REVIEW ARTICLE

Biological and therapeutic properties of the seaweed polysaccharides

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Abstract

Seaweed polysaccharides isolated from the cell walls of various species of algae, possess immunomodulatory, anti-inflammatory, antiviral, antitumor, antithrombotic, anticoagulant and antioxidant bioactivities.

Within the group of polysaccharides extracted from algae there are the phycocolloids. Colloids are extracted compounds that form colloidal solutions, an intermediate state between a solution and a suspension; they can be used commercially as thickeners, gelling agents and stabilizers for suspensions and emulsions. Hydrocolloids are carbohydrates that when dissolved in water form viscous solutions.

Sulfated galactans (e.g. agars, carrageenans, porphyrans) can be obtained from red algae, (e.g. ulvans) from green algae, alginates and other sulfated polysaccharides (e.g. ascophyllan, laminaran and fucoidan) are obtained from selected brown algae. The historic origin of the main phycocolloids and other seaweed polysaccharides (e.g. agar, carrageenan, alginic acid - alginates), their chemistry, uses and bioactivities are described in this review.

Keywords: Macroalgae; Hydrocolloids; Sulfated galactans; Sulfate content; Bioactivities

1. Introduction

Seaweeds (or macroalgae) are aquatic photosynthetic organisms belonging to the domain Eukarya and to kingdoms Plantae (green and red algae) and Chromista (brown algae). Although classification systems have varied greatly over time and according to the authors, it is generally agreed that:

a) the green algae are included in the phylum Chlorophyta and their pigmentation is identical to that of terrestrial plants (chlorophyll a, b and carotenoids);

b) the red algae belong to the phylum Rhodophyta and their photosynthetic pigments are chlorophyll a, phycobilins (R-phycocyanin and R-phycoerythrin) and caroteno-

ids, mostly β-carotene, lutein and zeaxanthin;

c) the brown algae are included in the phylum Ochrophyta (or Heterokontophyta), class Phaeophyceae and their pigments enclose chlorophylls a, c and carotenoids, dominated by fucoxanthin [1-3].

Macroalgae are known to be enriched in polysaccharides, with concentrations that can vary in the range of 4% to 76% of dry weight [4]. Globally, these are mainly structural cell wall polysaccharides, although considerable amounts of mucopolysaccharides and storage polysaccharides can occur in specific species [3,5,6].

The seaweed hydrocolloids, associated with the cell wall and intercellular spaces, are mainly produced by red and brown algae. Several species of red algae (Rhodophyta) produce galactans (e.g. carrageenans and agars) and of brown algae (Phaeophyceae) produce uronates (alginates) [7-10].

Sulfated galactans (e.g. agars and carrageenans) can be obtained from red algae, and alginates and other sulfated polysaccharides (e.g. laminaran and fucoidan) are obtained from brown algae. Phycocolloids are used in food industries as natural additives and have different European codes: E400 (alginic acid), E401 (sodium alginate), E402 (potassium alginate), E403 (ammonium alginate), E404 (calcium alginate), E405 (propylene glycol alginate), E406 (agar), E407 (carrageenan) and E407a (semi-refined carrageenan or "processed *Eucheuma* seaweed") [10]. Agar, alginates and carrageenans are the ones with the highest economic and commercial significance, since these polysaccharides exhibit high molecular weights, high viscosity and excellent gelling, stabilizing and emulsifying properties. They are extracted in fairly high amount from the algae. All these polysaccharides are water soluble and could be extracted with hot water or alkaline solution [11].

The historic origin of the main phycocolloids (agar, carrageenan, and alginate) and other seaweed polysaccharides (e.g. ulvan, porphyran, fucoidan, laminaran, ascophyllan), their chemistry, uses and bioactivities are described in this review.

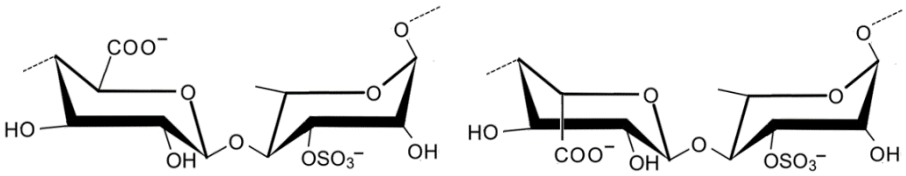
2. Polysaccharides from green algae (Chlorophyta)

2.1. Ulvan

Ulvan is a sulfated, water-soluble polysaccharide which have physio-chemical and biological features of great potentials in food, pharmaceutical, agricultural, and chemical applications [12,13].

Ulvan may represent 8 - 29% of algal dry weight and is produced by some species belonging to the Phylum Chlorophyta (green algae), mostly belonging to the Class Ulvophyceae [14]. It is mainly made up of disaccharide repeating sequences composed of sulfated rhamnose and glucuronic acid, iduronic acid, or xylose [15,16].

The two major repeating disaccharides are aldobiuronic acids designated as: type A, ulvanobiuronic acid 3-sulfate (A3s) and type B, ulvanobiuronic acid 3-sulfate (B3s) (Figure 1). Partially sulfated xylose residues at O-2 can also occur in place of uronic acids. Low proportions of galactose, glucose, mannose and protein were also generally described as components of ulvan. Additionally, minor repeating units were being reported to contain sulfated xylose, replacing the iduronic acid or glucuronic acid components [12,13,17].



(ulvanobiuronic acid – type A) (ulvanobiuronic acid – type B)

Figure 1 - Idealized structure of the chemical units of Ulvan.

Ulvan have exhibited strong antioxidant [18,19], anticoagulant, antithrombotic [20], antitumor [21], immune-stimulatory [22], anti-inflammatory [22,23], antiviral [24,25], antibacterial [26], antiprotozoal [12], anti-hypercholesterolemic [27], and antihyperlipidemic [28] activities; used also for hyperplasia prevention, gastrointestinal, regenerative and nanomedicine applications [29,30].

Alves et al. [31] determined the cytotoxicity of ulvan *via* MTT test (Methyl Thiazolyl Tetrazolium - colorimetric assay for assessing cell metabolic activity), using fibroblast-like cells were incubated with ulvan, *in vitro*. Quantified protein and total DNA stands data were directly correlated with hyaluronic acid use as control (non-cytotoxic); in the studies conducted by these researchers [31], ulvan showed encouraging results in terms of cytotoxicity. Meanwhile, cytotoxicity is one of the most important factors to determine the use of a biomaterial for medical purposes, as in tissue engineering and wound healing [32]. Ulvan can be used in biomedical applications, especially in tissue engineering. Biofunctionalized ulvan hydrogels with ALP (Alkaline Phosphatase) enzyme served as inducers of mineralization in osteoblastic differentiation [32,33] and the cytocompatibility of ulvan as well as it relative membrane are promising features for scaffold development [34,35]. The inter-molecular charges of ulvan and chitosan (i.e. anionic and cationic charges respectively) produced stable supramolecular structures via electrostatic interactions and could also form stabilized membranes. Ulvan/chitosan polyelectrolytes were found to mimics the extracellular matrix structure providing points of attachment for osteoblasts, believed to be enhanced by the nanofibrous structure of this construct. It is suggested that this construct could be a suitable scaffold for applications in tissue engineering [32,33].

3. Polysaccharides from red algae (Rhodophyta)

3.1. Carrageenans

Carrageenan has been used in Europe and Asia for centuries as a thickening and stabilizing agent in food. In Europe, the use of carrageenan began in Ireland more than 600 years ago when, in the small village on the southern coast of Ireland, called "Carraghen", the flans were made by cooking "Irish Moss" (Irish Moss - *Chondrus crispus*) in milk. The name carrageenin, the ancient name of carrageenan, was first used in 1862 to designate the extract obtained from *C. crispus*, in reference to the name of the small Irish town [36]. The procedure for the extraction of carrageenans was described for the first time by Schmidt in 1844 [37]. The name was later changed to carrageenan to comply with the "-an" suffix for the names of polysaccharides.

The Irish moss has been used in industry since the 19th Century, in the clarification of beer [39]. The industrial extraction of carrageenan had its start in 1930 in New-England, from *Chondrus crispus* and *Mastocarpus stellatus* thalli, for the preparation of chocolate milk. The interruption of agar imports during World War II, led to its replacement by carrageenan. This situation was the starting point of a booming industry [40].

Fractionation of crude carrageenan extracts started in the early 1950s [41,42], resulting in the characterization of the different carrageenan types. A Greek prefix was introduced to identify the different carrageenans. In the same period, the molecular structure of carrageenans was determined [43,44]. The structure of 3,6-anhydro-D-galactose in kappa (κ) carrageenan, as well as the type of linkages between galactose and anhydrogalactose rings, was determined.

Today, the industrial manufacture of carrageenan is no longer limited to extraction from *C. crispus*, and numerous red seaweed species (Gigartinales, Rhodophyta) are used. For a long period of time, these seaweeds have been harvested from naturally occurring populations. Seaweed farming started almost 200 years ago in Japan. Scientific information about the seaweed life cycles allowed artificial seeding in the 1950s. Today, lots of seaweed *taxa* are cultivated, lowering the pressure on naturally occurring populations [45].

The modern industry of carrageenans dates from the 1940's where it was found to be the ideal stabilizer for the suspension of cocoa in chocolate milk. In the last decades, due to its physical functional properties, such as gelling, thickening, emulsifying and stabilizing abilities, carrageenans have been employed in food industry to improve the texture of cottage cheese, puddings and dairy desserts, and in the manufacture of sausages, patties and low-fat hamburgers [7,9,37,46-48].

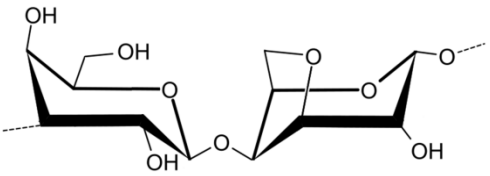
The most commonly used commercial carrageenans are extracted from *Kappaphycus alvarezii* and *Eucheuma denticulatum* [45]. Primarily wild-harvested genera such as *Chondrus, Furcellaria, Gigartina, Chondracanthus, Sarcothalia, Mazzaella, Iridaea, Mastocarpus,* and *Tichocarpus* are also mainly cultivated as carrageenan raw materials, and producing countries include Argentina, Canada, Chile, Denmark, France, Japan, Mexico, Morocco, Portugal, North Korea, South Korea, Spain, Russia, and the US [8,10,47].

The carrageenan market is the largest of the seaweed-derived, food hydrocolloids markets and currently holds the fourth largest share (in $ value) of the wider global hydrocolloids market, behind gelatin, starch and pectin [49]. In 2014, the global hydrocolloids market was valued at US$ 5.4 Billion but projected to reach US$ 7.6 Billion by 2020 [50]. Industry estimates for 2015/2016 put a value of US$ 600-700 Million on the global carrageenan market with a projected average growth of ≈ 3% per year (Compound Annual Growth Rate - CAGR). Most recent market research predicted that the global market for carrageenan would approach US$ 1 Billion by 2024 [49,51].

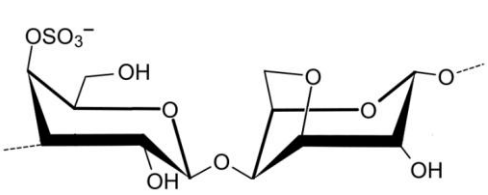
Current industry estimates for the global production capacity of carrageenan range from about 70,000-80,000 dry MT per year [52] to over 110,000 MT per year [53]. CyberColloids' (www.cybercolloids.net) estimate for current global usage, as based on end-sales data, is for 68,000 dry MT or approximately 80% of the estimated production capacity (Campbell and Hotchkiss 2017). Most of this carrageenan (≈ 90%) comes from species and strains of K*appaphycus* and *Eucheuma* or "cottonii" and "spinosum" respectively as they were referred to historically and still are by the

industry. In 2016, CyberColloids estimated the global production of *Kappaphycus* for the carrageenan industry to be in the region of 1.5 Million wet MT or 200,000 dry MT [51] with most cultivation occurring in Indonesia and the Philippines. *Eucheuma* is not cultivated on such a large-scale as *Kappaphycus*, current estimates put production for the carrageenan industry in the range of 0.3 Million wet MT or 40,000 dry MT [51]. It must be noted that a substantial market for sea vegetables also exists which is not included in these estimates. The remainder of the carrageenan produced comes from cold-water species that are harvested mostly in South America, approximately 15,000 dry MT and from the dwindling remnants of a small, traditional N. Atlantic industry which produces only a few hundred dry MT annually from *Chondrus crispus* [54].

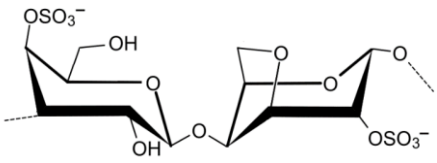
Main commercial carrageenans (Figure 2) are usually classified into κ (kappa), ι (iota), and λ (lambda) carrageenans [9].



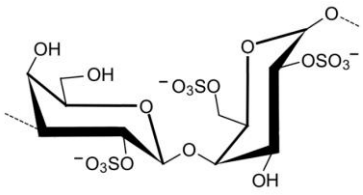
(β) Beta-carrageenan



(κ) Kappa-carrageenan



(ι) Iota-carrageenan



(λ) Lambda-carrageenan

Figure 2 - Idealized structure of the chemical units of the main types of Carrageenan.

3.2. Biological activities of Carrageenans

From a human health perspective, it has been reported that carrageenans have antioxidant, anticancer, antihyperlipidemic, anticoagulant, immunomodulatory, antifungal, antiviral properties, gastric discomfort management, and digestive health support [55-65]. Carrageenans are also classically used as agents for the induction of experimental inflammation and inflammatory pain [66].

Previous studies conducted by Panlasigui et al. [67], and by Dumelod et al. [68], showed that carrageenan incorporated into common Philippine foods such as "fishballs" and "arroz caldo" has hypoglycemic effects in normal subjects. A study done on another seaweed extract showed that when incorporated into "puto", "siomai" and a meal composed of rice and meatballs with sweet-and-sour sauce, agar had a glucose lowering effect in normal people [69]. The study conducted by Panlasigui et al. [57] indicates that regular inclusion of carrageenan in the diet may result in reduced blood cholesterol and lipid levels in human beings.

Historically, Irish Moss or Carrageen (*Chondrus crispus* and *Mastocarpus stellatus*) has many medical applications, some of which date from the 1830s. It is still used in Ireland to make traditional medicinal teas

and cough medicines to combat colds, bronchitis, and chronic coughs. It is said to be particularly useful for dislodging mucus and has antiviral properties. Carrageenans are also used as suspension agents and stabilizers in other drugs, lotions and medicinal creams. Other medical applications are as an anticoagulant in blood products and for the treatment of bowel problems such as diarrhea, constipation and dysentery. They are also used to make internal poultices to control stomach ulcers [70-72].

Many reports exist of anticoagulant activity and inhibited platelet aggregation of carrageenan [73-77]. Among the carrageenan types, λ-carrageenan (primarily from *C. crispus*) has approximately twice the activity of unfractionated carrageenan and four times the activity of κ-carrageenan (*Kappaphycus alvarezii* – formerly *Eucheuma cottoni*, and *E. denticulatum* – formerly *E. spinosum*). The most active carrageenan has approximately one fifteenth the activity of heparin [73], but the sulfated galactan from *Grateloupia indica* collected from Indian waters, exhibited anticoagulant activity as potent as heparin [78]. The principal basis of the anticoagulant activity of carrageenan appeared to be an antithrombotic property [73,74]. λ-carrageenan showed greater antithrombotic activity than κ-carrageenan, probably due to its higher sulfate content, whereas the activity of the unfractionated material remained between the two. It was found that toxicity of carrageenans depended on the molecular weight and not the sulfate content. Similar results were obtained with λ-carrageenan of *Coccotylus brodiei* (as *Phyllophora brodiaei*) which gave the highest blood anticoagulant activity. Carrageenan of *Grateloupia turuturu* also showed anticoagulant activity [78-80].

Chondrus ocellatus is distributed spontaneously in intertidal zone of the southeast seaside of China [59]. In ancient times the alga was not only edible, but was also used as a medicine to cure chronic constipation and bone fracture [81]. *C. ocellatus* is an important economic original alga that can produce carrageenan. Antitumor, antiviral, anticoagulant, and immunomodulatory activities of carrageenan have been found in this species, however, many evidences indicated that λ-carrageenan, which contained 1,4-linked D-galactose 2,6-disulfate units, and highly sulfated group, has more bioactivities than other carrageenan types [59,77,79].

Carrageenans extracted from several algae, i.e., *Chondrus crispus, Mastocarpus stellatus, Kappaphycus alvarezii, Eucheuma denticulatum*, Gigartinaceae and Phyllophoraceae, enter cough medicines, toothpastes, lotions, sun ray filterers, shaving creams, shampoos, hair conditioners, and deodorants. More than 20% of carrageenan production is used in pharmacy and cosmetology. They are said to be invaluable for the manufacture of wash-removable creams and ointments. Excipients of algal origin are used in vanishing creams—the rapid evaporation of the emulsion's aqueous phase on the skin leaves a thin protective medicated oily microfilm. Dried and pulverized *Lithothamnium* and *Phymatolithon* are used to make absorbent face and/or beauty masks [82,83].

Carrageenans are selective inhibitors of several enveloped and nonenveloped viruses and act predominantly by inhibiting the binding or internalization of virus into the host cells [84,85]. Carrageenans are exceptionally potent inhibitor of Human Papillomavirus (HPV) *in vitro* by inhibiting the initial stage of infection [84]. Notably, they are also extremely effective against a range of sexually transmitted HPV types that lead to cervical cancer and genital warts [86,87]. Several *in vitro* studies suggest that carrageenans may also have antiviral properties, inhibiting the replication of herpes and hepatitis A virus [86,88-90]. More recently, Schiller and coworkers demonstrated that carrageenan is an extremely potent infec-

tion inhibitor of a broad range of genital Human papillomaviruses (HPVs) and there are indications that carrageenan-based sexual lubricant gels may offer protection against HPV transmission [84,91,92].

The antimicrobial activities of numerous algae species have been tested and reported, presenting an extended spectrum of action against bacteria and fungi [93]. Carrageenans have proved to have effects against some bacterial strains such as *Salmonella enteritidis*, *S. typhimurium*, *Vibrio mimicus*, *Aeromonas hydrophila*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. The growth of all the bacterial strains except *L. monocytogenes* was significantly inhibited by them, particularly by the ι-carrageenan. A growth inhibition experiment using *S. enteritidis* showed that the inhibitory effect of the carrageenans was not bactericidal but bacteriostatic. Removal of the sulfate residues eliminated the bacteriostatic effect of ι-carrageenan, suggesting that the sulfate residues in carrageenan play an essential role in this effect [94]. In 2014, Sebaaly et al. [95] reported that carrageenans isolated from the red alga *Corallina* sp. exhibited antibacterial activity against *Staphylococcus epidermis*. Infrared spectroscopy (FTIR) showed that the isolated carrageenan was of λ-type [95].

Researchers from the Population Council [96] (http://www.popcouncil.org/), a non-profit research organization in the USA, conducted a clinical trial investigating whether a carrageenan-based sexual lubricant was effective as a topical microbicide by blocking Human Immunodeficiency virus (HIV) infection in women. The study was conducted from March 2004–2007 in South Africa and enrolled more than 6000 volunteers. The results demonstrated that the experimental microbicide gel was safe for use but does not provide protection against HIV [61,62,96]. These results are consistent with *in vitro* assays since carrageenan was active against HIV only at concentrations about 1000 times higher than those required to inhibit Papillomaviruses [84]. However, carrageenans may serve as models for designing novel anti-HIV agents, improving their therapeutic properties through chemical modifications [97].

