Marine Medicinal Foods: Implications and Applications, Macro and Microalgae
Sea vegetables are rich in soluble dietary fibers, proteins, minerals, vitamins, antioxidants, phytochemicals, and polyunsaturated fatty acids, with low caloric value. Polysaccharides from sea vegetables have been reported to possess biological activities with potential medicinal values in addition to their current status as a source of dietary fibers and prebiotics. Generally, sea vegetables are used as gelling agent and stabilizers in the food and pharmaceutical industries, but current research has proved their potential medicinal value against various diseases such as allergy, cancer, diabetes, hypertension, oxidative stress, inflammation, thrombosis, obesity, lipidemia, and other degenerative disorders. The human consumption of algal fiber promotes the growth and protection of the beneficial intestinal flora, greatly increases stool volume, and reduces the risk of colon cancer. Moreover, phytochemicals produced by algal species may potentially be bioactive compounds of interests in the food and nutraceutical industries. Thus, the value of sea vegetables as a new functional and health ingredient is gaining popularity. Further, they have the potential to provide the cosmetics and food market with valuable biomolecules such as highly unsaturated fatty acids, tannins, carotenoids, and sulfated polysaccharides. Hence, a large numbers of sea vegetable bioactives have been identified with potential uses in various areas including functional food, pharmaceutical, and cosmeceutical.

Sea vegetables are lower plants living attached to rocks or sand, contain pigments such as green chlorophyll, yellow carotenoids, and red phycobilins, and are classified as green, red, and brown algae depending on the pigments. Edible sea vegetables consist of 0.4% of green algae, 33% of red algae, and 66.5% of brown algae and are consumed primarily in East Asian countries including South Korea, Japan, and China. Sea vegetables are the most consumed food ingredient in South Korea and Japan occupying 75% and 45%, respectively of each country’s whole sea vegetable consumption. Sea vegetables that have ash content of 25% are alkaline food containing rich minerals such as calcium, potassium, phosphorous, copper, manganese, iron, iodine, etc. Recent research in natural products chemistry reveals a vast abundance of marine organisms, macro and micro, that contain novel compounds of interest to human health and industry. Among the marine organisms, marine macroalgae represent one of the richest sources of natural antioxidants and antimicrobials. They are also an excellent source of vitamins such as A, B1, B12, C, D, and E;
riboflavin; niacin; pantothenic acid; and folic acid as well as minerals such as Ca, P, Na, K, and I.

Dried organisms consist of 32–60% water soluble carbohydrates and 4–12% fiber, and when decomposing sticky viscous substances, they produce glucose and fructose. Moreover, they have lot of dietary fiber to lower blood cholesterol that they are effective in preventing hypertension, heart disease, arteriosclerosis, and iodine that improves metabolism—the smooth vascular and cardiac action, the body temperature and perspiration regulation.

This book is completed with the help of many invaluable contributors around the world. Therefore, I would like to thank them all for their valuable time and effort invested in this book. This book is an attempt to describe the present and future prospects of marine algae as medicinal food as the remedies containing natural ingredients from marine algae goes back to the ancient era. This book describes the nutritional elements, special ingredients (secondary metabolites), and biological activities centering around the reported information on marine algae may help to the personnel in academia and also for general public. This volume covers the use of marine algal based materials in terms of human health and personal care. Since the synthetically derived materials show some side effects, huge attention from general public and academia on the products from natural-based materials including marine macro- and microalgae has been gained due to their prominent biological activities and abundance. Hence, this book discusses the importance of biologically active compounds from algae under four sections.

The first section, which includes Chapters 1 and 2, familiarizes the readers with the prospects of marine-derived foods in relation to medicinal value. It covers the present and future prospects of marine algae. Second section (Chapters 3–16) looks at the medicinal foods from variety of marine sources. Individual chapters cover the importance and values of some frequently use marine algal species. The third section, containing Chapters 17–24 covers the biological implications of marine algae. Finally, the fourth section, containing Chapters 25–34, discusses the applications of marine algae-derived materials in medicinal and nutraceutical industries in relation to human health.

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Present and Future Prospects of Seaweeds in Developing Functional Foods

Eresha Mendis* and Se-Kwon Kim†,‡

Abstract

There has been a combined effort among scientists to explore and utilize varying food sources to develop functional foods to cater the ever-increasing demand from the consumers, who seek health-promoting roles of dietary compounds. Considering the diversity of biochemicals in seaweeds that are capable of exerting bioactivities,
a growing trend is developing across globe to employ seaweeds in functional food development. Proteins, peptides, amino acids, polysaccharides, phenolics, lipids, vitamins, and minerals in seaweeds and their functional properties provide insights into the success of potential functional food products that can be developed utilizing seaweeds. However, several factors need to be taken into consideration in designing seaweed-based functional foods to obtain the market success. This chapter elaborates on the prospects of seaweeds in developing seaweed-based functional food products.

I. FUNCTIONAL FOODS AND DISEASE PREVENTION

“Let food be thy medicine and medicine be thy food” Hippocrates, 460 BC. Identification of the relationship of foods and prevention from a range of diseases in human goes back to several centuries. It is noteworthy to comprehend that along with the ever-increasing consumer expectations toward convenient foods, the desire to maintain and improve health remains the key driver in the consumer goods market. Apparently, consumer awareness on the diet is gradually improving, and increasing health consciousness of modern consumers emphasizes on the broader idea of “wellness” obtained through optimum nutrition. Further, health challenges coming in the mode of chronic diseases that prevail mainly among the aging population compel the consumers to reevaluate their nutrition and lifestyle choices they adapted for years. Dietary transitions are becoming common as a result of this reevaluation process where consumers become proactive in reducing risk of the occurrence of chronic diseases and trying to manage the diseases without medical interventions. In this context, consumers are becoming highly receptive for functional foods and beverages seeking health-promoting roles of dietary compounds. Consequently, food industry has ramped up the development and marketing of diverse group of functional food products using different sources of foods on which biological assays have confirmed their beneficial effects related to the disease prevention and health promotion (Gray et al., 2003).

Functional foods can be developed in many forms. Conventional foods with bioactive components can be presented claiming positive health outcomes. Some may be fortified or enhanced foods, specifically created to reduce disease risk associated with a certain group of people. Although most foods have its own function, in this chapter “functionality” is ascribed to a specific phenomenon widely accepted by the scientific community involved in this field which is defined as “functional foods are foods and food components that provide a health benefit beyond basic nutrition for the
intended population. These substances provide essential nutrients often beyond quantities necessary for normal maintenance, growth and development, and/or other biologically active components that impart health benefits or desirable physiological effects” (Anonymous, 2005). Further, this definition emphasizes the positive health benefits of food components not considered nutrients in the traditional definition.

A. Emerging trends in the functional food industry

When surveying the world market for functional foods, it is evident that distinct trends are emerging in different parts of the world. Apparently, some functional food categories common to most regions are emerging faster and will significantly outperform during the years ahead (Farkas, 2000). Analyzing trends across globe, it is predicted that digestive health and heart health will be key areas of focus in developing functional food products for the years to come. Based on worldwide analysis of new introductions of functional food categories, products that are focused on digestive health are capturing the interest of a broader audience than products with a narrow focus such as products targeting specific illnesses.

In parallel to the ramped up need for the improvement of dietary health, foods with probiotics, prebiotics, and dietary fiber are capturing the interest of consumers and food manufacturers. Similarly, a great industry emphasis is given to development of designer beverages such as energy and sports drinks. This has a link to consumer preference to go for wide range of food or beverage products that claim “energy boosters,” “high vitamins,” “high minerals,” and “high antioxidants,” and products promoting their antioxidant capacity specifically are getting flooded to the consumer markets to meet this demand. There are products in the market claiming that they are made out of “super fruits,” fruits which are having higher antioxidant power as they contain phytochemicals responsible for antioxidative mechanisms. Further, among claims relating minerals, in food products “with calcium,” “high in calcium” type of health claims are increasingly exploited.

Though it is a focus of a narrow audience to look for products focusing heart health, products with low saturated fats and cholesterol are admired by the majority of the consumers. In the same arena, ω-3 fatty acids are still maintaining its recognition among consumers having identified its effects including protection against cardiovascular disease, various inflammatory and autoimmune conditions, and enhanced cognitive health. In recent years, there was concentration toward products targeting the women population composed of active ingredients capable of fighting against bone-related complications, pregnancy, or menopause-related issues. Wider coverage in research is given for phytoestrogens and phytosterols available in this category of products among other
phytochemicals. Further, soy protein inclusions are getting highly recognized by women population due to their ability to reduce the risk of heart diseases by lowering blood cholesterol levels, promoting bone health, and easing symptoms of menopause. Further, there is an increasing demand for products targeting children. Active ingredients are added in these products and are capable of supporting brain development of infants, immunity enhancement, and acting against allergy reactions in the body. Also some of these products are aiming at promoting healthier eating habits and active life styles among children to prevent the unprecedented growth of obesity and related complications. Functional protein, peptides, and amino acids from different food sources are also renowned, and among them, soy protein concentrates are gaining much popularity attributed to the functional properties specifically toward women population. Other than that, there are products that are becoming popular for their functional effects relating development of healthier skin, energy supplements, weight management, cognitive boosters, antihypertension, anticarcinogenic and antiallergic properties.

II. POTENTIAL OF SEAWEEDS AS A SOURCE TO DEVELOP FUNCTIONAL FOODS

There has been a combined effort among biochemists, biologists, food technologists, and nutritionists to explore and utilize varying food sources of both terrestrial and marine origin to cater the demand from the consumers who eagerly look forward to have optimum nutrition through their dietary interventions. Further, they quest for health benefits associated with these food sources knowing the current need for molecules with novel modes of action to face emerging diseases, seeking proactive approach than “firefighting” with medical interventions. When considering the sustainability of different sources, photosynthetic algae are the most heterogeneous group of organisms and considered the true survivors of the planet as they have been capable of facing the dramatic changes in climatic conditions for centuries and to occupy virtually all niches on earth with a ubiquitous distribution. Seaweeds are taxonomically classified as algae and they belong to four major seaweed classes, the rhodophyceae (red algae), the phaeophyceae (brown algae), the cyanophyceae (blue-green algae), and the chlorophyceae (green algae). A greater diversity in biochemical composition of seaweeds paves the path to explore variety of compounds in their bodily composition with a wide range of physiological and biochemical characteristics, many of which are rare or absent in other taxonomic groups (Holdt and Kraan, 2011).

Knowing the benefits associated with the seaweeds through the experience, seaweed has been used as an important dietary component for
centuries in countries like China, Japan, and Korea. However, seaweeds are attracting increasing attention as a valuable food source in other parts of Asia, Africa, and also other western parts of the world, and growing interest is developing to explore all possible seaweed interventions including functional food product development. For this purpose, several countries other than China, Japan, and South Korea have commercially exploited open and closed cultivation systems to grow seaweeds at large scale. These countries are expected to increase culturing of seaweeds dramatically over the years to come. Further advances in science and technology have provided researchers the required know-how and powerful analytical tools to better characterize the physiological roles of bioactive compounds from seaweeds in disease prevention and health promotion. Research currently underway at academic, industry, and government facilities will reveal how a myriad of substances from seaweed sources can be used as functional food products. Moreover, growing consumer interest in functional foods developed using marine sources has been seen as a significant business opportunity for the agri-food sector, and among them, greater potentials exists to promote the utilization of seaweeds in the functional food industry. Recognizing the market potential for functional foods, a number of firms all over the world have begun to capitalize on these emerging markets.

III. BIOCHEMICAL COMPOSITIONAL ANALYSIS OF SEAWEEDS THAT CATER TO THE POTENTIAL OF SEAWEEDS AS A SOURCE TO DEVELOP FUNCTIONAL FOODS

Scientific reports dealing with functional effects of seaweed proteins, peptides, amino acids, polysaccharides, phytochemicals, lipids, and minerals greatly endorse the efforts toward development of “health foods” using seaweeds. Table 1.1 provides some seaweed species studied and recognized for their richness in functionally important molecular groups. Evaluation of functional properties requires a clear idea about their biochemical composition, and it provides a platform to have an inspiration to decide on the molecules responsible for different biological activities.

