrasporophytes of *Gigartina skottsbergii* and subsequent treatment of the extract with alkaline borohydride resulted in conversion of the 4-linked residues to the 3,6-anhydride ring form, yielding θ-carrageenan with no detectable contamination of κ- or ι-carrageenans [15].

Natural carrageenans typically occur as mixtures of different hybrid types, such as κ/β-hybrids [16], κ/ι-hybrids [17–20], κ/μ-hybrids [21], or ν/ι-hybrids [22]. Additionally, methyl or pyruvic acid acetal constituents and the presence of small amount of other sugars can add to the structural complexity [23].

Figure 1. Repeating disaccharide units of different types of carrageenan and their transformation by treatment with alkali.



One of the best studied agarans is porphyran [24], obtained from *Porphyra* species of red algae including *Porphyra capensis* [25] and *P. haitanensis* [26,27]. Porphyran typical exhibits a linear backbone of alternating 3-linked β-D-galactose and 4-linked α-L-galactose-6-sulfate or 3,6-anhydro-α-L-galactose units. Sulfated agarans of a similar linear form are synthesized by *Polysiphonia* species, such as *P. strictissima*, *P. abscissoides* [28], *P. nigrescens* [29], and *P. atterima* [30]. The regular agaran backbone may be interrupted by different *O*-linked substitutions in addition to sulfate including methyl and xylosyl groups adding to the structural diversity. For example, Prado *et al.* recently reported that the sulfated agaran from *P. nigrescens* is highly substituted on the C-6 of β-D-galactose with sulfate, but methyl ether and β-D-xylose residues were also present [29]. Agaran from *Acanthophora spicifera*, is highly sulfated at the C-2 position of β-D-galactose units, with some of the residues being 4,6-pyruvylated [31]. This agaran also contains small amounts of xylose and sulfated xylose residues [31,32].

In addition to carrageenans and agarans, there are also red seaweed sulfated polysaccharides that have 4-linked D- and L-galactose sugars distributed within the same polysaccharide molecules, so-called DL-hybrids, and others with various substitutions involving sulfate groups, pyruvic acid ketals, and methoxyl groups [33]. Indeed, native polysaccharides are rarely in their uniform or "ideal" form. For example, we recently reported on the existence of a carrageenan-like sulfated galactan from *Furcellaria lumbricalis* composed of κ/β-carrageenan units, non-sulfated galactan units, and also smaller units containing 3-*O*-methyl-galactose [16]. Another example of non-ideal sulfated galactans are xylogalactans, first described in the red seaweed *Corallina officinalis* and termed corallinan [34], which are agarans that have β-D-xylosyl groups attached at the *O*-6 position of D-galactose units [35–37].

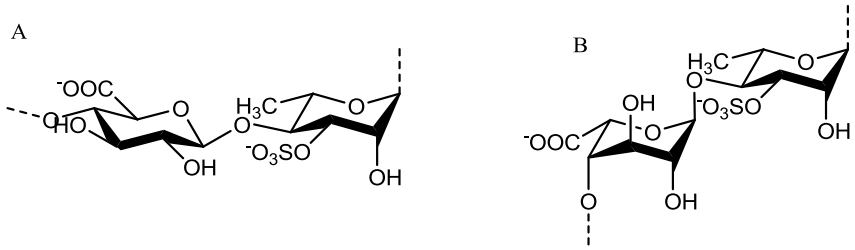
It should be noted that red seaweeds also produce other types of sulfated polysaccharides including those with mannose in their backbones [38,39]. For example, Mandal *et al.* described xylomannnan

from *Scinaia hatei* consisting primarily of a backbone of α-(1→3)-linked D-mannose residues substituted at C-6, C-4, and C-2 with β-D-xylosyl residues [39].

2.2. Sulfated Polysaccharides from Green Algae

Ulvan is the major water-soluble polysaccharide found in green seaweed of the order Ulvales (*Ulva* and *Enteromorpha* sp.) that has sulfate, rhamnose, xylose, iduronic and glucuronic acids as main constituents [40,41]. As reviewed by Lahaye and Robic, ulvan structure shows great complexity and variability as evidenced by the numerous oligosaccharide repeating structural units identified in native and chemically modified ulvan preparations [3]. The main repeating disaccharide units reported are ulvanobiouronic acid 3-sulfate types containing either glucuronic or iduronic acid (Figure 2). Additionally, minor repeat units have been reported that contain sulfated xylose replacing the uronic acid or glucuronic acid as a branch on *O*-2 of the rhamnose-3-sulfate [40,42].

Figure 2. The main repeating disaccharide units of ulvan. A. [→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*3S-(1→]$_n$; B. [→4)-α-L-Ido*p*-(1→4)-α-L-Rha*p*3S-(1→]$_n$.



Although the most common source of sulfated galactans is red macroalgae, some green algae, particularly *Codium* species, are a significant source of sulfated galactans [43–45]. Sulfated galactans from green algae tend to be more complex and heterogeneous in structure than their counterparts from red algae. For example, *C. fragile* and *C. cylindricum* contain sulfated arabinogalactan and sulfated glucogalactan, respectively [43,46]. Bilan *et al.* reported a highly ramified sulfated galactan from *C. yezoense* that contained a linear backbone of 3-linked β-D-galactopyranose residues containing short oligosaccharides branches through (1→6) linkages [47]. Sulfate groups were found mainly at C-4 and in minor amounts at C-6. Polysaccharides containing sulfated galactans from other green seaweeds including *Caulerpa* and *Ulva* have been reported [48,49], but the galactans are minor components.

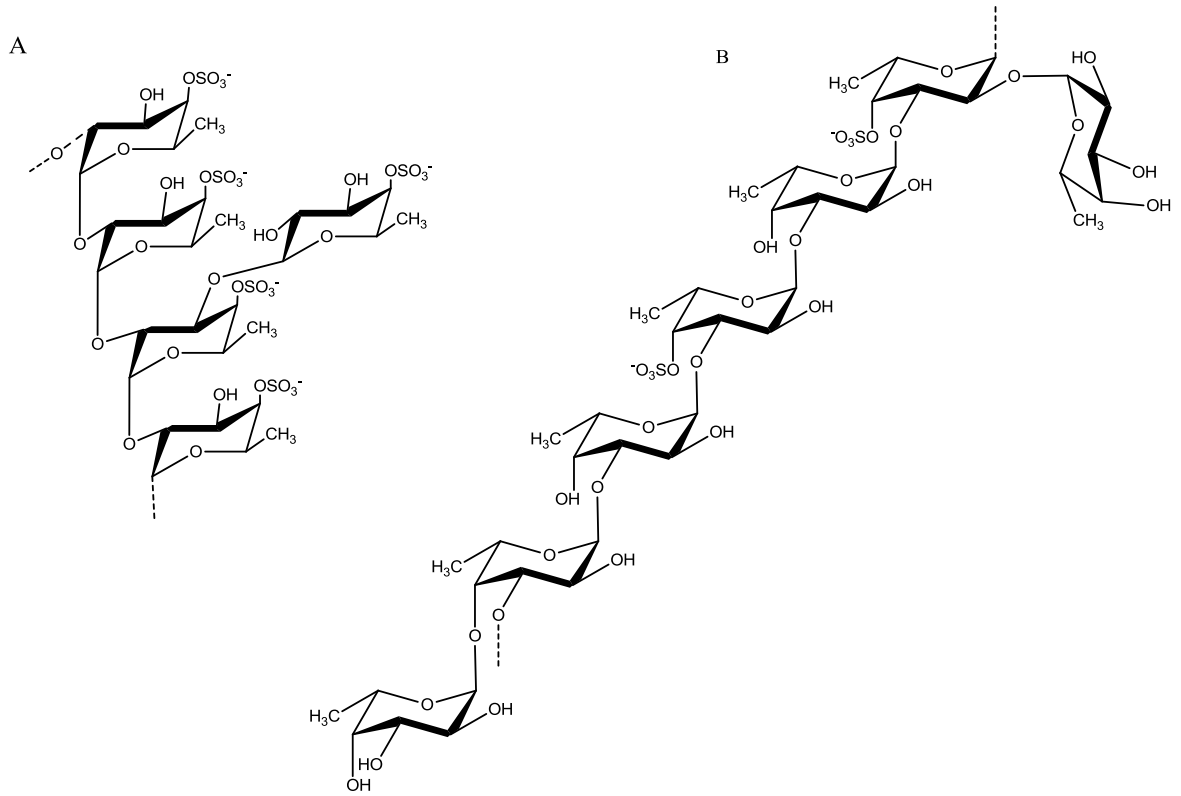
A variety of other forms of sulfated polysaccharides are synthesized by green seaweeds [41,50–53]. This includes, for example, a water-soluble heteroglycuronan from *Enteromorpha compressa*, composed of (1→2,4)-linked rhamnose, (1→4)-linked xylose, and (1→4)-linked glucuronic acid units [52]. Sulfate groups, when present, were situated at the C-3 of rhamnose and the C-2 of xylose. Recently, a rhamnan sulfate from *Monostroma nitidum* was shown to consist primarily of α-1,3-linked and α-1,2-linked rhamnose residues [51].

2.3. Fucose-containing Sulfated Polysaccharides from Brown Algae

Fucans are sulfated polysaccharides that are composed of a fucose backbone. One of the best studied fucans from brown algae is fucoidan, which was first isolated by Kylin in 1913 [54]. The

fucoidan from *Fucus vesiculosus* has been available commercially for decades (Sigma-Aldrich Chemical Company, St. Louis, MO, U.S.). Early work on its structure showed that it contained primarily (1→2) linked 4-*O*-sulfated fucopyranose residues [55]. However, 3-linked fucose with 4-sulfated groups were subsequently reported to be present on some of the fucose residues [56]. Additionally, it was determined to contain branches every 2–3 fucose residues. These early structures of fucoidan from *F. vesiculosus* are illustrated in Figure 3. Subsequently, Chevolot and colleagues reported that the fucoidan from *F. vesiculosus* and *Ascophyllum nodosum* contains a predominant disaccharide motif containing sulfate at the 2-position of the 3-linked fucose and sulfate groups on the 2- and 3-positions of the 4-linked fucose [57].

Figure 3. Structure of fucoidan prepared from *F. vesiculosus* by Percival (**A**) [55] and Patankar (**B**) [56].



Fucans can differ in structure among algal species and can vary even within the same species. Because of the heterogeneity in structures within seaweed, differing extraction conditions used by researchers can give rise to the isolation of distinct fucan forms [4]. Fucans have been classified into two groups [58]. One group includes the fucans from *Laminaria saccharina*, *L. digitata*, *Analipus japonicus*, *Cladosiphon okamuranus*, and *Chorda filum* that have their central chains composed of (1→3)-linked α-L-fucopyranose residues. A second group included fucans isolated from *Ascophyllum nodosum* and *Fucus* species that have their central chains composed of repeating (1→3)- and (1→4)-linked α-L-fucopyranose residues. However, many studies have revealed more complex fucans some with branching structures. A fucoidan isolated from *Turbinaria conoides* was shown to be highly complex, with 33–34% terminals, 27–28% linked and 21–22% branched in the (1→3)-linked main chain [59]. Representative fucan structures are illustrated in Table 1.

Table 1. Representative structures reported for fucans from brown algae.