Carlucci and colleagues [98,99] found that *λ*-type carrageenan is active against the replication of HSV upon its firm interaction that leads to inactivation of HSV virion [98]. They also discovered that the *λ*-carrageenan and moderately cyclized *μ*/*ι*-carrageenan isolated from *Gigartina skottsbergii* exert promising antiviral activities towards diverse strains of *Herpes simplex* virus HSV-1 and HSV-2, during virus attachment stage [90,99]. Surprisingly, similar results were reported by different group of researchers, who analyzed the chemical structure and antiviral activity of carrageenan (λ, κ, and ι) against HSV-2 infection [100,101]. The data obtained in the work of Soares [64] revealed that the viral infection by Lentivirus was reduced upon exposure to a pre-treatment with extracts from female gametophytes (FG) and tetrasporophytes (T) of *Chondracnthus teedei* var. *lusitanicus*. Although the inhibitions were not statistically significant, FG (producers of κ/ *ι* hybrid carrageenan) and T (producers of ξ-carrageenan) of *C. teedei* var. *lusitanicus* extracts was able to reduce 14% of the virus infection, and the Tetra extract was able to reduce, approximately, 35% of the virus infection [64].

A recent *in vitro* study conducted by Grassauer and colleagues [85] reported the inhibitory effects of *ι*-carrageenan against Human rhinovirus (HRV) proliferation by preventing the primary phases of virus replication. They have suggested that this effect is possibly attributed to the suppression of the allosteric activity of virus particles during their entry [85,102]. Additionally, *ι*-carrageenan was proven to be effective against dengue virus replication in mosquito and mammalian cells; however, the mode

of antiviral action of ι-carrageenan in both cell types was interestingly distinctive. In Vero cell line, the inhibitory activity has been exerted at early stage of virus adhesion probably due to some primary receptors, whereas in mosquito cell it affected the cell proliferation and protein synthesis [103,104].

Carrageenan from red marine algae is known to be also a potent inflammatory agent in rodents, primes mice leucocytes to produce tumor necrosis factor-α (TNF-α) in response to bacterial lipopolysaccharide [105]. Moreover, some types of carrageenans induce potent macrophage activation [106,107], while some carrageenans and fucoidan appear to inhibit macrophage functions [108,109]. However, sulfated polysaccharides may have potential biomedical applications in stimulating the immune system or in controlling macrophage activity to reduce associated negative effects [22].

Exposure of human (and other mammals) cells to ultraviolet (UV) light induces various deleterious responses. Damage to cells by UVA is thought to involve reactive oxygen species, including singlet oxygen, the superoxide and hydroxyl radicals, and hydrogen peroxide. Some of the major harmful effects are DNA damage, systemic immune suppression, and accelerated aging [110,111]. When thymic lymphocytes were exposed to a dose of 90 mJ/cm^2 UVA, their viability was decreased by about 60%. However, the viability of lymphocytes was significantly increased compared with the control group when carrageenan oligosaccharides and their derivatives were pre-administered to cells before UVA radiation. This phenomenon showed that carrageenan oligosaccharides and their derivatives can protect the lymphocytes against UVA injury. As like a H_2O_2 induced oxidative stress model, the chemical modification of carrageenan oligosaccharides also had no significant effect on their protective effect. So, the studies of Yuan et al. [112] suggest that carrageenan oligosaccharides and their derivatives show relevant antioxidant activity both *in vitro* and in a cell system.

According Sokolova et al. [113], antioxidant actions of λ-, ξ-, κ-, κ/β- and κ/ι-carrageenans against reactive oxygen/nitrogen species depend on polysaccharide concentration and such structural characteristics as presence of hydrophobic 3,6-anhydrogalactose unit, amount and position of sulfate groups, and an oxidant agent, on which sample antioxidant action is directed. The results of Sokova et al. [113] indicated that carrageenans possess antioxidant capacity *in vitro*, and this action notably depends on the structure of the polysaccharide itself than the reducing capacity of the polysaccharides.

Affording the works of Sokolova et al. [114], a carrageenan food supplement brings about the decrease in the level of cholesterol and low-density lipoprotein cholesterol, and the supplementation with carrageenans produces moderate modulation of all links of the immunity.

Until 1935, pneumonia was the leading recorded cause human death in the USA. 100 years ago, five of the top ten causes of death in men were respiratory diseases. Today, asthma is the leading cause of juvenile school absenteeism and is increasingly an affliction of adults. Red algae (carrageenophytes) containing carrageenan have been used for millennia as treatments for respiratory ailments, especially intractable sinus infections and lingering pneumonias. Asthma was not separated out as such in the old literature [115].

A recent *in vivo* study in mice has revealed that the low molecular weight carrageenans (3, 5, and 10 kDa), as well as acetylated and sulfated derivatives, have substantial inhibitory effects against influenza virus. Furthermore, the smallest *κ*-carrageenan with appropriate sulfation and acetylation degree

was the greatest antiviral candidate against influenza virus [116].

Acute viral upper respiratory tract infection, also known as common cold, is the most frequently observed infectious disease in human beings. Children get four to eight upper respiratory infections per year and adults suffer from two to four episodes per year [117]. In most of cases, common cold is caused by respiratory viruses such as rhinovirus, coronavirus, parainfluenza, influenza, respiratory syncytial virus, adenovirus, enterovirus and metapneumovirus [118-121]. According to the work of Koenighofer et al. [122], the administration of carrageenan nasal spray in children as well as in adults suffering from virus-confirmed common cold reduced duration of disease, increased viral clearance and reduced relapses of symptoms. Carrageenan nasal spray appeared as an effective treatment of common cold in children and adults [122-124].

A nasal spray containing ι-carrageenan only, or together with "Zanamivir", provides an easy to apply treatment of upper respiratory tract infections in patients under suspicion to be influenza A (H1N1) infected [125,126]. Patients would benefit from the fast and efficient treatment of uncomplicated influenza in the upper respiratory tract. Due to the faster influenza virus clearance from the upper respiratory tract and the independent antiviral mechanism of carrageenan and "Zanamivir" the likelihood to develop escape mutations against "Zanamivir" will be reduced. Both individual compounds can reduce severity and/or duration of the influenza illness and a combination is expected to work similarly. Additionally, due to the broad antiviral effectiveness of carrageenan, patients will receive in parallel a treatment of concomitant viral infections. Therefore, patients will benefit from a decreased probability to develop complications. In consideration of the complications known to accompany an influenza virus illness this combinational therapy meets an urgent medical need [125,126].

Marine algae are prolific sources of sulfated polysaccharides, which may explain the low incidence of certain cancers in countries that traditionally consume marine food. Breast cancer is one of the most common types of non-skin cancer in females. In the study made by Murad et al. [127], extracted sulfated carrageenan, predominantly consisting of ι-carrageenan extracted from the red alga *Palisada perforata* (as *Laurencia papillosa*), has demonstrated potential cytotoxic activity against the MDA-MB-231 cancer cell line. These findings suggest that sulfated carrageenan may serve as a potential therapeutic agent to target breast cancer *via* prompting apoptosis [127].

λ-Carrageenan is a seaweed polysaccharide which has been generally used as pro-inflammatory agent in the basic research, however, how the immunomodulating activity of λ-carrageenan affects tumor microenvironment remains unknown. Luo et al. [128] found that intratumoral injection of λ-carrageenan could inhibit tumor growth in B_{16}-F_{10} and ${}_4T_1$ bearing mice and enhance tumor immune response by increasing the number of tumor-infiltrating M_1 macrophages, DCs and more activated $CD_4^+CD_8^+$ T lymphocytes in spleen. In addition, λ-carrageenan could enhance the secretion of $IL_{17}A$ in spleen and significantly increase the level of TNF-α in tumor, most of which was secreted by infiltrating macrophages. Moreover, λ-carrageenan exhibited an efficient adjuvant effect in OVA-based preventative and therapeutic vaccine for cancer treatment, which significantly enhanced the production of anti-OVA antibody. The toxicity analysis suggested that λ-carrageenan was with a good safety profile. Thus, λ-carrageenan might be used both as a potent antitumor agent and an efficient adjuvant in cancer immunotherapy [128].

Carrageenans extracted from *Chondracanthus teedei* var. *lusitanicus* were studied in order to determine their potential antifungal activity [64,65]. FTIR-ATR and FT-Raman spectroscopic analysis confirmed the presence of a hybrid κ/ι-carrageenan belonging to the gametophyte phase and a hybrid ξ/θ-carrageenan in the tetrasporophyte phase [7,9,10,129]. κ/ι and ξ/θ-carrageenan induced the formation of swollen hyphal segments in *Alternaria infectoria*, upon exposure to 125 μg mL^{-1} and 60 μg mL^{-1}, respectively. The observed phenotype was similar to those induced by antifungals targeting the fungal cell wall. When exposed to 87.5 μg mL^{-1} of kappa/iota carrageenan, *Aspergillus fumigatus* hyphae became shortened and highly branched, a phenotype commonly observed in response to antifungals. These morphological alterations were associated with a decrease of the β-glucan content in *A. infectoria* after exposure to 150 μg mL^{-1} of kappa/iota and to 100 μg mL^{-1} of ξ/θ-carrageenan. On the other hand, the chitin cell wall content of *A. fumigatus* decreased significantly upon exposure to 150 μg mL^{-1} of both extracts, which triggered an increase in the content of β-glucan. Overall, the present work shows that carrageenans extracted from *C. teedei* var. *lusitanicus* cause alterations on the A. fumigatus *and A. infectoria* cell walls, indicating a marked antifungal activity [64,65].

According the works of Azizi et al. [130], the slow silver (Ag) release, κ-carrageenan/silver nanoparticles (Ag-NPs) presented good antibacterial activities against *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Peseudomonas aeruginosa* and *Escherichia coli* with maximum zones of inhibition 11 +/- 2 mm. Cytotoxicity study showed that the bio-nanocomposite hydrogels with non-toxic effect of concentration below 1000 μg/mL have great pharmacological potential and a suitable level of safety for use in the biological systems [130].

3.3. Agar

Agar is the Malay name for red algae, also called agar-agar and Kanten. It is an extract of red algae, sold in granular, powder form, as flakes or long strips. This colloid is widely used as a gelling agent and rarely eaten on its own. In its pure form, agar is a tasteless and odorless polysaccharide; which normally contains proteins, vitamins and minerals, as the red algae from which it is derived [83,131].

Kanten was discovered by accident. For more than a thousand years, the Japanese have eaten a dish called "Tokoroten", which is prepared for the abolition of red algae *Gelidium amansii* (called in Japan as "Tengusa"), and then letting the mixture stiffen. At some point toward the end of the 17^{th} Century, leftover "Tokoroten" away outside on a freezing cold day. When it was found later, it had become a dry, whitish solid [131,132].

Agar is light in color, semi-transparent, and very brittle when dry. When soaked it absorbs water and, in contrast to gelatin (prepared from animal origin), it must have allowed to swell up completely before the water is warmed to a temperature above its melting point of 85 °C. Agar can be used as a gelling agent as it cools [38,131].

Agar consists of a mixture of at least two polysaccharides, i.e. agarose and agaropectin [133]. Typically, agarose is the predominant fraction of agar (50-90%) [134,135] and the responsible for its gelling properties [135]. It consists of high molecular weight polysaccharides composed of repeating units of (1→3)-β-D-galactopyranosyl-(1→4)-3,6-anhydro-α-L galactopyranose (Figure 3), although some variations can occur, depending on factors such as the species of seaweed, as well as environmental and seasonal conditions [133]. In turn, agaropectin is a less clearly defined, it is a more complex polysaccharide of lower molecular weight than agarose and it has

thickening properties [38,133]. Its structure is essentially made up of alternating (1→3)-β-D-galactopyranose and of (1→4)-3,6-anhydro-α-L-galacto-pyranose residues [133,136].

The most important commercial agarophyte genera are *Gelidium, Pterocladiella, Gelidiella,* and *Gracilaria*. Agar has also been found in species of *Ceramium, Phyllophora, Ahnfeltia, Campylaephora, Acanthopholis,* and *Gracilariopsis* [137].

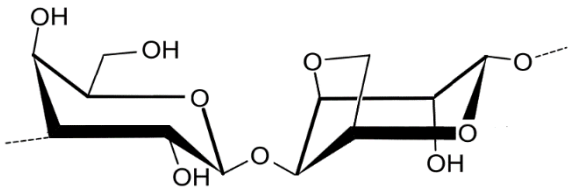


Figure 3 – Idealized structure of the chemical units of Agar

3.4. Biological activities of Agar

Agar has been used as functional dietary fiber for diabetics in the health food market because of its non-nutritive nature. Nevertheless, its α-glucosidase inhibitory ability should also be considered for retarding the carbohydrate digestion. Some inhibitors with pseudo-saccharide structure including acarbose and 1-deoxynojirimycin have been used very successfully in controlling diseases such as diabetes, obesity or hyperlipemia [138,139].

Agar has medicinal or pharmaceutical industrial applications including use as suspending agent for radiological solutions (barium sulfate), as a bulk laxative as it gives a smooth and non-irritating hydrated bulk in the digestive tract, and as a formative ingredient for tablets and capsules to carry and release drugs. Pharmaceutical grade agar has a viscous consistency. In microbiology, agar is the medium of choice for culturing bacteria on solid substrate. Agar is also used in some molecular microbiology techniques to obtain DNA information [140]. More recently, agar was used in a newly-developed medium, i.e., combined deactivators-supplemented agar medium (CDSAM), to evaluate the viability of dermatophytes in skin scales [141]. The experimental data from this clinical study indicate that CDSAM was more useful than standard media in accurately evaluating the efficacy of antifungal drugs. Agar proportion method for drug susceptibility tests has been used since 1957 [142]. More recently, the test has been replaced by more rapid tests [143].

The possibility to use agar and agarose beads for sustained release of water soluble drugs has been investigated [144]. Agarose has a significantly lower sulfate content, better optical clarity and increased gel strength with respect to agar, but it is considerably more expensive [145]. Agar beads containing phenobarbitone sodium as a water soluble and hypnotic drug were prepared [146]. The encapsulation procedure consists in dissolving the drug in a hot (around 70 °C) agar aqueous solution and then dropping the solution in a cold bath containing a non-solvent for agar (acetone or ethyl acetate). Agar beads instantaneously form by gelation. The results of dissolution and release studies indicated that agar beads could be useful for the preparation of sustained release dosage forms, although no many further studies have been developed [147].

Antitumor activity was found in an agar-type polysaccharide from cold water extraction of a *Gracilaria* species and hydrolysates of agar resulted in agaro-oligo-

saccharides with activity against α-glucosidase and antioxidant ability [139,148]. It has also been reported that agar leads to decreases in the concentration of blood glucose and exerts an anti-aggregation effect on red blood cells [4]. It has as well employed as an anti-rheumatic agent for prolonged treatments, and in the stabilization of cholesterol solutions [149].

Several antitumor oligosaccharides (GSO) include sulfated disaccharide and neoagarotetraose have been discovered in the Delesseriaceae (Ceramiales, Rhodophyta) [150]. These oligosaccharides were prepared by spray dried and proved the physiological activity by reducing the number of revertant colonies of *Salmonella typhimurim* and mildly inhibiting angiogenesis on the chicken chorioallantoic membrane (CAM). GSO was assumed to have a desmutagenic activity and no harmful side effects such as blood coagulation of fibrinolysis. GSO with high safety owed various physiological activity, which is expected to apply in future food and medical supplies [151].

Agarose can be separated from the agar with a yield of 42%, and the agar content varied seasonally from 26% - 42% in *Gelidium* spp. in another experiment [152,153].

In recent years, versatile biological activities of agaro-oligosaccharides have been observed, and the structure and bioactivity relationship research also revealed that the functionality is directly correlated to the degree of polymerization. Agaro-oligosaccharides were hydrolytically obtained from agar using hydrochloric acid, citric acid, and cationic exchange resin (solid acid) [139]. Agaro-oligosaccharides have been reported to display antioxidant effects, such as inhibiting the production of lipid peroxide radicals and inhibiting the production of nitric oxide (NO) [139]. Agaro-oligosaccharides have also been shown to suppress the production of a pro-inflammatory cytokine and an enzyme associated with the production of nitric oxide [154].

Among therapeutics for liver diseases, protective drugs have been attracted more and more attentions, such as antioxidant prevention approaches. The *in vitro* and *in vivo* antioxidative activities of agaro-oligosaccharides with the model related with liver disease was tested by [155]. Agaro-oligosaccharides derived from red seaweed polysaccharide have been reported to possess antioxidant activity. Overall, the results of the study made by Chen and colleagues [155] indicate that agaro-oligosaccharides can exert their *in vitro* and *in vivo* hepatoprotective effect through scavenging oxidative damage induced by reactive oxygen species (ROS).

Agaro-oligosaccharides and neoagaro-oligosaccharides have been reported to possess lots of biological activities, such as antioxidative activities [156], moisturizing effect on skin [157], and anti-inflammation effects [158], which suggested that these oligosaccharides merit further investigation as functional foods to control inflammation. Neoagaro-oligosaccharides derived from agarose have been demonstrated to possess a variety of biological activities, such as antibacterial and antioxidative activities [159].

3.5. Porphyran

Porphyran is a sulfated polysaccharide isolated from selected (red) algae of the Order Bangiales, Phylum Rhodophyta, especially from the genus *Porphyra*/*Pyropia* [160,161]. The chemical structure of porphyran contains 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 (Figure 4) [79,162].

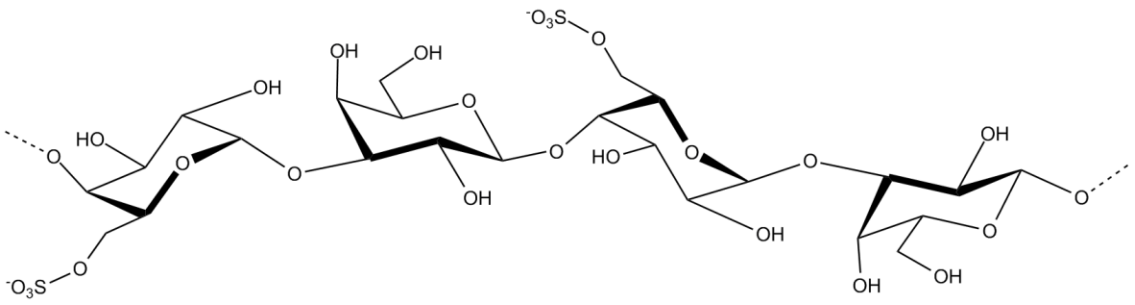


Figure 4 - Idealized structure of the chemical units of Porphyran (after [327]).

3.6. Biological activities of Porphyran

Non-starch polysaccharide (NSP) has been consumed for centuries and has been recognized as having nutritional benefits. Many reports have demonstrated that ingestion of NSP lowers serum cholesterol and lipoprotein levels [170-172], helps to normalize serum glucose and insulin levels [173] and promotes normal laxation [174]. Porphyran, which is considered an NSP, can be extracted with hot water from *Porphyra*/*Pyropia* spp. [161,175], edible species cultivated abundantly in eastern Asia. Porphyran is distributed in the intercellular matrix of the algal body and its basic structure is closely related to that of agar [176]. It has also been reported that porphyran also has health benefits as other NSPs: e.g., prebiotic activity [177], antitumor activity [178,179], and antihyperlipidemic activity [180]. The study in rats [180] suggested that dietary porphyran prevents hypercholesterolemia that is induced by feeding diet containing 1.5% cholesterol 0.5% bile salts. However, little is known about how dietary porphyran affects growth and lipid metabolism in the feeding of a normal diet [176].