A. Seaweed proteins, peptides, and amino acids

Percentage of proteins in seaweeds varies from about 10% to 40% (w/w) per dry weight, and it varies according to the season and the species (Murata and Nakazoe, 2001). Red algae are rich sources of proteins compared to other divisions of algae. Among the proteins present in
seaweeds, lectins, a group of hemagglutinin proteins that bind with carbohydrates, have captured the interest of researchers due to their ability to take part in host–pathogen interactions, cell–cell communication, recognizing and binding carbohydrates and to exert functional effects to induce apoptosis, metastasis, and cell differentiation in cancer cells, antibiotic, anti-inflammatory, anti-human immunodeficiency virus (anti-HIV) activity, and human platelet aggregation inhibition (Hori et al., 2000; Mori et al., 2005; Smit, 2004).

Other than the lectins, phycobiliproteins (phycocyanins and allphyocyanins) are popular for their potency to exert functional effects in the mode of anti-inflammatory, liver protecting, antiviral, antitumor, antiatherosclerosis, lipase activity inhibitor, serum lipid reducing agent, and antioxidant, and to obstruct absorption of environmental pollutants into the body (Sekar and Chandramohan, 2008). Seaweed peptides obtained through the enzymatic digestion process have shown several biological activities including antioxidant, antimicrobial, antithrombotic, immunomodulatory, and mineral binding activity (Smit, 2004). These peptides are inactive in the amino-acid sequence of the parental protein and become

TABLE 1.1 Seaweed species studied for their richness in specific bioactive compounds

<table>
<thead>
<tr>
<th>Bioactive compound(s)</th>
<th>Seaweed species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polysaccharides</td>
<td>Saccharina latissima, Sargassum pallidum</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Chondrus crispus, Eucheuma cottonii</td>
</tr>
<tr>
<td>Agar</td>
<td>Gracilaria cornea, Gracilaria domingensis</td>
</tr>
<tr>
<td>Algins/alginic acid</td>
<td>Laminaria digitata, Laminaria hyperborea</td>
</tr>
<tr>
<td>Fucoidan</td>
<td>Fucus vesiculosus, Ascophyllum nodosum</td>
</tr>
<tr>
<td>Laminarin</td>
<td>Fucus vesiculosus, Laminaria hyperborea</td>
</tr>
<tr>
<td>Ulvan</td>
<td>Ulva lactuca, Ulva rigida</td>
</tr>
<tr>
<td>Total protein</td>
<td>Undaria spp., Sargassum spp.</td>
</tr>
<tr>
<td>Lectins</td>
<td>Ulva sp., Eucheuma amakusaensis</td>
</tr>
<tr>
<td>Phycobiliproteins</td>
<td>Palmaria palmata, Gracilaria tikvahiae</td>
</tr>
<tr>
<td>Taurine</td>
<td>Saccharina latissima, Porphyra tenera</td>
</tr>
<tr>
<td>Kanoids (kainic and domoic acid)</td>
<td>Palmaria palmata, Digenea simplex</td>
</tr>
<tr>
<td>PUFA (o-3 fatty acids)</td>
<td>Laminaria digitata, Saccharina latissima</td>
</tr>
<tr>
<td>Phlorotannins</td>
<td>Ascophyllum, Fucus spp.</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Laminaria digitata, Fucus serratus</td>
</tr>
<tr>
<td>Iodine</td>
<td>Laminaria japonica, Laminaria digitata</td>
</tr>
<tr>
<td>Calcium</td>
<td>Porphyra tenera, Ulva lactuca</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>Ulva lactuca, Porphyra tenera</td>
</tr>
</tbody>
</table>

active upon release through the enzymatic digestion. *In vitro* and *in vivo* studies that have been carried out using water extracts of seaweeds have confirmed that dipeptides in extracts are capable of acting against hypertension through inhibition of angiotensin I converting enzyme (Sato *et al.*, 2002).

The free amino-acid fraction of seaweed is a mixture of amino acids and is mainly composed of taurine, alanine, amino butyric acid, omithine, citrulline, and hydroxyproline (Holdt and Kraan, 2011). Taurine is an amino acid present in high concentration in red algae. It also acts as an antioxidant and protects against toxicity of various heavy metals such as lead and cadmium by preventing their absorption in the stomach. Taurine has been shown to be effective in reducing the secretion of serum lipids and apolipoprotein B100, a structural component of low density lipoproteins, thereby reducing the risk of atherosclerosis and coronary heart disease. These finding have been followed and supported by several other research reports that taurine supplementation exerted a hypocholesterolemic effect in young overweight adults. Taurine has also shown its capability to relieve complications in people with congestive heart failure by increasing the force and effectiveness of heart–muscle contractions (Lourenço and Camilo, 2002; Mochizuki *et al.*, 1999). The kainoid amino acids, kainic, and domoic acids have also been found in numerous algal species. They act as central nervous system stimulants upon exceeding the safe levels and become neurotoxins. These compounds are currently used in research associated with neurophysiological disorders such as Alzheimer’s and Parkinson’s disease and epilepsy (Harnedy and FitzGerald, 2011).

### B. Polysaccharides

Presently sulfated polysaccharides are the group which is identified as economically most important among other ingredients found in algae that have been extensively used in the industry for food and medicinal purposes. Red algae and brown algae are the classes that produce these polysaccharides of interest in higher concentrations. These polysaccharides act as dietary fiber as they are not digested in the upper digestive tract but may be degraded by the colonic bacteria to some extent in the colon. Direct comparisons show that, in most of the seaweeds dietary, fiber amounts are similar or slightly elevated than the levels of total fiber in terrestrial foodstuffs. Edible seaweed contain 33–62% total fibers on a dry weight basis, which is higher than the levels found in higher plants, and these fibers are rich in soluble fractions (Dawczynski *et al.*, 2007; Lahaye, 1991). A growing interest can be seen among researchers to study the roles of polysaccharides in the human body particularly how they prevent the occurrence of certain diseases.
Carrageenans are generally identified as carbohydrate antigens and has the potency to promote the growth of connective tissues. Antiviral properties of few algal species have been studied extensively including Chondrus crispus and Gelidium cartilagineum, the species produce agar and carrageenan in higher concentrations. Researchers have concluded that this property is attributed to the galactan units available in agar and carrageenan of these algal species. Current research develops strong evidences to promote carrageenan as an useful antiviral agent that blocks the transmission of the HIV virus as well as other STD viruses such as gonorrhea, genital warts, and the herpes simplex virus (Buck et al., 2006). Carrageenan is also studied extensively in ulcer therapy, and it has been concluded that carrageenan is involved in developing protective layer by interacting with the mucoid lining of the stomach and thereby preventing enzymes and acid secretion (Emerson and Kerkut, 1974). Agar has similar structural and functional properties as carrageenans. Both agar and carrageenan have the ability to exert effects in modifying the adhesion and proliferation of normal and tumoral human colonic cells thereby affecting the process of metastasis (Zhou et al., 2006).

Other important polysaccharide, alginic acid, is present naturally in seaweeds as calcium or magnesium salts which are insoluble in water. Algins/alginates are extracted from brown seaweeds and are available in both acid and salt forms. Commercially alginic acid is extracted mainly from brown seaweeds as soluble sodium alginate. Sodium alginate is reported to serve as a coadjuvator in immunization against strain-specific influenza virus. Sodium alginate also has tried in the treatment of esophagitis and urolithiasis. It has the ability to function as a haemostatic agent which is capable of clotting blood in situ. Alginites have the capability to act like fibers and help clearing the digestive system to protect surface membranes of the stomach and intestine from potential carcinogens. Further, this feature has a link with its ability to prevent proliferation of implanted cancer cells in the stomach. Moreover, alginic acid and its derivatives are used for the production of drugs in the treatment of gastritis and gastroduodenal ulcers, as well as alginites are used as antiulcer remedies. The mechanism action of these materials has a link to its ability to effectively suppress postprandial acidic refluxes and binding of bile acids. Alginites are capable of reducing hypertension through several mechanisms including physical binding of sodium in the gastrointestinal tract and calcium channel blocker activity (Draget and Taylor, 2011).

The polysaccharide laminarin is commercially extracted mainly from kelp and fucoids and is a main form of food storage of brown algae. Sulfated laminarins have antilipidemic activities and capable of reducing serum cholesterol levels and total serum lipids (Kiriyama et al., 1969). The anticoagulant activity of this material is attributed to its antithrombotic
property, and laminarin only shows anticoagulant activity after structural modifications such as sulfation, reduction, or oxidation (Miao et al., 1999). Structural similarity of laminarin to barley, which is potent prebiotic, has prompted the study of laminarin as a prebiotic. Studies have proved that laminarin provides a substrate for prebiotic bacteria and promotes their growth and function in human (Deville et al., 2004). Further, laminarin has proved to be involved in modulating the gut environment and act as an immunostimulant. Further studies have revealed the potential of laminarin as a cancer therapeutic and as a tumor inhibiting agent (Miao et al., 1999).

Fucoidan, another polysaccharide of brown algae, is not found in other algae or in higher plants. Fucoidan has shown promising antiviral, immunomodulating, and antibacterial activities. Fucoidan inhibits the angiogenesis and promotes apoptosis in human cancer cells. Further, it inhibits the proliferation of tumor cells and thereby reduces the growth and the size of the tumor. Further, this compound has proven its capability to act as anti-inflammatory and anticoagulant agents. Further, fucoidan preparations have been proposed as an alternative to the injectable anticoagulant heparin considering its safety being free of viruses as they originate from plant matter and exert protective effects through direct inhibition of viral replication against HIV, hepatitis, and herpes viruses. Further, fucoidan has reputed for its ability to stimulate the immune system by acting as an immunomodulator directly on macrophage (Li et al., 2008).

Ulvan is a water soluble polysaccharide obtained from members of the Ulvales. Bioactive properties of ulvan such as cytotoxicity against colonic cancer cells through modification of the adhesion and proliferation of tumoral human colonic cells and modulating the expression of transforming growth factors related to cellular differentiation are reported. Further, there are reports to confirm that ulvan acts as an antiviral and antibacterial agent (Lahaye and Robic, 2007).

C. Phytochemicals

The secondary metabolites of seaweeds have always attracted the interest of biochemists because of their diversity as compared with those present in the leaves of higher plants. Isoprenoids (e.g., terpenes, carotenoids, steroids), polyketides (e.g., phlorotannins), amino-acid-derived natural products (e.g., alkaloids), and shikimates (e.g., flavonoids) are the major groups of secondary metabolites found in algae. Compared to other macroalgae, rhodophyta are richer sources of these secondary metabolites. Exceptionally, Phlorotannins, or polyphenols, are recognized as structural classes of polyketides found exclusively in brown algae. Phlorotannins are constructed through the polymerization of phloroglucinol units to form polyphloroglucinols. These polyphloroglucinols are
composed of six major groups: fucols, phlorethols, fucophlorethols, fuhalols, isofuhalols, and eckols. They possess strong antioxidative properties and act against oxidative stress (König and Wright, 1993). Certain polyphenols work as preventative medicines for problems such as cardiovascular diseases, cancers, arthritis, and autoimmune disorders that have a direct link with oxidative stress (Yuan et al., 2005). Further, phlorotannins has bactericidal activity (e.g., anti-Staphylococcus activity) together with other therapeutic perspectives.

Flavonoids and their glycosides present in green, brown, and red algae also have exhibited antioxidative properties and have demonstrated their capability to act against atherosclerosis and cancer. Fucoxanthin, β-carotene, and violaxanthin are carotenoids found in seaweeds and exhibit powerful antioxidant properties. Further, fucoxanthin has demonstrated strong anticancer effects and has demonstrated its capability to prevent obesity (Hosokawa et al., 1999). The correlation between a diet rich in carotenoids and a diminishing risk of cardiovascular diseases and ophthalmological diseases has been backed by the recent research carried out using different types of carotenoids in cellular systems and human intervention studies.

Halogenated compounds, another type of metabolites found in algae, are produced mainly by the brown and red algae, and among other halogenated compounds, polyhalogenated monoterpenes found in red algae have exhibited anticancer, antimicrobial, and antitubercular functionalities (Cabrita et al., 2010). A greater number of researches on varying type of secondary metabolites are progressing fast, and their biological mode of actions and efficacies in human dietary interventions are yet to be confirmed (König and Wright, 1993).

