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Novel Algal Polysaccharides from Marine Source: Porphyran

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ABSTRACT
Porphyran is a sulfated polysaccharide derived from *Porphyra* sp. It comprises of sulfate ester and 3, 6-anhydrogalactose whose arrangement is similar to agarose. Porphyran has enormous reported activities. It has potential pharmaceutical, medicinal and research applications. Porphyran a promising product is useful to produce antiallergic, anticancer, antioxidant, antitumour, antiviral, immunostimulant, antifatigue, metallic adsorption ability, improvement of intestinal flora, antibacterial, anticoagulant, antiviral, microphage promotion action, hepatoprotective agent, antihyperlipidemic, hypocholesterolemic, hypoglycemic, hypotensive, antiulcer and prebiotic activity. Despite of all this it is also used as gelling agent. Currently porphyran availabilty in the market is not known because of several problems such as its high molecular weight, high viscosity and availability of *Porphyra.* So it is very necessary to know about basic facts regarding *Porphyra* sp. before obtaining any novel compound from it.
KEY WORDS: Porphyran, Nori, Porphyra, Sulfated polysaccharides, Seaweeds, galactans.
Abbreviations: *IgE, Immunoglobulin; IFN-γ, interferon-γ; IgM, immunoglobulin M; IL-4, interleukin; PARP, poly(ADP-ribose) polymerase; HCMV, human cytomegalo virus; DENV, dengue virus; RSV, respiratory syncytial virus; SOD, super oxide dismutase; GSH-Px, glutathione peroxidase; CCl_4, carbon tetra chloride; IGF-1R, insulin growth factor- 1 receptor; 3, 6-AG, 3,6- anhydro galactan.*

INTRODUCTION
Porphyran is a sulphated polysaccharide isolated from seaweeds of order Bangiales especially from the genera *Porphyra* (1). It is obtained from red algae of Kingdom Rhodophyta. *Porphyra yezoensis*, *Porphyra tenera*, *Porphyra haitanensis* and *Porphyra suborbiculata* are traditionally used in Japan as food such as dry sheet laver or stewed laver in soy sauce. This algae is processed into a sheet type of dried food, "Nori" that contains major dietary fiber and constitutes nearly about 40% of mass (2). It is nutritious, having abundant protein, polysaccharide, vitamins, and minerals. *Porphyra* retard ageing in East Asia, is traditionally used to treat nephritic and cordis diseases in Chinese. So it is important to evaluate the nutritional and physiological functions of all of its constituents. Porphyran, *a*-sulfated polygalactan,
Chemically Porphyran is related to agarose, consists of linear backbone of alternating 3-linked β-D-galactose and 4-linked 3, 6-anhydro-α-L-galactose units. The L residues are mainly composed of α -L-galactosyl 6-sulfate units, and the 3, 6-anhydrogalactosyl units are minor. Porphyran is a major constituent of Nori which contains 78.2% of sugar, 49.5% of galactose, 17.5% of 3,6-anhydrogalactose and free and bound sulfur 2.0% and 5.6%, respectively, while the ash and protein content 9.1% and 0.9%, respectively (3-5).

PHARMACOLOGICAL PROFILE OF PORPHYRAN

Antiallergic effect
Porphyran is effective against contact hypersensitivity induced by 2, 4, 6 tri nitrochlorobenzene by suppressing the serum levels of IgE and IFN-γ. Porphyran has excellent inhibitory activity against hyaluronidase, responsible for the releasing of histamine from the mast cells (2).

Composed of the galactose and 3, 6 anhydrogalactose, Porphyran has been reported as, antioxidant, antitumour, immunostimulant, hypotensive, antifatigue, metallic adsorption ability improvement of intestinal flora antibacterial, anticoagulant, anticancer, antiviral, microphage promotion action, antihyperlipidemic, hepatoprotective and prebiotic activity which is reported in microorganism degrading Porphyran (3-5).