Porphyran produces hypocholesterolemic and hypolipidemic effects due to reduced cholesterol absorption in the gut, with increased fecal cholesterol content and a hypoglycemic response. It is also reported to reduce total cholesterol, free cholesterol and the levels of triglycerides and phospholipids in the liver. These substances are likely to be exploited by "nutraceutical" companies that market them as "health products" [57,68,176,180-187].

Porphyran is effective against contact hypersensitivity induced by 2, 4, 6 trinitrochlorobenzene. The mechanism is suggested to operate by suppressing the serum levels of IgE and IFN-γ. Porphyran has excellent inhibitory activities against hyaluronidase, responsible for the release of histamine from mast cells [188].

Some sulfated polysaccharides from red algae also showed anti-viral activities towards viruses responsible for human infectious diseases. Porphyran is reported to inhibit HIV reverse transcriptase *in vitro*. It has minimal effects on human DNA and RNA polymerase activity. Some agaroids, such as high molecular weight, galactan sulfate also have antiviral properties against the *Herpes simplex* virus, human cytomegalo virus (HCMV), dengue virus (DENV), respiratory syncytial virus (RSV) and influenza virus due to the inhibition of the initial viral attachment to host cells [189-194].

Upon the intraperitoneal administration of porphyran to aging mice, the activities of superoxide dismutase and glutathione peroxidase as well as the total antioxidant capacity were increased significantly and the level of lipid peroxidation was significantly decreased, suggesting that porphyran has antioxidant activity both *in vitro* and *in vivo* [195].

In human gastric cancer cells, porphyran displays antitumor properties, in particular the ester sulfates of the polysaccharides may be related to the antitumor activities reported. The degree of polyanionic properties may be intimately related to this activity. Porphyran reportedly induced apoptosis by caspase-3 or caspase-9 activation, while decreasing the Insulin-like, growth Factor-I receptor (IGF-IR) phosphorylation and down regulated a serine/threonine kinase (Akt) phosphorylation. The caspase-induce apoptosis was activated in a sequential cascade of cleavages from their inactive forms. Activation of caspase-3 leads to the cleavage of many proteins, one of which is poly (ADP-ribose) polymerase (PARP) [196]. The cleavage of the poly (ADP-ribose) polymerase is the hallmark of apoptosis. The expression level of antiapoptotic molecules, such as B-cell lymphoma 2 (Bcl-2), gradually decreased, whereas the proapoptotic molecule Bcl-2-associated death promoter (BAD), opposed the action of Bcl-2, which increased in response to levels of exposure porphyran. Porphyran decreased the expression levels in Gastric cancer cell lines (AGS) by negatively regulating the insulin-like, growth Factor-I receptor (IGF-IR) phosphorylation which is a potent mitogen and growth stimulatory factor for several kinds of cells (over-expression and enhanced activation of which is frequently observed in Human cancers) [196]. Thus, Insulin-like, growth Factor-I receptor (IGF-IR) phosphorylation was decreased in porphyran-treated AGS cells, which correlated with Akt activation. Porphyran down regulates Akt phosphorylation. The PI3- kinase/Akt pathway is viewed as a key player to cell survival in different systems. The IGF-I stimulation induced Akt activation decreased in cells treated with porphyran, which suggested that inhibition of Akt phosphorylation may be an important mechanism for porphyran-induced apoptosis [196,197].

Furthermore, polysaccharides prepared from *Pyropia yezoensis* (as *Porphyra yezoensis*) considerably inhibited human gastric and lung cancer cell growth, and degradation products of these polysaccharides resulting from an ultrasonic treatment significantly increased this antiproliferative eff ect against only human gastric cancer cells [198]. *Porphyra/ Pyropia* sp. also reportedly contains various anticancer compounds other than porphyrans [199-201]. In a human case-control study, it was reported that a high rate of intake of nori (*Porphyra/Pyropia* sp.) may decrease the risk of breast cancer [202].

Additionally, porphyrans reportedly improved lipid and glucose metabolism [203,204] and iron deficiency [205]. In the works of Wang et al. [206] the hypolipidemic and antioxidant effects of porphyran from the red algae *Pyropia haitanensis* (as *Porphyra haitanensis*) as a dietary supplement were evaluated in mice. The results suggested that porphyran could be used as functional foods and natural drugs in preventing the hyperlipidemia and this activity might be attributed to its antioxidant potential [206].

4. Polysaccharides from brown algae (Ochrophyta, Phaeophyceae)

4.1. Fucoidan

Fucans are sulfated polysaccharides which comprise a fucose backbone. One of the best studied fucans from the brown algae is fucoidan, which was first isolated by Kylin [207]. The fucoidan from *Fucus vesiculosus* has been available commercially for decades (Sigma-Aldrich Chemical Company, St. Louis, MO, US). Early work on its structure showed that it contained primarily (1→2) linked 4-O-sulfated fucopyranose residues (Figure 5). However, 3- linked fucose with 4-sulfated groups was subsequently reported to be present on some of

the fucose residues. Additionally, it was determined to contain branches every 2-3 fucose residues. Subsequently, Chevolot and colleagues reported that the fucoidan from *Fucus vesicul*osus and *Ascophyllum nodosum* contained a predominant disaccharide motif with sulfate at the 2-position of the 3-linked fucose and sulfate groups on the 2- and 3-positions of the 4-linked fucose [137,208].

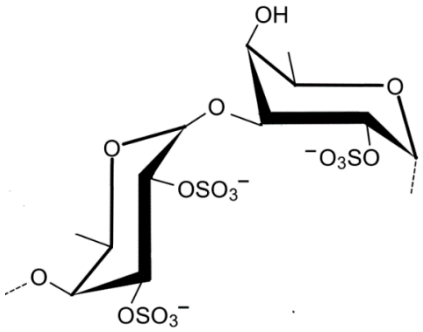


Figure 5 - Idealized structure of the chemical units of Fucoidan

4.2. Biological activities of Fucoidan

Fucoidan can be easily "cooked" out of most edible brown algae by simmering for 20-40 min. in water. When consumed, it seemed to reduce the intensity of the inflammatory response and promote more rapid tissue healing after wound or surgical trauma. This led to recommendations that brown seaweed broth be administered after auto-collision, sports injuries, bruising falls, muscle and joint damage and deep tissue cuts, including surgery [209].

However, the commercial importance of fucoidans is presently much lower than that of seaweed hydrocolloids; these polysaccharides are now attracting considerable attention because of the growing market for them as "bioactive polysaccharides" in a wide arena of diverse applications [210]. More recently, anticoagulant and antithrombotic activities are amongst the most studied biological effects of fucoidans. Commonly, the anti-coagulant activity of fucoidans is mediated through the activation of thrombin inhibitors, although direct thrombin inhibition and competitive binding of fibrinogen to block the actions of thrombin are also possible [210-212].

Several studies reported that the anticoagulant functionalities of fucoidans extracted from *Fucus vesiculosus* and *Eklonia cava* were due to thrombin-inhibition-mediated via plasma anti-thrombin-III; their anti-coagulant activity was like that of heparin [13,213,214]. Moreover, fucoidans from *Ascophyllum nodosum*, *Fucus distichous*, *F. evanescens*, *F. serratus*, *Laminaria digitata*, *Saccharina latissima* and *S. longissima* were also described to possess strong anticoagulant activities both *in vitro* and *in vivo* models [213,214].

In general, structural-bioactive studies suggested that the anticoagulant /anti-thrombin activities of fucoidans were mainly dependent on the content and/or positioning of sulfate groups, as well as the molecular weight of the polymers [213]. Likewise, their monomeric composition, types of linkages and branching might also exert moderate modulation on the biological properties of fucoidans [13,210,215]. In this context, it is possible that the greater anticoagulant/antithrombin activities exhibited by longer fucoidans are due to the higher content of fucose and sulfate groups [13,209,213,215,216], though this is still under debate [3,217].

Fucoidans are also reported to inhibit the replication of several enveloped viruses such as Human immune-deficiency and human cytomegalovirus, among others [13,213]. The mechanisms for such activity are thought to occur *via* inhibition of cell infection by viral sorption, or hampering of viral-induced, syncytium formation [13,215]. Fucoidans from *Saccharina japonica, Cladosiphon okamuranus, Adenocystis utricularis, Stoechospermum marginatum, Cystoseira indica, Dictyota mertensii, Lobophora variegata, Fucus vesiculosus, Spatoglossum schroederi* and *Undaria pinnatifida* showed impressive, positive results in both *in vitro* and *in vivo* models of infection by poliovirus III, adenovirus III, ECHO6 virus, coxsackie B3 virus, coxsackie A16, Newcastle disease virus (NDV), Herpes simplex virus (HSV-1, HSV-2), HIV and avian reverse transcriptase [13,212,215].

Fucoidan isolated from the brown seaweed *Fucus vesiculosus* [218] shows inhibitory effect on the replication of DNA viruses: Herpes virus (HSV-1, HSV-2) and Human cytomegalo virus (HCMV) [218-220]. These compounds are also active against RNA viruses: Vesicular stomatitis virus (VSV), Sinbis virus (SINV), and HIV-1 [221]. However, this compound is not active against Coxsackievirus, Poliovirus, and Parainfluenza virus. A water-soluble, non-carbohydrate component of fucoidan isolated from *F. vesiculosus* can inhibit HIV Reverse transcriptase (RT) *in vitro* at μg mL^{-1} [220]. Pre-incubation of cell-free virus to 200 μg mL^{-1} causes 100% reduction in the amount of HIV-1 p24 antigen released. Tests show that these effects are not due to the killing of target cells. In fact, fucoidan produced no adverse effects on cell proliferation and protein metabolism. The pre-incubation of target cells with fucoidan protects them from HIV-1 infection [220]. In addition to its antiviral activity, fucoidan possesses low anticoagulation properties [212,218-220,222].

Antitumor activities of fucoidans include the inhibition of tumor proliferation, the stimulation of tumor cell apoptosis, blocking of tumor cell metastasis and enhancement of various immune responses [223]. In this context, fucoidans from several macroalgal species (e.g. *Saccharina japonica, S. latissima, Laminaria digitata, Fucus serratus, F. distichus* and *F. vesiculosus*) proved to be useful and were regarded as good candidates for future cancer therapy [213,215]. Besides those, the commercial fucoidans branded "Tokida" (from cultured *Cladosiphon okamuranus*) and that from the Korean cultured sporophyte (Miyeokgui) of *U. pinnatifida* also revealed promising antitumoral activities, as tested in *in vitro* models [215].

Other important, demonstrated biological activities of fucoidans included antioxidant, anti-inflammatory and anti-allergic, although others cannot be forgotten, e.g. hepato-protection, cardio-protection, stomach protection and anti-obesity [13,213,215]. Examples of fucoidans showing promising antioxidant activities in *in vitro* models included those obtained from *Saccharina japonica, Canistrocarpus cervicornis, Fucus vesiculosus, Dictyota cervicornis, Sargassum filipendula* and *Dictyopteris delicatula* [13,213,215,224], while the anti-inflammatory activity of several fucoidans (*Laminaria digitata, Fucus evanescens, F. serratus, F. distichus, F. spiralis, Ascophyllum nodosum, Cladosiphon okamuranus, Padina gymnospora* and *Saccharina latissima*) have been described through inhibition of leucocyte recruitment, in an inflammation model in rats [225]. Moreover, inhibition of the expression of inducible nitric oxide synthase (iNOS) has also been demonstrated for fucoidans, such as that from the Sigma-Aldrich Chemical Co. (from *F. vesiculosus*). Furthermore, commercial fucoidans (from Mekabu and Sigma-Aldrich Chemical Co.) together with those isolated from *A. nodosum, F. evanescens, C. okamuranus* and from other several members

of the Order Laminariales (Phaeophyceae) were described to exhibit anti-complementary activities, rendering their potential as anti-allergen [13,121,213,215].

The sulfated and acetylated fucoidan fraction, containing fucose, galactose, mannose, glucose and uronic acid residues, was isolated from the brown alga *Padina boryana* [226]. The anticancer effect of native and modified fucoidan fractions was studied *in vitro* on the colorectal carcinoma cells DLD-1 and HCT-116. All fucoidans had no cytotoxicity under 400 μg/mL and inhibited colony formation of cancer cells at concentration of 200 μg/mL [226].

4.3. Laminaran

Laminarans are a category of small glucans present in either soluble or insoluble form. The first form is characterized by its complete solubility in cold water, while the other is only soluble in hot water [207,298]. This polysaccharide is composed of D-glucose with β-(1,3) linkages, with β-(1,6) intra-chain branching (Figure 6) [17,227-229]. Laminaran, also known as "laminarin" or "leucosin", was first isolated from members of the Laminariaceae (Phaeophyceae) by Schmiedeberg [230]. Laminaran is a food reserve of brown algae. It is located in vacuoles present in cells. Laminaran is found in the fronds of *Laminaria* and *Saccharina* species [231].

Laminarans are basically a class of low-molecular weight storage β-glucans, which are composed of (1,3)- β -D-glucan [232]. They consist of (1,3)- β -D-glucopyranose residues with some 6-O-branching in the main chain and some β-(1,6)-intra-chain links are also present. For example, laminaran from *Eisenia bicyclis* is composed of a linear chain of (1,3) and (1,6) linkage in a ratio of 2:1 (1,3)- β -D-glucans [233]. The molecular weight of laminaran is approximately 5 kDa and is dependent upon the degree of polymerization which is in the range of 20-25 glucose moieties [234,235]. The molecular weight of laminaran from *Saccharina longicruris* is reported to be in the range of 2.89 - 3.32 kDa depending on the extraction conditions [236], including the solvent type used [228]. Not all laminarans are the same characteristics, different species of raw material present different structures of molecules, therefore different biological properties [228,229].

In nature, laminarins exist in either soluble or insoluble forms. The soluble form is characterized by complete solubility in cold water, and the insoluble form can be dissolved only in hot-water solutions. The solubility of laminarins is determined by the branching factor: laminarins with the highest branching are easily dissolved in cold water in comparison with laminarins with less branching. Moreover, the degree of polymerization of water-soluble laminarins is approximately 20-25 glucose units [236,237].

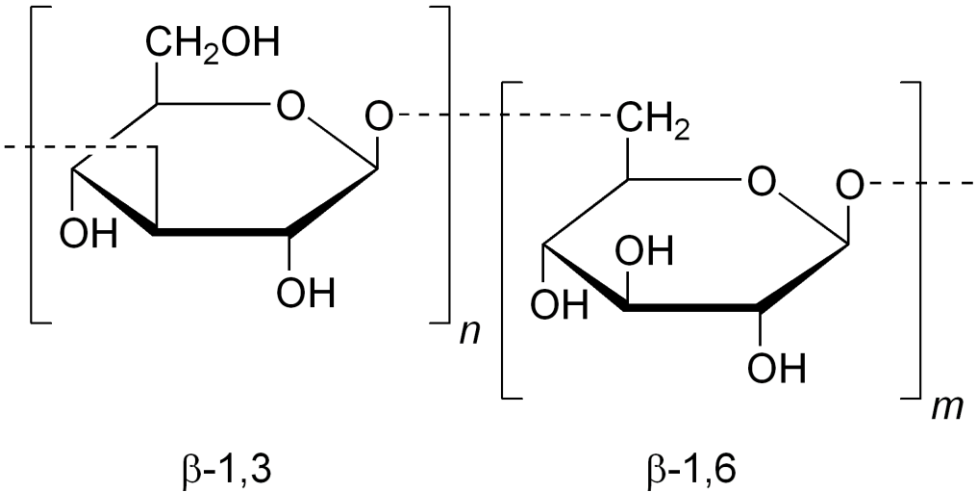


Figure 6 - Chemical structure of β-1,3-1,6-glucan (Laminarin, Laminaran) (after [328]).

4.4. Biological activities of Laminaran

The laminaran prepared from kelp by water extraction were reported to be able to effectively inhibit the adsorption of HIV on lymphocytes and the activity of HIV reverse transcriptase at the concentration of 50 μg mL^{-1}, which suggest that laminaran polysaccharides possess good inhibitory effect on HIV replication [238].

Laminaran has many reported bioactivities such as anticancer, anti-inflammatory, anticoagulant, and antioxidant effects [227]. Studies on feeding of laminaran-containing foods to animals (pigs) indicate that laminarans hold promise as a functional ingredient in food applications [239].

Algae polysaccharides have numerous biological activities because they enhance macrophage immune responses [240]. Similarly, laminaran can be used to achieve the activation of macrophages leading to immune-stimulatory, antitumor and wound-healing activities [241,242]. Thus, it is also termed as a biological response modifier [243]. The biological activities of laminarans can be enhanced or modified using various techniques including irradiation, sulfation, reduction and oxidation. During irradiation, the molecular weight of the laminarans investigated was reported to be significantly reduced with the formation of carbonyl groups. This is suggested to lead to enhanced antioxidant activities.

Ermakova et al. [245] demonstrated that water-soluble laminaran isolated from *Ecklonia bicyclis* (as *Eisenia bicyclis*) has no cytotoxic effect on Human malignant melanoma (SK-MEL-28) and Human colorectal adenocarcinoma cells (DLD-1) after treatment for 24, 48, and 72 h (1-200 μg/mL). However, the authors reported that incubation of laminaran with these cancer cells significantly inhibits colony formation (for SK-MEL-28 Human melanoma cells by 32% and 42% respectively at 100 and 200 μg/mL). Usoltseva and co-workers [246] also researched the inhibitory effects of laminarans isolated from *Alaria marginata* and *Alaria angusta* on colony formation by 3 cancer cell lines: Human colon adenocarcinoma cells (HT-29), Human breast cancer cells (T47D), and Human malignant melanoma (SK-Mel-28). Laminaran concentrations less than 400 μg/mL were not toxic to the tested cancer cell lines. However, the authors found that at the concentration of 100 μg/mL, laminaran has an inhibitory effect on colony formation on HT-29 cells compared to the control group (*A. angusta*

laminaran by 22% and *A. marginata* laminaran by 6%). In addition to these significant results, the authors reported that modifications of laminarans (deacetylation and desulfation) cause a loss of the anticancer activity under the tested conditions [237].

Ji and Ji [247] studied the anticancer effect of commercial laminaran on human colorectal adenocarcinoma (LoVo) cells. Their results revealed that incubation of cancer cells with laminaran (400-1600 μg/mL) for 72 h induces apoptosis in LoVo cells. Additionally, Ji et al. [248] studied anticancer activity of laminaran after modifying the sulfate content of laminaran by the chlorosulfonic-acid-pyridine method. The authors compared the anticancer effects of normal and modified laminaran on LoVo cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. At the concentration of 1600 μg/mL, modified laminaran could reduce the number of LoVo cells by 86%. However, at the same concentration, unmodified laminaran could reduce the cancer cell number by only 38%. Thus, the authors proposed that sulfation of the structure might increase bioactivities of laminaran [248].

In the works of Malyarenko et al. [249], the laminarans from brown seaweeds *Saccharina cichorioides*, *Saccharina japonica*, and *Fucus evanescens* were isolated and their sulfated derivatives were tested. The latter inhibited proliferation, colony formation, and migration of human colorectal adenocarcinoma, melanoma, and breast adenocarcinoma cells in different manners. The sulfated laminaran from *F. evanescens* possessed the highest anticancer activity *in vitro* and effectively prevented migration of breast adenocarcinoma cells by inhibiting of the Matrix Metalloproteinases 2 and 9 activity [249].

The effect of laminaran and fucoidan extracted from *Dictyota dichotoma* (40 μg/mL) on colony formation of colorectal adenocarcinoma (DLD-1) cancer cells after irradiation (4 Gy) showed a synergistic effect with X-ray irradiation against cancer cells, decreasing the amount and size of cancer cells colonies [250].