D. Lipids

In general, seaweeds are recognized to contain low amounts of lipid, however, polyunsaturated fatty acids (PUFAs) found in algae have attracted the attention due to their biological effects which have implications in human health. However, amounts and concentrations of these PUFAs are greatly varied according to environmental temperature, being the lower temperatures favoring their production. When comparing the two families of PUFAs found in the human diet (ω-6 fatty acid and ω-3 fatty acid), ω-3 PUFAs are of particular interest in the emerging field of functional food development. This is attributed to the properties of ω-3 PUFAs, eicosapentaenoic (EPA), and docosahexaenoic acids (DHA) that are linked to a range of biochemically and physiologically important functions in the human body. However, EPA has been reported as the predominant fatty acid in various seaweeds. A greater number of
scientific evidences support the efficacy of ω-3 PUFA as agents possessing antiartherosclerotic, antihypertensive, anti-inflammatory, and immunoregulatory effects (Khan et al., 2007; Plaza et al., 2008).

Reports provide evidence that the phospholipids are the prominent type in the composition of lipids in seaweeds and they provide better compounds for food applications than fish oil attributed to their greater resistance to oxidation and higher degree of bioavailability. Sterols are also an important part of seaweed lipids due to their different compositional and functional effects in the human body. The predominant types and amounts of sterols in seaweeds vary to a greater extent. Red algae contain primarily the cholesterol, and fucosterol is the predominant sterol type in brown algae. Fucosterol reduces the absorption of cholesterol into the bloodstream by restricting the solubility of cholesterol in bile acid (Ikeda et al., 1988). The sterol composition in green algae varies greatly among species, and isofucocholesterol, cholesterol, 24-methylene-cholesterol, and β-sitosterol are frequently found among others. Prolonged consumption of sterols from marine algae is reported to reduce the tendency to form a fatty liver and excessive fat deposition in the heart of human.

E. Minerals and vitamins

Seaweeds are rich sources of some important minerals and vitamins. In particular, seaweeds contain good amounts of iodine, calcium, and iron among others. Iodine content of seaweeds is incomparable with the highly consumed terrestrial vegetables as seaweeds are much better sources of iodine. However, amounts are varied with phylum, season, and environmental, geographical, and physiological variations. Brown algae have recognized as much important sources of iodine and have utilized extensively for the prevention and treatment of iodine deficiency goiter. Further, scientific reports link the potential of iodine in inhibiting tumorogenesis with the high amount of iodine in some seaweed species (Funahashi et al., 1999). In line with this capability of iodine in seaweeds, epidemiological studies suggest that high dietary seaweed content must have accounted for the low prevalence of breast cancer in some countries of Asia.

Seaweeds are also rich sources of calcium which provides a greater potential to be used in functional food developments attributed to their higher calcium concentration and easy assimilation (in the form of calcium carbonate) in to the body compared to calcium in cow’s milk (in the form of calcium phosphate). Further, seaweeds provide good sources of vitamins such as vitamin E, A, and B12 and have a greater potential to be exploited in functional food categories in demand (Berg et al., 1991).
IV. PRESENT SITUATION AND POTENTIAL OF SEAWEEDS FOR NOVEL FUNCTIONAL FOOD PRODUCT DEVELOPMENTS

Seaweeds have long been recognized as potential sources for the phycocolloid industry dealt with agar, carrageenan, furcellaran, and algin to use as food additives in the modes of stabilizers, texture enhancers, viscosity modulators, gelling agents, etc. Seaweeds have gained the popularity in the international trade specifically for these phycocolloids, dried seaweeds, and products of laminarin and fucoidans. With the increased understanding of the health beneficial properties of these seaweed compounds, considerable efforts have been exerted in discovering more direct therapeutic-related food applications, but, despite high expectations, no commercially successful product ranges have yet been developed utilizing these compounds targeting optimum health and nutrition of human. Very few seaweed-based functional food products can be seen covering a narrow market niches such as powder forms of alginates and carrageenans, phytocomplexes fortified with fucoidans, aligns, minerals and vitamins from seaweed sources, β-carotene as vitamin supplements, seaweed protein powders, fiber complexes fortified with phytochemical extractions from seaweeds, etc. However, their success as a functional food product in the market is not up to the expectation. Fortification of food products having higher consumer acceptance with seaweed bioactives would provide an opportune approach to popularize health benefits of seaweeds among consumers and very few such efforts are reported recently (Kadam and Prabhasankar, 2010; López-López et al., 2009). It is an agreeable fact that functional foods present major challenges for the food industry as they appear to be a new and unfamiliar territory for product developers in marketing and developing business strategies. This is mainly because translating scientific advances and nutritional innovations into consumer products is a costly and complex process. Sound science must underlie the development, marketing, and regulation of these new functional foods to gain success. Further, one needs to understand that the functional food trends are more heterogeneous than homogeneous, evolving and growing at different rates both within and across countries, owing to sociodemographic and sociocultural differences, and functional food products needs to be developed to match with the interests of the target populations (Wim, 2005).

Successful functional product innovations dealt with other food sources have been mainly launched targeting the markets for nonalcoholic beverages fortified with the vitamins or other functional ingredients, breakfast cereals, cholesterol-lowering spreads, confectionery, biscuits, cereal, cereal bars, soft drinks, probiotic and prebiotic dairy products,
isotonic drinks, bakery, and hypoallergenic baby foods. Further, ever-concerning chronic disease-related conditions such as cancer, high cholesterol, coronary heart diseases, atherosclerosis, stroke, hypertension, diabetes (type II), gastrointestinal disorders, osteoporosis, intestinal complications, and immune disorders including allergy have been used as prime focuses when developing these functional food products. When analyzing the supply structure of these functional foods, the main types of successful actors in the commercial functional food segment are multinational food companies with a broad product range and pharmaceutical or dietary products producing companies. Therefore, the combination of consumer acceptance, advances in science and technology, and scientifically backed evidence linking consumption of biochemical compounds in seaweed to disease and disease prevention can be taken as unprecedented opportunity for these food marketers to develop seaweed-based functional products to address nutritional and health-promoting demands of consumers. Hence, they can take the challenge of developing novel food products using seaweeds with their wider experience in handling other functional food categories. Today’s science and technology can be used to provide many additional functional foods, and future scientific and technological advances promise an even greater range of health benefits for consumers through seaweed-based functional food innovations. Other than these main factors, the extent of cultivation needs to be expanded to which raw seaweed demands can be met at competitive prices, and further efforts are needed to explore new sources of algae so far neglected. Further, in obtaining the success in the market, strategic planning is required to enhance the knowledge and awareness of the consumers about health effects of seaweeds-related functional ingredients. Moreover, the long-term success of a functional food for health and well-being depends on perspective and the alignment of a number of interests and different stakeholders such as health sector, food industry, technologists, scientists, regulators, and even environmentalists. Therefore, a dialog needs to be initiated among researchers, industry, regulators, and other important stakeholders to initiate strategies to promote this invaluable natural resource of food to develop successful functional food products to the market.

REFERENCES


CHAPTER 2

Nutritional and Digestive Health Benefits of Seaweed

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Abstract

Seaweed is a famous delicacy in some parts of the Asia and also a well-known source of important food hydrocolloids, such as agar, alginates, and carrageenan. In addition to the food value of seaweed,

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several health benefits have also been reported to be present in this valuable food source. It is presumed that the unique features of the marine environment, where the seaweeds are grown, are mainly responsible for most of its properties. Among the functional effects of the seaweed, nutritional and health-related benefits have been widely studied. Compared to the terrestrial plants and animal-based foods, seaweed is rich in some health-promoting molecules and materials such as, dietary fiber, $\omega-3$ fatty acids, essential amino acids, and vitamins A, B, C, and E. In this chapter, the nutritive value of seaweed and the functional effects of its soluble fiber are discussed with a special reference to the digestive health promotion of human.

I. SEAWEED AS A FOOD

Seaweed, also called as algae, is taxonomically classified under four groups namely: red algae (rhodophyta), brown algae (phaeophyta), green algae (chlorophyta), and blue-green algae (cyanophyta). Macroalgae, which include above three groups of seaweed other than blue-green algae, have a long history of utilization as direct or processed food across the globe. In Asian countries, seaweed is directly used for several culinary purposes, whereas in the west, it is exclusively used for the extraction of important food hydrocolloids including agar, carrageenan, and alginates. Availability almost throughout the year and relatively easy collection potential make macroalgae an inexpensive food source. With the advancement of biological and marine sciences, identification and large-scale culturing of edible microalgae (blue-green algae) have also become a reality, and later they have been introduced into different food applications.

Seaweed is a rich source of nutrients included in Asians traditional cuisine and is being extensively explored for its other merits as a food. Apart from its proven nutritional properties, bioactive molecules found in seaweeds have attracted the interest of health conscious societies, as seaweed is regarded as a remarkable marine medicinal food.

II. INTERVENTION OF SEAWEED TO ENHANCE HUMAN NUTRITION

Acquirement of a good mental and physical health through optimum nutrition is a key to wellness of humans. The nutrients in our daily diet or those synthesized in the human body using the precursor molecules play a vital role in regulating the bodily functions, essential for normal growth and development. Carbohydrates, proteins, lipids, and vitamins
are provided to the human body through different food sources. Like most of the terrestrial plants, marine algae are also a rich source of above nutritional elements. In comparison with many common vegetables, high levels of fiber, minerals, ω-3 fatty acids, and moderate concentrations of lipids and proteins available in most of the edible seaweed help it to be considered as an important food source for human nutrition. However, the available amounts of the above nutrients may vary basically depending on the variety, season, and the area of production (Murata and Nakazoe, 2001).

A. Carbohydrates

Seaweed contains a large amount of carbohydrate as structural, storage, and functional polysaccharides, and the total carbohydrate content may ranges from 20% to 76% of dry weight (Holdt and Kraan, 2011) depending on the species. Though the carbohydrate content in seaweed is considerably high, its greater portion is available as polysaccharide dietary fiber, which is not taken up by the human body. Therefore, seaweed is not a good source of carbohydrate in terms of bioavailability. Little, but absorbable, forms of carbohydrate present in seaweed comprise glucose, mannose, and galactose.

B. Proteins

In general, seaweed protein is rich in glycine, arginine, alanine, and glutamic acid, and contains all the essential amino acids, the levels of which are comparable to those of the FAO/WHO requirements of dietary proteins (Anonymous, 2006). However, when compared with the other protein-rich food sources, seaweed is appeared to be limiting with lysine and cystine. With respect to the protein level and amino acid composition, the amino acid score and the essential amino acid index were higher in red seaweed than those in brown and green seaweeds (Holdt and Kraan, 2011). The amino acid score of the proteins in some red seaweed such as Porphyra spp. and Undaria spp. was 91 and 100, respectively, the same as that in animal-derived foods (Murata and Nakazoe, 2001). Red seaweed contains the highest protein content, which is comparable in quantitative terms to legumes at 30–40% of dry matter, and brown and green seaweeds contain only 15% and 30%, respectively (Murata and Nakazoe, 2001). A comparative study carried out with several red and brown seaweeds revealed that protein content of red seaweed species Porphyra palmate and Porphyra tenera ranged from 21% to 47% and that in brown seaweeds Laminaria japonica and Undaria pinnatifida ranged from 7% to 16% (Marsham et al., 2007). Therefore, most of the edible red seaweeds can be considered as a good source of protein to be included in the diet.
However, aspartic and glutamic acid that exhibit interesting properties in flavor development are less in red seaweed compared to that in brown seaweed. In addition, the blue-green alga, *Spirulina*, is well known for its very high protein content which is close to 70% of the dry matter. The *in vivo* digestibility of seaweed proteins is not well documented. However, the extractability and the *in vitro* digestibility of seaweed protein attain more than 80% irrespective of the species (*Fleurence, 1999*).

C. Lipids

Seaweed has a very little lipid content, ranging from 1% to 5% of dry matter (*Khotimchenko, 2005*). Neutral lipids and glycolipids are the major lipid classes in all seaweeds, and the proportion of essential fatty acids in seaweed is higher than that in land plants. Seaweed synthesizes higher amounts of polyunsaturated fatty acids (PUFAs) especially under the cool climates, and the total lipid content is elevated during the hot seasons (*Narayan et al., 2006*). However, the content and the composition of fat can be greatly varied depending on the type of seaweed.

PUFAs in seaweeds contain substantial amount of ω-3 fatty acids as the major component. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two important fatty acids of marine origin belonging to ω-3 fatty acids that are responsible for a number of health benefits in the human body. ω-Linolenic acid is the precursor of both EPA and DHA and is not synthesized in mammals. However, ω-linolenic acid can be converted into EPA and DHA upon consumption by the human. The major PUFA in most seaweed is EPA and the content of which can be even closer to 30% of the total fatty acid content. Though the red seaweed is rich in EPA and ω-6 fatty acids such as arachidonic acid, as a whole, all seaweeds are a balanced source of ω-3 and ω-6 acids. Therefore, seaweed is a good source of health-promoting PUFA compared to the other foods derived from plant and animal sources. The amount of phospholipids in seaweed is about 4–10% of the total lipid. Moreover, phospholipids in the diet act as an emulsifier and ease the digestion and absorption of fatty acids enhancing the nutritive value of the food. Moreover, seaweed contains many essential fatty acids, which may add to their efficacy as a part of a balanced diet.