Species	Fucoidan structures	Reference
Analipus japonicus	3 (4Fuc*p*) and 1 (2Fuc*p*) per ten (1→3)-α-L-Fuc*p*(2/4SO_3^-) residues	Bilan *et al.*, 2007 [60]
Ascophyllum nodosum	→3)-α-L-Fuc*p*(2SO_3^-)-(1→4)-α-L-Fuc*p*(2,3-diSO_3^-)-(1→	Chevolot *et al.*, 2001 [57]
A. nodosum	(1→3)-α-L-Fuc*p* and a few (1→4)-α-L-Fuc*p* with (1→3)-α-L-(2 and/or 4 Fuc*p*)	Marais *et al.*, 2001 [61]
Chorda filum	-[→3)-α-L-Fuc*p*-(1-]$_3$→3)-α-L-Fuc*p*(2Fuc*p*)-(1→	Chizhov *et al.*, 1999 [62]
Fucus distichus L.	→3)-α-L-Fuc*p*-(2,4-diSO_3^-)-(1→4)-α-L-Fuc*p*-(2SO_3^-)-(1→	Bilan *et al.*, 2004 [63]
F. evanescens	→3)-α-L-Fuc*p*(2SO_3^-)-(1→4)-α-L-Fuc*p*(2SO_3^-)-(1→	Bilan *et al.*, 2002 [64]
F. serratus L	→3)-α-L-Fuc*p*(2R_1,4R_2)-(1→4)-α-L-Fuc*p*(2SO_3^-)-(1→ a. (~50%): R_1 = SO_3^-, R_2 = H b. (~50%): R_1 = H, R_2 = α-L-Fuc*p*-(1→4)-α-L-Fuc*p*(2SO_3^-)-(1→3)-α-L-Fuc*p*(2SO_3^-)-(1→	Bilan *et al.*, 2006 [65]
Laminaria saccharina	→3)-α-L-Fuc*p*(4SO_3^-)-(1→ and additionally →3)-α-L-Fuc*p*(4SO_3^- or 2Fuc*p*)-(1→	Usov *et al.*, 1998 [66]
Stoechospermum marginatum	→3)-α-L-Fuc*p*(2/4SO_3^-)-(1→ and →4)-α-L-Fuc*p*(2SO_3^-)-(1→	Adhikari *et al.*, 2006 [67]

Many fucans from brown algae contain small amounts of other monosaccharides, including glucose [68], galactose [69], mannose [70–72], xylose [69,73], uronic acids [68,74] and also acetyl groups [75].

3. Approaches in Structural Analysis of Algal Sulfated Polysaccharides

3.1. Desulfation and Methylation for Structure Analysis

Structure analysis of sulfated polysaccharides requires determination of the attached sulfate esters along their backbones and the glycosidic linkage types. Methylation is used to determine linkages between monsaccharides. By comparing the methylation of native polysaccharides to that of their desulfated counterparts, the positions of the sulfate groups can be determined. Thus, hydrolysis of the permethylated, desulfated polysaccharides, yields partially methylated monosaccharides, which following acetylation are separated and identified on gas chromatography/mass spectra [76].

Accurate structural determination requires desulfation of the polysaccharide without cleavage of the polysaccharides chain linkages. Often a solvolytic desulfation procedure is used wherein the polysaccharide as a pyridinium salt is heated in dimethyl sulfoxide [47,77,78]. Desulfation of fucoidan by methyl sulfoxide-pyridine is rapid and complete, resulting in higher yields with little degradation. However, a method using chlorotrimethylsilane (CTMS) for treatment of pyridinium salts is more appropriate for desulfation of sulfated galactans of both the agaran and carrageenan families [79]. Other approaches for desulfation that have been used involve methanolic hydrogen chloride [80], silylating reagents [81], and pyromellitic acid [82]. Chemical desulfation is relatively non-specific and usually results in a significant loss of sample material. The use of sulfatases represents a more specific approach to desulfation and would be advantageous in structural studies, yet such enzymatic

approaches do not appear to be a frequent method of choice [7,83]. The reason for this is unclear but could be due to the lack of commercially available enzymes of the required specificity.

The typical methylation procedure is straightforward, involving treating the desulfated polysaccharide sample with methyl iodide in the presence of solid base, usually sodium hydroxide, in methyl sulfoxide and the procedure can be repeated to obtain a complete methylation [51,62,77,84,85].

3.2. Structural Analysis by Using NMR and MS

A powerful tool for the structural elucidation of sulfated polysaccharides is NMR spectroscopy, which can provide structural details such as the monosaccharide components, linkages, anomeric configurations, and positions of branching or sulfations. This can be done by combining various 1D and 2D-NMR techniques. The use of NMR in the structural analysis of red algal galactans and green algal ulvans has been considerable [3,86]. In part, this has been due to the relative high proportion of repeating sequences (identical sulfation pattern) in these polysaccharides that make them amenable to analysis by ^{13}C-NMR. For example, Gonçalves and colleagues described the structural elucidation by NMR of positional isomers of sulfated oligosaccharides obtained from agarans and carrageenans [87]. This entailed partial reductive hydrolysis to produce oligosaccharides from repetitive galactans followed by separation by anion exchange and gel-filtration chromatography prior to 1D and 2D NMR analysis.

Effort has also been made to elucidate the structures of fucans by NMR. Usov *et al.*, by 1D-NMR, found the sulfated fucans from *Saccharina latissima* (formerly *Laminaria saccharina*) to be 1→3 linked α-L-fucopyranose with a sulfate group at C4 and branched at C2 [66]. More recently, they confirmed the complex structure of this fucoidan with a more detailed structural investigation by 2D-NMR [88]. These studies also revealed the presence of three additional sulfated polysaccharide types, a fucogalactan, a fucoglucuronomannan and a fucoglucuronan, that appear to occur in minor amounts in their preparation.

Mass spectrometry (MS) is valuable in the structural analysis of polysaccharides, as it generates accurate molecular mass data for oligosaccharides and it can also provide sequence information. Compared with other analytical techniques, mass spectrometric methods have several advantages, including low sample consumption (e.g., picomole quantities) and short analysis time. While analysis of sulfated polysaccharides by MS can be problematic due to the labile nature of the sulfate groups, approaches based on electrospray ionization and matrix-assisted laser desorption/ionization (MALDI) are increasingly being developed to characterize sulfated oligosaccharides [89–92]. Negative-ion ESI-CID-MS/MS was used to characterize oligosaccharide fragments derived from mild hydrolysis of κ-carrageenan that revealed highly ordered disaccharide repeats leading to a complete series of exclusively odd-numbered oligosaccharides [92]. Similarly, fucan oligosaccharides from *A. nodosum*, including a highly sulfated pentasaccharide, were analyzed successfully by negative ion ESI-MS [89].

3.3. Oversulfation of Algal Polysaccharides

Structure modification of sulfated polysaccharides, such as desulfation, oversulfation, acetylation and benzoylation, would allow the development of new and possibly more effective derivatives of naturally occurring polysaccharides [93–95]. For example, benzoylated derivatives of native ulvan

from *Ulva pertusa* exhibited enhanced antioxidant properties [94]. Certainly, the most frequent structural modification to sulfate polysaccharides is oversulfation due to the typically strong positive correlation between their sulfate content and biological activity (discussed further in Section 4). A number of methods have been developed for polysaccharide oversulfation, such as treatment with sulfuric acid, sulfur trioxide-pyridine, chlorosulfonic acid-pyridine, dimethylformamide and sulfur trioxide-dimethylamine [96–102].

3.4. Molecular Size Modification of Algal Sulfated Polysaccharides

Lower molecular weight algal sulfated polysaccharides can be prepared by chemical, physical or enzymatic means to obtain oligosaccharides with more diverse bioactivities. For example, linear or branched sulfated galactans and fucans can be cleaved by mild acid hydrolysis [99,102] or by a radical process involving a hydrogen peroxide-cupric redox system [98,101]. The chemical methods are easy to perform but lack specificity. Minor changes in temperature or acidity can lead to variations in oligosaccharide sizes and sulfation patterns, while strong acid may alter the sulfation pattern or destroy the polysaccharide chain [97]. Enzymatic degradation of sulfated polysaccharides can be achieved by selecting enzymes such as hydrolases, fucoidanases (EC 3.2.1.44), α-L-fucosidases (EC 3.2.1.51) and galactosidases [96,103] to target glycosidic bonds while preserving the sulfate groups [100]. Fucoidanases and galactosidases have been identified in marine invertebrates and microorganisms [83,104–108]. Some bioactivities of lower molecular weight oligosaccharides prepared from algal sulfated polysaccharides are highlighted in Table 2.

Table 2. Biological activities of selected low molecular weight oligosaccharides derived from algal sulfated polysaccharides.

Modification methods	Source	Applications	Reference
Chemical	*Ascophyllum nodosum*	Antithrombotic activity	Colliec-Jouault *et al.*, 2003 [109]
	Botryocladia occidentalis	Anti-venom activity	Toyama *et al.*, 2010 [110]
	Furcellaria lumbricalis	Immunostimulation activity	Yang *et al.*, 2011 [16]
	Solieria chordalis	Immunostimulation activity	Bondu *et al.*, 2010 [111]
Enzymatic	*Chondrus ocellatus*	Anti-tumor activity	Mou *et al.*, 2003 [112]
	Nemacystus decipieus	Anticoagulant activity	Kitamura *et al.*, 1992 [113]
	Pelvetia canaliculata	Antiviral activity	Klarzynski *et al.*, 2003 [114]
	Undaria pinnatifida	Anticoagulant activity	Kim *et al.*, 2010 [115]

4. Bioactivity and Structure-Activity Relationship

Due to the difficulties in identifying the chemical structure of algal sulfated polysaccharides, the relation between their structures and biological activities is not completely understood. One of the research approaches in establishing structure-function relationships has been to make inferences based on information obtained from studies of invertebrate sulfated polysaccharides that have a regular structure and so are more easily studied. For example, Pereira and colleagues compared branched fucans from brown seaweeds with the more simple linear versions obtained from sea cucumber and sea urchins [116]. The anticoagulant activity of seaweed fucans was shown to depend on their molecular

weight, the extent of sulfation, and the distribution of sulfate groups in the repeating units. Interestingly, the algal fucans were found to inhibit coagulation through a direct inhibition of thrombin whereas the invertebrate fucans inhibited the enzyme indirectly with a requirement for antithrombin and heparin cofactor II.

In this section, we will highlight the key biological activities that have been reported for algal sulfated polysaccharides, the current knowledge regarding their mode of action and the structural requirements necessary to elicit these effects.

4.1. Anticoagulant and Antithrombotic Activities

Probably the most widely recognized and studied bioactivity of marine sulfated polysaccharides is the heparin-like anticoagulant activity exhibited by fucoidans and other fucans of brown seaweeds. This was first reported for fucoidan isolated from *F. vesiculosus* by Springer and colleagues who found inhibition of fibrin clot formation and antithrombin activity [117,118]. Since then, studies on fucans from various seaweeds have revealed anticoagulant and antithrombotic activities and are discussed in several recent reviews [1,4,6].

The basis for these activities is not completely understood, but a number of investigations suggest more than one mechanism of action including direct and indirect inhibition of thrombin through the activation of thrombin inhibitors (e.g antithrombin and heparin cofactor II) [116,119–121]. Recently, Cumashi and colleagues reported that fucans from 10 brown seaweeds each prolonged the clotting time of human plasma; however, only five of these fucans had significant activity against thrombin-induced platelet aggregation [122]. While the results of the latter assay are suggestive of a direct action of certain fucans on thrombin, the authors pointed out that an interfering action of thrombin binding to its receptors on platelets could not be ruled out.