4.5. Alginates

"Alginate" is the term usually used for the salts of alginic acid, but it can also refer to all the derivatives of alginic acid and alginic acid itself; in some publications the term "algin" is used instead of alginate. Chemically, alginates (Figure 7) are linear copolymers of β-D-mannuronic acid (M) and α-L-guluronic acid (G) (1-4)-linked residues, arranged either in heteropolymeric (MG) and/or homopolymeric (M or G) blocks [251-253].

Alginic acid was discovered in 1883 by E.C.C. Stanford, a British pharmacist who called it algin. Alginic acid is extracted as a mixed salt of sodium and/or potassium, calcium and magnesium. Since Stanford discovered algin, the name has been applied to a number of substances, e.g. alginic acid and all alginates, derived from alginic acid. The extraction process is based on the conversion of an insoluble mixture of alginic acid salts of the cell wall in a soluble salt (alginate) which is appropriate for the water extraction [254,255]. Alginic acid is present in the cell walls of brown seaweeds, where it is partially responsible for their flexibility [256].

While any brown seaweed could be used as a source of alginate, the actual chemical structure of the alginate varies from one genus to another, and similar variability is found in the properties of the alginate that is extracted from the seaweed. Since the main applications of alginate are in thickening aqueous solutions and forming gels, its quality is judged on how well it performs in these uses [256].

25 to 30 years ago almost all extraction of alginates took place in Europe, USA, and Japan. The major change in the alginates industry over the last decade has been the emergence of producers in China in the 1980s. Initially, production was limited to low cost, low quality alginate for the internal, industrial markets produced from the locally cultivated *Saccharina japonica*. By the 1990s, Chinese producers were competing in western industrial markets to sell alginates, primarily based on low [38,257].

A high-quality alginate forms strong gels and gives thick, aqueous solutions. A good raw material for alginate extraction should also give a high yield of alginate. Brown seaweeds that fulfill the above criteria are species of *Ascophyllum, Durvillaea, Ecklonia, Fucus, Laminaria, Lessonia, Macrocystis* and *Sargassum*. However, *Sargassum* is only used when nothing else is available: its alginate is usually borderline quality and the yield usually low [258].

The goal of the extraction process is to obtain dry, powdered, sodium alginate. The calcium and magnesium salts do not dissolve in water; the sodium salt does. The rationale behind the extraction of alginate from the seaweed is to convert all the alginate salts to the sodium salt, dissolve this in water, and remove the seaweed residue by filtration [256].

Water-in-oil emulsions such as mayonnaise and salad dressings are less likely to separate into their original oil and water phases if thickened with alginate. Sodium alginate is not useful when the emulsion is acidic, because insoluble alginic acid forms; for these applications propylene glycol alginate (PGA) is used since this is stable in mild acid conditions. Alginate improves the texture, body and sheen of yoghurt, but PGA is also used in the stabilization of milk proteins under acidic conditions, as found in some yoghurts. Some fruit drinks have fruit pulp added and it is preferable to keep this in suspension; addition of sodium alginate, or PGA in acidic conditions, can prevent sedimentation of the pulp and to create foams. In chocolate milk, the cocoa can be kept in suspension by an alginate/phosphate mixture, although in this application it faces strong competition from carrageenan. Small amounts of alginate can thicken and stabilize whipped cream [135,259].

Alginates have several commercial applications based on their thickening, gelling, emulsifier and stabilizing abilities. They are used in the food industry for improving the textural quality of numerous products such as salad dressing, ice-cream, beer, jelly and lactic drinks, but also in cosmetics, pharmaceuticals, textiles and painting industries [5,38,260].

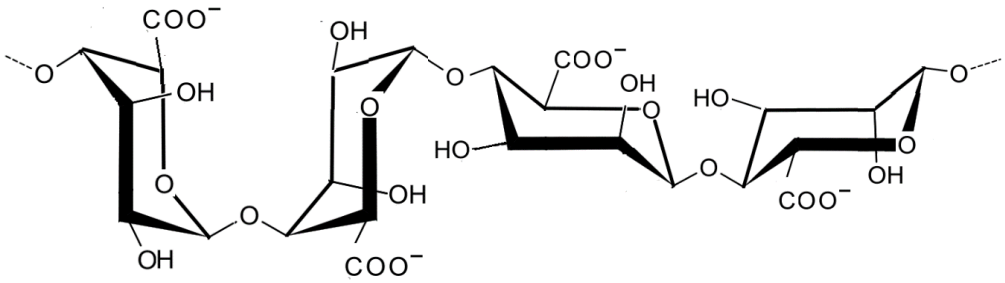


Figure 7 - Idealized structure of the chemical units of alginic acid

4.5. Biological activities of Alginates

Alginates have anticancer properties [5,147,261] and a bioactive food additive "Detoxal", containing calcium alginate, has anti-toxic effects on hepatitis. This drug decreases the content of lipid peroxidation products and normalizes the concentrations of lipids and glycogen in the liver [71,262].

Alginate molecules are playing vital role in the fields of pharmacy and medicine. Due to its ability to retain water, is mainly used as gelling and viscosifying agent. In controlled drug delivery systems, alginates can be used. In this case, the rate of drug release depends on the type and molecular weight of alginates used. Alginates have been mostly used marine derived polymers in the preparation of drug delivery particles with the shape of spheres of different sizes [263]. Alginate instantaneously forms gel-spheres at pH > 6 by ionotropic gelation with divalent cations such as Ca^{2+} [264], Ba^{2+}, or Zn^{2+} and for this it is widely used for microencapsulation of drugs. On the other hand, at low pH, hydration of alginic acid leads to the formation of a high-viscosity "acid gel". The ability of alginate to form two types of gel dependent on pH, i.e., an acid gel and an ionotropic gel, gives the polymer unique properties compared to neutral macromolecules, and it can be tailor-made for many applications [147].

The microencapsulation technique has been developed particularly for the oral delivery of proteins, as they are quickly denatured and degraded in the hostile environment of the stomach. The protein is encapsulated in a core material that, in turn, is coated with a biocompatible, semi permeable membrane, which controls the release rate of the protein while protecting it from biodegradation. Due to its mild gelation conditions at neutral pH, alginate gel can act as core material in this application, while poly(ethylene glycol) (PEG), which exhibits properties such as protein resistance, low toxicity and immunogenicity [265], together with the ability of preserving the biological properties of proteins [266,267], can act as a coating membrane. A chitosan/PEG-alginate microencapsulation process [268], applied to biological macromolecules such as albumin or hirudin [269], was reported to be a good candidate for oral delivery of bioactive peptides.

Several examples of alginate-encapsulated drugs, other than proteins, can also be found in literature. Qurrat-ul-Ain et al. [270] reported that alginate microparticles showed better drug bioavailability and reduction of systemic side effects compared with free drugs in the treatment of tuberculosis. Polyelectrolyte coating of alginate microspheres showed to be a promising tool to achieve release systems characterized by approximately zero-order release kinetics, release up to 100% of entrapped drug (dexamethasone) within 1 month, and improved biocompatibility [271]. Composites technology has been applied to alginate for drug delivery purposes. As an example, montmorillonite-alginate nanocomposites have been recently proposed as a system for sustained release of B_1 and B_6 vitamins [272]. The vitamins intercalated in the nanocrystals of the inorganic phase, and successively the hybrid B_1/B_6 montmorillonite (MMT) is further used for the synthesis of B_1/B_6-MMT-alginate nanocomposite.

In their simplest design, oral controlled-release dosage forms made from alginates are monolithic tablets in which the drug is homogeneously dispersed. Drug release is controlled by the formation of a hydrated viscous layer around the tablet, which acts as a diffusional barrier to drug diffusion and water penetration. Water soluble drugs are mainly released by diffusion of dissolved drug molecules across the gel layer, while poorly soluble drugs are mainly released by erosion mechanisms. Modulation of drug release rate has been achieved by incorporating pH-independent hydrocolloids gel-

ling agents or adding polycationic hydrocolloids such as chitosan [273,274]. Several mucoadhesive systems based on alginate have been developed [275,276]. The main shortcoming of alginates consists in their rapid erosion at neutral pH; furthermore, the adhesion to mucosal tissues is reduced when cross-linked with divalent cations. Alginates have been extensively used to modify the performances of other polysaccharides, such as chitosan, through the realization of alginate coated chitosan microspheres [277]. In the literature, it is also possible to find acrylic modified polysaccharides developed with the aim to obtain a finer control over release rate or to improve adhesive properties [278,279].

Due to the large variety of possible chemical compositions and molecular weights of alginate preparations resulted in different effects on biological systems. A biological effect of alginate initially was hinted at in the transplantation trails of encapsulated Langerhans islets for diabetes control. Over growth of alginate capsule by phagocytes and fibroblasts resembling foreign body/inflammatory reaction was reported. The main hurdles to the widespread use of islet transplantation for the treatment of type 1 diabetes continue to be the insufficient number of appropriate donors and the need for immunosuppression. Microencapsulation has been proposed to protect transplanted islets from the host's immune system. The study made by Qi et al. [280] investigated the function of human pancreatic islets encapsulated in Ca^{2+}/Ba^{2+}– alginate micro beads intraperitoneally transplanted in diabetic Balb/c mice and concluded that The Ca^{2+}/Ba^{2+}– alginate microbeads can protect human islets from xenogeneic rejection in immune competent mice without immunosuppression. However, grafts ultimately failed likely secondary to a macrophage-mediated foreign body reaction [281].

Alginate forms mild gelation by addition of divalent cation Ca^{2+} and have been extensively studied and applied as a biomaterial in wound healing, tissue engineering [282], orthopedics, and dental implant surgery because of its low toxicity, relatively low cost, good biocompatibility, and osteoconductivity [32,283]. The thickening, gel-forming and stabilizing property of alginate adds it amongst the most widely used biopolymer with broader range of applications including tissue engineering, drug delivery, biosensor, and wound dressing [284].

Composite materials of alginate with ceramics play a major role in increasing the mechanical strength of extracellular matrix. Injectable calcium phosphate-alginate hydrogel-umbilical cord mesenchymal stem cell (UCMSC) paste reported by Zhao et al. [285] showed remarkable osteogenic differentiation with alkaline phosphatase activity (ALP), osteocalcin (OC), collagen I, and mineralization expressions. Bouhadir et al. [286] introduced hydrogel containing chondrocytes in to the dorsal region of mice. After 7 weeks the mice were dissected; hydrogel-containing chondrocytes scaffolds uncovered and removed. A white opalescence was observed in which appearance of native cartilage was confirmed by standard trichrome blue staining. Reduction in weight of the hydrogel implant within a span of 7 weeks was attributed to the degradation of the hydrogel and release of oxidized alginate much faster than *in vitro* studies on the same construct revealed. These results were in contrast with the unmodified alginate and chondrocytes in which small cartilage-like tissues were surrounded by huge amount of residues from the alginate. This injectable scaffold could find way in regeneration of cartilage like tissues. Moshaverinia et al. [287] developed an injectable and biodegradable oxidized alginate microbeads encapsulation to periodontal ligament and gingival mesenchymal stem cells (GMSCs) and *in vitro* study

showed high level osteo-differentiation and adipo-differentiation [32,287].

Alginates, fucoidans and laminaran have also antibacterial effect. Extracts were tested against nine bacteria, including *Escerichia coli, Salmonella, Staphycococcus* and *Listeria*. They appeared to be effective against *E. coli* and *Staphylococococcus*. Sodium alginate seemed to demonstrate a strong antibacterial element. It not only binds but also kills the bacteria. Studies conducted on seaweed extracts found that fucoidan appeared to function as a good prebiotic (a substance that encourages the growth of beneficial bacteria in the intestines). An anti-inflammatory effect from some of the extracts has also been found, and so far, no toxic effects have emerged in use for human health [281,288,289].

A prominent marine polysaccharide drug named "911" derived from alginate polysaccharide exhibited promising activity against Human Immunodeficiency virus (HIV-1) at both chronic infection of Human Cutaneous T lymphocyte (H9) cells and acute infection of Human Tcell leukemia (MT4) cells *in vitro* and *in vivo* [102]. These special effects revealed that "911" drug inhibited the viral replication of HIV via significantly decrementing the activity of reverse transcriptase (RTase), discontinuing the virus adsorption, and improving the defense mechanisms of the host cells [290-292]. Alternative inhibitory result was also reported later for hepatitis B virus (HBV) that "911" drug could inhibit the virus replication by suppressing the activity of DNA polymerase activity [293]. Wang and colleagues discovered that the sulfated polymannuroguluronate (SPMG) [294], the sulfated form of alginate, is a characteristic anti-AIDS drug candidate, as it caused the inhibition of HIV-1 infection mainly through the robust attachment of virus gp120 protein with CD4 (the cluster of differentiation 4 is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells) molecules on the surface of lymphocyte (T) cells. Furthermore, there is huge correlation between the size of the sulfated SPMG oligosaccharides and their inhibitory significance that the octasaccharide will be the minimal active fragment preventing syncytium formation and reducing the p24 core antigen level in HIV-IIIB-infected CEM cells (a cell line derived from human T cells) [295,296].

Moreover, alginates with molecular weights greater than or equal to 50 kDa could prevent obesity, hypocholesterolemia and diabetes [281,297-300]. Clinical observations of volunteers who were 25-30% overweight showed that alginate, a drug containing alginic acid, significantly decreased body weight [301,302]. Cooking into the bread does not reduce the molecular size of the alginate or affect its inhibition properties. These data demonstrate the robustness of alginates lipase inhibition despite the cooking process and digestion. Therefore, adding alginate to a bread vehicle may have the potential in the treatment for obesity [303]. In type II diabetes treatment, taking 5 g of sodium alginate, every morning was found to prevent a postprandial increase of glucose, insulin, and C-peptide levels and slowed down gastric transit [304]. Meal supplemented with 5% Kelp alginates decreased glucose absorption balance over 8 h in pigs. Similar studies have been done on rats and humans [305,306].

Another health effect is that the binding property of alginic acid to divalent metallic ions is correlated to the degree of the gelation or precipitation in the range of Ba<Pb<Cu<Sr<Cd<Ca<Zn<Ni<Co<Mn<Fe<Mg. No intestinal enzymes can digest alginic acid. This means that heavy metals taken into the human body are gelated or rendered insoluble by alginic acid in the intestines and cannot be absorbed into the body tissue [307]. In several countries such as the Germany, Japan, Belgium, USA and

Canada, the use of alginic acid and its derivatives for the treatment of gastritis and gastroduodenal ulcers, as well as the use of alginates as anti-ulcer remedies, is protected by patents [3,281,308-310]. Several products of alginate containing drugs have been shown to effectively suppress postprandial (after eating) and acidic refluxes, binding of bile acids and duodenal ulcers in humans [310]. Examples are "Gaviscon" (sodium alginate, sodium bicarbonate, and calcium carbonate), "Algitec" (sodium alginate and cimetidine, an H2 antagonist) and "Gastralgin" (alginic acid, sodium alginate, aluminum hydroxide, magnesium hydroxide and calcium carbonate) [262,281,311-313]. Clinical trials showed that sodium alginate promotes regeneration of the mucous membrane in the stomach, suppresses inflammation, eradicates colonies of *Helicobacter pylori* in the mucous membrane and normalizes non-specific resistance of the latter in 4 to 15-year-old children. It also promotes restoration of the intestinal biocenosis [71,281,314]. Other studies show positive dietary effects of alginates on fecal microbial fauna, changes in concentrations of compounds and acids, and prebiotic properties that can promote health [71,315, 316].

A study conducted by Zaharudin et al. [317] investigated the potential of dried edible seaweed extracts and alginates for α-amylase inhibitory effects. Alginates found in *Laminaria digitata* and *Undaria pinnatifida* (Phaeophyceae) appeared to be potent inhibitors of α-amylase activity with an IC_{50} of (0.075 +/- 0.010-0.103 +/- 0.017) mg/mL, also a mixed-type inhibition. Overall, the findings provide information that crude extracts of brown edible seaweeds, phenolic compounds and alginates are potent α-amylase inhibitors, thereby potentially retarding glucose liberation from starches and alleviation of postprandial hyperglycemia [317].

4.6. Ascophyllan

Ascophyllan isolated from the brown alga *Ascophyllum nodosum* (Phaeophyceae) is a fucose-containing sulfated polysaccharide, which has similar but distinct characteristic monosaccharide composition and entire chemical structure to fucoidan [322]. Ascophyllan (xylofucoglycuronan) has similar but obviously distinct composition characteristics from those of fucoidans isolated from *A. nodosum* and *Fucus vesiculosus* [323,324]. Specifically, ascophyllan has fucose and xylose in about equimolecular proportion, whereas fucoidans have much higher ratio of fucose than of xylose. The sulfate levels of ascophyllan and fucoidans isolated from *A. nodosum* and *F. vesiculosus* were 9.6, 19.4, and 22.6%, respectively [325]. In addition, the apparent molecular mass of ascophyllan estimated by gel filtration chromatography was about 390 kDa, which is much higher than that of fucoidan [326].

4.7. Biological activities of Ascophyllan

Mohsin et al. [318] characterize and evaluate the antioxidant activity of a sulfated polysaccharide ascophyllan isolated from marine brown algae *Padina tetrastromatica*. The results showed that one of the ascophyllan fractions showed stronger free-radical-scavenging abilities and had good antioxidant effect. Available data obtained by *in vitro* models suggest that there is a correlation between the sulfate content and antioxidant activity.

In work carried out by Jiang et al. [319], ascophyllan and crude extract administered *via* the oral route showed greater antitumor effects than via IP (Intraperitoneal) route, and the tumor sizes in mice treated with ascophyllan and crude extract were reduced by a mean of 68.7% and 42.4% by the oral route, and 41.4% and 13.6% by IP route compared to control mice. Splenic natural

killer cell activity in the mice treated with ascophyllan and crude extract by IP route was significantly enhanced, while only a slight increase of this activity was observed in orally-treated mice. Furthermore, increase in spleen weight of tumor-bearing mice was slightly suppressed by oral administration of ascophyllan, whereas IP administration resulted in further enlargement. Analysis of serum cytokines revealed that oral treatment with ascophyllan resulted in significant increase of tumor necrosis factor-α and interleukin-12 levels. Since ascophyllan showed no direct cytotoxic effect on sarcoma-180 cells, orally-administered ascophyllan is suggested to exhibit its antitumor activity through the activation of the host immune system [319].

Zhang et al. [320] investigated the effect of ascophyllan, a sulfated polysaccharide purified from *Ascophyllum nodosum*, on the maturation of mouse dendritic cells (DCs) *in vitro* and *in vivo*. Ascophyllan induced up-regulation of co-stimulatory molecules and production of pro-inflammatory cytokines in bone marrow-derived DCs (BMDCs). Interestingly, ascophyllan induced a higher degree of co-stimulatory molecule up-regulation and pro-inflammatory cytokine production than fucoidan, a marine-derived polysaccharide with well-defined effect for promoting DCs maturation. Ascophyllan also promoted the generation of IFNγ (Interferon gamma)-producing Th1 (murine Type 1 helper T cells) and Tc1 (Type 1 cytotoxic T lymphocytes) cells in the presence of DCs in an IL (cytokines)-12-dependent manner. Finally, myeloid differentiation primary response 88 (MyD88) signaling pathway was essential for DCs maturation induced by ascophyllan. Taken together, these results demonstrate that ascophyllan induces DCs maturation, and consequently enhances Th1 and Tc1 responses *in vivo*. This knowledge could facilitate the development of novel therapeutic strategies to combat infectious diseases and cancer [320,329].