CHEMICAL PROFILE OF PORPHYRAN
Rees and Conway (1962) studied that *Porphyra* contains sulfated ester ranging from 6% to 11%, and 3, 6-anhydrogalactose from 5% to 19%. Polysaccharide from the *Porphyra* are 4-linked 6-*O*-sulfo-α-L-galactopyranose and 3-linked 6-*O*-methyl-β-D-galactopyranose

Immunomodulating activity -
Porphyran has immunostimulating activity. It elevates primary antibody response (IgM) and stimulates macrophages. Being undigestible polysaccharides they are utilized by the intestinal bacteria and hence modify the immune activity by elevating the cecal level of short chain fatty acids, lowering the cecum pH and modifying the intestine flora. Porphyran can suppress Th-2 type immune system without influencing Th-1 type immune system. It has been reported that 2% Porphyran reduces the IgE, IgG2α and cytokines such as interferon-γ (IFN-γ), IL-4, IL-10 are not affected. Besides of Porphyran there are many sulfated polysaccharides such as fucoidan, carrgeenan and funoran which are having a marked effect on immunomodulation activities (2).

Anticoagulant
Algal polysaccharides, Porphyran are also known to act as blood anticoagulants. Porphyran may be useful to reduce

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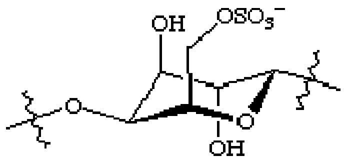
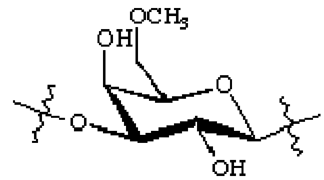


4-linked 6-*O*-sulfo-α-L-galactopyranose



3-linked 6-*O*-methyl-ß-D-galactopyranose

serum lipid levels and prevent thrombosis. Recently, a mixture of seaweeds has been developed as a health supplement, it effects on serum lipid levels and platelet aggregation (5).

Antiaging activity

Porphyra is an important food source in many parts of the world. In East Asia, *Porphyra* is regarded as a vegetable having power to retard aging and improve the vitality. Porphyran can increase the activity of glutathione peroxidase and superoxide dismutase an antioxidant enzyme, which metabolizes potentially damaging molecules to non-toxic products. In addition, Porphyrans also inhibit lipid peroxidation and increase total antioxidant capacity (6).

Antiviral Agent

Some sulphated polysaccharides from red algae show antiviral activities towards viruses responsible for human infectious diseases. Porphyran inhibits HIV reverse transcriptase *in vitro*. It has minimal effect on human DNA and RNA polymerase activity. Some agaroids such as high molecular weight galactan sulphate have antiviral properties against herpes simplex virus, human cytomegalo virus (HCMV), dengue virus (DENV), respiratory syncytial virus (RSV), and influenza virus due to the inhibition of the initial virus attachment to the host cell (8-12).

Hepatoprotective agent

Porphyran plays a protective action against hepatotoxicity induced by CCl_4 in mice. Its mechanisms may be related to free radical scavenging, increasing SOD, GSH-Px activity and anti-lipid peroxide (13-17).

***In vivo* antioxidant activity**

Porphyran exhibit *in vivo* antioxidant activity by enhancing the activity of SOD and GSH-Px and inhibiting lipid peroxidation. It has been reported that these two enzymes decrease the consequence of ageing. Porphyran is a high molecular weight polysaccharide featuring high viscosity, which may limit its pharmaceutical application. Lower molecular weight Porphyran exhibits high antioxidant activity. Low molecular weight Porphyran products contain carboxyl groups, which may have a very important effect on free radicals (18, 19).

Antihyperlipidemic, Hypocholesterolemic, Hypoglycemic, Hypotensive activities

Porphyran produce hypocholesterolemic and hypolipidemic effect due to reduced cholesterol absorption in the gut with an increase in faecal cholesterol content and a hypoglycaemic response. It also reduces total cholesterol, free cholesterol, triglyceride and phospholipid in the liver. These substances are likely to be exploited by 'nutraceutical' companies that market them as Health products (20-30).