The general structural features of fucans that are important in their anticoagulation activity include sugar composition, molecular weight, sulfation level and the position of sulfate groups on the sugar backbone [70,123–125]. For example, Nishino and colleagues found that a higher content of fucose and sulfate groups coincided with higher anticoagulant activities in sulfated polysaccharide fractions from *E. kurome* [70]. They also showed that anticoagulation activity of fucans was positively correlated with sulfate content and that only fucans with a sulfate-total sugar residue ratio greater than one possessed significant activity [93,123]. Higher molecular weight fucans (e.g., 27 and 58 kDa) showed greater anticoagulant activity than lower molecular weight ones (~10 kDa) [126]. The relationship between molecular weight of sulfated polysaccharides and their anticoagulant activity was also considered by Pomin and colleagues who reported that linear, sulfated fucan required significantly longer chains than mammalian glycosaminoglycans to achieve anticoagulant activity [125]. Selective cleavage to reduce molecular size of the fucan by only a modest amount dramatically reduced its effect on thrombin inactivation mediated by heparin cofactor II. Lower molecular weight fucans appear to bind to heparin cofactor II but, unlike the native (full length) fucan, were unable to effectively facilitate the heparin cofactor II interaction with thrombin [125]. Chevolot *et al.* reported on the importance of sulfate group location on the sugar residues for anticoagulant activity [57,62]. Studying the fucoidan from *A. nodosum*, they found that anticoagulant activity required 2-*O*-sulfated and 2,3-*O* disulfated fucose residues, whereas sulfation at the *O*-4 position did not appear necessary.

Marine sulfated polysaccharides other than fucans have also been shown to possess anticoagulant activities. Reports include sulfated galactan and ulvan-like sulfated polysaccharides obtained from green algae, in particular from species of *Codium* and *Ulva* [44,45,49,127,128]. For example, Mao *et al.* described a sulfated polysaccharide from *U. conglobata* with high rhamnose content and 35% sulfate ester that prolonged clotting time through what appeared to be direct inhibition of thrombin and modulation of heparin cofactor II [49]. Hayakawa *et al.* tested sulfated polysaccharides from 23 green algae species for anticoagulant activity and discovered a high rhamnose-containing sulfated polysaccharide from *Monostroma nitidum*, the purified version of which was more potent than standard heparin [127].

Red seaweeds have also yielded a number of sulfated polysaccharides with potent anticoagulant activities [129–131]. Studies on a sulfated galactan from the red seaweed *Botryocladia occidentalis* are particularly illustrative. Farias *et al.* reported that a 2,3-di-*O*-sulfated D-galactan from *B. occidentalis* exhibited anticoagulant activity, comparable to heparin, which appeared to be due to inhibition of thrombin and factor X. Its activity was more potent than similar sulfated galactans, from invertebrate sources, that had only one sulfate per galactose residue [129]. A similar polysaccharide chain from *G. crinale*, but with lower amounts of 2, 3-di-*O*-sulfated D-galactose, was less potent in a clotting time assay when compared with that from *B. occidentalis* [131]. The two sulfated polysaccharides did not differ in thrombin inhibition mediated by antithrombin; however, in assays where heparin cofactor II was used in place of antithrombin, the sulfated galactan from *G. crinale* was less inhibitory than that from *B. occidentalis.* Yet the sulfated galactan from *G. crinale* was a more potent anticoagulant than that from *B. occidentalis* when Factor X was the target protease. These observations suggested that the proportion and/or the distribution of 2,3-di-sulfated galactose along the polysaccharide chain modulate the interaction of the polysaccharides with specific proteases in the coagulation system. Recently, Glauser *et al.* showed that the 2,3-disulfated galactan from *B. occidentalis* inhibits intrinsic tenase and prothrombinase complexes that are critical for factor Xa and thrombin generation, respectively [130]. The sulfated galactan interacts with the heparin-binding site on the heavy chain of factor Xa. Interestingly, the anticoagulant activities associated with the sulfated galactan and that of heparin are modulated differently by heparin cofactor II; heparin anticoagulant activity was enhanced in plasma devoid of heparin cofactor II, whereas the activity of the sulfated galactan was independent of this cofactor.

Heparin is used extensively for the prevention of venous thrombosis and the treatment of other thromboembolic disorders due to its inhibition of thrombin and other enzymes in the coagulation system. To overcome the obvious potential side-effect of bleeding, researchers have investigated means of reducing the anticoagulant activities of heparin while enhancing its anti-thrombotic activities including chemical modification and fractionation of native heparin to lower molecular forms [132–134]. Nevertheless, the development of antithrombotic algal polysaccharides would be advantageous since their use would avoid the potential for contamination with prions or viruses in commercial heparins, which are obtained from pig and bovine intestine. Moreover, with more specific activities and/or targets, the algal sulfated polysaccharides could find applications complementary to heparin [132]. To this end, one of the approaches has been to develop low molecular weight (LMW) fucoidans [121,135]. For example, a LMW fraction of approximately 8,000 derived from the fucoidan of *A. nodosum* reduced mean thrombus weight by 80% *vs.* control saline injection in a rabbit model of

venous thrombosis [135]. This LMW fucoidan and related derivatives [136] are promising since they show lower effects in coagulation tests when compared to the commercial LMW heparin, dalterparin (Fragmen®, Pfizer Inc.). More recently, Rocha and colleagues reported that a sulfated galactofucan from the brown seaweed *Spatoglossum schoederi* has potent antithrombotic activity in a rat model of venous thrombosis [69]. Unlike heparin, which produces a rapid but transient antithrombotic effect, the *in vivo* action of this sulfated galactofucan progressed slowly, showing maximal effectiveness about eight hours post injection. When tested *in vitro* using endothelial cells, it was discovered that the galactofucan stimulates the production of heparan sulfate leading to the hypothesis that its delayed action *in vivo* is tied to the need for an accumulation of the heparan sulfate on blood vessel surfaces. Despite its high sulfation level, the galactofucan lacks significant anticoagulation activity, making it an ideal candidate as an antithrombotic agent [69].

4.2. Antiviral Activity

The ability of sulfated polysaccharides from seaweeds to inhibit the replication of enveloped viruses including herpes simplex virus (HSV), human immunodeficiency virus (HIV), human cytomegalovirus, dengue virus and respiratory syncytial virus is well established [137–140]. The original observations on antiviral activities of seaweed constituents go back more than 50 years to the observation that seaweed extracts protected chicken embryos against influenza B and mumps [141].

It was discovered a little later, somewhat serendipitously, that heparin inhibited HSV in leukocyte cultures [142], an effect hypothesized to be due to electrostatic interference with viral attachment to the cell surfaces. This spurred research into the antiviral effects of various polyanionic substances including sulfated polysaccharides from a number of seaweed species. Interest around the antiviral effects of algal polysaccharides has grown substantially in recent years with the mounting evidence that their effects on viral replication occurs by a number of mechanisms that involve specific structural qualities of the polysaccharides and not simply through non-specific interactions [137,143]. We will discuss some of the more recent findings regarding the structural requirements of algal polysaccharides for antiviral activity and the underlying modes of action.

A fucan from *Cladosiphon okamuranus* composed of glucuronic acid and sulfated fucose units potently inhibited infection of BHK-21 cells with dengue virus type 2 (DENV-2), but showed little effect on the other three serotypes of the virus [144]. Sulfation of the fucan was necessary for this activity; surprisingly, carboxyl-reduction of the glucuronic acids to glucose units also abolished the fucans' antiviral properties. Analysis of the structure of the envelope glycoproteins from the four serotypes of dengue virus suggested that arginine-323 in DENV-2, which is proximal to the putative heparin binding site, was critical for the interaction with the fucan.

Similarly, Talarico and colleagues reported that two sulfated polysaccharides from red seaweeds, a carrageenan from *Gymnogongrus griffithsiae* and a galactan from *Cryptonemia crenulata*, inhibited DENV-2 multiplication in Vero cells [145–147]. Their effects were less potent against DENV-3 and DENV-4 and were completely inactive against DENV-1 infection [147]. These polysaccharides were shown to interfere with both DENV-2 adsorption and internalization into the cells and were only effective if added together with the virus or shortly after infection. For example, no inhibition of virus

multiplication occurred when the normal viral entry process was bypassed by DENV-2 RNA transfection into the cells [145,146].

Recently, light has also been shed on the antiviral activity of marine sulfated polysaccharides against HSV types 1 and 2 (HSV-1, HSV-2) [67,143,148,149]. Sulfated xylomannans from the red seaweed *Sebdenia polydactyla* inhibited the propagation of HSV-1 in Vero cells [148]. The activity was abolished by desulfation of the xylomannan and, conversely, oversulfated derivatives exhibited enhanced potency. Mohsen reported that sulfated polysaccharide fractions isolated from *Sargassum latifolium* inhibited HSV-1 in the plaque assay with the most effective fraction having greater sulfate ester content and molecular weight compared to the other fractions studied [150]. It has been generally observed that antiviral activity of sulfated polysaccharides increases with their molecular weight [137]. Representative polysaccharides from brown and red seaweeds differing in structure (galactans, fucans and galactofucans), sulfation level and molecular weight were shown to inhibit HSV-1 and HSV-2 infection [149] reinforcing the view that, as with other activities, antiviral activity of sulfated polysaccharides is due to a complex interplay of structural features including sulfation level, distribution of sulfate groups along the polysaccharide backbone, molecular weight, sugar residue composition, and stereochemistry [139,143]. A subset of these sulfated polysaccharides also showed an ability to inactivate HSV-2 directly through incubation with the virus. This virucidal activity has significance since it is associated with augmentation of antiviral activity *in vivo* [151].

4.3. Immuno-Inflammatory Activity

Sulfated polysaccharides, including those from algae, have been shown to possess immunomodulatory activities that may be of potential application in stimulating the immune response or in controlling immune cell activity to mitigate associated negative effects such as inflammation [152]. Sulfated polysaccharides may affect multiple targets in the immune and inflammatory systems that can have impact on disease progression and outcome including tumor progression and metastasis [153].

One of the interests in algal sulfated polysaccharides as anti-inflammatory agents is the growing body of evidence illustrating their ability to interfere with the migration of leukocytes to sites of inflammation. For example, in a rabbit model of bacterial meningitis, leukocyte rolling was markedly reduced by intravenous infusion of fucoidan [154]. Similarly, intravenous addition of fucoidan reduced, in a dose-dependent manner, leukocyte recruitment to peritoneum in a rat model of peritoneal inflammation [155]. These effects were ascribed to the binding of fucoidan to L- and P selectins, cell adhesion molecules essential in the recruitment process. Both of these studies used the fucoidan from Sigma-Aldrich Chemical Co (St. Louis, MO, U.S.) that is sourced from *F. vesiculosus*. Fucans from other seaweeds including *Laminaria spp*., *Fucus spp*., *A. nodosum*, and *C. okamuranus* also inhibit leukocyte recruitment to the abdominal cavity during acute peritonitis in rats [122]. In addition to impairing the action of selectins, algal sulfated polysaccharides inhibit tissue degradative enzymes such as heparanase and elastases that are involved in the breakdown of basement membrane integrity during inflammation [156,157].

One of the major and potentially promising activities is the potent inhibitory effect of sulfated fucans on human complement activation. The original observations showed that fucoidan fractions

from *A. nodosum* potently inhibit both the classical and alternative pathways in human serum [158]. Tissot and colleagues have extensively studied this activity [159–162]. It was determined that low molecular weight fucoidan fractions bind to the C1q subunit of the C1 complex that triggers complement through recognition and binding of immune complexes [162]. The binding of fucoidan appears to interfere with the ability of C1q to fully trigger C1 activation [160]. Fucoidan also binds C4, thereby preventing its breakdown and generation of its cleavage product C4b, the latter being required for the formation of C3 convertase and the propagation of complement [162]. Furthermore, it was found that fucoidan binds C1q globular heads and may interfere with C1q recognition of IgG [161]. Recently, using NMR, it was found that branched fucoidan oligosaccharides are better at inhibiting complement compared to linear structures [159].