D. Vitamins

Seaweed contains several vitamins both water soluble such as B and C and lipid soluble such as A and E at varying levels. Brown seaweed, *U. pinnatifida*, contained 14.5 mg/100 g of vitamin E and that was much higher than the vitamin E content (10 mg/100 g) in peanut (*Anonymous, 2004*). This high vitamin E content helps to protect PUFA in seaweed and
to maintain their nutritional benefits. Red and brown seaweeds are rich in carotenes (provitamin A) and vitamin C, and their amounts may range from 20 to 170 ppm and 500 to 3000 ppm, respectively. They are also considered as good sources of vitamin B12, which is not found in most land plants but present in a few vegetables in considerable amounts (Bender, 1980).

E. Minerals

Generally, seaweed contains high ash content indicating appreciable amounts of minerals. Mineral content of seaweed can account for up to 36% of its dry mass and mineral macronutrients include sodium, calcium, magnesium, potassium, chlorine, sulfur, and phosphorus whereas the micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel, and cobalt. Among these minerals, calcium holds 4–7% of dry matter. At 7% calcium, a typical daily portion size of seaweed (8 g dry weight) provides 560 mg of calcium which is a considerable amount compared to its recommended daily allowance (800–1000 mg) (Anonymous, 2004). In seaweeds, calcium is available as calcium phosphate, and that is more bioavailable than the form of calcium in milk, which is calcium carbonate.

Seaweed is a primary source of iodine, and in some seaweed, iodine content exceeds its dietary minimum requirement (150 µg/day). The highest iodine content is found in brown algae (1500–8000 ppm), and in most instances, red and green algae have lower contents. Iodine amount in the seaweed remains comparatively high than that in the land plants. Since animal- and plant-derived foods are very low in iodine, seaweeds can be considered as the best inexpensive food to fulfill the iodine requirement of human.

Interestingly, seaweed contains considerably high amounts of iron and copper compared to food sources renowned to contain those minerals such as, meat and spinach (Holland et al., 1993). In addition, a normal portion size of brown seaweed, which includes species such as Laminaria and Undaria, provides more than 50% of the recommended daily allowance of magnesium. Therefore, seaweed can be used as a food supplement to fulfill most of the important mineral requirements of the body.

III. DIETARY FIBER IN SEAWEED HELPS TO AMELIORATE DIGESTIVE HEALTH

Dietary fiber, a group of non-starch carbohydrates basically of plant origin found in various vegetables, fruits, grains, nuts, and root crops, is an essential part of a healthy diet. Since the dietary fiber is not digested by
the digestive enzymes, it cannot perform a direct nutritional effect in the human body. However, dietary fiber indirectly supports the human nutrition by involving in some important functions to promote the digestive health during its passage through the gastrointestinal track. These functions include reduction of incidences of colorectal cancers, suppression of bowel inflammations and related abdominal disorders, facilitation of bowel movement, and growth promotion of health-promoting gut microflora.

In comparison to the fiber content of the foods derived from terrestrial plants, seaweed has similar or even higher levels of dietary fiber. The average total dietary fiber content in seaweed can be varied from 36% to 60% based on its dry matter (Lahaye, 1991; Rasmussen and Morrissey, 2007). Nearly, 55–70% of its total dietary fiber is represented by the soluble fiber fraction which mainly comprises agar, alginates, and carrageenan at varying amounts depending on the type of seaweed (Table 2.1) and the growing conditions. In addition, some other important sulfated polysaccharides such as fucoidans, laminarin, porphyran, and ulvan are also available at relatively low quantities in seaweed. *U. pinnatifida*, *Chondrus*, and *Porphyra* have the highest content of soluble dietary fiber, and *Fucus* and *Laminaria* have the highest content of insoluble dietary fiber among the other common seaweed used in the food industry (Fleury and Lahaye, 1991). The recommended average daily intake of dietary fiber in the United States and in the United Kingdom is about 25–30 g and more than 18 g, respectively. The typical daily portion size of the seaweeds consumed in Asian cuisines on dry matter basis is about 8 g (MacArtain et al., 2007). Therefore, 12–15% of daily dietary requirement of fiber can be fulfilled by adding seaweed in the diet. This is considerably a large amount compared to that of other food sources on weight-for-weight basis.

**TABLE 2.1** Different types of soluble fiber available in seaweed

<table>
<thead>
<tr>
<th>Soluble fiber (hydrocolloid)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>Red seaweeds (<em>Gracilaria, Gelidium, Pterocladia</em>)</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Red seaweeds (<em>Eucheuma, Chondrus, Hypnea, Gigartina</em>)</td>
</tr>
<tr>
<td>Alginates</td>
<td>Brown seaweeds (<em>Macroystis, Laminaria, Ascophyllum</em>)</td>
</tr>
<tr>
<td>Fucoidan</td>
<td>Brown seaweeds (<em>Laminaria religiosa, Nemacystus decipiens</em>)</td>
</tr>
<tr>
<td>Laminarin</td>
<td>Brown seaweeds (<em>Laminaria japonica, Saccharina latissima</em>)</td>
</tr>
<tr>
<td>Porphyran</td>
<td>Red seaweeds (<em>Porphyra spp.</em>)</td>
</tr>
<tr>
<td>Ulvan</td>
<td>Green seaweeds (<em>Ulva lactuca, Enteromorpha spp.</em>)</td>
</tr>
</tbody>
</table>
A. Reduces the risk of colorectal cancer

Colorectal cancer, which is characterized by neoplasia in the colon, rectum, or vermiform appendix, is the third most commonly diagnosed cancer in the world. More than half of the deaths of colorectal cancer are reported from the developed regions of the world. Several studies suggested that diets high in red and processed meat, as well as those low in fiber, are associated with an increased risk of colorectal cancer (Chao et al., 2005; Wakai et al., 2007). Dietary fiber has been hypothesized to involve in reducing the risk of colorectal cancer through several protective mechanisms including dilution of fecal carcinogens, reduction of transit time of feces through the bowel, production of short chain fatty acids which promote anticarcinogenic action, and binding of carcinogenic bile acids (Lipkin et al., 1999).

Soluble fiber in seaweed can bind with water 20 times of their own volume exhibiting strong hydrocolloidal properties of its network structure. Therefore, seaweed added to the diet can enhance water binding to the food pellet in the gut and facilitate stool bulking, and decrease transit time in the colon, that act as positive factors to prevent colon cancer (Brownlee et al., 2005). The viscous indigestible masses of fiber in the gut trap toxins and other cancerous material in the digested food, and those are then expelled through the feces. Thereby, they help to protect the surface membrane of the digestive tract against potential carcinogens. Studies carried out using the laboratory animal models revealed that some seaweed fibers are effective in controlling chemically induced gut cancer. Different diets consisting 0.05–0.2% seaweed powders of Eisenia bicyclis, Laminaria angustata, and P. tenera were tested in intestinal tumor-induced rats with potent intestinal carcinogen, 1,2-dimethylhydrazine, and after 20 days, the tumor incidence was clearly reduced at varying degrees (Yamamoto and Maruyama, 1985). Moreover, porphyran showed appreciable antitumor activity against Meth-A fibrosarcoma in rats (Noda, 1993). Development of colorectal epithelium into carcinoma is associated with a progressive inhibition of apoptosis and it further contributes to tumor growth. A study was carried out to determine apoptosis-inducing activity of fucoidan in cultured HT-29 and HCT116 human colon cancer cells and revealed that fucoidan can reduce the viability of tested cells in a dose-dependent manner through the inhibition of both tumor necrosis factor and caspase-induced cell signaling (Kim et al., 2010).

B. Suppresses gastrointestinal inflammation

Seaweed polysaccharide fiber in the diet has shown to be effective in suppressing inflammation in the stomach and reducing the risk of gastro-duodenal ulcers. Most of the soluble types of fiber in algae help to develop a
viscous layer next to the epithelial margin of the upper digestive tract displaying a protective and coating effect against the digestive enzymes and low pH environment. Therefore, the chances of inflaming the epithelial layer either by chemicals or pathogenic microorganisms are minimized. In addition, some seaweed dietary fiber contributes to regenerate the damaged mucous membrane. Clinical trials showed that sodium alginate promotes the regeneration of mucous membrane in the stomach, suppresses inflammation, and eradicates colonies of *Helicobacter pylori* in the mucous membrane (Khotimchenko et al., 2001). In support of the above observations, the effects of alginic acid and its derivatives for the treatment of gastritis and gastroduodenal ulcers were also studied and positive results were obtained.

Other than the reparative and sheathing effects of these polysaccharides, some agaro-oligosaccharides suppressed the production of proinflammatory cytokines and enzymes associated with the production of nitric oxide in the tissues of the digestive track, controlling the inflammatory reactions at cellular level (Enoki et al., 2003).

C. Encourages the action of probiotics

Though the seaweed fiber is not digested by the enzymes in the upper digestive track (stomach and duodenum) of humans, it is partially degraded by the microflora in the colon, the lower segment of the digestive system. The colonic microflora is a complex and co-existing microbial ecosystem of potentially pathogenic and beneficial bacteria associated with gut lymphoid tissue. Probiotics, potentially health-promoting bacteria in the gut lymphoid tissue, and prebiotics, the fermentable substrates of such bacteria including dietary fiber, play a key role in promoting digestive health and in nutrition by salvaging nutrient and energy producing end metabolic products, like short chain fatty acids. Dietary modulation of the intestinal microflora can be achieved via either oral administration of probiotics or prebiotic compounds. Fermentation of fiber from brown seaweed with human fecal bacteria has indicated that probiotics follow their original fermentation pathways as exhibited with prebiotics from some other non-seaweed food sources (Mabeau and Fleurence, 1993). This fermentable fiber stimulates the growth of bifidobacteria and lactobacilli, which are the most important probiotic genera in humans, and maintains a more favorable balance among the colonic microflora.

Laminarin, a less viscous phycocolloid amply found in *Laminaria* and *Saccharina*, has shown its capability to promote higher production of butyric acid through bacterial fermentation (Deville et al., 2004). Butyrates are important energy-yielding metabolites for the colonial epithelial cells and account for about 70% of the energy requirement of the colon (Reilly et al., 2008). Prebiotic effects of laminarin studied in animal models reported that 1% dietary supplementation resulted in an increase in
Bifidobacterium counts in the cecum of rats compared to a control diet, but there was no significant difference in Lactobacillus counts (Kuda et al., 2005). Studies carried out on seaweed extracts found that fucoidan also functions as a good prebiotic. Several other studies have also confirmed the positive dietary effects of alginates encouraging the growth of beneficial microbial fauna in fecal matter (Wang et al., 2006).

Laminarin and fucoidan may offer a dietary means to modulate the gut environment and immunity, and thereby reducing the risk of pathogenic microorganisms in the gut. Inclusion of brown seaweed, Ascophyllum nodosum, to the diet of weanling pigs resulted in lower numbers of Escherichia coli in the small intestine (Dierick et al., 2009). Moreover, sodium alginate seemed to demonstrate a strong antibacterial element by decreasing enterobacteriaceae, enterococci, and lecithinase negative clostridia, indicating a potentially beneficial shift in the microbial ecosystem in the gut.

IV. NUTRITION-RELATED OTHER HEALTH BENEFITS OF SEAWEED

In addition to the above discussed nutrients, diverse amount of phenolic molecules has been identified from seaweed and is classified under different groups of phytochemicals. Those molecules virtually do not play the roles of nutrients and proven to have different bioactive properties associated with enhancing physical fitness to refrain from diseases or to exert therapeutic effects against certain illnesses. However, the medicinal effects of such molecules are not discussed in this chapter. Non-communicable diseases such as diabetes, obesity, and cardiovascular diseases have a strong relationship with dietary habits and nutritional profiles of the food. Therefore, next few sections of this chapter address the nutrition-related several health effects of seaweed fiber, other than previously mentioned, and its contribution to enhance digestive health.