Anticancer activity

Porphyran display antitumor properties. In particular, the ester sulfates of the polysaccharides may be related to the antitumor activity. The degree of polyanionic properties may be intimately related to this activity. Porphyran induces apoptosis by caspase-3 or caspase-9 activation, decreasing the IGF-1R phosphorylation, and down regulation of Akt phosphorylation. The caspase induce apoptosis are activated in a sequential cascade of their cleavages from their inactive forms. Activation of caspase-3 leads to the cleavage of a number of proteins, one of which is poly (ADP-ribose) polymerase (PARP). The cleavage of PARP is the hallmark of apoptosis. The expression level of anti-apoptotic molecules such as Bcl-2 gradually decreased, whereas the pro-apoptotic molecule Bad, which opposes the action of Bcl-2, increased in response to Porphyran exposure.

Porphyran decreases the expression levels in AGS gastric cancer cells by negatively regulating insulin-like growth factor-I receptor (IGF-IR) phosphorylation which is a potent mitogen and growth stimulatory factor for several kinds of cells, of which, over expression and enhanced activation is frequently observed in human cancers. 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 suggests that inhibition of Akt phosphorylation may be an important mechanism of Porphyran induced apoptosis (31-45).

Antiulcer activity

Porphyran inhibits gram negative bacterium *Helicobacter pylori* colonization. This substance can eliminate specifically *H. pylori* from the stomach and used in the prevention or treatment of gastritis, gastric ulcers, duodenal ulcers and gastric cancer. Oral administration of Porphyran prevent the adhesion of the urease on the *H. pylori* cells so as to prevent several diseases associated with it (46).

Other Therapeutic Uses Of Porphyran

Porphyran and its hydrated form 2-O-methylated derivatives and 2-O-sulfated derivatives exhibits antidiabetic, anti-rheumatic, anti-inflammatory, anti-α-glycosidase, anti-prostaglandin synthesis effect, anti-endotoxin shock effect, and inducing heme oxygenase production. This soluble sulfated polymer as in Porphyran also has capability in removing nucleic acid synthesis inhibitory substances thereby amplifying a nucleic acid in a sample efficiently (47, 48).

In cosmetic field

Porphyran, its viscosity and effect of retaining moisture were focused so that the use was aimed to improve skin condition after applying it to skin: providing smooth or moisturized skin, or removing overproduced oil. Recently, knowledge referring to physiological activities of Porphyran as cosmetics has been found, whereas little has been elucidated as to its specific physiological activity and biochemical activity on skin (49).

Heavy metals absorption

Algae cell wall contains number of sulfated galactan such as Porphyran which has remarkable heavy metals bioabsorption capability. The enormous amounts of polysacharide matrix are embedded in the cell wall of Porphyra. This characteristic, combined with their well known ability to bind metals, makes them potentially excellent heavy metal biosorbents. The passive removal of toxic heavy metals such as Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, and Hg^{2+} by inexpensive biomaterials, termed biosorption, requires that the substrate displays high metal uptake and selectivity, as well as suitable mechanical properties for applied remediation scenario. In recent years, many low cost sorbents have been investigated, but the brown algae have since proven to be the most effective and promising substrates. It is their basic biochemical constitution that is responsible for this enhanced performance among biomaterials (50).