The interaction of algal sulfated polysaccharides with the complement system suggests that they may have utility in influencing innate immunity to reduce the pro-inflammatory state or other detrimental conditions such as allergic reactions arising during the innate immune response. In addition, there is a growing body of evidence that algal polysaccharides can regulate the innate immune response directly by binding to pattern recognition receptors (PRRs) such as the mannose receptor and toll-like receptors on phagocytic cells including macrophages [152]. For example, λ-carrageenan stimulated mouse T cell cultures in a toll-like receptor-4 (TLR4) dependent manner [163] generating a T helper 1 (Th1) patterned cytokine response. However, splenocytes prepared from TLR4-deficient mice still retained some ability to produce interferon-γ in response to λ-carrageenan suggesting that PRRs other than TLR4 were also elicited. In mice immunized with ovalbumin to produce an allergic reaction, oral dosing with λ-carrageenan lead to a reduction in ovalbumin-specific IgE and serum histamine release, suggesting that λ-carrageenan might be used to ameliorate allergic reactions. Similar results were reported for mekabu fucoidan from *U. pinnatifida* [164].

Direct stimulatory effects of algal polysaccharides on immune cells results in production of nitric oxide through induction of inducible nitric oxide synthase (iNOS) and a pro-inflammatory cytokine/chemokine profile [165]. Depending on the situation, the interaction of sulfated polysaccharides with other effectors may result in reduced inflammation. For example, fucoidan from *F. vesiculosus* induced iNOS in RAW264.7 macrophage cells leading to enhanced production of nitric oxide [166,167]. Yet, in the presence of lipopolysaccharide (LPS), the fucoidan impaired LPS-induced expression of iNOS and nitric oxide production [167]. Similarly, fucoidan suppresses interferon gamma-induced iNOS expression in macrophage and glial cell types [168].

These and other reports of algal sulfated polysaccharides directly stimulating the innate immune system [165,168–170] suggests that they may find therapeutic use in opposing T helper 2 (Th2)-based pathologies such as autoimmune disorders and allergy. Additionally, there is evidence that algal sulfated polysaccharides including fucoidans and carrageenans increase the cytotoxicity of natural killer cells, lymphocytes and macrophages against tumors [169,171].

The structural requirements for this immunostimulatory activity of algal sulfated polysaccharides have not been greatly studied. One report, by Leiro and colleagues, has shown greatly diminished immunostimulatory activity of ulvan-like polysaccharides from *U. rigida* when they were desulfated [165].

4.4. Antioxidant Activities

Algal sulfated polysaccharides, until recently, were largely ignored as sources of antioxidant activity. Studies over the last several years reveal that sulfated polysaccharides from a number of seaweeds have appreciable antioxidant capability [95,172–177]. For example, fucans from *F. vesiculosus* exhibited considerable ferric reducing/antioxidant power [172] and superoxide radical scavenging ability [173]. Fucan fractions from *L. japonica* also showed significant antioxidant capabilities in superoxide radical and hydroxyl radical scavenging assays [174,176,177]. Superoxide radical scavenging activity correlated positively with the sulfate content of the polysaccharide fractions [173,176]. Antioxidant properties of carrageenans [173] and ulvans [94] also appeared related to sulfate content. In the latter study, high sulfate content derivatives of ulvan showed improved antioxidant activities [94]. Interestingly, metal chelating, free radical and hydroxyl radical scavenging activities of fucan fractions appear to relate to their ratio of sulfate content/fucose [176].

4.5. Antilipidemic Effects

Algal sulfated polysaccharides exert lipid-lowering and other beneficial properties in hyperlipidemic animal models [178–180]. An extract from *F. vesiculosus*, in a dose-dependent manner, effectively reduced the elevation in serum triglyceride and total cholesterol levels in triton-induced-hyperlipemic rats. In rats fed a high cholesterol diet for 21 days, supplementation of the diet with ulvan from *U. pertusa* led to reductions in serum total cholesterol and LDL-cholesterol with no significant alteration in serum triglycerides [180]. The effects of ulvan were modified when it was degraded into lower molecular weight fractions. Ulvan derivatives of lower molecular weight and intrinsic viscosity did not reduce serum cholesterol but did normalize the hypertriglyceridemia of these animals and raised HDL-cholesterol. The underlying mechanisms of these actions are unclear but it does not appear to involve bile acid sequestration since ulvan and its lower molecular weight derivatives increased bile excretion to a similar extent.

Recently, it was reported that fucoidan from *L. japonica* reduced serum total and LDL-cholesterol and triglycerides and raised HDL-cholesterol in a hyperlipidemic rat model [179]. The treatment also increased the activities of lipoprotein lipase (LPL), hepatic lipase (HL) and lecithin cholesterol acyltranferase (LCAT) in serum. These changes in enzyme activities could be the direct result of fucoidan treatment or an indirect effect associated with improvement in lipid profile. Certainly fucoidan and other algal sulfated polysaccharides may influence LPL and HL through interaction with well-characterized heparin-binding sites on these enzymes. Consistent with this is the observation that fucoidan from *F. vesiculosus* releases LPL from cell surface binding sites and stabilizes LPL activity in culture medium [181].

Algal sulfated polysaccharides are showing promising effects in addressing the hyperlipidemia associated with certain drug toxicities. Fucans from *S. polycystum* were shown to have significant preventive effects on the elevation of cholesterol and triglycerides in serum and liver tissue resulting from acetaminophen-induced toxic hepatitis [182]. Treatment also partially reversed the reduction in hepatic LCAT and HL and improved overall histological appearance of the liver. Similarly, a sulfated polysaccharide from *S. wightii* reduced hyperlipidemia and normalized LPL and LCAT in plasma in

cyclosporine A-induced nephrotoxicity [183]. The excretion of urea, uric acid, and creatinine were normalized by the sulfated polysaccharide treatment. In addition, the susceptibility of LDL to oxidation was reduced, suggesting that the antioxidant activity of the sulfated polysaccharide was also playing a role and may contribute to its renoprotective activity.

5. Future Perspectives

Algal sulfated polysaccharides are a source of numerous biological activities that may find therapeutic benefit. They are structurally diverse and heterogeneous, which makes studies of their structures challenging, and may also have hindered their development as therapeutic agents to date. The production of a standardized commercial product based on algal sulfated polysaccharide constituents will be a challenge since their structural and pharmacological features may vary depending on species and on location and time of harvest. For example, Bourgougnon and colleagues reported that there was a significant annual variation in the composition and the *in vitro* anti-HIV-1 activity of a water-soluble sulfated glucuronogalactan from *Schizymenia dubyi* [184]. Another issue to the therapeutic use of algal polysaccharides is their potentially low bioavailability given their often high molecular weights. It is likely, based on observations with heparin [185], that algal sulfated polysaccharides will display some, albeit low, degree of oral bioavailability. A recent pilot study in humans reported that fucoidan was ineffective as an oral anticoagulant agent [186], which underscores the issue. It also emphasizes the importance of understanding the structural requirements for biological activity and whether low molecular weight derivatives, which are potentially more bioavailable, remain active. For some applications, low bioavailability may not be a concern. First, some of the hypolipidemic effects of seaweed sulfated polysaccharides arise through effects on bile acid sequestration in the intestinal lumen. Second, for some immunomodulatory activities, the site of activation of the immune system may also be within the intestinal lumen (e.g., at Peyer's patches) as has been hypothesized for immunomodulatory effects of polysaccharide constituents from *Chlorella pyrenoidosa* [187]. Finally, algal sulfated polysaccharides are already used topically in cosmetics and there is significant interest in further development for cosmetics and cosmeceuticals products [188].

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References

1. Kusaykin, M.; Bakunina, I.; Sova, V.; Ermakova, S.; Kuznetsova, T.; Besednova, N.; Zaporozhets, T.; Zvyagintseva, T. Structure, biological activity, and enzymatic transformation of fucoidans from the brown seaweeds. *Biotechnol. J.* **2008**, *3*, 904–915.

2. Lahaye, M. Developments on gelling algal galactans, their structure and physico-chemistry. *J. Appl. Phycol.* **2001**, *13*, 173–184.
3. Lahaye, M.; Robic, A. Structure and functional properties of ulvan, a polysaccharide from green seaweeds. *Biomacromolecules* **2007**, *8*, 1765–1774.
4. Li, B.; Lu, F.; Wei, X.; Zhao, R. Fucoidan: structure and bioactivity. *Molecules* **2008**, *13*, 1671–1695.
5. Pomin, V.H. Structural and functional insights into sulfated galactans: A systematic review. *Glycoconj. J.* **2010**, *27*, 1–12.
6. Pomin, V.H.; Mourao, P.A.S. Structure, biology, evolution, and medical importance of sulfated fucans and galactans. *Glycobiology* **2008**, *18*, 1016–1027.
7. Usov, A.I.; Bilan, I. Fucoidans-Sulfated polysaccharides of brown algae. *Russ. Chem. Rev.* **2009**, *78*, 785–799.
8. Wijesekara, I.; Pangestuti, R.; Kim, S.-K. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydr. Polym.* **2010**, in press.
9. Berteau, O.; Mulloy, B. Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology* **2003**, *13*, 29R–40R.
10. Knutsen, S.H.; Myslabodski, D.E.; Larsen, B.; Usov, A.I. A modified system of nomenclature for red algal galactans. *Bot. Mar.* **1994**, *37*, 163–170.
11. Anderson, N.S.; Dolan, T.C.S.; Rees, D.A. Carrageenans. Part VII. Polysaccharides from *Eucheuma spinosum* and *Eucheuma cottonii*. The covalent structure of L-carrageenan. *J. Chem. Soc. Perkin Trans. I* **1973**, 2173–2176.
12. Estevez, J.M.; Ciancia, M.; Cerezo, A.S. The system of low-molecular-weight carrageenans and agaroids from the room-temperature-extracted fraction of *Kappaphycus alvarezii*. *Carbohydr. Res.* **2000**, *325*, 287–299.
13. Funami, T.; Hiroe, M.; Noda, S.; Asai, I.; Ikeda, S.; Nishinari, K. Influence of molecular structure imaged with atomic force microscopy on the rheological behavior of carrageenan aqueous systems in the presence or absence of cations. *Food Hydrocolloids* **2007**, *21*, 617–629.
14. Zhou, G.; Sheng, W.; Yao, W.; Wang, C. Effect of low molecular [lambda]-carrageenan from *Chondrus ocellatus* on antitumor H-22 activity of 5-Fu. *Pharmacol. Res.* **2006**, *53*, 129–134.
15. Doyle, J.P.; Giannouli, P.; Rudolph, B.; Morris, E.R. Preparation, authentication, rheology and conformation of theta carrageenan. *Carbohydr. Polym.* **2010**, *80*, 648–654.
16. Yang, B.; Yu, G.; Zhao, X.; Ren, W.; Jiao, G.; Fang, L.; Wang, Y.; Du, G.; Tiller, C.; Girouard, G.; Barrow, C.J.; Ewart, H.S.; Zhang, J. Structural characterisation and bioactivities of hybrid carrageenan-like sulphated galactan from red alga *Furcellaria lumbricalis*. *Food Chem.* **2011**, *124*, 50–57.
17. van de Velde, F.; Antipova, A.S.; Rollema, H.S.; Burova, T.V.; Grinberg, N.V.; Pereira, L.; Gilsenan, P.M.; Tromp, R.H.; Rudolph, B.; Grinberg, V.Y. The structure of kappa/iota-hybrid carrageenans II. Coil-helix transition as a function of chain composition. *Carbohydr. Res.* **2005**, *340*, 1113–1129.