A. Reduction of obesity by bringing down the caloric value of the diet

Dietary obesity and obesity-related diseases are among the widely occurring nutritional health problems in most of the developed nations in the Western world. Seaweed fiber in the diet helps to control weight gain in different ways. Adding considerable amount of seaweed to the diet enables to keep the dieter feel fuller quickly and to reduce the appetite dramatically for further eating. Moreover, most of the dietary fiber in seaweed is not taken up by the human body and provides a low caloric value to the diet. In addition, this soluble fiber forms a viscous mass in the
gut and traps digestive enzymes and some other nutrients, slowing down the digestibility of food and the absorption of nutrients in the intestine. A recent study carried out with a drug developed using alginic acid revealed that volunteers who were 25–30% overweight significantly decreased their body weight after treating with the drug (Zee, 1991). In addition to the dietary fiber, polyphenols in the seaweed extracts of *Ascophyllum* and *A. nodosum* inhibited α-amylase and α-glucosidase activities (Nwosua *et al*., 2011).

**B. Reduction of lipid absorption and cardiovascular diseases**

Reduction of the risk of cardiovascular diseases by consuming seaweed is suggested due to its modifying effects on the gastrointestinal tract such as emulsification of bile acid and interfering with lipid micelle formation, dilution of lipase concentration, binding with cholesterol, and slowing down of lipid absorption. Studies carried out using rats reported that alginic acid leads to a decrease in the concentration of cholesterol and is often coupled with an increase in the fecal cholesterol content and a hypocholesterolemic response (Dumelod *et al*., 1999). Moreover, porphyran significantly lowered the artificially enhanced level of hypertension and blood cholesterol in rats conserving cardiac health (Noda, 1993).

**C. Influence on glycemic control**

Dilution and slowing down the action of carbohydrases in the gut by seaweed fiber would have a positive impact on regulating the blood glucose level. Therefore, control of starch digestion in the diet can help to control blood glucose in type II diabetes. Five grams of sodium alginate administered daily to type II diabetic patients was found to prevent a postprandial increase of glucose and insulin, and to slow down gastric transit (Torsdottir *et al*., 1991). Hydrolysates of agar resulted in agaro-oligosaccharides possessing an activity against α-glucosidase (Chen *et al*., 2005). Moreover, *Ascophyllum* extracts at 50 mg/ml completely inhibited amylase activity. A meal supplemented with 5% alginites from brown seaweed decreased glucose absorption balance over 8 h in pigs, and much similar studies have been done on rats and humans (Vaugelade *et al*., 2000). The above findings suggest that seaweed fiber has an effective influence in inhibiting starch digestive enzymes at a very low level and maintains glycemic control in vivo.

Taking all the above discussed dietary functions of seaweed into consideration, it can be concluded that seaweed is a potential food to be added to the diet to enhance the human nutrition and digestive health.
REFERENCES


CHAPTER

Marine Edible Algae as Disease Preventers

Claudia Mariana Gomez-Gutierrez, Graciela Guerra-Rivas,1 Ima Esthela Soria-Mercado, and Nahara Ernestina Ayala-Sánchez

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Abstract

As modern lifestyles and new feeding habits settle in the world, noncommunicable diseases (NCDs) have evolved to be major causes of disability in developing as well as developed countries. As a concomitant effect, there is a growing interest in natural, healthy food and an increasing awareness of risk factors and determinants of disease. This chapter describes some nutritional facts about seaweeds, which have been used as food since ancient times in China, Japan, Egypt, and India and comments on the potential utilization of marine algae as functional foods.

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This concept and the description of metabolic syndrome are used as a basis to comprehension of seaweeds against two dreadful illnesses of our times: high blood pressure and cancer.

I. INTRODUCTION

For centuries, mankind has relied on food as a main source of well-being and health. An improved nutrition and better healthcare are among major factors determining life expectancy, which has increased all over the world (WHO, 2011). However, modern lifestyle and current feeding behavior have fostered the development of illnesses due to physical inactivity; overweight and obesity, and other diet-related factors; and tobacco and alcohol-related risks. The evolution of the human diet over the past years has adversely affected health, and chronic diseases cause substantial disability and death, disease prevalence being one major challenge. Chronic diseases, including obesity, diabetes mellitus, cardiovascular disease (CVD), hypertension and stroke, and some types of cancer, have become the target in a fight where marine algae may play a major role.

II. MARINE ALGAE AS FOOD HEALTH PROMOTERS

Ancient times witnessed the health benefits linked to seaweed consumption by Eastern countries. This utilization of marine algae can be traced back to the fourth century in Japan and the sixth century in China, although archeological evidences also indicate that seaweeds were included in folk medicine for many thousands of years in Japan, China, Egypt, and India (Fakoya et al., 2011). Today, Japan, China, and the Republic of Korea are the largest consumers of seaweeds as food (FAO, 2003).

Despite the scarce knowledge on composition and nutritional value of marine algae, oriental communities included them in their dietetic habits centuries ago, and nowadays, they have a long traditional use of marine algae as food. As more information about algae components is being accumulated, more people around the world are becoming familiarized with “sea vegetables” as part of a regular diet and, slow but steadily, cooking recipes are incorporated in Western countries.

In the past three decades, many authors have published on the chemical composition of diverse species of algae; however, there is still a lack of studies on the nutritional properties of a great variety of species, and even the most popular edible seaweeds are incompletely known which is understandable, given the enormous number of species that occur worldwide, around 12,000. Despite this incompleteness, data on major edible
seaweeds might be used to point out some representative nutritional facts to make some statements on their value as foodstuff. A comparison can be made using published data on the composition of edible seaweeds and the amounts of typical intakes through both western and eastern diets, applying typical nutritional indicators. Daily consumption of seaweed is difficult to establish since they are used in a variety of ways: noodles, soups, snacks, salads are some of the dishes that can be prepared. However, some approaches are useful to determine a common measure and be able to compare on a portion basis. According to the third Korean National Health and Nutrition Survey, the daily intake of seaweed is 8.5 g/d. In Japan, a daily typical consumption in that country is up to 10 g/d (Teas et al., 2004). For Koreans, diet is based on Porphyra sp., Undaria pinnatifida, and Laminaria sp. seaweeds which constitute over 95% of seaweed consumption in Korea. In Japan and China, Monostroma sp., Hizikia fusiformis, Ulva sp., and Palmaria palmata are also used as food, and all of them are among the most commonly consumed algae which are being incorporated into Western dietetic habits (FAO, 2003). Well known are also species of Gracilaria, Gellidium, Sargassum, Caulerpa, and Ascophyllum. Using this information, a comparison is made using common measures (portions) of usual foods in an occidental diet (Table 3.1).

III. ALGAE AS FUNCTIONAL FOOD: THE PREVENTING DISEASE POTENTIAL

Experts around the world have joined efforts to provide tools for international organisms as the Food and Agriculture Organization of the United Nations and the World Health Organization to make recommendations regarding the prevention of chronic diseases and the reduction of their impact. Information has been gathered, reviewed, and systematized in order to have an overview of the so-called noncommunicable diseases (NCD) (WHO, 2003). According to their report, changes in dietary and lifestyle patterns, chronic NCDs—including obesity, diabetes mellitus, CVD, hypertension and stroke, and some types of cancer—are becoming increasingly significant causes of disability and premature death. According to WHO statistics, in 2008, NCDs caused an estimated 36 million deaths worldwide, up from 35 million in 2004 (WHO, 2011). Among the causes of main chronic disease epidemics, unhealthy diet, excessive energy intake, over weight, and obesity are widely documented as major modifiable factors. As the global burden of chronic diseases have steadily increasing, there is growing interest on prevention to keep the world far from the leading global risks for mortality in the world: high blood pressure (BP), tobacco use, high blood glucose, physical inactivity,
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Nutritional facts $^{a,b}$</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Fiber</td>
<td>Fiber in <em>Ulva lactuca</em> (5.3 g) is as high as one of papaya (5.2 g) and in <em>Ulva rigida</em> (4 g) is similar to bananas (3.9 g). <em>Laminaria digitata</em> and <em>Enteromorpha</em> sp. contain (3.6 g) slightly more fiber than a portion of rice brown (3.5 g), blueberries (3.5 g), or cooked mushrooms (3.4 g). Fiber in a portion of <em>Porphyra umbilicalis, Porphyra tenera, Palmaria palmata, Ascophyllum nodosum, or Undaria pinnatifida</em> is almost the same (3.3 g) as a portion of raw carrots (3.1 g), raw mangos (3.3 g), oranges (3.1 g), strawberries (3.3 g), or dates (3.3 g). In these algae, fiber content is higher than in a portion of nuts (2.8 g), wheat bran muffin (2.8 g), or a slice of both wheat and rye bread (2.8 g) or multigrain bread (1.9 g).</td>
<td>Wong and Cheung (2000), Taboada (2002)</td>
</tr>
<tr>
<td>Calcium</td>
<td>A portion of <em>Caulerpa veravelensis</em> provides 395 mg of calcium, an amount similar to the amount provided by a portion of a milk shake with thick chocolate (396 mg) or one portion of low fat milk (305 mg). <em>Sargassum polycystum</em> contains (360 mg) more calcium than a portion of low fat yogurt with fruit (345 mg), one of low fat milk enriched with A and D vitamins (305 mg) or one of plain yogurt made of whole milk (275 mg). <em>Ulva lactuca</em>, in one portion, has more calcium (257 mg) than a portion of cheese: swiss (224 mg), provolone (214 mg), (207 mg) or cheddar (204 mg).</td>
<td>Mac Artain <em>et al.</em> (2007), Matanjun <em>et al.</em> (2009), Kumar <em>et al.</em> (2011)</td>
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<tr>
<td>Vitamin C</td>
<td><em>Gelidiella aerosa</em> has a vitamin C content (311 mg) higher than a glass of concentrated, undiluted, orange juice, which is the highest reported content of vitamin C (293.7 mg). <em>Padina pavonica</em> and <em>Ulva reticulata</em> have 242.25 and 232 mg respectively, higher than other raw juices: papaya (185 mg), orange(124 mg), grapefruit (94 mg), or a portion of strawberries (97.6 mg).</td>
<td>Chang <em>et al.</em>, 1997, Matanjun <em>et al.</em> (2009), Mac Artain (2007), Anantharaman <em>et al.</em> (2011)</td>
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<td>ω-3 PUFA</td>
<td>Seaweeds have small levels of lipids (1–5%), but they have high contents of n-3 and n-6 fatty acids; also have an n-6:n-3 ratio around 1. <em>Sargassum polycystum</em> provides 77.5 mg of ω-3 and 75.6 of ω-6; in a portion of <em>Eucheuma cottonii</em>, 256 and 26 mg of n-3 and n-6 respectively. <em>Ulva lobata</em> has 95 mg of n-3, 4 mg of n-6, and a 0.04 ratio. <em>Palmaria palmate</em> provides 85.5 mg of n-3 and 1.2 mg of n-6.</td>
<td>Matanjun et al. (2009), Nelson et al. (2002), van Ginneken et al., 2011</td>
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<td>Potassium/Sodium Ratio</td>
<td>Seaweeds with a ratio (K/Na) equal to or higher than 2.0 and high potassium content (K): <em>Laminaria digitata</em> (3.2; K, 1159 mg), <em>Himanthalia elongata</em> (2.25; K, 360 mg), <em>Porphyra umbilicalis</em> (2.5; K, 252 mg), <em>Palmaria palmata</em> (4.6; K, 694 mg), <em>Enteromorpha</em> sp. (6.8; K, 257 mg), <em>Gracilaria corticata</em> (3.3; K, 1334 mg), <em>G. pudumadensis</em> (1.9; K, 1087 mg), <em>Sargassum myriocystum</em> (2.2; K, 1153 mg) and <em>S. polycystum</em> (6.1; K, 8371 mg).</td>
<td>Sivakumar and Arunkumar (2009), Matanjun et al. (2009)</td>
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*a* A Portion 9.5 g/d (dry matter) for seaweed.

and overweight and obesity since they are considered to be responsible for raising the risk of chronic diseases, such as heart disease and cancers.