RECENT DEVELOPMENTS AND MARKET VALUE

Porphyra, known as Nori in Japan, Kim in China red algae components are getting pace especially red algal polymers such as agar and carrageenan, and mainly Porphyran in nori. All of these polymers are sulfated galactans. Day by day these polysaccharides are gaining importance and come with new invention. Porphyran is not commercially available currently; though it was once tried to be extracted from *Porphyra* marine algae and used and it was also sold in 1980's. Although high cost of laver was cited as a reason of commercial unavailability. Porphyran is abundantly contained in laver with inferior quality, therefore the difficulty with material cost has been solved. As to the reason why Porphyran has not yet been applied in practical use even when the difficulties with material cost has been already solved. The market price of Porphyrans varies with the purity grades. The pharmaceutical grade product reaches US$ 13-15 per kg and the food grade US$ 6.5-11.0 kg (51). Porphyran with modified bioactivity and physical property is produced by dissociating a salt from a sulphate group or by dissociating a salt from sulphate group in Porphyran, removing it, and then altering the sulphate group potential utility value. The modified Porphyran also can be obtained by converting a salt of a sulphate group in Porphyran into a sulphate salt of a given salt by ion exchange. The modified Porphyran can be added to cosmetics, food, and drink, and used as cosmetic, food and drink having inhibitory activity against hyaluronidase activity (52, 53). The sulfated phycocolloids such as agar, Porphyran and carageenan have a wide range of uses as viscosifiers, emulsifiers and lubricants in the food, paper, textile, drug, and cosmetic industries. Porphyran which is also a gelling agent or water soluble polymer provides gel strength in various formulations such as tooth paste and serving the purpose of thickeners and binders with several purposes. In present days polysaccharide such as Porphyran also has effect on stabilizing the tear film on the eyeball surface over a prolonged period of time and is usable as an artificial tear fluid. In these fields, technologies for imparting brand new texture, coating properties, biocompatibility, drug retention and pharmacological effect by properly controlling the sol/gel state have been demanded.

Degradation of Porphyran

As it is known that Porphyan has very high molecular weight (higher than 100,000). So with such a high molecular weight it is very hard to absorb and tend not to affect physiological function to the body. Considerable reports have shown that the particle size of polysaccharide fractions have more advantages over unfractioned polysaccharides, such as greater bioavailabilty and improved efficacy to safety ratio. Microorganism such as *B-agarase*-1, *Alteromonas*, *Vibrio*, *Pseudomonas*, *Bacillus cereus*, and *Thalassompnas* are responsible for the degradation of oligosaccharides. Most of the microorganisms are present on the thalii of Porphyran. Porphyran when subjected to alkaline or acid treatment, the structures of 3, 6-anhydro-L-galactose and 6-O-methyl-L-galactose might be changed and unexpected products might be made by such treatments. Therefore, milder treatments are needed for oligomerization, such as enzymolysis. Degradation also was done with the help of radicals such as ascorbate and hydrogen peroxide. It is possible to prepare desired Porphyran products with different molecular weight by adjusting ascorbate to hydrogen peroxide proportions and their concentrations. Novel two types of *B-agarases* derived from a marine bacterium screened and isolated, include *B-agarases* A, of molecular weight of about 90,000 and *B-agarases* B of molecular weight of about 98,000. *B-agarases* A has an enzyme activity of hydrolyzing agarose and mainly into neoagarotetraose and neoagarohexaose. *B-agarases* B has an enzyme activity of hydrolyzing agarose and Porphyran mainly into neoagarobiose. These novel two types of *B-agarases* are used for preparing an oligosaccharide and for preparing the protoplast of seaweed cells (54).

Present scenario in the world market

Currently there are 42 countries in the world with reports of commercial seaweed activity. China holds with first rank in sea weed production, with *Porphyra* sp. accounting for most of its production, followed by Japan, Philippines, Chile, Norway, Indonesia, USA and India. These top ten countries contribute about 95% of the world commercial seaweed volume. About 90% seaweed production comes from culture based practices the most valuable crop is the red alga Nori (*Porphyra* sp. mainly *Porphyra yezzoensis*), used as food in Japan, China and Pacific. East and South East Asian countries contribute almost 99% cultured production, with half of the production (3 millions tons) supplied by China. Most output is used domestically for food, but there is growing international trade. The *Porphyra* cultivation in Japan is the biggest seaweed industry, with a turnover of more than US $1.8 billion per annum. Total EU imports of seaweed in 2001 amounted to 61,000 metric tonnes with the Philippines, Chile and Indonesia as the biggest suppliers. The seaweed industry

***Porphyra* sp. growing at different sites and time (% w/w of dry weight) (5).**

Place of collection	Time of collection	Yield	Total sugar	Sulfate	3,6-AG
Swakopmund, Namibia	August	25.4	73.3	11.5	12.3
	November 1998	18.8	78.7	11.5	12.9
	January 1999	23.4	77.4	9.7	12.9
Cape Town, South Africa	January 1998	17.1	76.6	9.6	10.1
	November 1998	22.3	70.7	10.1	11.0

Annual yield of sea weed in India (56).