18. Chopin, T.; Kerin, B.F.; Mazerolle, R. Phycocolloid chemistry as a taxonomic indicator of phylogeny in the Gigartinales, Rhodophyceae: A review and current developments using Fourier transform infrared diffuse reflectance spectroscopy. *Pharmacol. Res.* **1999**, *47*, 167–188.
19. Hilliou, L.; Larotonda, F.D.S.; Abreu, P.; Ramos, A.M.; Sereno, A.M.; Gonealves, M.P. Effect of extraction parameters on the chemical structure and gel properties of kappa/iota-hybrid carrageenans obtained from *Mastocarpus stellatus*. *Biomol. Eng.* **2006**, *23*, 201–208.
20. Hilliou, L.; Wilhelm, M.; Yamanoi, M.; Gonclves, M.P. Structural and mechanical characterization of [kappa]/[iota]-hybrid carrageenan gels in potassium salt using Fourier Transform rheology. *Food Hydrocolloids* **2009**, *23*, 2322–2330.
21. Jouanneau, D.; Guibet, M.; Boulenguer, P.; Mazoyer, J.; Smietana, M.; Helbert, W. New insights into the structure of hybrid [kappa]-/[mu]-carrageenan and its alkaline conversion. *Food Hydrocolloids* **2010**, *24*, 452–461.
22. van de Velde, F. Structure and function of hybrid carrageenans. *Food Hydrocolloids* **2008**, *22*, 727–734.
23. Yu, G.; Hu, Y.; Yang, B.; Zhao, X.; Wang, P.; Ji, G.; Wu, J.; Guan, H. Extraction, isolation and structural characterization of polysaccharides from a red alga *Gloiopeltis furcata*. *J. Ocean Univ. China Nat. Sci.* **2010**, *9*, 193–197.
24. Morrice, L.M.; McLean, M.W.; Long, W.F.; Williamson, F.B. Porphyran primary structure. *Hydrobiologia* **1984**, *116–117*, 572–575.
25. Zhang, Q.; Qi, H.; Zhao, T.; Deslandes, E.; Ismaeli, N.M.; Molloy, F.; Critchley, A.T. Chemical characteristics of a polysaccharide from *Porphyra capensis* (Rhodophyta). *Carbohydr. Res.* **2005**, *340*, 2447–2450.
26. Zhang, Z.; Zhang, Q.; Wang, J.; Zhang, H.; Niu, X.; Li, P. Preparation of the different derivatives of the low-molecular-weight porphyran from *Porphyra haitanensis* and their antioxidant activities *in vitro*. *Int. J. Biol. Macromol.* **2009**, *45*, 22–26.
27. Zhang, Q.; Li, N.; Liu, X.; Zhao, Z.; Li, Z.; Xu, Z. The structure of a sulfated galactan from *Porphyra haitanensis* and its *in vivo* antioxidant activity. *Carbohydr. Res.* **2004**, *339*, 105–111.
28. Miller, I.J.; Furneaux, R.H. The structural determination of the agaroid polysaccharides from four New Zealand algae in the order Ceramiales by means of ^{13}C-NMR Spectroscopy. *Bot. Mar.* **1997**, *40*, 333–340.
29. Prado, H.J.; Ciancia, M.; Matulewicz, M.C. Agarans from the red seaweed *Polysiphonia nigrescens* (Rhodomelaceae, Ceramiales). *Carbohydr. Res.* **2008**, *343*, 711–718.
30. Miller, I.J. Evaluation of the structures of polysaccharides from two New Zealand members of the Ceramiaceae. *Bot. Mar.* **2003**, *46*, 378–385.
31. Gonçalves, A.G.; Ducatti, D.R.B.; Duarte, M.E.R.; Noseda, M.D. Sulfated and pyruvylated disaccharide alditols obtained from a red seaweed galactan: ESIMS and NMR approaches. *Carbohydr. Res.* **2002**, *337*, 2443–2453.
32. Duarte, M.E.R.; Cauduro, J.P.; Noseda, D.G.; Noseda, M.D.; Gonçalves, A.G.; Pujol, C.A.; Damonte, E.B.; Cerezo, A.S. The structure of the agaran sulfate from *Acanthophora spicifera* (Rhodomelaceae, Ceramiales) and its antiviral activity. Relation between structure and antiviral activity in agarans. *Carbohydr. Res.* **2004**, *339*, 335–347.

33. Stortz, C.A.; Cerezo, A.S. Novel findings in carrageenans, agaroids and "hybrids" red seaweed galactans. *Curr. Top. Phytochem.* **2000**, *4*, 121–134.
34. Cases, M.R.; Stortz, C.A.; Cerezo, A.S. Structure of the 'corallinans'-sulfated xylogalactans from *Corallina officinalis*. *Int. J. Biol. Macromol.* **1994**, *16*, 93–97.
35. Navarro, D.A.; Ricci, A.M.; Rodr ́guez, M.C.; Stortz, C.A. Xylogalactans from *Lithothamnion heterocladum*; a crustose member of the Corallinales (Rhodophyta). *Carbohydr. Polym., In Press*.
36. Navarro, D.A.; Stortz, C.A. The system of xylogalactans from the red seaweed *Jania rubens* (Corallinales, Rhodophyta). *Carbohydr. Res.* **2008**, *343*, 2613–2622.
37. Martone, P.T.; Navarro, D.A.; Stortz, C.A.; Estevez, J.M. Differences in polysaccharide structure between calcified and uncalcified segments in the coralline *Calliarthron cheilosporioides* (Corallinales, Rhodaphyta). *J. Phycol.* **2010**, *46*, 507–515.
38. Lim, B.L.; Ryu, I.H. Purification, structural characterization, and antioxidant activity of antioxidant substance from the red seaweed *Gloiopeltis tenax*. *J. Med. Food* **2009**, *12*, 442–451.
39. Mandal, P.; Pujol, C.A.; Carlucci, M.J.; Chattopadhyay, K.; Damonte, E.B.; Ray, B. Anti-herpetic activity of a sulfated xylomannan from *Scinaia hatei*. *Phytochemistry* **2008**, *69*, 2193–2199.
40. Lahaye, M.; Ray, B. Cell-wall polysaccharides from the marine green alga *Ulva rigida* (Ulvales, Chlorophyta)-NMR analysis of ulvan oligosaccharides. *Carbohydr. Res.* **1996**, *283*, 161–173.
41. Percival, E.; McDowell, R.H. *Chemistry and Enzymology of Marine Algal Polysaccharides*; Academic Press: New York, NY, USA, 1967; p. 219.
42. Lahaye, M.; Brunel, M.; Bonnin, E. Fine chemical structure analysis of oligosaccharides produced by an ulvan-lyase degradation of the water-soluble cell-wall polysaccharides from *Ulva* sp. (Ulvales, Chlorophyta). *Carbohydr. Res.* **1997**, *304*, 325–333.
43. Love, J.; Percival, E. The polysaccharides of the green seaweed *Codium fragile*. Part II. The water-soluble sulphated polysaccharides. *J. Chem. Soc.* **1964**, 3338–3345.
44. Matsubara, K.; Matsuura, Y.; Bacic, A.; Liao, M.L.; Hori, K.; Miyazawa, K. Anticoagulant properties of a sulfated galactan preparation from a marine green alga, *Codium cylindricum*. *Int. J. Biol. Macromol.* **2001**, *28*, 395–399.
45. Farias, E.H.C.; Pomin, V.H.; Valente, A.P.; Nader, H.B.; Rocha, H.A.O.; Mourao, P.A.S. A preponderantly 4-sulfated, 3-linked galactan from the green alga *Codium isthmocladum*. *Glycobiology* **2008**, *18*, 250–259.
46. Matsubara, K.; Matsuura, Y.; Bacic, A.; Liao, M.L.; Hori, K.; Miyazawa, K. Anticoagulant properties of a sulfated galactan preparation from a marine green alga, *Codium cylindricum*. *Int. J. Biol. Macromol.* **2001**, *28*, 395–399.
47. Bilan, M.I.; Vinogradova, E.V.; Shashkov, A.S.; Usov, A.I. Structure of a highly pyruvylated galactan sulfate from the Pacific green alga *Codium yezoense* (Bryopsidales, Chlorophyta). *Carbohydr. Res.* **2007**, *342*, 586–596.
48. Shevchenko, N.; Burtseva, Y.; Zvyagintseva, T.; Makar′eva, T.; Sergeeva, O.; Zakharenko, A.; Isakov, V.; Thi Linh, N.; Xuan Hoa, N.; Minh Ly, B.; Van Huyen, P. Polysaccharides and sterols from green algae *Caulerpa lentillifera* and *C. sertularioides*. *Chem. Nat. Compd.* **2009**, *45*, 1–5.
49. Mao, W.; Zang, X.; Li, Y.; Zhang, H. Sulfated polysaccharides from marine green algae *Ulva conglobata* and their anticoagulant activity. *J. Appl. Phycol.* **2006**, *18*, 9–14.

50. Ghosh, P.; Adhikari, U.; Ghosal, P.K.; Pujol, C.A.; Carlucci, M.J.; Damonte, E.B.; Ray, B. *In vitro* anti-herpetic activity of sulfated polysaccharide fractions from *Caulerpa racemosa*. *Phytochemistry* **2004**, *65*, 3151–3157.
51. Lee, J.-B.; Koizumi, S.; Hayashi, K.; Hayashi, T. Structure of rhamnan sulfate from the green alga *Monostroma nitidum* and its anti-herpetic effect. *Carbohydr. Polym.* **2010**, in press.
52. Ray, B. Polysaccharides from *Enteromorpha compressa*: Isolation, purification and structural features. *Carbohydr. Polym.* **2006**, *66*, 408–416.
53. Harada, N.; Maeda, M. Chemical structure of antithrombin-active Rhamnan sulfate from *Monostrom nitidum*. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1647–1652.
54. Kylin, H. biochemistry of sea algae. *Phys. Chem.* **1913**, *83*, 171–197.
55. Conchie, J.; Percival, E.G.V. Fucoidin. Part II. The hydrolysis of a methylated fucoidin prepared from *Fucus vesiculosus*. *J. Chem. Soc.* **1950**, 827–832.
56. Patankar, M.S.; Oehninger, S.; Barnett, T.; Williams, R.L.; Clark, G.F. A revised structure for fucoidan may explain some of its biological activities. *J. Biol. Chem.* **1993**, *268*, 21770–21776.
57. Chevolot, L.; Mulloy, B.; Ratiskol, J.; Foucault, A.; Colliec-Jouault, S. A disaccharide repeat unit is the major structure in fucoidans from two species of brown algae. *Carbohydr. Res.* **2001**, *330*, 529–535.
58. Ushakova, N.A.; Morozevich, G.E.; Ustyuzhanina, N.E.; Bilan, M.I.; Usov, A.I.; Nifantiev, N.E.; Preobrazhenskaya, M.E. Anticoagulant activity of fucoidans from brown algae. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry* **2009**, *3*, 77–83.
59. Chattopadhyay, N.; Ghosh, T.; Sinha, S.; Chattopadhyay, K.; Karmakar, P.; Ray, B. Polysaccharides from *Turbinaria conoides*: Structural features and antioxidant capacity. *Food Chem.* **2010**, *118*, 823–829.
60. Bilan, M.I.; Zakharova, A.N.; Grachev, A.A.; Shashkov, A.S.; Nifant'ev, N.E.; Usov, A.I. Polysaccharides of algae: 60. Fucoidan from the Pacific brown alga *Analipus japonicus* (Harv.) Winne (Ectocarpales, Scytosiphonaceae). *Bioorg. Khim.* **2007**, *33*, 44–53.
61. Marais, M.F.; Joseleau, J.P. A fucoidan fraction from *Ascophyllum nodosum*. *Carbohydr. Res.* **2001**, *336*, 155–159.
62. Chizhov, A.O.; Dell, A.; Morris, H.R.; Haslam, S.M.; McDowell, R.A.; Shashkov, A.S.; Nifant'ev, N.E.; Khatuntseva, E.A.; Usov, A.I. A study of fucoidan from the brown seaweed *Chorda filum*. *Carbohydr. Res.* **1999**, *320*, 108–119.
63. Bilan, M.I.; Grachev, A.A.; Ustuzhanina, N.E.; Shashkov, A.S.; Nifantiev, N.E.; Usov, A.I. A highly regular fraction of a fucoidan from the brown seaweed *Fucus distichus* L. *Carbohydr. Res.* **2004**, *339*, 511–517.
64. Bilan, M.I.; Grachev, A.A.; Ustuzhanina, N.E.; Shashkov, A.S.; Nifantiev, N.E.; Usov, A.I. Structure of a fucoidan from the brown seaweed *Fucus evanescens* C.Ag. *Carbohydr. Res.* **2002**, *337*, 719–730.
65. Bilan, M.I.; Grachev, A.A.; Shashkov, A.S.; Nifantiev, N.E.; Usov, A.I. Structure of a fucoidan from the brown seaweed *Fucus serratus* L. *Carbohydr. Res.* **2006**, *341*, 238–245.
66. Usov, A.I.; Smirnova, G.P.; Bilan, M.I.; Shashkov, A.S. Polysaccharides of algae: 53. Brown alga *Laminaria saccharina* (L.) Lam. as a source of fucoidan. *Bioorg. Khim.* **1998**, *24*, 382–389.