Later, the same team [321] evaluated ascophyllan as an adjuvant for its therapeutic and preventive effect on tumor in a mouse melanoma model. Ascophyllan induced migration of DCs to spleen and tumor-draining lymph node (drLN) in a mouse B16 melanoma model. Moreover, ascophyllan induced activation of dendritic cells (DCs), and promoted IFN-γ- and TNF-α-producing murine type 1 helper T cells (Th1) immune responses in tumor-bearing mice. In addition, treatment with a combination of ascophyllan and ovalbumin (OVA) in the tumor-bearing mice promoted proliferation of OVA-specific CD4 and CD8 T cells and migration of those cells into the tumor, consequently inhibiting the tumor growth. Immunization with the combination of ascophyllan and OVA caused enhanced OVA-specific antibody production and memory T cell responses compared to OVA immunization alone, and almost completely prevented B16-OVA tumor growth upon subsequent tumor challenge. Lastly, the combination of ascophyllan and OVA prevented B16-OVA tumor invasion and metastasis into the liver. Therefore, these results demonstrate that ascophyllan can function as an adjuvant to induce DCs activation, antigen specific cytotoxic T lymphocyte (CTL) activation, Th1 immune response and antibody production, and hence may be useful as a therapeutic and preventive tumor vaccine [321].

5. Conclusion

Currently, seaweeds are used in many countries for very different purposes. Most of macroalgae are used directly for human consumption, while the remaining portion is used for industrial exploitation of macroalgae derived products, among which polysaccharides have many uses. The polysaccharides produced by the marine macroalgae have varied bioactivities, in which they stand out the antioxidant, anticoagulant, antithrombotic, antitumor, anti-inflamma-

tory, antiviral, antiprotozoal, and other activities [329]. Among the various bioactivities listed in this review, polysaccharides may be used as potential ingredients in future pharmaceutical products.

Acknowledgements

This work had the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UID/MAR/04292/2013 granted to MARE. This work had the support from the European Structural & Investment Funds through the COMPETE Programme and from National Funds through FCT – Fundação para a Ciência e a Tecnologia under the Programme grant SAICTPAC/0019/2015. This work is also co-financed by the European Regional Development Fund through the Interreg Atlantic Area Programme, under the project NASPA.

References

1. Pereira L. Guia ilustrado das macroalgas - conhecer e reconhecer algumas espécies da flora Portuguesa (Illustrated guide of macroalgae - to know and recognize some species of the Portuguese flora). Imprensa da Universidade de Coimbra, Coimbra, Portugal, **2009**, 90 pp. (*in Portuguese*).

2. Pereira L. Chapter 4 - Cytological and cytochemical aspects in selected carrageenophytes (Gigartinales, Rhodophyta). *In*: Heimann, K. and Katsaros, C. (eds.), Advances in Algal Cell Biology. De Gruyter, Berlin, **2012**, pp. 81-104.

3. Cardoso SM, Loïc GC, Paulo JS, Rodrigues MS, Pereira OR, Pereira L. Bioproducts from macroalgae: A review with focus on the Iberian Peninsula. *Current Organic Chemistry* **2014**, *18*(7), 896-917.

4. Holdt SL, Kraan S. Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, **2011**, *23*(3), 543-597.

5. Murata M, Nakazoe J. 2001. Production and use of marine algae in Japan. Japan *Agricultural Research Quartery*, **2001**, *35*, 281-290.

6. Chandini SK, Ganesan P, Bhaskar N. *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chem*, **2008**, *107*(2), 707-713.

7. Pereira L, Amado AM, Critchley AT, van de Velde F, Ribeiro-Claro PJA. Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). *Food Hydrocolloids*, **2009**, *23*, 1903-1909.

8. Bixler HJ, Porse H. A decade of change in the seaweed hydrocolloid industry. *Journal of Applied Phycology*, **2011**, *23*, 321-335.

9. Pereira L, van de Velde F. Portuguese carrageenophytes: carrageenan composition and geographic distribution of eight species (Gigartinales, Rhodophyta). *Carbohydrate Polymers*, **2011**, *84*, 614-623.

10. Pereira L. Population studies and carrageenan properties in eight Gigartinales (Rhodophyta) from western coast of Portugal. *The Scientific World Journal*, **2013**, 11 pp. https://www.hindawi.com/journals/tswj/2013/939830/

11. Minghou J. Processing and extraction of phycocolloids, pp 85-96. *In*: FAC/ACA. Technical Resource Papers. Regional Workshop on the Culture and Utilization of Seaweeds. Vol. II (Regional Sea Farming Development & Demonstration Project RAS/ 901002. 27-31 August 1990. Cebu City, Philippines. Regional Sea Farming Development & Demonstration Project/ Network of Aquaculture Centres in Asia. Bangkok, Thailand, **1990**.

12. Lahaye M, Robic A. Structure and functional properties of Ulvan, a polysaccharide from green seaweeds. *Biomacromolecules*, **2007**, *8*(6), 1765-1774.

13. Jiao GL, Yu GL, Zhang JZ, Ewart SH. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Marine Drugs*, **2011**, *9*(2), 196-223.

14. Robic A, Rondeau-Mouro C, Sassi JF, Lerat Y, Lahaye M. Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae). *Carbohydrate Polymers*, **2009**, *77*(2), 206-216.

15. Percival EJV, McDowell RH. Chemistry and enzymology of marine algal polysaccharides. Academic Press, London, New York, **1967**, 219 pp.

16. Quemener B, Lahaye M, Bobin-Dubigeon C. Sugar determination in ulvans by a chemical-enzymatic method coupled to high performance anion exchange chromatography. *Journal of Applied Phycology*, **1997**, *9*, 179-188.

17. Pereira L, Ribeiro-Claro PJA. Analysis by vibrational spectroscopy of seaweed with potential use in food, pharmaceutical and cosmetic, pp. 225-247. *In*: Leonel Pereira and João M. Neto (eds.), Marine Algae - Biodiversity, Taxonomy, Environmental Assessment and Biotechnology. Science Publishers, an Imprint of CRC Press / Taylor & Francis Group, Boca Raton, FL, **2015**.

18. Qi H, Zhang Q, Zhao T, Chen R, Zhang H, Niu X. Antioxidant activity of different sulphate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta*) in vitro. International Journal of Biological Macromolecules*, **2005**, *37*(4), 195-199.

19. 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). *Bioorganic Medicinal Chemistry Letters*, **2006**, *16*(9), 2441-2445.

20. Zhang HJ, Mao WJ, Fang F, Li HY, Sun HH, Gehen Y, Qi XH. Chemical characteristics and anticoagulant activities of a sulphated polysaccharide and its fragments from *Monostroma latissimum*. *Carbohydrate Polymers*, **2008**, *71*(3), 428-434.

21. Kaeffer B, Benard C, Lahaye M, Blottiere HM, Cherbut C. Biological properties of ulvan, a new source of green seaweed sulphated polysaccharides, on cultured normal and cancerous colonic epithelial cells, *Planta Medica*, **1999**, *65*(6), 527-531.

22. Leiro JM, R. Castro R, Arranz JA, Lamas J. 2007. Immunomodulating

activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh. *International Immunopharmacology*, **2007**, *7*, 879-888.

23. Chiellini F, Morelli A. Ulvan: A versatile platform of biomaterials from renewable resources. *In*: R. Pignatello (ed.). Biomaterials - Physics and Chemistry. INTECH Open Access Publisher, **2011**. Available online at: https://www.intechopen.com/books/biomaterials-physics-and-chemistry/ulvan-a-versatile-platform-of-biomaterials-from-renewable-resources (accessed 2018)

24. Ivanova V, Rouseva R, Kolarova M, Serkedjieva J, Rachev R, Manolova N. Isolation of a polysaccharide with antiviral effect from *Ulva lactuca*. *Preparative Biochemistry*, **1994**, *24*, 83-97.

25. Karnjanapratum S, Tabarsa M, Cho M, You S. Characterisation and immunomodulatory activities of sulfated polysaccharides from *Capsosiphon fulvescens*. *International Journal of Biological Macromolecules*, **2012**, *51*, 720-729.

26. Choi J-S, Ha Y-M, Lee B-B, Moon HE, Cho KK, Choi IS. Seasonal variation of antibacterial activities in the green alga *Ulva pertusa* Kjellman. *Journal of Environmental Biology*, **2013**, *35*, 341-344.

27. Rizk MZ, Aly HF Matloub AA, Fouad GI. The anti-hypercholesterolemic effect of ulvan polysaccharide extracted from the green alga *Ulva fasciata* on aged hypercholesterolemic rats. *Asian Journal of Pharmaceutical and Clinical Research*, **2016**, *9*(3), 11 pp.

28. Taboada C, Millán R, Míguez I. Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. *Journal of the Science of Food and Agriculture*, **2010**, *90*, 445-449.

29. Patel S. Therapeutic importance of sulfated polysaccharides from seaweed: Updating the recent findings. *3 Biotech*, **2012**, *2*, 10. 71-185.

30. Cunha L, Grenha A. Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs*, **2016**, *14*(3), 42.

31. Alves A, Sousa RA, Reis RL. *In vitro* cytotoxicity assessment of ulvan, a polysaccharide extracted from green algae. *Phytotherapy Research*, **2013**, *27*, 1143-1148.

32. Venkatesan J, Lowe B, Anil S, Manivasagan P, Al Kheraif AA, Kang KH, Kim SK. Seaweed polysaccharides and their potential biomedical applications. *Starch-Starke*, **2015**, *67*(5-6), 381-390.

33. Toskas, G, Heinemann S, Heinemann C, Cherif C, Hund RD, V. Roussis V, Hanke T. Ulvan and ulvan/chitosan polyelectrolyte nanofibrous membranes as a potential substrate material for the cultivation of osteoblasts. *Carbohydrate Polymers*, **2012**, *89*(3), 997-1002.

34. Alves A, Sousa RA, Reis RL. Processing of degradable ulvan 3D porous structures for biomedical applications. *Journal of Biomedical Materials Research Part A*, **2013**, *101*(4), 998-1006.

35. Rossi F, van Griensven M. Polymer functionalization as a powerful tool to improve scaffold performances. *Tissue Engineering Part A*, **2014**, *20*(15-16), 2043-2051.

36. Tseng CK. The terminology of seaweed colloids. *Science*, **1945**, *101*(2633), 597-602.

37. van de Velde F, de Ruiter GA. Carrageenan, pp. 245-274. *In*: Vandamme, E.J., S.D. Baets and A. Steinbèuchel (eds.) Biopolymers V6, Polysaccharides II, Polysaccharides from Eukaryotes. Wiley, Weinheim, **2002**.

38. Pereira L. A review of the nutrient composition of selected edible seaweeds, pp. 15-47. *In*: Vitor H. Pomin (ed.) Seaweed: Ecology, Nutrient Composition and Medicinal Uses. Nova Science Publishers Inc., New York, **2011**.

39. Therkelsen GH. Carrageenan, pp. 145-180. *In*: Whistler, R.L. and J.N. Bemiller (eds.). Industrial Gums: Polysaccharides and Their Derivatives. Academic Press, San Diego, **1993**.

40. Ribier J, Godineau JC. Les algues. La Maison Rustique, Flammarion, France, **1984**, pp. 15-26.

41. Smith DB, Cook HW. Physical studies on carrageenin and carrageenin fractions. *Archives of Biochemistry and Biophysics*, **1954**, *45*, 223 -233.

42. Smith DB, Oneill AN, Perlin AS. 1955. Studies on the heterogeneity of carrageenin. *Canadian Journal of Chemistry*, **1955**, *33*(8), 1352-1360.

43. O'neill AN. Derivatives of 4-O-Beta-D-galactopyranosyl-3,6-anhydro-D-galactose from κ-carrageenin. *Journal of the American Chemical Society*, **1955**, *77*(23), 6324-6326.

44. O'neill AN. 3,6-anhydro-D-galactose as a constituent of κ-carrageenin. *Journal of the American Chemical Society*, **1955**, *77*(10), 2837-2839.

45. Hurtado A, Critchley A, Neish I (eds.). Tropical Seaweed Farming Trends, Problems and Opportunities. Developments in Applied Phycology, *9*. Springer International Publishing AG, Gewerbestrasse, Cham, Switzerland, **2017**.

46. van de Velde F, Pereira L, Rollema HS. The revised NMR chemical shift data of carrageenans. *Carbohydrate Research*, **2004**, *339*, 2309-2313.

47. Pereira L, Critchley AT, Amado AM, Ribeiro-Claro PJA. A comparative analysis of phycocolloids produced by underutilized *versus* industrially utilized carrageenophytes (Gigartinales, Rhodophyta). *Journal of Applied Phycology*, **2009**, *21*(5): 599-605.

48. Li L, Ni R, Shao Y, Mao S. Carrageenan, and its applications in drug delivery. *Carbohydrate Polymers*, **2014**, *103*, 1-11.

49. Seisun D. Food Hydrocolloids. IMR International Hydrocolloid Conference, Barcelona, Spain, **2016**, April 2016.

50. Markets and Markets. Hydrocolloids Market Global Forecast by Type (Gelatin, Xanthan, Carrageenan, Alginate, Agar, Pectin, Guar, Locust Bean, Gum Arabic, and CMC), Function (Thickener, Stabilizer,

Gelling, Fat Replacer, and Coating), Source, Application, & by Region - Global Forecast to 2020. Markets and Markets, **2016**, February 2016

51. Hotchkiss S, Campbell R, Hepburn C. Carrageenan: sources and extraction methods, pp. 1-293. *In*: Pereira, L. (ed.). Carrageenans: Sources and Extraction Methods, Molecular Structure, Bioactive Properties and Health Effects. Nova Science Publications Inc., NY, **2016**.

52. Porse H, Ladenburg S. Seaweed value chain final report. Report submitted to Smart-Fish Indonesia under the UNIDO Seaweed Value Chain Programme. Jakarta, Indonesia, **2015**.

53. Neish IC. A diagnostic analysis of seaweed value chains in Sumenep Regency, Madura Indonesia. Report submitted for UNIDO Project no 140140 "preparatory assistance to develop a seaweed value chain development project for Madura", Indonesia, **2015**.

54. Campbell R, Hotchkiss S. Carrageenan Industry Market Overview. *In*: Hurtado A., Critchley A., Neish I. (eds.) Tropical Seaweed Farming Trends, Problems and Opportunities. Developments in Applied Phycology, *9*. Springer International Publishing AG, Gewerbestrasse, Cham, Switzerland, **2017**.

55. Cáceres PJ, Carlucci MJ, Damonte EB, Matsuhiro B, Zúñiga EA. Carrageenans from Chilean samples of *Stenogramme interrupta* (Phyllophoraceae): Structural analysis and biological activity. *Phytochemistry*, **2000**, *53*(1), 81-86.

56. Vlieghe P, Clerc T, Pannecouque C, Witvrouw M, De Clercq E, Salles J, Kraus JL. Synthesis of new covalently bound kappa-carrageenan–AZT conjugates with improved anti-HIV activities. *Journal of Medicinal Chemistry*, **2002**, *45*, 1275-1283.

57. Panlasigui LN, Baello OQ, Dimatangal JM, Dumelod BD. Blood cholesterol and lipid lowering effects of carrageenan on human volunteers. *Asia Pacific Journal of Clinical Nutrition*, **2003,** *12*, 209-214.

58. 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*. *Pharmacological Research*, **2004**, *50*, 47-53.

59. 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. *Pharmacological Research*, **2006**, *53*(2), 129-134.

60. Yuan H, Song J. Preparation, structural characterization and *in vitro* antitumor activity of kappa-carrageenan oligosaccharide fraction from *Kappaphycus striatum*. *Journal of Applied Phycology*, **2005**, *17*, 7-13.

61. Skoler-Karpoff S, Ramjee G, Ahmed K, Altini L, Plagianos MG, Friedland B, Govender S, De Kock A, Cassim N, Palanee T, Dozier G, Maguire R, Lahteenmaki P. Efficacy of Carraguard for prevention of HIV infection in women in South Africa: a randomised, double-blind, placebo-controlled trial. *The Lancet*, **2008**, *372*(9654), 1977-1987.

62. Campo VL, Kawano DE, da Silva Jr DB, Carvalho I. Carrageenans: Biological properties, chemical modifications and structural analysis – a review. *Carbohydrate Polymers*, **2009**, *77*(2), 167-180.

63. Wijesekara I, Pangestuti R, Kim S-K. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydrate Polymers*, **2011**, *84*, 14-21.

64. Soares F. Antifungal, antibacterial and antiviral activity of *Chondracathus teedei* var. *lusitanicus* (Gigartinaceae, Rhodophyta). MsC Thesis, University of Coimbra, Coimbra, Portugal, **2015**, 154 pp.

65. Soares F, Fernandes C, Silva P, Pereira L, Gonçalves T. Antifungal activity of carrageenan extracts from the red alga *Chondracanthus teedei* var. *lusitanicus*. *Journal of Applied Phycology*, **2016**, *28*(5), 2991-2998.

66. Morris CJ. Carrageenan-induced paw edema in the rat and mouse, pp. 115-121. *In*: Paul G. Winyard and Derek A. Willoughby (eds.). Inflammation Protocols. Humana, Totowa, **2003**, NJ.

67. Panlasigui LN, Tiangson CLP, Quijano AMM, Arugay HT, Dumelod BD. 1997. Blood glucose response of normal subjects to a carrageenan-incorporated Filipino food. *J Nutr Diet Assoc Phil*, **1997**, *11*, 87-92.

68. Dumelod BD, Ramirez RP, Tiangson CL, Barrios EB, Panlasigui LN. Carbohydrate availability of *arroz caldo* with lambda-carrageenan. International *Journal of Food Sciences and Nutrition*, **1999**, *50*, 283-289.

69. Panlasigui LN, Dumelod BD, Alonde L, Torres JB, Basilio AL, Concepcion MT, Mercado L, Olermo C. Metabolic effects of local seaweed extracts on I. Blood glucose levels. Terminal Report. HERDIN - Health Research and Development Information Network, Manila, Philippines, 1998.

70. Morrissey J, Kraan S, Guiry MD. A guide to commercially important seaweeds on the Irish coast. Bord Iascaigh Mhara/Irish Fisheries Board, Dublin, **2001**, pp. 5-40.

71. Kraan S. Algal polysaccharides, novel applications and outlook, pp. 489-532. *In*: Chang C-F (ed.). Biochemistry, Genetics and Molecular Biology - Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology. INTECH, Science, Technology and Medicine open access publisher, **2012**. Available online at: http://www.intechopen.com/books/carbohydrates-comprehensive-studies-on-glycobiology-and-glycotechnology/algal-polysaccharides-novel-applications-and-outlook (accessed 2018)

72. Pereira L, Correia F. Algas marinhas da costa Portuguesa - ecologia, biodiversidade e utilizações. Nota de Rodapé Edições, Paris, **2015**, 340 pp. (*in Portuguese*).

73. Hawkins WW, Leonard VG. Antipeptic and antithrombic properties of carrageenin. *Journal of Laboratory and Clinical Medicine*, **1962**, *60*, 641-648.

74. Hawkins WW, Leonard VG. Antithrombotic activity of carrageenan in human blood. *Canadian Journal of*

Biochemistry and Physiology, **1963**, *41*(5), 1325-1327.

75. Kindness G, Long WF, Williamson FB, Boyd J. Effects of carrageenans on the aggregation of human blood platelets. *Thrombosis Research*, **1979**, *15*(1-2): 3-15.

76. Kindness, G., W.F. Long and F.B. Williamson. Anticoagulant effects of sulphated polysaccharides in normal and antithrombin III-deficient plasmas. *British Journal of Pharmacology*, **1980**, *69*(4), 675-677.

77. Güven K, Özsoy Y, Ulutin O. Anticoagulant, fibrinolytic and antiaggregant activity of carrageenans and alginic acid. *Botanica Marina*, **1991**, *34*(5), 429-432.