A. Risk factors for chronic disease: The metabolic syndrome (MetS)

The metabolic syndrome (MetS) is a combination of medical disorders integrated to have a useful description of related cardiovascular risk factors which also predict the risk of developing diabetes. Although there have been arguments against the use of a minimalistic view for these diseases, there is currently a unifying definition for the MetS (Fig. 3.1). According to this (Alberti et al., 2006), obesity and insulin resistance appear to be the causative factors in the development of the MetS. General features include obesity, insulin resistance (correlated with the risk of Type 2 diabetes and CVD), atherogenic dyslipidemia (increased triglycerides and HDL cholesterol), elevated BP, elevated C-reactive protein (CRP; proinflammatory state), and a prothrombic state, associated with coagulation and fibrinolytic proteins. Scientific effort has been done to clarify associations between MetS factors, and after decades of effort, some facts can be stated:

![FIGURE 3.1 General features of MetS. Obesity and insulin resistance are causative factors in the development of the MetS. Insulin resistance and elevated blood pressure are related to cardiovascular diseases.](image-url)
• Causes of atherosclerosis include obesity, age, smoking, hypertension, hypotension, abnormalities of bone and mineral homeostasis, and disturbances of lipid metabolism and insulin resistance.
• Insulin resistance is part of the natural history of Type 2 diabetes and may be present years before the clinical diagnosis; cardiovascular risk factors are strongly related to insulin resistance.
• Atherogenic dislipidemia, characterized by high triglycerides and low high density lipoprotein, leads to coronary heart disease (CHD).
• BP and cardiovascular risk related; elevated BP is one of the major risk factors of CVDs worldwide.
• BP and CVD (CHD, stroke, heart failure, etc.) are strongly related.
• A proinflammatory state means elevated CRP values, and this strongly predicts future coronary events.
• Inflammation and thrombosis influence the pathogenesis of CHD. The inflammation may favor blood clot formation.

B. Metabolic syndrome prevention

The WHO, in the 2009 report, states that high BP ranked first in the list of leading global risks for mortality and accounted for 7.5 million deaths in the world in 2004. According to MetS concept, elevated BP is clustered with other symptoms, including obesity, dyslipidemia, and glucose dysregulation. For treating these disorders, clinical guidelines such as the National High Blood Pressure Education Program recommend, among others, weight loss, dietary sodium reduction (no more than 2.4 g/d), adequate potassium intake (more than 3.5 g/d), and consumption of a diet rich in fruit and vegetables. In its initial stage, one of the major concerns is on preventing disease worsening, and shifting to more adequate foods is crucial. In this context, functional foods play a relevant role if they supply what is needed to comply with dietary guidelines.

Several pieces of knowledge support the importance of marine algae as a functional food, defined this as a food that provides a health benefit beyond basic nutrition, that is, that has health-promoting benefits and/or prevent diseases (Barker and Meletis, 2004), by offering health benefits to reduce the risk of chronic diseases. Probably, the best illustration of marine algae as functional foods is the case of wakame, brown seaweed widely consumed by oriental people.

Undaria pinnatifida (wakame) is a very popular food in the Asiatic countries (Suetsuna et al., 2004). Wakame has a fiber content similar to rice (3.4% wet weight) and proportionate the same beneficial sensation of satiety and aid digestive transit through their bulking capacity without the starchy carbohydrate (Mac Artain et al., 2007). Besides, wakame has antihypertensive effect through inhibition of angiotensin I-converting...
enzyme (ACE-I) by peptides isolated from hot water extracts (Suetsuna et al., 2004; Suetsuna and Nakano, 2000). Inhibition of ACE-I is considered to be a useful therapeutic approach in the treatment of hypertension and inhibitors such as captopril, enalapril, alacepril, and lisinopril, which are currently used in the treatment of essential hypertension and heart failure in humans. Peptides from wakame can be a better alternative, as natural products might have less secondary effects. Another beneficial effect of wakame has been reported to be against the development of stroke in stroke-prone spontaneously hypertensive rats putatively due to fucoxanthin (Ikeda et al., 2003). Other studies suggest that wakame and dietary fish oil synergistically decrease rat serum and liver triacylglycerol (Murata et al., 2002), effect attributable to stimulation of enzymes involved in hepatic fatty acid oxidation (Murata et al., 1999). Of particular interest in clinical studies are dose dependency investigations, which are reported for sodium alginate, polysaccharide obtained from brown seaweeds. The results of this study indicated that the optimum dose would be 1.25–2.5 g/day (Takamitsu et al., 2006). Another potential therapeutic application is the reduction of increased uptake cholesterol and glucose on individuals with high body mass index by fibers of alginate in treatments with 1.5 g dose, which was demonstrated by Paxman et al. (2008). More recently, in a randomized double-blinded placebo-controlled trial, Teas et al. (2009) achieved excellent results on systolic pressure decrease by 10.5 mm Hg and waist circumference reduction of 3.9 cm after consecutive treatments of 4 g/d for 1 month and 6 g/d of Undaria pinnatifida in 14 women (aged 45.6 ± 12.2 year) with at least one symptom of the MetS.

C. Cancer prevention

Another evidence to prove seaweeds as functional foods comes from the studies on the role of nutrition in cancer etiology, a line of research that converges with the use of seaweeds as a breast cancer anticarcinogen. Both lines can be traced back to first recommendations of using seaweeds as remedies to treat tumors by ancient Egyptians in the Ebers Papyrus (1500 BC), to the more recently suggestions on the use of seaweeds as a breast cancer anticarcinogen and the conclusive results of cause–effect relationships on cancer matters. Today, there is evidence of an inverse association between endometrial cancer and dietary fiber through epidemiological studies as the one by Bandera et al. (2007). In this study, authors performed the first systematic literature review and made an analysis of the role of dietary fiber intake on endometrial cancer, concluding that current evidence supports the idea of reducing risks in endometrial cancer through fiber intake. Other findings were obtained from a cohort study of more than 180,000 postmenopausal women, which suggests that dietary fiber also can play a role in preventing breast cancer, the
most common cancer in women worldwide (Park et al., 2009). Fiber consumption, in turn, is associated to seaweed intake in a study of pattern of dietary habits among the Japanese general population where it was demonstrated the high contribution of seaweed to dietary fiber intake (Fukuda et al., 2007). Japanese women with a traditional consumption of fiber from seaweeds have a good excretion of estradiol, which in serum high level increases the risk of developing breast cancer. It has been shown that Alaria esculenta, a brown seaweed related to Undaria pinnatifida, modulates serum estradiol levels and urinary excretion of estrogen metabolites and phytoestrogens. This investigation led to the conclusion that seaweed fiber reaching the colon may be a prebiotic, providing substrate for specific bacteria favoring the healthy ones (Teas et al., 2009). Recently, a case–control study investigated 362 women with confirmed breast cancer, concluded that high intake of gim (Porphyra sp.) may decrease the risk of breast cancer (Yang et al., 2010). Evidence for polysaccharides from marine algae as prebiotics has provided and there are claims that fucoidan, laminarin, alginate, and their derivatives could be exploited as prebiotic functional ingredients for human health applications since they have demonstrated in a specific manner an antiproliferative activity against several cellular lines of cancer, among them, human colon adenocarcinoma, human neuroblastoma, rat basophilic leukemia, and Chinese hamster fibroblasts (O’Sullivan et al., 2010).

IV. CONCLUSION

As research is being performed and results are presented, the possibility for marine algae as functional food is increasing. Wakame and alginate studies are only a small piece of knowledge showing us that sea remains until now as an untapped reservoir of raw materials for a better health in a variety of ways.

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Potential Role of Marine Algae on Female Health, Beauty, and Longevity

Se-Kwon Kim*†,1 and Ratih Pangestuti*

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Abstract

Marine environment has been known as a rich source of chemical structures with numerous health benefit effects. Among marine organisms, marine algae have been identified as an underexploited plant resource although they have long been recognized as valuable sources of structurally diverse bioactive compounds. Presently, several lines of studies have provided insight into biological activities of marine algae in promoting female health, beauty, and longevity. Hence, marine algae have a great potential to be used as

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a part of pharmaceuticals, nutraceuticals, and functional foods. This contribution presents an overview of marine algal potential effect in promoting female health, beauty, and longevity.

I. INTRODUCTION

More than 70% of the Earth’s surface is covered by oceans with marine species comprising approximately half of the total global biodiversity (Kim and Wijesekara, 2010; Swing, 2003). Hence, marine organisms are being recognized as rich sources of functional materials which are elicited by marine organisms to help them survive in the hostile ocean environment (Shahidi, 2008; Shahidi and Janak Kamil, 2001). Among marine organisms, marine algae are still identified as an underexploited plant resources; although they have been used as food sources since ancient times (Heo et al., 2009b; Pangestuti and Kim, 2011).

In Asian culture, marine algae have always been of particular interest as marine food sources (Khan et al., 2010). Edible marine algae or sometimes referred as seaweeds or sea vegetables accounted for more than 10% of Japanese diet with average consumption reaching an average of 1.4 kg/person/year (Burtin, 2003). In Korea, 37 days after delivering their babies, new mothers served with miyeok-guk which is a hot and spicy marine algae soup (Dennis et al., 2007). Korean believes that miyeok-guk provide nutrition and help the new mother to regain their energy. Marine algae have been demonstrated as rich sources of structurally diverse biologically active compounds with great pharmaceutical and biomedical potential; therefore, it represents one of the most nutritious plant foods. Several epidemiologic studies provided evidence that marine algae consumption correlates with low breast cancer rates in East Asia. As an example, 1 year prevalence case of breast cancer rates per 100,000 populations in Japan and China are 42.2 and 13.1, respectively, versus 125.9 and 106.2 cases in North America and Europe, respectively (Pisani et al., 2002; Yuan and Walsh, 2006).

In recent years, biological activities, nutritional value, and potential health benefits of marine algae have been intensively investigated and reviewed. In spite of extensive studies and reviews on nutritional value and potential health benefits of marine algae for human, there is little available literature that focuses on potential benefits of marine algae for female subject. Therefore, this chapter focuses on biological roles of marine algae and presents an overview of their potential benefits for female health and beauty.
II. POTENTIAL ROLE OF MARINE ALGAE ON FEMALE HEALTH, BEAUTY, AND LONGEVITY

A. Anticancer activity

Breast cancer is the leading cause of cancer-related death among females worldwide (Geyer et al., 2006; Parkin, 2001). Globally, more than 1.1 million females are diagnosed each year, representing around 10% of all newly diagnosed cancer cases (Anderson et al., 2006). The mortality rate for premenopausal breast cancer is almost four times greater in the Western world, compared with East Asia nation. In breast cancer etiology, genetics are thought to play a smaller role compared to environmental factors such as food diets. One important difference in the diet of East Asian populations compared to Western populations is higher amount of fish and marine algae consumption. As mentioned earlier, ancient tradition of marine algae consumption has made a large number of epidemiological researches showing the health benefit in females linked to marine algae consumption.

Three decades ago, Teas et al. investigated effect of *Laminaria angustata* consumption and development of breast cancer in female Sprague-Dawley rats induced with the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA), a widely used rat mammary cancer model (Huggins et al., 1961; Teas et al., 1984). Diet containing 5% *L. angustata* was found to be effective in delaying the time of DMBA-induced tumor developments. Although the mechanism for *L. angustata* activity is not elucidated yet, the authors argue that bioactivity of *L. angustata* might bring by their nutrient content such as polyphenols, sulfated polysaccharides, vitamins, minerals, carotenoids, etc. In accordance, *wakame* (*Undaria pinnatifida*) and *mekabu* (sporophyll of wakame) have been demonstrated to reduce the incidence, multiplicity, and size of breast tumors in female Sprague-Dawley rats induced with DMBA (Funahashi et al., 1999, 2001). Considering that *wakame* and *mekabu* are particularly rich in iodine, the investigators suggested that the cancer inhibition was brought about by the iodine. More recently, statistical correlations between dietary intake of iodine and breast cancers have been carried out; however, their exact mechanisms of action are not yet completely understood (Ellerker, 1955; Majem et al., 1988).

Apoptosis or programmed cell death is a key process in cancer development and progression which can be characterized through distinct set of morphological and biochemical progresses. Inactivation of apoptosis has been considered to be one of the six fundamental hallmarks of cancer; therefore, apoptosis is a major target of cancer therapy development up to present (Brown and Attardi, 2005). Dioxinodehydroeckol, a phloroglucinol derivative from *Ecklonia cava*, has a potential inhibitory effect against growth of human breast cancer cells MCF-7 via induction of apoptosis.
Further, 1 μg/ml mekabu strongly induced apoptosis in three human breast cancer cell line (MCF-7, T-47D, and MDA-MB-231), the induction of apoptosis even greater than 5-fluorouracil, a chemotherapeutic agent frequently used in human breast cancer clinics. Hence, developing of novel molecules derived from marine algae which promote apoptosis in breast cancer cells by targeting both the intrinsic and extrinsic apoptotic pathways may lead to the development of effective breast cancer therapies.