67. Adhikari, U.; Mateu, C.G.; Chattopadhyay, K.; Pujol, C.A.; Damonte, E.B.; Ray, B. Structure and antiviral activity of sulfated fucans from Stoechospermum marginatum. *Phytochemistry* **2006**, *67*, 2474–2482.
68. Nagaoka, M.; Shibata, H.; Kimura-Takagi, I.; Hashimoto, S.; Kimura, K.; Makino, T.; Aiyama, R.; Ueyama, S.; Yokokura, T. Structural study of fucoidan from *Cladosiphon okamuranus* TOKIDA. *Glycoconj. J.* **1999**, *16*, 19–26.
69. Rocha, H.A.O.; Moraes, F.A.; Trindade, E.S.; Franco, C.R.C.; Torquato, R.J.S.; Veiga, S.S.; Valente, A.P.; Mourao, P.A.S.; Leite, E.L.; Nader, H.B.; Dietrich, C.P. Structural and hemostatic activities of a sulfated galactofucan from the brown alga *Spagtoglossum schroederi*: An ideal antithrombotic agent? *J. Biol. Chem.* **2005**, *280*, 41278–41288.
70. Nishino, T.; Yokoyama, G.; Dobashi, K.; Fujihara, M.; Nagumo, T. Isolation, purification, and characterization of fucose-containing sulfated polysaccharides from the brown seaweed *Ecklonia kurome* and their blood-anticoagulant activities. *Carbohydr. Res.* **1989**, *186*, 119–129.
71. Duarte, M.E.R.; Cardoso, M.A.; Noseda, M.D.; Cerezo, A.S. Structural studies on fucoidans from the brown seaweed *Sargassum stenophyllum*. *Carbohydr. Res.* **2001**, *333*, 281–293.
72. Li, B.; Wei, X.J.; Sun, J.L.; Xu, S.Y. Structural investigation of a fucoidan containing a fucose-free core from the brown seaweed, *Hizikia fusiforme*. *Carbohydr. Res.* **2006**, *341*, 1135–1146.
73. Leite, E.L.; Medeiros, M.G.L.; Rocha, H.A.O.; Farias, G.G.M.; da Silva, L.F.; Chavante, S.F.; de Abreu, L.D.; Dietrich, C.P.; Nader, H.B. Structure and pharmacological activities of a sulfated xylofucoglucuronan from the alga *Spatoglossum schroderi*. *Plant Sci.* **1998**, *132*, 215–228.
74. Ponce, N.M.A.; Pujol, C.A.; Damonte, E.B.; Flores, M.L.; Stortz, C.A. Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antiviral activity and structural studies. *Carbohydr. Res.* **2003**, *338*, 153–165.
75. Teruya, T.; Tatemoto, H.; Konishi, T.; Tako, M. Structural characteristics and *in vitro* macrophage activation of acetyl fucoidan from *Cladosiphon okamuranus*. *Glycoconj. J.* **2009**, *26*, 1019–1028.
76. Bjorndal, H.; Hellerqvist, C.G.; Lindberg, B.; Svensson, S. Gas-liquid chromatography and mass spectrometry in methylation analysis of polysaccharides. *Angew. Chem. Int. Ed. Engl.* **1970**, *9*, 610–619.
77. Bilan, M.I.; Vinogradova, E.V.; Tsvetkova, E.A.; Grachev, A.A.; Shashkov, A.S.; Nifantiev, N.E.; Usov, A.I. A sulfated glucuronofucan containing both fucofuranose and fucopyranose residues from the brown alga *Chordaria flagelliformis*. *Carbohydr. Res.* **2008**, *343*, 2605–2612.
78. Usov, A.I.; Adamyants, K.S.; Miroshnikova, L.I.; Shaposhnikova, A.A.; Kochetkov, N.K. Solvolytic desulphation of sulphated carbohydrates. *Carbohydr. Res.* **1971**, *18*, 336–338.
79. Kolender, A.A.; Matulewicz, M.C. Desulfation of sulfated galactans with chlorotrimethylsilane. Characterization of beta-carrageenan by 1H NMR spectroscopy. *Carbohydr. Res.* **2004**, *339*, 1619–1629.
80. Kantor, T.G.; Schubert, M. A method for the desulfation of Chondroitin Sulfate[1]. *J. Am. Chem. Soc.* **1957**, *79*, 152–153.
81. Takano, R.; Matsuo, M.; Kamei-Hayashi, K.; Hara, S.; Hirase, S. A novel regioselective desulfation method specific to carbohydrate 6-sulfate using silylating reagents. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 1577–1580.

82. Miller, I.J.; Blunt, J.W. Desulfation of algal galactans. *Carbohydr. Res.* **1998**, *309*, 39–43.
83. Kusaykin, M.I.; Chizhov, A.O.; Grachev, A.A.; Alekseeva, S.A.; Bakunina, I.Y.; Nedashkovskaya, O.I.; Sova, V.V.; Zvyagintseva, T.N. A comparative study of specificity of fucoidanases from marine microorganisms and invertebrates. *J. Appl. Phycol.* **2006**, *18*, 369–373.
84. Ciucanu, I.; Kerek, F. A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.* **1984**, *131*, 209–217.
85. Zibetti, R.G.M.; Duarte, M.E.R.; Noseda, M.D.; Colodi, F.G.; Ducatti, D.R.B.; Ferreira, L.G.; Cardoso, M.A.; Cerezo, A.S. Galactans from *Cryptonemia* species. Part II: Studies on the system of galactans of *Cryptonemia seminervis* (Halymeniales) and on the structure of major fractions. *Carbohydr. Res.* **2009**, *344*, 2364–2374.
86. Usov, A.I.; Yarotsky, S.V.; Shashkov, A.S. ^{13}C-NMR spectroscopy of red algal galactans. *Biopolymers* **1980**, *19*, 977–990.
87. Gonçalves, A.G.; Ducatti, D.R.B.; Paranha, R.G.; Eugênia, M.; Duarte, R.; Noseda, M.D. Positional isomers of sulfated oligosaccharides obtained from agarans and carrageenans: preparation and capillary electrophoresis separation. *Carbohydr. Res.* **2005**, *340*, 2123–2134.
88. Bilan, M.I.; Grachev, A.A.; Shashkov, A.S.; Kelly, M.; Sanderson, C.J.; Nifantiev, N.E.; Usov, A.I. Further studies on the composition and structure of a fucoidan preparation from the brown alga *Saccharina latissima*. *Carbohydr. Res.* **2010**, *345*, 2038–2047.
89. Daniel, R.; Chevolot, L.; Carrascal, M.; Tissot, B.; Mourao, P.A.S.; Abian, J. Electrospray ionization mass spectrometry of oligosaccharides derived from fucoidan of *Ascophyllum nodosum*. *Carbohydr. Res.* **2007**, *342*, 826–834.
90. Fatema, M.K.; Nonami, H.; Ducatti, D.R.B.; Gonçalves, A.G.; Duarte, M.E.R.; Noseda, M.D.; Cerezo, A.S.; Erra-Balsells, R.; Matulewicz, M.C. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis of oligosaccharides and oligosaccharide alditols obtained by hydrolysis of agaroses and carrageenans, two important types of red seaweed polysaccharides. *Carbohydr. Res.* **2010**, *345*, 275–283.
91. Goncalves, A.G.; Ducatti, D.R.; Grindley, T.B.; Duarte, M.E.; Noseda, M.D. ESI-MS differential fragmentation of positional isomers of sulfated oligosaccharides derived from carrageenans and agarans. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1404–1416.
92. Yang, B.; Yu, G.; Zhao, X.; Jiao, G.; Ren, S.; Chai, W. Mechanism of mild acid hydrolysis of galactan polysaccharides with highly ordered disaccharide repeats leading to a complete series of exclusively odd-numbered oligosaccharides. *FEBS J.* **2009**, *276*, 2125–2137.
93. Nishino, T.; Nagumo, T. Anticoagulant and antithrombin activities of oversulfated fucans. *Carbohydr. Res.* **1992**, *229*, 355–362.
94. Qi, H.; Zhang, Q.; Zhao, T.; Chen, R.; Zhang, H.; Niu, X.; Li, Z. Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) *in vitro*. *Int. J. Biol. Macromol.* **2005**, *37*, 195–199.
95. Qi, H.; Zhang, Q.; Zhao, T.; Hu, R.; Zhang, K.; Li, Z. In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2441–2445.
96. Bhattacharyya, S. Carrageenan-induced innate immune response is modified by enzymes that hydrolyze distinct galactosidic bonds. *J. Nutr. Biochem.* **2010**, *21*, 906–913.