78. Sen AK, Das AK, Banerji N, Siddhanta AK, Mody KH, Ramavat BK, Chauhan VD, Vedasiromoni JR, Ganguly DK. A new sulfated polysaccharide with potent blood anticoagulant activity from the red seaweed *Grateloupia indica*. *International Journal of Biological Macromolecules*, **1994**, *16*, 279-280.

79. Anderson W, Duncan JGC. The anticoagulant activity of carrageenan. *Journal of Pharmacy and Pharmacology*, **1965**, *17*, 647-654.

80. Efinmov VS, Usov AI, Ol'skaya TS, Baliunis A, Rozkin M. Comparative study of the anticoagulant activity of sulfated polysaccharides from red marine algae. *Farmakologiia I Toksikologiia (Moscow)*, **1985**, *46*(3): 61-67 (*in Russian*).

81. Fengwu J, Yushun. The dictionary of Chinese oceanic medicine. Ocean Press, **1993**, 160 pp.

82. Charlier HR, Chaineux M-CP. The healing sea: a sustainable coastal ocean resource: thalassotherapy. *Journal of Coastal Research*, **2009**, *25*(4), 838-856.

83. Pereira L. Littoral of Viana do Castelo – ALGAE: uses in agriculture, gastronomy and food industry. Câmara Municipal de Viana do Castelo, Portugal, **2010**, 68 pp. (*Bilingual*)

84. Buck CB, Thompson CD, Roberts JN, Muller M, Lowy DR, Schiller JT. Carrageenan is a potent inhibitor of Papillomavirus infection. PLoS Pathogens, **2006**, *2*, 671-680.

85. Grassauer A, Weinmuellner R, Meier C, Pretsch A, Prieschl-Grassauer E aUnger H. 2008. Iota-carrageenan is a potent inhibitor of rhinovirus infection. *Virology Journal*, **2008**, *5*(10), 13 pp.

86. Gonzalez ME, Alarcon B, Carrasco L. Polysaccharides as antiviral agents: Antiviral activity of carrageenan. *Antimicrobial Agents and Chemotherapy*, **1987**, *31*, 1388-1393.

87. Zeitlin L, Whaley KJ, Hegarty TA, Moench TR, Cone RA. Tests of vaginal microbicides in the mouse genital herpes model. *Contraception*, **1997**, *56*(5), 329-335.

88. Girond S, Crance JM, Van Cuyck-Gandre H, Renaudet J and Deloince R. Antiviral activity of carrageenan on hepatitis A virus replication in cell culture. *Research in Virology*, **1991**, *142*, 261-270.

89. Marchetti M, Pisani S, Pietropaolo V, Seganti L, Nicoletti R, Orsi N. Inhibition of *Herpes simplex* virus

infection by negatively charged and neutral carbohydrate polymers. *Journal of Chemotherapy*, **1995**, *7*, 90-96.

90. Carlucci MJ, Scolaro LA, Damonte EB. Inhibitory action of natural carrageenans on *Herpes simplex* virus infection of mouse astrocytes. *Chemotherapy*, **1999**, *45*, 429-436.

91. Roberts JN, Buck CB, Thompson CD, Kines R, Bernardo M, Choyke PL, Lowy DR, Schiller JT. Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. *Nature Medicine*, **2007**, *13*, 857-861.

92. Rodríguez A, Kleinbeck K, Mizenina O, Kizima L, Levendosky K, Jean-Pierre N, Villegas G, Ford BE, Cooney ML, Teleshova N, Robbiani M, Herold BC, Zydowsky T, Fernandez Romero JA. 2014. *In vitro* and *in vivo* evaluation of two carrageenan-based formulations to prevent HPV acquisition. *Antiviral Research*, **2014**, *108*, 88-93.

93. Guedes EA, Araujo MA, Souza AK, de Souza LI, de Barros LD, Maranhao FC, Sant'Ana AE. Antifungal activities of different extracts of marine macroalgae against dermatophytes and *Candida* species. *Mycopathologia*, **2012**, *174*, 223-232.

94. Venugopal V (ed.). Marine products for healthcare: functional and bioactive nutraceutical compounds from the ocean. CRC Press, Taylor & Francis Group, Boca Raton, FL, **2008**, 528 pp.

95. Sebaaly C, Kassem S, Grishina E, Kanaan H, Sweidan A, Chmit MS, Kanaan HM. Anticoagulant and antibacterial activities of polysaccharides of red algae *Corallina* collected from Lebanese coast. *Journal of Applied Pharmaceutical Science*, **2014**, *4*(4), 30-37.

96. Population Council. Microbicides program. Online Population Council News, **2018**. Available online at: http://www.popcouncil.org/research/results?projects=yes&experts=yes&resources=yes&keywords=carrageenan (accessed 2018)

97. Ahmed ABA, Kumar RA, Taha RMM. Chapter 36 - Role of nanocomposites and nanostructured biomaterials in biomedical and nanobiotechnology, pp. 717-736. *In*: Kim S-K (ed.), Marine Biomaterials: Characterization, Isolation and Applications. CRC Press / Taylor & Francis Group, Boca Raton, FL, **2017**.

98. Carlucci MJ, Scolaro LA, Damonte EB. *Herpes simplex* virus type 1 variants arising after selection with an antiviral carrageenan: lack of correlation between drug susceptibility and synphenotype. *Journal of Medical Virology*, **2002**, *68*(1): 92-98.

99. Carlucci MJ, Scolaro LA, Noseda MD, Cerezo AS, Damonte EB. Protective effect of a natural carrageenan on genital *Herpes simplex* virus infection in mice. *Antiviral Research*, **2004**, *64*(2), 137-141.

100. Zacharopoulos VR, Phillips DM. Vaginal formulations of carrageenan protect mice from herpes simplex virus infection. *Clinical and Diagnostic Laboratory Immunology*, **1997**, *4*(4), 465-468.

101. De SF-Tischer PC, Talarico LB, Noseda MD, Guimarães SMPB,

Damonte, Duarte MER. Chemical structure and antiviral activity of carrageenans from *Meristiella gelidium* against *Herpes simplex* and Dengue virus. *Carbohydrate Polymers*, **2006**, *63*(4), 459-465.

102. Ahmadi A, Zorofchian Moghadamtousi S, Abubakar S, Zandi Z. Antiviral potential of algae polysaccharides isolated from marine sources: a review. *BioMed Research International*, **2015**, *825203*, 10 pp.

103. Talarico LB, Damonte EB. Interference in Dengue virus adsorption and uncoating by carrageenans. *Virology*, **2007**, *363*(2), 473-485.

104. Talarico, L.B., M.D. Noseda, D.R.B. Ducatti, M.E. Duarte and E.B. Damonte. Differential inhibition of dengue virus infection in mammalian and mosquito cells by iota-carrageenan. *Journal of General Virology*, **2011**, *92*(6), 1332-1342.

105. Ogata M, Matsui T, Kita T, Shigematsu A. Carrageenan primes leukocytes to enhance lipopolysaccharide-induced tumor necrosis factor alpha production. *Infection and Immunity*, **1999**, *67*, 3284-3289.

106. Nacife VP, Soeiro M, Araújo-Jorge TC, Neto H, Meirelles M. Ultrastructural, immunocytochemical and flow cytometry study of mouse peritoneal cells stimulated with carrageenan. *Cell Structure and Function*, **2000**, *25*, 337-350.

107. Nacife VP, Soeiro M, Gomes RN, D'Avila H, Neto H, Meirelles M. Morphological and biochemical characterization of macrophages activated by carrageenan and lipopolysaccharide *in vivo*. *Cell Structure and Function*, **2004**, *29*, 27-34.

108. Van Rooijen N, Sanders A. Elimination, blocking, and activation of macrophages: three of a kind? *Journal of Leukocyte Biology*, **1997**, *62*, 702-709.

109. Yang JW, Yoon SY, Oh SJ, Kim SK, Kang KW. Bifunctional effects of fucoidan on the expression of inducible nitric oxide synthase. *Biochemical and Biophysical Research Communications*, **2006**, *346*, 345-350.

110. Didier C, Kerblat I, Drouet C, Favier A, Beani JC, Richard MJ. Induction of thioredoxin by ultraviolet-a radiation prevents oxidative-mediated cell death in human skin fibroblasts. *Free Radical Biology & Medicine*, **2001**, *31*(5), 585-598.

111. Chignell CF, Sik RH. A photochemical study of cells loaded with 2',7'-dichlorofluorescin: Implications for the detection of reactive oxygen species generated during UVA irradiation. *Free Radical Biology & Medicine*, **2003**, *34*(8), 1029-1034.

112. Yuan HM, Song JM, Zhang WW, Li XG, Li N, Gao XL. Antioxidant activity and cytoprotective effect of kappa-carrageenan oligosaccharides and their different derivatives. *Bioorganic & Medicinal Chemistry Letters*, **2006**, *16*, 1329-1334.

113. Sokolova EV, Barabanova AO, Bogdanovich RN, Khomenko VA, Solov'eva TF, Yermak IM. *In vitro* antioxidant properties of red algal polysaccharides. *Biomedicine & Preventive Nutrition*, **2011**, *1*(3), 161-167.

114. Sokolova EV, Bogdanovich LN, Ivanova TB, Byankina AO, Kryzhanovskiy SP, Yermak IM. Effect of carrageenan food supplement on patients with cardiovascular disease results in normalization of lipid profile and moderate modulation of immunity system markers. *PharmaNutrition*, **2014**, *2*, 33-37.

115. Drum R. Medicinal uses of seaweeds, 2018. Available online at: http://www.ryandrum.com/seaweeds.htm (accessed 2018)

116. Tang F, Chen F, Li F. Preparation and potential *in vivo* anti-influenza virus activity of low molecular-weight κ-carrageenans and their derivatives. *Journal of Applied Polymer Science*, **2013**, *127*(3), 2110-2115.

117. Goldmann DA. Epidemiology and prevention of pediatric viral respiratory infections in health-care institutions. *Emerging Infectious Diseases*, **2001**, *7*, 249-253.

118. Monto AS, Sullivan KM. Acute respiratory illness in the community. Frequency of illness and the agents involved. *Epidemiology and Infection*, **1993**, *110*, 145-160.

119. Makela MJ, Puhakka T, Ruuskanen O, Leinonen M, Saikku P, Kimpimaki M, Blomqvist S, Hyypia T, Arstila P. Viruses and bacteria in the etiology of the common cold. *Journal of Clinical Microbiology*, **1998**, *36*, 539-542.

120. Monto AS, Fendrick AM, Sarnes MW. Respiratory illness caused by picornavirus infection: a review of clinical outcomes. *Clinical Therapeutics*, **2001**, *23*, 1615-1627.

121. Monto AS. Epidemiology of viral respiratory infections. *American Journal of Medicine*, **2002**, *112*, 4S-12S.

122. Koenighofer M, Lion T, Bodenteich A, Prieschl-Grassauer E, Grassauer A, Unger H, Mueller CA, Fazekas T. Carrageenan nasal spray in virus confirmed common cold: individual patient data analysis of two randomized controlled trials. *Multidisciplinary Respiratory Medicine*, **2014**, *9*(57), 12 pp.

123. Eccles R, Meier C, Jawad M, Weinmullner R, Grassauer A, Prieschl-Grassauer E. Efficacy and safety of an antiviral iota-carrageenan nasal spray: A randomized, double-blind, placebo-controlled exploratory study in volunteers with early symptoms of the common cold. *Respiratory Research*, **2010**, *11(*108),10 pp.

124. Eccles R, Winther B, Johnston SL, Robinson P, Trampisch M, Koelsch S. Efficacy and safety of iota-carrageenan nasal spray versus placebo in early treatment of the common cold in adults: The ICICC trial. *Respiratory Research*, **2015**, *16*(1), 11 pp.

125. Leibbrandt A, Meier C, Konig-Schuster M, Weinmullner R, Kalthoff D, B. Pflugfelder B, P. Graf P, B. Frank-Gehrke B, M. Beer M, T. Fazekas T, H. Unger H, Prieschl-Grassauer E, Grassauer A. Iota-carrageenan is a potent inhibitor of Influenza A Virus Infection. *PLoS One*, **2010**, *5*(12), 0014320.

126. Morokutti-Kurz M, Konig-Schuster M, Koller C, Graf C, Graf P, Kirchoff N, Reutterer B, Seifert JM, Unger H, Grassauer A, Prieschl-Grassauer E, Nakowitsch S. The intranasal application of Zanamivir and carrageenan is synergistically active against influenza A virus in the murine model. *PLoS One*, **2015**, *10*(6), 1-16.

127. Murad H, Ghannam A, Al-Ktaifani M, Abbas A, Hawat M. Algal sulfated carrageenan inhibits proliferation of MDA-MB-231 cells *via* apoptosis regulatory genes. *Molecular Medicine Reports*, **2015**, *11*(3), 2153-8258.

128. Luo M, Shao B, Nie W, X. Wei XW, Li YL, Wang BL, He ZY, Liang X, Ye TH, Wei YQ. Antitumor and adjuvant activity of lambda-carrageenan by stimulating immune response in cancer immunotherapy. *Scientific Reports*, **2015**, *5*(11062), 12 pp.

129. Pereira L, Mesquita JF. Population studies and carrageenan properties of *Chondracanthus teedei* var. *lusitanicus* (Gigartinaceae, Rhodophyta). *Journal of Applied Phycology*, **2004**, *16*, 369-383.

130. Azizi S, Mohamad R, Abdul Rahim R, Mohammadinejad R, Bin Ariff A. Hydrogel beads bionanocomposite based on kappa-carrageenan and green synthesized silver nanoparticles for biomedical applications. *International Journal of Biological Macromolecules*, **2017**, *104*, 423-431.

131. Mouritsen OG. Seaweeds: Edible, available & sustainable. University of Chicago Press, Chicago & London, **2013**, 304 pp.

132. Shimamura N. Agar. *In:* Rediscovering the treasures of food. The Tokyo Foundation, Tokyo, **2010**. Available online at: http://www.tokyofoundation.org/en/topics/japanese-traditional-foods/vol.-4-agar (accessed 2018)

133. Armisen R, Galatas F. 2000. Agar. *In*: Phillips, G. and P. Williams (eds.) Handbook of Hydrocolloids. CRC Press, Taylor & Francis Group, Boca Raton, FL, **2000**, pp. 21-40.

134. Araki CH. Acetylation of agar like substance of *Gelidium amansii*. *Journal of the Chemical Society*, **1937**, *58*, 1338-1350.

135. Nussinovitich A. Hydrocolloid applications: gum technology in the food and other Industries, Blackie Academic & Professional, London, **1997**, pp. 1-18.

136. Qi H., Li D, Zhang J, Liu L, Zhang Q. Study on extraction of agaropectin from *Gelidium amansii* and its anticoagulant activity. Chinese Journal of Oceanology and Limnology, **2008**, *26*(2), 186-189.

137. Pereira L, Gheda FS, Ribeiro-Claro PJA. Analysis by vibrational spectroscopy of seaweed polysaccharides with potential use in food, pharmaceutical and cosmetic industries. *International Journal of Carbohydrate Chemistry*, vol. **2013**, Article ID 537202, 7 pp.

138. Asano N, Nash RJ, Molyneux RJ, Fleet GWJ. Sugar-mimic glycosidase inhibitors: Natural occurrence, biological activity and prospects for therapeutic application. *Tetrahedron: Asymmetry*, **2000**, *11*, 1645-1681.

139. Chen H-M, Zheng L, Yan X-J. Agaro-bioactivity research of oligosaccharides. *Food Technology and Biotechnology*, **2005**, *43*(1), 29-36.

140. Dumitriu S. Polysaccharides: structural diversity and functional versatility, 2nd edition. CRC Press, Taylor & Francis Group, Boca Raton, FL, **2004**, 1224 pp.

141. Adachi M, Watanabe S. Evaluation of combined deactivators-supplemented agar medium (CDSAM) for recovery of dermatophytes from patients with tinea pedis. *Medical Mycology*, **2007**, *45*, 347-349.

142. Knox R, Woodroffe R. Semi-solid agar media for rapid drug sensitivity tests on cultures of *Mycobacterium tuberculosis*. *Journal of General Microbiology*, 1**957**, *16*, 647-659.

143. Yew WW, Tonb S, Lui KS, Leung SK, Chau CH, Wang EP. Comparison of B/BacT system and agar proportion method in drug susceptibility testing of *Mycobacterium tuberculosis*. *Diagnostic Microbiology and Infectious Disease*, **2001**, *39*, 229-232.

144. Nakano M, Nakamur Y, Takikawa K, Kouketsu M, Arita I. Sustained release of sulfamethizole from agar beads. *Journal of Pharmacy and Pharmacology*, **1979**, *31*, 869-872.

145. Kojima T, Hashida M, Muranishi S, Sezaki H. Antitumor activity of timed-release derivative of mitomycin C, agarose bead conjugate. *Chemical and Pharmaceutical Bulletin*, **1978**, *26*, 1818-1824.

146. El-Raheem AE-H, El-Said Y. Preparation and characterization of agar beads containing phenobarbitone sodium. *Journal of Microencapsulation*, **1988**, *5*, 159-163.

147. Laurienzo P. Marine polysaccharides in pharmaceutical applications: an overview. *Marine Drugs*, **2010**, *8*(9), 2435-2465.

148. Fernandez LE, Valiente OG, Mainardi V, Bello JL, Velez H, Rosado A. Isolation and characterization of an antitumor active agar-type polysaccharide of *Gracilaria dominguensis*. *Carbohydrate Research*, **1989**, *190*(1), 77-83.

149. Armisen R, Galatas F. Production, properties and uses of agar. *In*: McHugh DJ (ed.). Food and Agriculture Organization of the United Nations (FAO) Fisheries Technical Paper, Food and Agriculture Organization of the United Nations (FAO), Rome, **1987**, *288*, 1-57.

150. Osumi Y, Kawai M, Amano H, Noda H. Physiological activities of oligosaccharides derived from marine algae *Porphyra yezoensis* porphyran. *Fisheries Science-Tokyo*, **2002**, *68*, 1441-1444.

151. Ji J, Wang L-C, Wu H, Luan H-M. Bio-function summary of marine oligosaccharides. *International Journal of Biology*, **2011**, *3*(1), 74-86.

152. Mouradi-Givernaud A, Givernaud T, Morvan H, Cosson J. Agar from *Gelidium latifolium* (Rhodophyceae, Gelidiales) - biochemical composition and seasonal variations. *Botanica Marina*, **1992**, *35*, 153-159.

153. Jeon Y-J, Athukorala Y and Lee J. 2005. Characterization of agarose

product from agar using DMSO. *Algae*, **2005**, *20*, 61-67.

154. Enoki T, Sagawa H, Tominaga T, Nishiyama E, Komyama N, Sakai T, Yu F-G, Ikai K, Kato I. Drugs, foods or drinks with the use of algae-derived physiologically active substances. Patent US6475990 B1, **2003**.

155. Chen H, Yan X, Zhu P, Lin J. Antioxidant activity and hepatoprotective potential of agaro-oligosaccharides in *vitro* and in *vivo*. *Nutrition Journal*, **2006**, *5*(31), 1-12.

156. Wang JX, Mou HJ, Jiang XL, Guan HS. Biological activities of a neutral water-soluble agar polysaccharide prepared by agarose degradation. *High Technology Letters*, **2005**, *11*, 415-420.

157. Ohta Y, Hatada Y, Nogi Y, Liz, Ito S, Horikoshi K. Cloning, expression, and characterization of a glycoside hydrolase family 86 β-agarase from a deep-sea microbulbifer-like isolate. *Applied Microbiology Biotechnology*, **2004**, *6*6, 266-275.