Area	Annual yield (tonnes fresh wt.)
Gujarat	1,20,000
Maharastra	20, 315
Goa	2, 000
Karnataka	Negligible
Kerala	Negligible
Tamilnadu	1, 10,000
Andhra pradesh	Not available
Orissa	5
Andman and Nicobar	120

in India is mainly a cottage industry. The production of total seaweeds in India in 2000 was approximately 600,000 tons. Furthermore many alga based industries have been established in different places in maritimes states of Tamilnadu, Andhra pradesh, Kerala, Karnataka and Gujarat the seaweed industry is certainly on its way towards establishing itself in India. Porphyran yield depend on the type of extraction and purification methods applied over it. Mariculture of Porphyra also plays an important role in the production of *Porphyra*. Seaweed mariculture is carried out only in Asia, where there is a high demand for Nori products and burgeoning populations to create market growth. Cultivation of seaweeds in Asia is relatively low technology business (55). The chemical composition of polysaccharides extracted from algae growing at different sites and collected at different times is shown in Table 1 (5).

Challenges and Prospects

The major challenge in the seaweed industry is the over exploration leading to a scarcity of raw material. There is great potential for the Rhodophyta cultivation because of the low availabilty from the wild stock due to over exploration. Many edible seaweeds are available on the Indian coast; attempts should be made to develop products suitable for the Indian plateau and to popularize them amongst the public. Rhodophyta of Indian coast can be utilized for the production of many pharmaceutical products through extraction of bioactive compounds. Attention should be given towards developing *Porphyra* sp. with superior growth and nutritional characterstics, as the same has been proved successful in counteries like Japan. Rather opting for high volume low value seaweeds, culture of high value seaweeds such as *Porphyra* should be aimed for, as part of integerated coastal and national development programmes.

Out of estimated around US $ 3 billion global phycocolloid and biochemical business, India share meager. We can surely grab a bigger part in this lucrative buisness with since efforts towards large scale cultivation of commercialy important speices and processing. To facilitate this, more technologically sophisticated extraction plants with easy access to markets and marketing organization need to establish nearby cultivation areas to utilize the resources efficiently with greater profits. *Porphyra* sp. has a very important role to play towards betterment of coastal fishing communities and as a valuable foreign exchange earner. Government, research institutes, seaweed industry, Marine Products Export Development Authority and local angios, to adopt commercially viable large scale culture technologies, to provide them with good marketing facilities through proper channel (57).

Conclusion

Among the various sources for the development of new drugs, compounds from living organisms, so called natural products, are of particular significance. In recent years, a significant novel compound such as “Porphyran” with potent pharmacological properties has been discovered from the red algae. Porphyrans a natural red galactan related to agarose is derived from “*Porphyra*” the futuristically promising plant used in pharmaceuticals, cosmetics, nutritional supplements, molecular probes, fine chemicals and agrochemicals. In order to harness the rich potential of this seaweed it is necessary to focus sight on their active components. Research into the pharmacological properties of *Porphyra* sp. has led to the discovery of Porphyran considered worthy of clinical application. In the past pharmaceutical utility was limited due to its high molecular weight and high viscosity. Recently several inventions have been made on the degradation of this compound to improve its utility in pharmaceutical field. Porphyran now a day is gaining momentum in the pharmaceutical field as like other red algal galactans agar, carrageenan, alginate and fucoidan having peculiar pharmacological, gelling and thickening properties. Although there are only a few Porphyran products currently in the market, several robust new compounds derived from *Porphyra* products are now in the clinical pipeline, with more clinical development. But still there are so many problems come into the path while working on marine algae. Thus, the major break through in drug discovery from the sea has so far not happened but is still eagerly awaited by those active in the field. What are plausible reasons and obstacles that have slowed down marine drug development and what could be promising research avenues that will possibly provide solutions for the future?

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