97. Holtkamp, A.D.; Kelly, S.; Ulber, R.; Lang, S. Fucoidans and fucoidanases-focus on techniques for molecular structure elucidation and modification of marine polysaccharides. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 1–11.
98. Nardella, A.; Chaubet, F.; Boisson-Vidal, C.; Blondin, C.; Durand, P.; Jozefonvicz, J. Anticoagulant low molecular weight fucans produced by radical process and ion exchange chromatography of high molecular weight fucans extracted from the brown seaweed *Ascophyllum nodosum*. *Carbohydr. Res.* **1996**, *289*, 201–208.
99. Pomin, V.H.; Valente, A.P.; Pereira, M.S.; Mourao, P.A.S. Mild acid hydrolysis of sulfated fucans: A selective 2-desulfation reaction and an alternative approach for preparing tailored sulfated oligosaccharides. *Glycobiology* **2005**, *15*, 1376–1385.
100. Qiu, X.; Amarasekara, A.; Doctor, V. Effect of oversulfation on the chemical and biological properties of fucoidan. *Carbohydr. Polym.* **2006**, *63*, 224–228.
101. Tao, S.; Huina, T.; Jing, X.; Shuo, Z.; Xin, X. Degradation and antioxidant activity of kappa-carrageenans. *J. Appl. Phycol.* **2010**, *117*, 194–199.
102. Teruya, T.; Takeda, S.; Tamaki, Y.; Tako, M. Fucoidan isolated from *Laminaria angustata* var. longissima induced macrophage activation. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 1960–1962.
103. Michel, G.; Nyval-Collen, P.; Barbeyron, T.; Czjzek, M.; Helbert, W. Bioconversion of red seaweed galactans: A focus on bacterial agarases and carrageenases. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 23–33.
104. Barbeyron, T.; L'Haridon, S.; Michel, G.; Czjzek, M. Mariniflexile fucanivorans sp. nov., a marine member of the Flavobacteriaceae that degrades sulphated fucans from brown algae. *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 2107–2113.
105. Barbeyron, T.; Michel, G.; Potin, P.; Henrissat, B.; Kloareg, B. Iota-Carrageenases constitute a novel family of glycoside hydrolases, unrelated to that of kappa-carrageenases. *J. Biol. Chem.* **2000**, *275*, 35499–35505.
106. Burtseva, Y.V.; Kusaikin, M.I.; Sova, V.V.; Shevchenko, N.M.; Skobun, A.S.; Zvyagintseva, T.N. Distribution of fucoidan hydrolases and some glycosidases among marine invertebrates. *Russ. J. Mar. Biol.* **2000**, *26*, 453–456.
107. Guibet, M.; Colin, S.; Barbeyron, T.; Genicot, S.; Kloareg, B.; Michel, G.; Helbert, W. Degradation of lambda-carrageenan by *Pseudoalteromonas carrageenovora* lambda-carrageenase: A new family of glycoside hydrolases unrelated to kappa-and iota-carrageenases. *Biochem. J.* **2007**, *404*, 105–114.
108. Lemoine, M.; Collen, P.N.; Helbert, W. Physical state of iota-carrageenan modulates the mode of action of iota-carrageenase from *Pseudoalteromonas carrageenovora*. *Biochem. J.* **2009**, *419*, 545–553.
109. Colliec-Jouault, S.; Millet, J.; Helley, D.; Sinquin, C.; Fischer, A.M. Effect of low-molecular-weight fucoidan on experimental arterial thrombosis in the rabbit and rat. *J. Thromb. Haemost.* **2003**, *1*, 1114–1115.
110. Toyama, M.H.; Toyama, D.O.; Torres, V.M.; Pontes, G.C.; Farias, W.R.L.; Melo, F.R.; Oliveira, S.C.B.; Fagundes, F.H.R.; Diz Filho, E.B.S.; Cavada, B.S. Effects of Low Molecular Weight Sulfated Galactan Fragments From *Botryocladia occidentalis* on the Pharmacological and Enzymatic Activity of Spla2 From Crotalus *Durissus Cascavella*. *Protein J.* **2010**, 1–5.

111. Bondu, S.; Deslandes, E.; Fabre, M.S.; Berthou, C.; Yu, G. Carrageenan from *Solieria chordalis* (Gigartinales): Structural analysis and immunological activities of the low molecular weight fractions. *Carbohydr. Polym.* **2010**, *81*, 448–460.
112. Mou, H.; Xiaolu, J.; Huashi, G. A kappa-carrageenan derived oligosaccharide prepared by enzymatic degradation containing anti-tumor activity. *J. Appl. Phycol.* **2003**, *15*, 297–303.
113. Kitamura, K.; Matsuo, M.; Yasui, T. Enzymic degradation of fucoidan by fucoidanase from the hepatopancreas of *Patinopecten yessoensis*. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 490–494.
114. Klarzynski, O.; Descamps, V.; Plesse, B.; Yvin, J.C.; Kloareg, B.; Fritig, B. Sulfated fucan oligosaccharides elicit defense responses in tobacco and local and systemic resistance against tobacco mosaic virus. *Mol. Plant Microbe Interact.* **2003**, *16*, 115–122.
115. Kim, K.J.; Lee, O.H.; Lee, H.H.; Lee, B.Y. A 4-week repeated oral dose toxicity study of fucoidan from the Sporophyll of *Undaria pinnatifida* in Sprague-Dawley rats. *Toxicology* **2010**, *267*, 154–158.
116. Pereira, M.S.; Mulloy, B.; Mourao, P.A.S. Structure and anticoagulant activity of sulfated fucans. Comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. *J. Biol. Chem.* **1999**, *274*, 7656–7667.
117. Bernardi, G.; Springer, G.F. Properties of highly purified fucan. *J. Biol. Chem.* **1962**, *237*, 75–80.
118. Springer, G.F.; Wurzel, H.A.; McNeal, G.M., Jr.; Ansell, N.J.; Doughty, M.F. Isolation of anticoagulant fractions from crude fucoidin. *Proc. Soc. Exp. Biol. Med.* **1957**, *94*, 404–409.
119. Grauffel, V.; Kloareg, B.; Mabeau, S.; Durand, P.; Jozefonvicz, J. New natural polysaccharides with potent antithrombic activity: Fucans from brown algae. *Biomaterials* **1989**, *10*, 363–368.
120. Kuznetsova, T.A.; Besednova, N.N.; Mamaev, A.N.; Momot, A.P.; Shevchenko, N.M.; Zvyagintseva, T.N. Anticoagulant activity of fucoidan from brown algae *Fucus evanescens* of the Okhotsk Sea. *Bull. Exp. Biol. Med.* **2003**, *136*, 471–473.
121. Mauray, S.; Sternberg, C.; Theveniaux, J.; Millet, J.; Sinquin, C.; Tapon-Bretaudiere, J.; Fischer, A.M. Venous antithrombotic and anticoagulant activities of a fucoidan fraction. *Thromb. Haemost.* **1995**, *74*, 1280–1285.
122. Cumashi, A.; Ushakova, N.A.; Preobrazhenskaya, M.E.; D'Incecco, A.; Piccoli, A.; Totani, L.; Tinari, N.; Morozevich, G.E.; Berman, A.E.; Bilan, M.I.; Usov, A.I.; Ustyuzhanina, N.E.; Grachev, A.A.; Sanderson, C.J.; Kelly, M.; Rabinovich, G.A.; Iacobelli, S.; Nifantiev, N.E. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology* **2007**, *17*, 541–552.
123. Nishino, T.; Nagumo, T. The sulfate-content dependence of the anticoagulant activity of a fucan sulfate from the brown seaweed *Ecklonia kurome*. *Carbohydr. Res.* **1991**, *214*, 193–197.
124. Nishino, T.; Kiyohara, H.; Yamada, H.; Nagumo, T. An anticoagulant fucoidan from the brown seaweed Ecklonia kurome. *Phytochemistry* **1991**, *30*, 535–539.
125. Pomin, V.H.; Pereira, M.S.; Valente, A.P.; Tollefsen, D.M.; Pavao, M.S.G.; Mourao, P.A.S. Selective cleavage and anticoagulant activity of a sulfated fucan: Stereospecific removal of a 2-sulfate ester from the polysaccharide by mild acid hydrolysis, preparation of oligosaccharides, and heparin cofactor II-dependent anticoagulant activity. *Glycobiology* **2005**, *15*, 369–381.

126. Nishino, T.; Aizu, Y.; Nagumo, T. The influence of sulfate content and molecular weight of a fucan sulfate from the brown seaweed *Ecklonia kurome* on its antithrombin activity. *Thromb. Res.* **1991**, *64*, 723–731.
127. Hayakawa, Y.; Hayashi, T.; Lee, J.-B.; Srisomporn, P.; Maeda, M.; Ozawa, T.; Sakuragawa, N. Inhibition of thrombin by sulfated polysaccharides isolated from green algae. *Biochim. Biophys. Acta* **2000**, *1543*, 86–94.
128. Shanmugam, M.; Mody, K.H. Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. *Curr. Sci.* **2000**, *79*, 1672–1683.
129. Farias, W.R.L.; Valente, A.P.; Pereira, M.S.; Mourao, P.A.S. Structure and anticoagulant activity of sulfated galactans. Isolation of a unique sulfated galactan from the red algae *Botryocladia occidentalis* and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. *J. Biol. Chem.* **2000**, *275*, 29299–29307.
130. Glauser, B.F.; Rezende, R.M.; Melo, F.R.; Pereira, M.S.; Francischetti, I.M.B.; Monteiro, R.Q.; Rezaie, A.R.; Mourao, P.A.S. Anticoagulant activity of a sulfated galactan: Serpin-independent effect and specific interaction with factor Xa. *Thromb. Haemost.* **2009**, *102*, 1183–1193.
131. Pereira, M.G.; Benevides, N.M.B.; Melo, M.R.S.; Valente, A.P.; Melo, F.R.; Mourao, P.A.S. Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? *Carbohydr. Res.* **2005**, *340*, 2015–2023.
132. Mourao, P.A.S.; Pereira, M.S. Searching for alternatives to heparin: Sulfated fucans from marine invertebrates. *Trends Cardiovasc. Med.* **1999**, *9*, 225–232.
133. Anderson, J.A.; Fredenburgh, J.C.; Stafford, A.R.; Guo, Y.S.; Hirsh, J.; Ghazarossian, V.; Weitz, J.I. Hypersulfated low molecular weight heparin with reduced affinity for antithrombin acts as an anticoagulant by inhibiting intrinsic tenase and prothrombinase. *J. Biol. Chem.* **2001**, *276*, 9755–9761.
134. Barrow, R.T.; Parker, E.T.; Krishnaswamy, S.; Lollar, P. Inhibition by heparin of the human blood coagulation intrinsic pathway factor X activator. *J. Biol. Chem.* **1994**, *269*, 26796–26800.
135. Millet, J.; Jouault, S.C.; Mauray, S.; Theveniaux, J.; Sternberg, C.; Vidal, C.B.; Fischer, A.M. Antithrombotic and anticoagulant activities of a low molecular weight fucoidan by the subcutaneous route. *Thromb. Haemost.* **1999**, *81*, 391–395.
136. Boisson-Vidal, C.; Chaubet, F.; Chevolot, L.; Sinquin, C.; Theveniaux, J.; Millet, J.; Sternberg, C.; Mulloy, B.; Fischer, A.M. Relationship between antithrombotic activities of fucans and their structure. *Drug Dev. Res.* **2000**, *51*, 216–224.
137. Witvrouw, M.; De Clercq, E. Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *Gen. Pharmacol.* **1997**, *29*, 497–511.
138. Schaeffer, D.J.; Krylov, V.S. Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicol. Environ. Saf.* **2000**, *45*, 208–227.
139. Damonte, E.B.; Matulewicz, M.C.; Cerezo, A.S. Sulfated seaweed polysaccharides as antiviral agents. *Curr. Med. Chem.* **2004**, *11*, 2399–2419.
140. Luescher-Mattli, M. Algae, A Possible Source for New Drugs in the Treatment of HIV and Other Viral Diseases. *Curr. Med. Chem.* **2003**, *2*, 219–225.