158. Yun EJ, Lee S, Kim JH, Kim BB, Kim HT, Lee SH, Pelton JG, Kim KH. Enzymatic production of 3,6-anhydro-L-galactose from agarose and its purification and *in vitro* skin whitening and anti-inflammatory activities. *Applied Microbiology Biotechnology*, **2013**, *97*(7), 2961-2970.

159. Wang W, Liu P, Hao C, Wu L, Wan W, Mao X- X. 2017a. Neoagaro-oligosaccharide monomers inhibit inflammation in LPS-stimulated macrophages through suppression of MAPK and NF-κB pathways. *Scientific Reports*, **2017**,*7*, 44252.

160. Villarroel LH, Zanlungo AB. Structural studies on the Porphyran from *Porphyra columbina*. *Carbohydrate Research*, **1981**, *88*, 139-145.

161. Bhatia S, Sharma A, Sharma K, Kavale M, Chaugule BB, Dhalwal K, Namdeo AG, Mahadik KR. Novel algal polysaccharides from marine source: porphyran. *Pharmacognosy Reviews*, **2008**, *2*(4), 271-276.

162. Zhang QB, Qi HM, Zhao TT, Deslandes E, Ismaeli NM, Molloy F, Critchley AT. chemical characteristics of a polysaccharide from *Porphyra capensis* (Rhodophyta). *Carbohydrate Research*, **2005**, *340*(15), 2447-2450.

170. Marlett JA. Sites and mechanisms for the hypocholesterolemic actions of soluble dietary fiber sources, pp. 109-121. *In*: Kritchevsky D, Bonfield C (eds.). Dietary Fiber in Health and Disease. Plenum Press, New York, **1997**.

171. Anderson JW, Jones AE, Mason SR. Ten different dietary fibers have significantly different effects on serum and liver lipids of cholesterol-fed rats. *The Journal of Nutrition*, **1994**, *124*, 78-83.

172. Buhman KK, Furumoto EJ, Donkin SS, Story JA. Dietary psyllium increases fecal bile acid excretion, total steroid excretion and bile acid biosynthesis in rats. *The Journal of Nutrition*, **1998**, *128*,1199-1203.

173. Haack VS, Chesters JG, Vollendorf NW, Story JA, Marlett JA. Increasing amounts of dietary fiber provided by foods normalizes physi-

ologic response of the large bowel without altering calcium balance or fecal steroid excretion. *The American Journal of Clinical Nutrition*, **1998**, *68*, 615-622.

174. Cummings JH. The effect of dietary fiber on fecal weight and composition, pp. 263-349. *In*: Spiller GA (ed.), CRC Handbook of Dietary Fiber in Human Nutrition, 2nd ed. CRC Press, Boca Raton, FL, **1993**.

175. Peat S, Turvey JR, Rees DA. Carbohydrates of the red alga, *Porphyra umbilicalis*. *Journal of the Chemical Society*, **1961**, 1590-1595.

176. Tsuge K, Okabe M, Yoshimura T, Sumi T, Tachibana H, Yamada K. Dietary effects of porphyran from *Porphyra yezoensis* on growth and lipid metabolism of Sprague-Dawley Rats. *Food Science and Technology Research*, **2004**, *10*(2), 147-151.

177. Kawadu D, Tanaka M, Fujii T. Effect of polysaccharides of "Susabinori" *Porphyra yezoensis* on intestinal flora. *Nippon Suisan Gakkaishi*, **1995**, *61*, 59-69.

178. Yoshizawa Y, Enomoto A, Todoh H, Ametani A, Kaminogawa S. Activation of murine macrophage by polysaccharide fractions from a marine alga (*Porphyra yezoensis*). *Bioscience, Biotechnology, and Biochemistry*, **1993**, *57*,1862-1866.

179. Yoshizawa Y, Ametani A, Tsunehiro J, Nomura K, Itoh M, Fukui F, Kaminogawa S. Macrophage stimulation activity of the polysaccharide fraction from a marine alga (*Porphyra yezoensis*): structure-function relationships and improved solubility. *Bioscience, Biotechnology, and Biochemistry*, **1995**, *59*, 1933-1937.

180. Ren DW, Noda H, Amano H, Nishino T, Nishizana K. Study on hypertensive and antihyperlipidemic effect of marine algae. *Fisheries Science*, **1994**, *60*, 83-88.

181. Kiriyama S, Okazaki Y, Yoshida A. Hypocholesterolemic effect of polysaccharides and polysaccharide foodstuffs in cholesterol fed rats. *Journal of Nutrition*, **1968**, *97*, 382-88.

182. Ito K, Tsuchida Y. 1972. The effect of algal polysaccharides on depressing of plasma cholesterol level in rats. *Proceedings of International Seaweed Symposium*, **1972**, *7*, 451-55.

183. Lamela M, Anca J, Villar R, Otero J, Calleja JM. Hypoglycemic activity of several seaweed extracts. *Journal of Ethnopharmacology*, **1989**, *27*, 35-43.

184. Nishide E, Anzai H, Uchida H. Effects of alginates on the ingestion and excretion of cholesterol in the rat. *Journal of Applied Phycology*, **1993**, *5*, 207-211.

185. Ren DW, Noda H, Amano H, Nishino T, Nishizana K. Antihypertensive and antihyperlipidemic effects of funoran. *Fisheries Science*, **1994**, *60*, 423-427.

186. Ara J, Sultana V, Qasim VU. Hypolipidemic activity of seaweed from Karachi coast. *Phytotherapy Research*, **2002**, *16*, 479-483.

187. Nishide E, Uchida H. Effects of *Ulva* powder on the ingestion and excretion of cholesterol in rats. pp.

165-168. *In*: Chapman AR, Anderson RJ, Vreeland VJ, Davison IR (eds.). Proceedings of the 17th International Seaweed Symposium, Oxford University Press, Oxford, **2003**.

188. Ishihara K, Oyamada S, Matsushima R, Murata M, Muraoka T Nakashima. Inhibitory effect of porphyran, prepared from dried Nori, on contact hypersensitivity in mice. *Bioscience, Biotechnology, and Biochemistry*, **2005**, *69*(10), 1824-1830.

189. Nakashima H, Kido Y, Kobayashi N, Motoki Y, Neushul M, Yamamoto N. Antiretroviral activity in a marine red alga; reverse transcriptase inhibition by an aqueous extract of *Schizymenia pacifica*. *Journal of Cancer Research and Clinical Oncology*, **1987**, *113*, 413-416.

190. Nakashima H, Kido Y, Kobayashi N, Motoki Y, Neushul M, Yamamoto N. Purification and characterization of an avian myeloblastosis and Human immunodeficiency virus reverse transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. *Antimicrobial Agents and Chemotherapy*, **1987**, *31*, 1524-1528.

191. Mazumder S, Ghosal PK, Pujol CA, Carlucci MJ, Damonte EB, Ray B. Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticata* (Gracilariaceae, Rhodophyta). *International Journal of Biological Macromolecules*, **2002**, *31*, 87-95.

192. Zhu W, Ooi VEC, Chan PKS, Ang PO. Inhibitory effect of extracts of marine algae from Hong Kong against *Herpes simplex* viruses, pp. 159-164. *In*: Chapman ARO, Anderson RJ, Vreeland VJ, Davison IR (eds.), Proceedings of the 17th International Seaweed Symposium. Oxford University Press, Oxford, **2003**.

193. Zhu W, Ooi VEC, Chan PKS, Ang PO. Inhibitory Effect of extracts of marine algae from Hong Kong against *Herpes simplex* viruses, pp. 164-166. *In:* Chapman ARO, Anderson RJ, Vreeland VJ, Davison IR (eds.). Proceedings of the 17th International Seaweed Symposium. Oxford University Press, Oxford, **2003**.

194. Smit JA. Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, **2004**, *16*, 245-262.

195. Zhang Q, Li N, Zhou G, Lu X, Xu Z, Li Z. *In vivo* antioxidant activity of polysaccharide fraction from *Porphyra haitanesis* (Rhodophyta) in aging mice. *Pharmacological Research*, **2003**, *48*, 151-155.

196. Kwon M-J, Nam T-J. Porphyran induces apoptosis related signal pathway in AGS gastric cancer cell lines. *Life Sciences*, **2006**, *79*(20), 1956-1962.

197. Wang L, Chen Y, Sternberg P, Cai J. Essential roles of the PI3 kinase/Akt pathway in regulating Nrf2-dependent antioxidant functions in the RPE. *Investigative Ophthalmology & Visual Science*, **2008**, *49*, 1671-1678.

198. Yu X, Zhou C, Yang H, Huang X, Ma H, Qin X, Hu J. Effect of ultrasonic treatment on the degradation and inhibition cancer cell lines of polysaccharides from *Porphyra yezoensis*. *Carbohydrate Polymers*, **2015**, *117*, 650-656.

199. Okai Y, Higashi-Okai K, Nakamura S, Yano Y, Otani S. Suppressive effects of the extracts of Japanese edible seaweeds on mutagen-induced umu C gene expression in *Salmonella typhimurium* (TA 1535/pSK 1002) and tumor promotor-dependent ornithine decarboxylase induction in BALB/c 3T3 fibroblast cells. *Cancer Letters*, **1994**, *87*, 25-32.

200. Eitsuka T, Nakagawa K, Igarashi M, Miyazawa T. Telomerase inhibition by sulfoquinovosyldiacylglycerol from edible purple laver (*Porphyra yezoensis*). *Cancer Letters*, **2004**, *212*, 15-20.

201. Kazlowska K, Lin HTV, Chang SH, Tsai GJ. *In vitro* and *in vivo* anticancer effects of sterol fraction from red algae *Porphyra dentata*. *Evidence-Based Complementary and Alternative Medicine*, **2013**, Article ID 493869, 10 pp.

202. Yang YJ, Nam SJ, Kong G, Kim MK. A case-control study on seaweed consumption and the risk of breast cancer. *British Journal of Nutrition*, **2010**, *103*, 1345-1353.

203. Inoue N, Yamano N, Sakata K, Nagao K, Hama Y, Yanagita T. The sulfated polysaccharide porphyran reduces apolipoprotein B 100 secretion and lipid synthesis in HepG2 cells. *Bioscience, Biotechnology, and Biochemistry*, **2009**, *73*, 447-449.

204. Kitano Y, Murazumi K, Duan J, Kurose K, Kobayashi S, Sugawara T, Hirata T. Effect of dietary porphyran from the red alge, *Porphyra yezoensis*, on glucose metabolism in diabetic KK-Ay mice. *Journal of Nutritional Science and Vitaminology*, **2012**, *58*, 14-19.

205. Zhang ZS, Wang XM, Han ZP, Yin L, Zhao MX, Yu SC. Physicochemical properties and inhibition effect on iron deficiency anemia of a novel polysaccharide-iron complex (LPPC). *Bioorganic & Medicinal Chemistry Letters*, **2012**, *22*, 489-492.

206. Wang XM, Li WD, Xiao L, Liu CD, Qi HM, Zhang ZS. *In vivo* antihyperlipidemic and antioxidant activity of porphyran in hyperlipidemic mice. *Carbohydrate Polymers*, **2017**, *174*, 417-420.

207. Kylin H. Biochemistry of sea algae. *Zeitschrift für Physikalische Chemie*, **1913**, *83*, 171-197.

208. 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. *Carbohydrate Research*, **2001**, *330*(4), 529-535.

209. Fitton JH. Therapies from Fucoidan; multifunctional marine polymers. *Marine Drugs*, **2011**, *9*(10), 1731-1760.

210. Lorbeer AJ, Tham R, Zhang W. Potential products from the highly diverse and endemic macroalgae of southern Australia and pathways for their sustainable production. *Journal of Applied Phycology*, **2013**, *25*(3), 717-732.

211. Kuznetsova TA, Besednova NN, Mamaev AN, Momot AP, Shevchenko NM, Zvyagintseva TN. Anticoagulant activity of fucoidan from brown algae *Fucus evanescens* of the Okhotsk Sea. *Bulletin of Expe-*

rimental Biology and Medicine, **2003**, *136*(5): 471-473.

212. Wu L, Sun J, Su X, Yu Q, Yu Q, Zhang P. A review about the development of fucoidan in antitumor activity: progress and challenges. *Carbohydrate Polymers*, **2016**, *154*, 96-111.

213. Li B, Lu F, Wei XJ, Zhao RX. Fucoidan: structure and bioactivity. *Molecules*, **2008**, *13*(8), 1671-1695.

214. Holtkamp AD, Kelly S, Ulber R, Lang S. Fucoidans and fucoidanases-focus on techniques for molecular structure elucidation and modification of marine polysaccharides. *Applied Microbiology and Biotechnology*, **2009**, *82*(1), 1-11.

215. Thanh-Sang V, Kim S-K. Fucoidans as a natural bioactive ingredient for functional foods. *Journal of Functional Foods*, **2013**, *5*(1), 16-27.

216. Fitton JH, Stringer DN, Karpiniec SS. Therapies from fucoidan: an update. *Marine Drugs*, **2015**, *13*(9), 5920-5946.

217. Cardoso SM, Pereira OR, Seca AM, Pinto D, Silva AM. Seaweeds as preventive agents for cardiovascular diseases: From nutrients to functional Foods. *Marine Drugs*, **2015**, *13*, 6838-6865.

218. Béress A., Wassermann O, Bruhn T, Béress L, Kraiselburd EN, Gonzalez LV, de Motta GE, Chavez PI. A new procedure for the isolation of anti-HIV compounds (polysaccharides and polyphenols) from the marine alga *Fucus vesiculosus*. *Journal of Natural Products*, **1993**, *56*, 478-488.

219. Baba M, Nakajima M, Schols D, Pauwels R, Balzarini J, De Clercq E. Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent *in vitro*. *Antiviral Research*, **1988**, *9*, 335-343.

220. Moen LK, Clark GF. A novel reverse transcriptase inhibitor from *Fucus vesiculosus*. *International AIDS Conference*, **1993**, *9*, 145-161.

221. Baba M, Snoeck R, Pauwels R, de Clercq E. Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including Herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrobial Agents and Chemotherapy*, **1988**, *32*, 1742-1745.

222. Yasuhara-Bell J, Lu Y. Marine compounds and their antiviral activities. *Antiviral Research*, **2010**, *86*(3), 231-240.

223. Koyanagi S, Tanigawa N, Nakagawa H, Soeda S, Shimeno H. Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. *Biochemical Pharmacology*, **2003**, *65*(2), 173-179.

224. Kim IH, Nam TJ. Fucoidan downregulates insulin-like growth factor-I receptor levels in HT-29 human colon cancer cells. *Oncology Reports*, **2018**, *39*, 1516-1522.

225. Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'Incecco A, Piccoli A, Totani L, Tinari N, Morozevich GE, Berman AE, Bilan MI, Usov AI, Ustyuzhanina NE, Grachev AA, Sanderson CJ, Kelly M, Rabinovich GA, Iacobelli S, Nifantiev NE. A comparative study of the anti-inflammatory, anticoagulant,

antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology*, **2007**, *17*(5), 541-552.

226. Usoltseva RV, Anastyuk SD, Ishina IA, Isakov VV, Zvyagintseva TN, Thinh PD, Zadorozhny PA, Dmitrenok PS, Ermakova SP. Structural characteristics and anticancer activity *in vitro* of fucoidan from brown alga *Padina boryana*. *Carbohydrate Polymers*, **2018**, *184*, 260-268.

227. Kadam SU, Tiwari BK, O'Donnell CP. Extraction, structure and biofunctional activities of laminarin from brown algae. *International Journal of Food Science & Technology*, **2014**, *50*, 24-31.

228. Kadam SU, Brijesh, Tiwari K, O'Donnell CP. Extraction, structure and biofunctional activities of laminarin from brown algae. *International Journal of Food Science & Technology*, **2015**, *50*(1), 24-31.

229. Kadam SU, O'Donnell CP, Rai DK, Hossain MB, Burgess CM, Walsh D, Tiwari BK. Laminarin from Irish brown seaweeds *Ascophyllum nodosum* and *Laminaria hyperborea*: ultrasound assisted extraction, characterization and bioactivity. *Marine Drugs*, **2015**, *13*(7), 4270-4280.

230. Schmiedeberg JEO. 1885. Gesellschaft deutscher naturforscher und arzte: *Leipzig. Tageblatt der Versammlung*, **1885**, *58*, 427 (*in German*).

231. Kawai H, Henry EC. Phaeophyta. *In*: Archibald J, Simpson A, Slamovits C (eds.). Handbook of the Protists. Springer, Cham, **2017**.

232. Rioux LE, Turgeon SL, Beaulieu M. 2007. Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers*, **2007**, *69*(3), 530-537.

233. Shin HJ, Oh SJ, Kim SI, Won Kim H, Son, J-H Nelson. Conformational characteristics of β-Glucan in laminarin probed by terahertz spectroscopy. *Applied Physics Letters*, **2009**, *94*(11), 111911-111911-3.

234. Nelson TE, Lewis BA. Separation and characterization of the soluble and insoluble components of insoluble laminaran. *Carbohydrate Research*, **1974**, *33*, 63-74.

235. Alderkamp A-C, Van Rijssel M, Bolhuis H. Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. *FEMS Microbiology Ecology*, **2007**, *59*, 108-117.

236. Rioux LE, Turgeon SL, Beaulieu M. Structural characterization of laminaran and galactofucan extracted from the brown seaweed *Saccharina longicruris*. *Phytochemistry*, **2010**, *71*, 1586-1595.

237. Sanjeewa KKA, Lee JS, Kim WS, eon YJ. The potential of brown-algae polysaccharides for the development of anticancer agents: an update on anticancer effects reported for fucoidan and laminaran. *Carbohydrate Polymers*, **2017**, *177*, 451-459.

238. Muto S, Niimura K, Oohara M, Oguchi Y, Matsunaga K, Hirose K, Kakuchi J, Sugita N, Furusho T. Polysaccharides from marine algae and antiviral drugs containing the same

as active ingredients. EP295956. Eur. Patent, **1988**.

239. Lynch MB, Sweeney T, Callan JJ, O'Sullivan JT, O'Doherty JV. The effect of dietary Laminaria-derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. *Journal of the Science of Food and Agriculture*, **2010**, *90*(3), 430-437.

240. Schepetkin IA, Quinn MT. Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. *International Immunopharmacology*, **2006**, *6*, 317-333.

241. Ibrahim AMM, Mostafa MH, El-Masry MH, El-Naggar MMA. Active biological materials inhibiting tumor initiation extracted from marine algae. *Egyptian Journal of Aquatic Research*, **2005**, *31*, 146-155.

242. Lee JY, Kim YJ, Kim HJ, Kim YS, Park W. Immunostimulatory effect of laminarin on RAW 264.7 mouse macrophages. *Molecules*, **2012**, *17*, 5404-5411.

243. Bohn JA, BeMiller JN. (1→3)-β-d-Glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers*, **1995**, *28*, 3-14.

244. Choi J-I, Kim H-J, Lee J-W. Structural feature and antioxidant activity of low molecular weight laminarin degraded by gamma irradiation. *Food Chemistry*, **2011**, *129*, 520-523.

245. Ermakova S, Men'shova R, Vishchuk O, Kim S-M, Um B-H, Isakov V, Zvyagintseva T. Water-soluble polysaccharides from the brown alga *Eisenia bicyclis*: structural characteristics and antitumor activity. *Algal Research*, **2013**, *2*, 51-58.

246. Usoltseva RV, Anastyuk SD, Shevchenko NM, Zvyagintseva TN, Ermakova SP. The comparison of structure and anticancer activity *in vitro* of polysaccharides from brown algae *Alaria marginata* and *A. angusta*. *Carbohydrate Polymers*, **2016**, *153*, 258-265.

247. Ji C-F, Ji Y-B. Laminaran-induced apoptosis in human colon cancer LoVo cells. *Oncology Letters*, **2014**, *7*(5), 1728-1732.