Estrogen-dependent cancers such as breast, endometrial, and ovarian cancer are among the leading causes of morbidity and mortality in American females (Kramer and Wells, 1996). Increased incidence of these cancers is predicted in the future, and the need for primary prevention is clear. Epidemiological studies demonstrated that incidence rates of estrogen-dependent cancers are among the highest in Western, industrialized countries, while rates are much lower in China and Japan (Parkin et al., 1999, 2002). Due to some research study, low estrogen-dependent cancer rates have been attributed to the soy-rich and marine algae diets inherent among Asian populations (Teas et al., 2009). As an example, dietary intake of Alaria esculenta and soy protein has been reported to modify estrogen and phytoestrogen metabolism in healthy postmenopausal females (Teas et al., 2009). In another female pilot study, Skibola (2004) demonstrated that intake of Fucus vesiculosus (bladderwrack) significantly increased the total number of days of the menstrual cycle, reduced circulating 17β-estradiol levels, and elevated serum progesterone levels in premenopausal women with abnormal menstrual cycling histories (Skibola, 2004). Moreover, F. vesiculosus have been demonstrated to modulate endocrine hormones in female Sprague-Dawley rats and human luteinized granulose cells (Skibola et al., 2005). Hence, it may assumed that intake of marine algae may contribute to the lower estrogen circulating level which may correlate to the lower incidence of hormone-dependent cancers in females.

Cervical cancer is the second most common cancer in females worldwide and more females die annually because of cervical cancer rather than from AIDS (ElHage, 2005; Kaplan-Myrth and Dollin, 2007; Munoz et al., 2003). It is the principal cancer of female in most developing countries, where 80% cases occur (Munoz et al., 2003). Recent reports demonstrated that several marine algae species: Palmaria palmate (dulse), Laminaria setchellii, Macrocystis integrifolia, Nereocystis lutkeana, Udotea flabellum, and Udotea conglutinate extracts were able to inhibit cervical cancer cell proliferations in vitro (Moo-Puc et al., 2009; Yuan and Walsh, 2006; Yuan et al., 2005). The goal of most current cancer therapy is to reduce the number of tumor cells and to prevent their further accumulation. Hence, antiproliferative activity of marine algae in cervical cancer cells demonstrated potential of marine algae as therapeutic agent for cervical cancer treatment.
In addition, formation of cancer cells in the human body can be directly induced by free radicals and natural anticancer drugs as chemopreventive agents have gained a positive popularity in treatment of cancer. Therefore, marine algal radical scavenging compounds such as phlorotannins, sulfated polysaccharides, carotenoids, carbamol derivatives can be used indirectly to reduce cancer formation in the female body.

Taken together, marine algae and their secondary metabolites have shown promising anticancer activities, and hence, marine algae have a great potential to improve female health and longevity by being a part of anticancer medicinal foods and nutraceuticals. However, future studies are needed focusing on the synergistic benefits of consuming different marine algae species, recommended doses, and timing of intake and preparation methods for marine algae in order to maximize the desired effect in the prevention of cancer, particularly cancer which occurs mainly in female subject.

B. Antiviral activity

Infection by certain human papilloma virus (HPV) types in female genital has been associated with cervical cancer, hence HPV prevention has received great attention from scientific studies (Lehtinen and Dillner, 2002). The first generation of HPV vaccine is currently available on the market to prevent HPV infection (Paczos et al., 2010). However, high cost of vaccine has been a cause for concern and will be too expensive for use in the developing world. Therefore, the search for potential anti-HPV candidates containing higher inhibitory activity and fewer prices has rise great interest in pharmaceutical industries. In this regard, natural bioactive compounds and their derivatives are potential source for the development of functional foods as new generation anti-HPV therapeutics which is more effective, less side effects, and less expensive.

A large number of marine algae contain significant quantities of complex structural sulfated polysaccharides which have been demonstrated as potent inhibitors of wide variety of viruses, such as HPV (Campo et al., 2009; Pujol et al., 2007; Witvrouw and De Clercq, 1997). Carrageenan, a sulfated polysaccharides of D-galactose and 3,6-anhydro-D-galactose extracted from the Rhodophyceae, has been used in food products for centuries. Recently, carrageenan has been shown to bear anti-HPV activity in vitro (Campo et al., 2009). Buck et al. noted that carrageenan, particularly i-carrageenan, inhibits HPV three orders magnitude more potent than heparin, a highly effective model for HPV inhibitor (Buck et al., 2006). Carrageenan acts primarily by preventing the binding of HPV virions to cells and blocks HPV infection through a second, postattachment heparin sulfate-independent effect. Those mechanism is consistent by the fact that carrageenan resembles heparin sulfate, which is known as HPV-cell
attachment factor. Further, some of milk-based products which contain carrageenan block HPV infectivity in vitro, even when diluted million-fold (Buck et al., 2006). In another study, carrageenan has been reported to inhibit genital transmission of HPV in female mouse model of cervicovaginal (Roberts et al., 2007; Schiller and Davies, 2004). In addition, carrageenan was able to generate antigen-specific immune responses and antitumor effects in female (C57BL/6) mice vaccinated with HPV-16 E7 peptide vaccine (Zhang et al., 2010).

Based on these findings, carrageenan can be an alternative source of novel therapeutic candidate for HPV by being a part of food additives. There are numerous advantages of carrageenan over other classes of antiviral agents, such as relatively low production costs, broad spectrum of antiviral properties, low cytotoxicity, safety, wide acceptability, and novel modes of action, suggesting that carrageenan are promising candidates in the near future. However, further studies with clinical trials are needed for their anti-HPV activity in female subject.

C. Antiobesity activity

Obesity may occur in any gender, however, it is more likely to occur in females (Popkin and Doak, 1998; Rennie and Jebb, 2005). Obesity among females (from teens and seniors) continues to increase in many industrialized and developing countries, which cause a worrying health trend (Kelishadi, 2007). A detrimental effect of obesity on female reproductive system has been demonstrated consistently (Pettigrew and Hamilton-Fairley, 1997). Further, it is reported that media and sociocultural influence continue to pressure young female to be thin which promotes body dissatisfaction, eating disturbance, depression, and negative effect in young female (Stice et al., 2003). Therefore, female may pay a higher health price for obesity than male. Accordingly, many categories of natural and synthetic compounds which demonstrated as antiobesity drugs have been used by female to reduce their weight. However, synthetic antiobesity agents are believed to have certain side effects such as unacceptable tachycardia, hypertension, improve lipid blood levels, improve glucose metabolism, disturbance of female reproductive system, etc. (Bays, 2004). Hence, more scientific efforts have been dedicated to study medicinal foods that can act as antiobesity agents.

In the past four decades, researchers have found that soluble dietary fibers are negatively associated with obesity. Marine algae is particularly rich in two different types of fiber, soluble and insoluble (Table 4.1; Lahaye, 1991). Eisenia bicyclis, sometimes referred as Arame, contained more than 50% soluble fiber of its dry weight; the other brown algae species, F. vesiculosus, contained around 40% insoluble fiber per dry weight (Lahaye, 1991; Ruperez and Saura-Calixto, 2001). In human
<table>
<thead>
<tr>
<th>Marine algae</th>
<th>Soluble fiber</th>
<th>Insoluble fiber</th>
<th>Total fiber</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorophyceae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ulva lactuca</em> (Sea lettuces)</td>
<td>21.3</td>
<td>16.8</td>
<td>38.1</td>
<td>Burtin (2003)</td>
</tr>
<tr>
<td><em>Enteromorpha</em> sp. (Ao nori)</td>
<td>17.2</td>
<td>16.2</td>
<td>33.4</td>
<td>Burtin (2003)</td>
</tr>
<tr>
<td><strong>Rhodophyceae</strong></td>
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</tr>
<tr>
<td><em>Porphyra teneri</em> (Nori)</td>
<td>14.56</td>
<td>19.22</td>
<td>33.78</td>
<td>Ruperez and Saura-Calixto (2001)</td>
</tr>
<tr>
<td><em>Chondrus crispus</em> (Irish moss)</td>
<td>22.25</td>
<td>12.04</td>
<td>34.29</td>
<td>Ruperez and Saura-Calixto (2001)</td>
</tr>
<tr>
<td><strong>Phaeophyceae</strong></td>
<td></td>
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<tr>
<td><em>Hijiki fusiformis</em> (Hijiki)</td>
<td>16.3</td>
<td>32.9</td>
<td>49.2</td>
<td>Lahaye (1991)</td>
</tr>
<tr>
<td><em>Himanthalia elongate</em> (Sea spaghetti)</td>
<td>25.7</td>
<td>7.0</td>
<td>32.7</td>
<td>Lahaye (1991)</td>
</tr>
<tr>
<td><em>Eisenia bicyclis</em> (Arame)</td>
<td>59.7</td>
<td>14.9</td>
<td>74.6</td>
<td>Lahaye (1991)</td>
</tr>
<tr>
<td><em>Undaria pinnatifida</em> (Wakame)</td>
<td>17.31</td>
<td>16.26</td>
<td>33.58</td>
<td>Ruperez and Saura-Calixto (2001)</td>
</tr>
<tr>
<td><em>Laminaria digitata</em> (Kombu)</td>
<td>9.15</td>
<td>26.98</td>
<td>36.12</td>
<td>Ruperez and Saura-Calixto (2001)</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> (Bladderwrack)</td>
<td>9.80</td>
<td>40.29</td>
<td>50.09</td>
<td>Ruperez and Saura-Calixto (2001)</td>
</tr>
<tr>
<td><em>Durvillaea antarctica</em></td>
<td>27.7</td>
<td>43.7</td>
<td>71.4</td>
<td>Ortiz <em>et al.</em> (2006)</td>
</tr>
</tbody>
</table>
body, soluble and insoluble fiber acts in a very different way. Consumption of marine algae soluble fiber such as carrageenan, agar, alginate are primarily associated with hypocholesterolemic and hypoglycemic effects (Panlasigui et al., 2003). In example, alginates have been shown to modulate appetite and energy intake in models of acute feeding. Upon reaction with gastric acid (acid-soluble calcium source), alginates undergo ionic gelation to form an alginate gel that can slow gastric emptying, stimulate gastric stretch receptors, reduce intestinal nutrient uptake, and influence the glycaemic response (Dettmar et al., 2011). In accordance, ingesting calcium-gelled, alginate-pectin twice per day has been reported to reduce spontaneous food intake in overweight and obese females (Pelkman et al., 2007). Further, insoluble fiber such as cellulose, xylans, mannans are associated with excretion of bile acids, increase fecal bulk, and decrease intestinal transit time (Burtin, 2003; Moore et al., 1998).

More recently, Maeda et al. reported that dietary intake of fucoxanthin significantly attenuates the weight gain of white adipose tissue (WAT) and expressed Uncoupling Protein 1 (UCP1) in diabetic/obese KKAY female mice (Maeda et al., 2005, 2007). The potential involvement of fucoxanthin in attenuating the weight gain of WAT may correlate to the presence of unusual double allenic bonds at C-7' position (Miyashita and Hosokawa, 2009). WAT is the predominant type of adipose tissue and commonly called “fat” in mammals (Trayhurn and Wood, 2005). Besides its role in energy storage, WAT is now recognized as an endocrine and active secretory organ through its production of biologically active mediators termed adipokines (Curat et al., 2006). Excess production of adipokines including proinflammatory factors and chemokines has been linked with obesity and plays an important role in the development of obesity-related disease (Trayhurn and Wood, 2005). Therefore, fucoxanthin activity to attenuate the weight gain of WAT in female mice demonstrated potential of fucoxanthin for the prevention and treatment of obesity and diabetes particularly in female subject. Dioxinodehydroeckol and 1-(3',5'-dihydroxyphenoxy)-7-(2",4",6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin, two phloroglucinol derivatives isolated from E. cava, have significantly inhibits adipocyte differentiation in 3T3-L1 cells suggesting its potential use as a functional ingredient in obesity management.

According to those findings, marine algae may serve as a potential candidate for functional foodstuffs with health benefits, especially for obesity management. Hence, negative effect in female subjects, particularly in young females, caused by pressures to be thin can be minimized by the application of marine algae in foods, pharmaceuticals, etc. Additionally, marine algae would develop a new approach for the treatment of obesity in addition to currently available antiobesity agents. Therefore,
marine algae would be a potent natural source for the development of foods and pharmaceuticals for the management of obesity.