141. Gerber, P.; Dutcher, J.D.; Adams, E.V.; Sherman, J.H. Protective effect of seaweed extracts for chicken embryos infected with influenza B or mumps virus. *Proc. Soc. Exp. Biol. Med.* **1958**, *99*, 590–593.
142. Nahmias, A.J.; Kibrick, S. Inhibitory effect of heparin on herpes simplex virus. *J. Bacteriol.* **1964**, *87*, 1060–1066.
143. Ghosh, T.; Chattopadhyay, K.; Marschall, M.; Karmakar, P.; Mandal, P.; Ray, B. Focus on antivirally active sulfated polysaccharides: From structure-activity analysis to clinical evaluation. *Glycobiology* **2009**, *19*, 2–15.
144. Hidari, K.I.P.J.; Takahashi, N.; Arihara, M.; Nagaoka, M.; Morita, K.; Suzuki, T. Structure and anti-dengue virus activity of sulfated polysaccharide from a marine alga. *Biochem. Biophys. Res. Commun.* **2008**, *376*, 91–95.
145. Talarico, L.B.; Duarte, M.E.R.; Zibetti, R.G.M.; Noseda, M.D.; Damonte, E.B. An algal-derived DL-galactan hybrid is an efficient preventing agent for *in vitro* dengue virus infection. *Planta Med.* **2007**, *73*, 1464–1468.
146. Talarico, L.B.; Damonte, E.B. Interference in dengue virus adsorption and uncoating by carrageenans. *Virology* **2007**, *363*, 473–485.
147. Talarico, L.B.; Pujol, C.A.; Zibetti, R.G.M.; Farea, P.C.S.; Noseda, M.D.; Duarte, M.E.R.; Damonte, E.B. The antiviral activity of sulfated polysaccharides against dengue virus is dependent on virus serotype and host cell. *Antiviral Res.* **2005**, *66*, 103–110.
148. Ghosh, T.; Pujol, C.A.; Damonte, E.B.; Sinha, S.; Ray, B. Sulfated xylomannans from the red seaweed *Sebdenia polydactyla*: structural features, chemical modification and antiviral activity. *Antivir. Chem. Chemother.* **2009**, *19*, 235–242.
149. Harden, E.A.; Falshaw, R.; Carnachan, S.M.; Kern, E.R.; Prichard, M.N. Virucidal activity of polysaccharide extracts from four algal species against herpes simplex virus. *Antiviral Res.* **2009**, *83*, 282–289.
150. Mohsen, M.S.A.; Mohamed, S.F.; Ali, F.M.; El-Sayed, O.H. Chemical Structure and Antiviral Activity of Water-soluble Sulfated Polysaccharides from *Sargassum latifolium*. *J. Appl. Sci. Res.* **2007**, *3*, 1178–1185.
151. Carlucci, M.J.; Scolaro, L.A.; Noseda, M.D.; Cerezo, A.S.; Damonte, E.B. Protective effect of a natural carrageenan on genital herpes simplex virus infection in mice. *Antiviral Res.* **2004**, *64*, 137–141.
152. Chen, D.; Wu, X.Z.; Wen, Z.Y. Sulfated polysaccharides and immune response: promoter or inhibitor? *Panminerva Med.* **2008**, *50*, 177–183.
153. Groth, I.; Grunewald, N.; Alban, S. Pharmacological profiles of animal- and nonanimal-derived sulfated polysaccharides--comparison of unfractionated heparin, the semisynthetic glucan sulfate PS3, and the sulfated polysaccharide fraction isolated from Delesseria sanguinea. *Glycobiology* **2009**, *19*, 408–417.
154. Granert, C.; Raud, J.; Xie, X.; Lindquist, L.; Lindbom, L. Inhibition of leukocyte rolling with polysaccharide fucoidin prevents pleocytosis in experimental meningitis in the rabbit. *J. Clin. Invest.* **1994**, *93*, 929–936.
155. Preobrazhenskaya, M.E.; Berman, A.E.; Mikhailov, V.I.; Ushakova, N.A.; Mazurov, A.V.; Semenov, A.V.; Usov, A.I.; Nifant'ev, N.E.; Bovin, N.V. Fucoidan inhibits leukocyte recruitment

in a model peritoneal inflammation in rat and blocks interaction of P-selectin with its carbohydrate ligand. *Biochem. Mol. Biol. Int.* **1997**, *43*, 443–451.

156. Senni, K.; Gueniche, F.; Foucault-Bertaud, A.; Igondjo-Tchen, S.; Fioretti, F.; Colliec-Jouault, S.; Durand, P.; Guezennec, J.; Godeau, G.; Letourneur, D. Fucoidan a sulfated polysaccharide from brown algae is a potent modulator of connective tissue proteolysis. *Arch. Biochem. Biophys.* **2006**, *445*, 56–64.
157. Parish, C.R.; Freeman, C.; Hulett, M.D. Heparanase: a key enzyme involved in cell invasion. *Biochim. Biophys. Acta* **2001**, *1471*, M99–M108.
158. Blondin, C.; Fischer, E.; Boisson-Vidal, C.; Kazatchkine, M.D.; Jozefonvicz, J. Inhibition of complement activation by natural sulfated polysaccharides (fucans) from brown seaweed. *Mol. Immunol.* **1994**, *31*, 247–253.
159. Clement, M.J.; Tissot, B.; Chevolot, L.; Adjadj, E.; Du, Y.; Curmi, P.A.; Daniel, R. NMR characterization and molecular modeling of fucoidan showing the importance of oligosaccharide branching in its anticomplementary activity. *Glycobiology* **2010**, *20*, 883–894.
160. Tissot, B.; Daniel, R. Biological properties of sulfated fucans: The potent inhibiting activity of algal fucoidan against the human complement system. *Glycobiology* **2003**, *13*, 29G–31G.
161. Tissot, B.; Gonnet, F.; Iborra, A.; Berthou, C.; Thielens, N.; Arlaud, G.J.; Daniel, R. Mass spectrometry analysis of the oligomeric C1q protein reveals the B chain as the target of trypsin cleavage and interaction with fucoidan. *Biochemistry* **2005**, *44*, 2602–2609.
162. Tissot, B.; Montdargent, B.; Chevolot, L.; Varenne, A.; Descroix, S.; Gareil, P.; Daniel, R. Interaction of fucoidan with the proteins of the complement classical pathway. *Biochim. Biophys. Acta* **2003**, *1651*, 5–16.
163. Tsuji, R.F.; Hoshino, K.; Noro, Y.; Tsuji, N.M.; Kurokawa, T.; Masuda, T.; Akira, S.; Nowak, B. Suppression of allergic reaction by lambda-carrageenan: toll-like receptor 4/MyD88-dependent and -independent modulation of immunity. *Clin. Exp. Allergy* **2003**, *33*, 249–258.
164. Maruyama, H.; Tamauchi, H.; Hashimoto, M.; Nakano, T. Suppression of Th2 immune responses by mekabu fucoidan from *Undaria pinnatifida* sporophylls. *Int. Arch. Allergy Immunol.* **2005**, *137*, 289–294.
165. Leiro, J.M.; Castro, R.; Arranz, J.A.; Lamas, J. Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh. *Int. Immunopharmacol.* **2007**, *7*, 879–888.
166. Nakamura, T.; Suzuki, H.; Wada, Y.; Kodama, T.; Doi, T. Fucoidan induces nitric oxide production via p38 mitogen-activated protein kinase and NF-kB-dependent signaling pathways through macrophage scavenger receptors. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 286–294.
167. Yang, J.W.; Yoon, S.Y.; Oh, S.J.; Kim, S.K.; Kang, K.W. Bifunctional effects of fucoidan on the expression of inducible nitric oxide synthase. *Biochem. Biophys. Res. Commun.* **2006**, *346*, 345–350.
168. Do, H.; Pyo, S.; Sohn, E.H. Suppression of iNOS expression by fucoidan is mediated by regulation of p38 MAPK, JAK/STAT, AP-1 and IRF-1, and depends on up-regulation of scavenger receptor B1 expression in TNF-alpha- and IFN-gamma-stimulated C6 glioma cells. *J. Nutr. Biochem.* **2010**, *21*, 671.

169. Choi, E.M.; Kim, A.J.; Kim, Y.O.; Hwang, J.K. Immunomodulating activity of arabinogalactan and focoidan *in vitro*. *J. Med. Food* **2005**, *8*, 446–453.
170. Kim, M.-H.; Joo, H.-G. Immunostimulatory effects of fucoidan on bone marrow-derived dendritic cells. *Immunol. Lett.* **2008**, *115*, 138–143.
171. Zhou, G.; Sun, Y.; Xin, H.; Zhang, Y.; Li, Z.; Xu, Z. In vivo antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from *Chondrus ocellatus*. *Pharmacol. Res.* **2004**, *50*, 47–53.
172. Ruparez, P.; Ahrazem, O.; Leal, J.A. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *J. Agric. Food Chem.* **2002**, *50*, 840–845.
173. Rocha de Souza, M.; Marques, C.; Guerra Dore, C.; Ferreira da Silva, F.; Oliveira Rocha, H.; Leite, E. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. *J. Appl. Phycol.* **2007**, *19*, 153–160.
174. Zhao, X.; Xue, C.; Cai, Y.; Wang, D.; Fang, Y. Study of antioxidant activities of fucoidan from *Laminaria japonica*. *High Tech. Lett.* **2005**, *11*, 91–94.
175. Costa, L.S.; Fidelis, G.P.; Cordeiro, S.L.; Oliveira, R.M.; Sabry, D.A.; Ciara, R.B.G.; Nobre, L.T.D.B.; Costa, M.S.S.P.; Almeida-Lima, J.; Farias, E.H.C.; Leite, E.L.; Rocha, H.A.O. Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed. Pharmacother.* **2010**, *64*, 21–28.
176. Wang, J.; Zhang, Q.; Zhang, Z.; Song, H.; Li, P. Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. *Int. J. Biol. Macromol.* **2010**, *46*, 6–12.
177. Wang, J.; Liu, L.; Zhang, Q.; Zhang, Z.; Qi, H.; Li, P. Synthesized oversulphated, acetylated and benzoylated derivatives of fucoidan extracted from *Laminaria japonica* and their potential antioxidant activity *in vitro*. *Food Chem.* **2009**, *114*, 1285–1290.
178. Vaquez-Freire, M.J.; Lamela, M.; Calleja, J.M. Hypolipidaemic activity of a polysaccharide extract from *Fucus vesiculosus Phytother. Res.* **1996**, *10*, 647–650.
179. Huang, L.; Wen, K.; Gao, X.; Liu, Y. Hypolipidemic effect of fucoidan from *Laminaria japonica* in hyperlipidemic rats. *Pharm. Biol.* **2010**, *48*, 422–426.
180. Pengzhan, Y.; Ning, L.; Xiguang, L.; Gefei, Z.; Quanbin, Z.; Pengcheng, L. Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta). *Pharmacol. Res.* **2003**, *48*, 543–549.
181. Yokota, T.; Nagashima, M.; Ghazizadeh, M.; Kawanami, O. Increased effect of fucoidan on lipoprotein lipase secretion in adipocytes. *Life Sci.* **2009**, *84*, 523–529.
182. Raghavendran, H.R.; Sathivel, A.; Devaki, T. Effect of *Sargassum polycystum* (Phaeophyceae)-sulphated polysaccharide extract against acetaminophen-induced hyperlipidemia during toxic hepatitis in experimental rats. *Mol. Cell Biochem.* **2005**, *276*, 89–96.
183. Josephine, A.; Veena, C.K.; Amudha, G.; Preetha, S.P.; Varalakshmi, P. Protective role of sulphated polysaccharides in abating the hyperlipidemic nephropathy provoked by cyclosporine A. *Arch. Toxicol.* **2007**, *81*, 371–379.
184. Bourgougnon, N.; Lahaye, M.; Quemener, B.; Chermann, J.C.; Rimbert, M.; Cormaci, M.; Furnari, G.; Kornprobst, J.M. Annual variation in composition and *in vitro* anti-HIV-1 activity of

the sulfated glucuronogalactan from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *J. Appl. Phycol.* **1996**, *8*, 155–161.
185. Hiebert, L.M. Oral heparins. *Clin. Lab.* **2002**, *48*, 111–116.
186. Irhimeh, M.R.; Fitton, J.H.; Lowenthal, R.M. Pilot clinical study to evaluate the anticoagulant activity of fucoidan. *Blood Coagul. Fibrin.* **2009**, *20*, 607–610.
187. Ewart, H.S.; Bloch, O.; Girouard, G.S.; Kralovec, J.; Barrow, C.J.; Ben-Yehudah, G.; Suárez, E.R.; Rapoport, M.J. Stimulation of cytokine production in human peripheral blood mononuclear cells by an aqueous *Chlorella* extract. *Planta Med.* **2007**, *73*, 762–768.
188. Fitton, J.H.; Irimeh, M.; Falk, N. Macroalgal fucoidan extracts: a new opportunity for marine cosmetics. *Cosmet. Toiletries* **2007**, *122*, 55–64.

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