248. Ji C-F, Ji Y-B, Meng D-Y. Sulfated modification and anti-tumor activity of laminaran. *Experimental and Therapeutic Medicine*, **2013**, *6*(5), 1259-1264.

249. Malyarenko OS, Usoltseva RV, Shevchenko NM, Isakov VV, Zvyagintseva TN, Ermakova SP. *In vitro* anticancer activity of the laminarans from Far Eastern brown seaweeds and their sulfated derivatives. *Journal of Applied Phycology*, **2017**, *29*, 543-553.

250. Usoltseva RV, Shevchenko NM, Malyarenko OS, Ishina IA, Ivannikova SI, Ermakova SP. Structure and anticancer activity of native and modified polysaccharides from brown alga *Dictyota dichotoma*. *Carbohydrate Polymers*, **2018**, *180*, 21-28.

251. Larsen B, Salem DM, Sallam MA, Mishrikey MM, Beltagy AI. Characterization of the alginates from algae harvested at the Egyptian Red Sea coast. *Carbohydrate Research*, **2003**, *338*, 2325-2336.

252. Pereira L, Sousa A, Coelho H, Amado AM, Ribeiro-Claro PJA. Use of FTIR, FT-Raman and ^{13}C-NMR spectroscopy for identification of some seaweed phycocolloids. *Biomolecular Engineering*, **2003**, *20*, 223-228.

253. Leal D, Matsuhiro B, Rossi M, Caruso F. FT-IR spectra of alginic acid block fractions in three species of brown seaweeds. *Carbohydrate Research*, **2008**, *343*, 308-316.

254. Lobban CS, Chapman DL, Kremer BP. Experimental phycology: a laboratory manual. Phycological Society of America. Cambridge University Press, Cambridge, UK, **1988**, 320 pp.

255. Lahaye M. Chemistry and physicochemistry of phycocolloids. *Cahiers de Biologie Marine*, **2001**, *42*(1-2), 137-157.

256. McHugh DJ. A guide to the seaweed industry. *FAO, Fisheries Technical Paper*, **2003**, *441*, 73-90.

257. Pereira L. Soares, F, Freitas AC, Duarte AC, Ribeiro-Claro P. Chapter 3 - Extraction, characterization, and use of carrageenans, pp. 34-89. *In*: Parappurath Narayanan Sudha (ed.). Industrial Applications of Marine Biopolymers, CRC Press, Taylor & Francis Group, Boca Raton, FL, **2017**.

258. Pereira L. As algas marinhas e respectivas utilidades. Monografias, **2008**. Available online at: http://br.monografias.com/trabalhos913/algas-marinhas-utilidades/algas-marinhas-utilidades.pdf (*in Portuguese*) (accessed 2018).

259. Onsoyen E. Alginates. pp. 22-44. *In*: Imeson A (ed.). Thickening and Gelling Agents for Food. Blackie Academic and Professional, London, UK, **1997**.

260. Pereira L. Edible seaweeds of the world. Science Publishers, an Imprint of CRC Press / Taylor & Francis Group, Boca Raton, FL, **2016**, 453 pp.

261. Hu X, Jiang X, Hwang H, Liu S, Guan H. Antitumour activities of alginate-derived oligosaccharides and their sulphated substitution derivatives. *European Journal of Phycology*, **2004**, *39*(1), 67-71.

262. Khotimchenko YS, Kovalev VV, Savchenko OV, Ziganshina OA. Physicalchemical properties, physiological activity, and usage of alginates, the polysaccharides of brown algae. *Russian Journal of Marine Biology*, **2001**, *27*(1), 53-64.

263. Tonnesen HH, Karlsen J. Alginate in drug delivery systems. *Drug Development and Industrial Pharmacy*, **2002**, *2*(6), 621-630.

264. Russo R, Malinconico M, Santagata G. Effect on cross-linking with calcium ions on the physical properties of alginate films. *Biomacromolecules*, **2007**, *8*, 3193-3197.

265. Merrill EW, Salzman EW. Polyethylene oxide as a biomaterial. *American Society for Artificial Internal Organs Journal*, **1983**, *6*, 60-64.

266. Han DK, Park KD, Ahn KD, Jeong SY, Kim YH. Preparation and surface characterization of PEO-grafted and heparin-immobilized polyure-

thanes. *Journal of Biomedical Materials Research*, **1989**, *23*, 87-104.

267. Tu R., Lu CL, Thyagarajan K, Wang E, Nguyen H, Shen S, Hata C, Quijano RC. Kinetic study of collagen fixation with polyepoxy fixatives. *Journal of Biomedical Materials Research*, **1993**, *27*, 3-9.

268. Chen JP, Chu IM, Shiao MY, Hsu BRS, Fu SH. Microencapsulation of islets in PEG-amine modified alginate-poly(l-lysine)-alginate microcapsules for constructing bioartificial pancreas. *Journal of Fermentation and Bioengineering*, **1998**, *86*, 185-190.

269. Chandy T, Mooradian DL, Rao GHR. Chitosan/polyethylene glycol-alginate microcapsules for oral delivery of hirudin. *Journal of Applied Polymer Science*, **1998**, *70*, 2143-2153.

270. Qurrat-ul-Ain SS, Khuller GK, Garg SK. Alginate-based oral drug delivery system for tuberculosis: pharmacokinetics and therapeutic effects. *Journal of Antimicrobial Chemotherapy*, **2003**, *51*(4), 931-938.

271. Jayant RD, McShane MJ, Srivastava R. Polyelectrolyte-coated alginate microspheres as drug delivery carriers for dexamethasone release. *Drug Delivery*, **2009**, *16*, 331-340.

272. Kevadiya BD, Joshi GV, Patel HA, Ingole PG, Mody HM, Bajaj HC. Montmorillonite-alginate nanocomposites as a drug delivery system: Intercalation and *in vitro* release of vitamin B_1 and vitamin B_6. *Journal of Biomaterials Applications*, **2010**, *25*, 161-177.

273. Miyazaki S., Nakayama A, Oda M, Takada M, Attwood D. Chitosan and sodium alginate based bioadhesive tablets for intraoral drug delivery. *Biological and Pharmaceutical Bulletin*, **1994**, *17*, 745-747.

274. Tapia C, Escobar Z, Costa E, Sapag-Hagar J, Valenzuela F, Basualto C, Gai MN, Yazdani-Pedram M. Comparative studies on polyelectrolyte complexes and mixtures of chitosan-alginate and chitosan-carrageenan as prolonged diltiazem clorohydrate release systems. *European Journal of Pharmaceutics and Biopharmaceutics*, **2004**, *57*, 65-75.

275. Gavini E, Sanna V, Juliano C, Bonferoni MC, Giunchedi P. Mucoadhesive vaginal tablets as veterinary delivery system for the controlled release of an antimicrobial drug, Acriflavine. *AAPS PharmSciTech*, **2002**, *3*(3), E20.

276. El-Kamel A., Sokar M, Naggar V, Al Gamal S. Chitosan and sodium alginate-based bioadhesive vaginal tablets. *AAPS PharmSciTech*, **2002**, *4*, E44.

277. Strand BL, Gaserod O, Kulseng B, Espevik T, Skjak-Braek G. Alginate-polylisine-alginate microcapsules: effect of size reduction on capsule properties. *Journal of Microencapsulation*, **2002**, *19*, 612-630.

278. Mumper RJ, Hoffman AS, Puolakkainen PA, Bouchard LS, Gombotz WR. Calcium-alginate beads for the oral delivery of transforming growth factor-β_1 (TGF-β_1): stabilization of TGF-β_1 by the addition of polyacrylic acid within acid-treated beads. *Journal of Controlled Release*, **1994**, *30*, 241-251.

279. Shojaei AH, Paulson J, Honary S. Evaluation of poly(acrylic acid-co-ethylhexyl acrylate) films for mucoadhesive transbuccal drug delivery: factors affecting the force of mucoadhesion. *Journal of Controlled Release*, **2000**, *67*, 223-232.

280. Qi M., Morch Y, Lacik I, Formo K, Marchese E, Wang Y, Danielson KK, Kinzer K, Wang S, Barbaro B, Kollarikova G, Chorvat D, Hunkeler D, Skjak-Braek G, Oberholzer J, Strand BL. Survival of human islets in microbeads containing high guluronic acid alginate crosslinked with Ca^{2+} and Ba^{2+}. *Xenotransplantation*, **2012**, *19*(6), 355-364.

281. Nalamothu N, Potluriand A, Muppalla MB. Review on marine alginates and its applications. *Indo American Journal of Pharmaceutical Research*, **2014**, *4*(10), 4006-4015.

282. Lee KY, Mooney DJ. Alginate: Properties and biomedical applications. Progress in *Polymer Science*, **2012**, *37*, 106-126.

283. Lin L, Chow KL, Leng Y. Study of hydroxyapatite osteoinductivity with an osteogenic differentiation of mesenchymal stem cells. *Journal of Biomedical Materials Research Part A*, **2009**, *89*, 326-335.

284. Valente J, Valente T, Alves P, Ferreira P, Silva A, Correia IJ. Alginate based scaffolds for bone tissue engineering. *Materials Science and Engineering*, **2012**, *C32*, 2596-2603.

285. Zhao L, Weir MD, Xu HH. An injectable calcium phosphate-alginate hydrogel-umbilical cord mesenchymal stem cell paste for bone tissue engineering. *Biomaterials*, **2010**, *31*(25), 6502-6510.

286. Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ. Degradation of partially oxidized alginate and its potential application for tissue engineering. *Biotechnology Progress*, **2001**, *17*(5), 945-950.

287. Moshaverinia A, Chen C, Akiyama K, Ansari S, Xu X, Chee WW, Schricker SR, Shi S. Alginate hydrogel as a promising scaffold for dental-derived stem cells: An *in vitro* study. *Journal of Materials Science: Materials in Medicine*, **2012**, *23*, 3041-3051.

288. Hennequart F. Seaweed applications in human health/nutrition: the example of algal extracts as functional ingredients in novel beverages. 4th International Symposium Health and Sea, Granville, France, **2007**.

289. Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science and Technology*, **2011**, *22*, 315-326.

290. Xin XL, Ding H, Geng MY, Liang PF, Li YX, Guan HS. Studies of the anti-AIDS effects of marine polysaccharide drug 911 and its related mechanisms of action. *Chinese Journal of Marine Drugs*, **2000**, *6*, 4-8.

291. Xin XL, Geng MY, Guan HS, Li ZL. Study on the mechanism of inhibitory action of 911 on replication of HIV-1 *in vitro*. *Chinese Journal of Marine Drugs*, **2000**, *19*, 15-18.

292. Xianliang X, Meiyu G, Huashi G, Zelin L. Study on the mechanismof

inhibitory actionof 911 onreplication of HIV-1 *in vitro*. *Chinese Journal of Marine Drugs*, **2000**, *19*(4), 15-22.

293. Jiang B, Xu X, Li L, Yuan W. Study on '911' anti-HBV effect in HepG2.2.15 cells culture. *Modern Preventive Medicine*, **2003**, *30*, 517-518.

294. Wang W, Wang S-X, Guan H-S. The antiviral activities and mechanisms of marine polysaccharides: an overview. *Marine Drugs*, **2012**, *10*(12), 2795-2816.

295. Meiyu G, Fuchuan L, Xianliang X, Jing L, Zuowei Y, Huashi G. The potential molecular targets of marine sulfated polymannuroguluronate interfering with HIV-1 entry: interaction between SPMG and HIV-1 rgp120 and CD4 molecule. *Antiviral Research*, **2003**, *59*(2), 127-135.

296. Liu HY, Geng MY, Xin XL, Li FC, Chen HX, Guan HS, Ding J. Multiple and multivalent interactions of novel anti-AIDS drug candidates, sulfated polymannuronate (SPMG)-derived oligosaccharides, with gp120 and their anti-HIV activities. *Glycobiology*, **2005**, *15*, 501-510.

297. Kimura Y, Watanabe K, Okuda H. Effects of soluble sodium alginate on cholesterol excretion and glucose tolerance in rats. *Journal of Ethnopharmacology*, **1996**, *54*, 47-54.

298. Lordan S, Ross RP, Stanton C. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Marine Drugs*, **2011**, *9*(6), 1056-1100.

299. Harden CJ, Richardson JC, Dettmar PW, Corfe BM, Paxman JR. An ionic-gelling alginate drink attenuates postprandial glycaemia in males. *Journal of Functional Foods*, **2012**, *4*(1), 122-128.

300. El Khoury D, Goff HD, Berengut S, Kubant R, Anderson GH. Effect of sodium alginate addition to chocolate milk on glycemia, insulin, appetite and food intake in healthy adult men. *European Journal of Clinical Nutrition*, **2014**, *68*(5), 613-618.

301. Zee S. Body weight loss with the aid of alginic acid. *Archives of Internal Medicine*, **1991**, *3-4*, 113-114.

302. Georg JM, Kristensen M, Astrup A. Effect of alginate supplementation on weight loss in obese subjects completing a 12-Wk energy-restricted diet: a randomized controlled trial. *The American Journal of Clinical Nutrition*, **2012**, *96*(1), 5-13.

303. Houghton D, Wilcox MD, Chater PI, Brownlee IA, Seal CJ and Pearson JP. Biological activity of alginate and its effect on pancreatic lipase inhibition as a potential treatment for obesity. *Food Hydrocolloids*, **2015**, *49*, 18-24.

304. Torsdottir I, Alpsten M, Holm G, Sandberg, Tolli J. A small dose of soluble alginate-fiber affects postprandial glycemia and gastric-emptying in humans with diabetes. *Journal of Nutrition*, **1991**, *121*(6), 795-799.

305. Vaugelade P, Hoebler C, Bernard F, Guillon F, Lahaye M, Duee PH, Darcy-Vrillon B. Non-starch polysaccharides extracted from seaweed can modulate intestinal absorption

of glucose and insulin response in the pig. *Reproduction Nutrition Development*, **2000**, *40*, 33-47.

306. El Khoury D, Goff HD, Anderson GH. The role of alginates in regulation of food intake and glycemia: a gastroenterological perspective. *Critical Reviews in Food Science and Nutrition*, **2015**, *55*(10), 1406-1424.

307. Arasaki S, Arasaki T. Low calorie, high nutrition vegetables from the sea. To help you look and feel better. Japan Publications Inc., Tokyo, **1983**, 196 pp.

308. Sheth BB. Alginate containing antacid compositions. US Patetent 3,326,755, **1967**.

309. Bogentoff CB. Antacid composition for neutralizing stomach acid. Germany Patent 2,722,484, **1981**.

310. Nicolai JJ, Speelman P, Tytgat GN, van der Stadt J. 1981. Comparison of the combination of cholestyramine/alginates with placebo in the treatment of postgastrectomy biliary reflux gastritis. *European Journal of Clinical Pharmacology*, **1981**, *21*(3), 189-194.

311. Brailski Kh, Dimitrov B. Treatment of duodenal ulcer with the Bulgarian preparation Gastralgin. *Vutreshni Bolesti*, **1987**, *26*(2), 79-82.

312. Washington N, Denton G. Effect of alginate and alginate-cimetidine combination therapy on stimulated postprandial gastro-oesophageal reflux. *Journal of Pharmacy and Pharmacology*, **1995**, *47*(11), 879-882.

313. Kwiatek MA, Roman S, Fareeduddin A, Pandolfino JE, Kahrilas PJ. An alginate-antacid formulation (Gaviscon Double Action Liquid®) can eliminate the postprandial "acid pocket" in symptomatic GERD patients. *Alimentary Pharmacology & Therapeutics*, **2011**, *34*(1), 59-66.

314. Raposo MF, de Morais A, de Morais R. Marine polysaccharides from algae with potential biomedical applications. *Marine Drugs*, **2015**, *13*(5), 2967-3028.

315. Terada A, Hara, Mitsuoka T. Effect of dietary alginate on the faecal microbiota and faecal metabolic activity in humans. *Microbial Ecology in Health and Disease*, **1995**, *8*(6), 259-266.

316. Wang Y, Han F, Hu B, Li JB, Yu WG. *In vivo* prebiotic properties of alginate oligosaccharides prepared through enzymatic hydrolysis of alginate. *Nutrition Research*, **2006**, *26*(11), 597-603.

317. Zaharudin N, Salmean AA, Dragsted LO. Inhibitory effects of edible seaweeds, polyphenolics and alginates on the activities of porcine pancreatic alpha-amylase. *Food Chemistry*, **2018**, *245*, 1196-1203.

318. Mohsin S, Mahadevan R, Muraleedhara Kurup G. Free-radical-scavenging activity and antioxidant effect of ascophyllan from marine brown algae *Padina tetrastromatica*. *Biomedicine & Preventive Nutrition*, **2014**, *4*(1), 75-79.

319. Jiang Z, Abu R, Isaka S, Nakazono S, Ueno M, Okimura T, Yamaguchi K, Oda T. Inhibitory effect of orally-administered sulfated polysaccharide ascophyllan isolated from *As-*

cophyllum nodosum on the growth of sarcoma-180 solid tumor in mice. *Anticancer Research*, **2014**, *34*(4), 1663-1671.

320. Zhang WJ-YD, Jiang Z, Okimura T, Oda T, Yu Q, Jin J-O. Ascophyllan purified from *Ascophyllum nodosum* induces Th1 and Tc1 immune responses by promoting dendritic cell maturation. *Marine Drugs*, **2014**, *12*, 4148-4416.

321. Zhang W, Okimura T, Xu L, Zhang LJ, Oda T, Kwak M, Yu Q, Jin JO. Ascophyllan functions as an adjuvant to promote anti-cancer effect by dendritic cell activation. *Oncotarget*, **2016**, *7*, 19284-19298.

322. Jiang Z., Okimura T, Yamaguchi K, Oda T. The potent activity of sulfated polysaccharide, ascophyllan, isolated from *Ascophyllum nodosum* to induce nitric oxide and cytokine production from mouse macrophage RAW264.7 cells: Comparison between ascophyllan and fucoidan. *Nitric Oxide*, **2011**, *25*, 407-415.

323. Larsen B, Haug A, Painter T. Sulphated polysaccharides in brown algae. 3. The native state of fucoidan in *Ascophyllum nodosum* and *Fucus vesiculosus*. *Acta Chemica Scandinavica*, **1970**, *24*(3), 3339-3352.

324. Kloareg B, Demarty M, Mabeau S. Polyanionic characteristics of purified sulphated homofucans from brown algae. *International Journal of Biological Macromolecules*, **1986**, *8*, 380-386.

325. Nakayasu S, Soegima R, Yamaguchi K, Oda T. Biological activities of fucose-containing polysaccharide ascophyllan isolated from the brown alga *Ascophyllum nodosum*. *Bioscience, Biotechnology, and Biochemistry*, **2009**, *73*, 961-964.

326. Wang Y, Jiang Z, Kim D, Ueno M, Okimura T, Yamaguchi K, Oda T. Stimulatory effect of the sulfated polysaccharide Ascophyllan on the respiratory burst in Raw264.7 macrophages. *International Journal of Biological Macromolecules*, **2013**, *52*, 164-169.

327. Eklof J. Chemical structure of Porphyran, **2012** (image in public domain). Available online at: https://www.cazypedia.org/index.php/File:Porphyran.png (accessed 2018)

328. Edgar G. Laminaran/laminarin - chemical structure of β-1,3-1,6-glucan, 2008 (image in public domain). Available online at: https://commons.wikimedia.org/w/index.php?curid=5274129 (accessed 2018)

329. Pereira L. Therapeutic and Nutritional Uses of Algae. Science Publishers' (SP), An Imprint of CRC Press / Taylor & Francis Group, Boca Raton, FL, **2018**, 560 pp.