D. Antiosteoporosis activity

Osteoporosis is a skeletal condition characterized by decreased bone mineral density (BMD) (mass/volume unit) that leads to an increased risk of fractures (Beikler and Flemmig, 2003). A number of studies have identified that osteoporosis occurs much more frequently in females compared to males (Cadarette et al., 2000; Hannan et al., 2000; Schuit et al., 2004). There are a number of reasons for the high prevalence of osteoporosis in females. First, at skeletal maturity, males have 30–50% bone mass compared to females (Christiansen, 1993; Nieves et al., 2005). Second, although decreased BMD occurs in both males and females with age, the decreased of BMD is substantially greater in females after menopause (Kanis et al., 1997; Riggs et al., 2004, 2008). Therefore, it is very important to help postmenopausal females to prevent them from progressing to osteoporosis.

Fujita et al. (1996) indicated that active absorbable algal calcium (AAA Ca) was effective for improving BMD in elderly subject. AAA Ca is a mixture of active absorbable calcium (AA Ca) and heated algal ingredients prepared by heating cleaned oyster and marine algae (Cystophyllum fusiforme) submaximally under reduced pressure (Fujita et al., 1996). Further, mineral-rich extract from red marine algae Lithothamnion calcareum has been demonstrated to increased mineral content and bone strength in female mice on a western-style diet (Aslam et al., 2010). However, it is not clear yet by which minerals on the algal extracts act to preserve bone structure and function in female mice. The algal extract is currently available as a food supplement under the name Aquamin (GRAS 000028) which currently used in various products for human consumption in Europe, Asia, Australia, and North America.

In addition, Das et al. demonstrated the effects of fucoxanthin on osteoclastogenesis. Treatment with 2.5 M fucoxanthin also induced apoptosis accompanied by activation of caspase-3 in osteoclast-like cells. Those in vitro studies suggest that fucoxanthin suppresses osteoclastogenesis via the inhibition of osteoclast differentiation and the induction of apoptosis in osteoclasts (Das et al., 2010). Hence, dietary fucoxanthin may be useful for the prevention of bone diseases such as osteoporosis and rheumatoid arthritis, which are known to be related to bone resorption.

Collectively, it may assume that marine algae would be a potent natural source for the development of functional foods and pharmaceuticals to prevent osteoporosis. Moreover, it is important to evaluate other marine algae species which may have a great potential as antiosteoporosis agent.
E. Skin whitening activity

Skin whitening has been in practice around the world with Asia as the largest market. As much Asian female preferred more fair skin tone, skin whitening product has become and continues to be the best selling skin care products in Asia (Wang et al., 1997). Tyrosinase inhibition is the most common approach to achieve skin hypo-pigmentation as this enzyme catalyzes the rate-limiting step of pigmentation. Despite the large number of tyrosinase inhibitors in vitro, only a few are able to show induced effects in clinical trials. In this chapter, we review some potential marine organisms with its effects on pigmentation of skin focusing mainly on tyrosinase inhibitors. Hence, development of novel tyrosinase inhibitors from natural resources continues to arouse great attention, and in recent years, marine algae have attracted great attention in the search of natural tyrosinase inhibitor agents (Solano et al., 2006).

Recently, Cha et al. investigated 43 indigenous marine algae for tyrosinase inhibiting activity (Cha et al., 2010). They reported that extracts from Ecklonia cava and Sargassum silquastrum exhibited excellent inhibitory effects on the pigmentation of zebra fish, which is due to their potential tyrosinase inhibitory activity. Fucoxanthin isolated from Laminaria japonica has been reported to suppress tyrosinase activity in UVB-irradiated guinea pig and melanogenesis in UVB-irradiated mice. Oral treatment of fucoxanthin significantly suppressed skin mRNA expression related to melanogenesis, suggesting that fucoxanthin negatively regulated melanogenesis factor at transcriptional level (Shimoda et al., 2010). Fucoxanthin and astaxanthin have been demonstrated to possess photoprotective properties in human fibroblast cells via inhibition of DNA damage and enhance antioxidant activity (Heo and Jeon, 2009). Further, potential whitening effects of diphlorethohydroxycarmalol isolated from Ishige okamurae have been reported (Heo et al., 2009a, 2010). Phloroglucinol derivatives, a common secondary metabolite constituents of brown algae, possess tyrosinase inhibitory activity due to its ability to chelate copper in this enzyme (Kang et al., 2004). Some phlorotannins such as 7-phloroeckol and dioxinodehydroeckol have been described to inhibit tyrosinase activity stronger than arbutin and kojic acids (Yoon et al., 2009). Flavonoid glycoside derived from Hizikia fusiformis has been reported as potential tyrosinase inhibitor. H. fusiformis is one of the most common edible brown macroalgae belonging to the Sargassaceae family (Ranathunga et al., 2006).

These evidences suggest that bioactive compounds derived from marine algae have a promising potential to be used as skin whitening agents. There are numerous advantages of marine algae, such as relatively low production costs, broad spectrum of skin whitening properties, low cytotoxicity, safety, wide acceptability, and novel modes of action,
suggesting marine algae as nutritious food which can be used to restore female beauty; however, further studies are needed with clinical trials for their whitening effects.

III. CONCLUSIONS

The wide range of biological activities associated with natural compounds derived from marine algae such as phlorotannins, alginates, sulfated polysaccharides, and carotenoids have potential to expand its nutritional and health beneficial values of marine algae in food industries. Further, the wide diversity of marine algae and numerous undiscovered unique metabolites present in marine algae are interesting sources to increase numbers of novel functional foods, which is beneficial for female health, beauty, and longevity. Accordingly, possibilities of designing new medicinal foods or nutraceuticals and pharmaceuticals derived from marine algae are promising. However, clinical trials are needed to confirm anticancer, antiviral, antiosteoporosis, and antiobesity activity of marine algae. In addition, further research studies are needed in order to investigate marine algae activities in female subjects.

REFERENCES


CHAPTER 5

Sea Lettuces: Culinary Uses and Nutritional Value

Se-Kwon Kim,*†,1 Ratih Pangestuti,* and Puji Rahmadi‡

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Abstract

In many countries, sea lettuces are commonly consumed as food by human since the beginning of times. Sea lettuces contain significant amount of nutrients which are essential for human body. Moreover, several studies have provided insight into biological activities and health promoting effects of sea lettuces. Despite having so much health beneficial effects, sea lettuces are still identified as an underexploited plant resources for food purposes. Hence, sea lettuces have a great potential for further development as products in foods and pharmaceutical areas. Further, potential applications of polysaccharides, protein and amino acid, lipid and fatty

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acid, mineral and vitamin contents may increase the sea lettuces value. This contributions presents information on the currently culinary use of sea lettuces worldwide and nutritional aspects of sea lettuces.

I. INTRODUCTION

The ancient tradition and everyday habits of seaweeds consumption have made possible large numbers of epidemiological studies showing the health benefits linked to seaweeds consumption. When considering in combination with international diet-related chronic disease incidences, a significant environmental factors including dietary difference between populations varying in seaweeds consumption have revealed. For example, several epidemiologic studies showed that seaweeds consumption correlates with low breast cancer rates in Japan and China compared with North America and Europe (Pisani et al., 2002; Yuan and Walsh, 2006). Further, Jorm and Jolley reported that neurodegenerative cases in East Asian countries were lower than that of Europe ($p < 0.0004$; Jorm and Jolley, 1998). Studies showed that low cancer and neurodegenerative disease cases in Eastern hemisphere are associated with high amount of seaweeds consumption. In Asian culture, seaweeds have always been of particular interest as food sources (Khan et al., 2010; Pangestuti and Kim, 2011). Edible marine algae accounted for more than 10% of Japanese diet with average consumption reaching an average of 1.4 kg per person per year (Burtin, 2003).

Seaweeds represent one of the most nutritious plant foods, and general utilization of seaweeds in food products has grown steadily since the early 1980s (Besada et al., 2009). In recent years, consumers in developed countries are turning to more natural and nutritional products such as seaweeds (Van Netten et al., 2000). Seaweeds have recently been approved in France for human consumption, thus opening for the food and fisheries industries. During 2003, it was estimated that about 1 million tons of seaweeds were harvested in 35 countries mainly as food sources (Garcia-Casal et al., 2009).

Several seaweed species are consumed by human directly after only minor preprocessing such as drying. Porphyra sp. which is commercially known as nori or lavers are the most widely consumed among edible red seaweeds worldwide (Watanabe et al., 1999). Among green seaweeds, sea lettuces are the most common, ubiquitous, and environmentally important genera (Tan et al., 1999). Sea lettuces comprise the genus Ulva, a group of edible green seaweeds which is widely distributed along the coasts of the world’s oceans and often found in the mid and upper tidal zones. Sea lettuces or sometimes termed as green laver are found in tidal and near
tidal seawater worldwide, generally anchored to rocks or other algae. They are easily identified by its paper-thin, semitranslucent, and vibrant green color. Most sea lettuces are gathered wild as it grows prolifically wherever there are sufficient nutrients, but some is farmed. Many species of sea lettuces are reported to be tolerant of organic and metal pollution; hence, if we consume, we need to make sure that they are collected far from any potential sources of pollution.

There are a number of reviews available on the pharmaceuticals and medicinal bioactive compounds derived from marine algae. This chapter focuses specifically on the culinary use, nutritional value of sea lettuces, and emphasizing their associated health promoting effects. Further, it is important to acknowledge that there are gaps in our knowledge of local names for sea lettuces, which some also lack common names in English. Hence, in this chapter, we present several local names for sea lettuces in several countries.

II. CULINARY USES OF SEA LETTUCES

Sea lettuces are eaten by a number of different sea animals, including manatees or sometimes referred as sea cows and the sea slugs known as sea hares (Carefoot, 1979). Not only consumed by marine fauna, many species of sea lettuces are consumed by humans in Scandinavia, Great Britain, Ireland, China, Korea, and Japan. As a food for humans, sea lettuces are eaten raw in salads, dried, toasted, cooked in soups, as a garnish, etc. Sea lettuces taste better when harvested early in the spring growing season (Misheer et al., 2006).

In Japan, sea lettuces are known as *aosa nori* (Murata and Nakazoe, 2001). The texture of these sea lettuces closely resembles *nori* (*Porphyra* sp.); sea lettuces, however, have milder in flavor and less expensive. It is used in dried form for flavoring some of Japanese foods. Sea lettuces used in Japan in the same way as parsley and lettuces are often employed by American as a garnishment for foods. Commonly, they are used by sprinkling the sea lettuces powder on the hot food to obtain specific aroma. Sea lettuces are widely used as a savory seasoning in processed Japanese foods like fried noodle (*Yakisoba* or *yakiudon*), *okonomiyaki* (Japanese pancake), *takoyaki* (octopus dumpling ball), potato chips, rice cracker, etc. Japan and Korea use sea lettuces as side dishes (known as *banchan*) which help to compliment Korean dishes, by adding special flavors to the meal (Kwak et al., 2005).

Hawaiian name for sea lettuces is *pālahalaha* which means spread out. Hawaiians commonly eat fresh sea lettuces after chopping and sometimes mix with other seaweeds (Novaczek, 2002). In Cuba, sea lettuces were boiled and then drunk as a juice to kill intestinal worms. Sea lettuces have
also been used as worm medicine and folk remedy for gout treatment in Pacific (Novaczek, 2002). Local name for sea lettuces in Philippines is gam gamet-Ilokano; which is the most popular and highly prized sea vegetables in the Ilocandia, Philippines (Novaczek, 2002). In Philippines, the most common food preparation of sea lettuces is salad. Sea lettuces collected from the sea were washed and then added to prepare salad or soup. The translucent, slightly bitter leaves make these seaweeds perfect as salad ingredients. After washing and blanching with lukewarm water, the sea lettuces are mixed with crushed ripe tomatoes, sliced green mango, and sliced onion, complemented with native fermented fish to taste. Similar in Philippines, the most common food preparation of sea lettuces in Indonesia is salad (Istini et al., 1998).

In British Isles, sea lettuces are commonly toasted (Kenicer et al., 2000). To prepare toasted sea lettuces, salt and sesame oil were mixed and rubbed on the sea lettuces. Around six leaves were laid on top of one another and each leaf was cooked separately in a hot pan over low heat until crisp. Laverbread are the most common sea lettuces preparation in Wales (Fitton, 2003). To make laverbread, the sea lettuces are boiled for several hours and then minced or grinded into puree. Laverbread are traditionally eaten fried with bacon and cockles for breakfast. It can also be used to make a sauce to accompany lamb, crab, monkfish, etc. Further, in Scotland, sea lettuces have been eaten for hundreds of years and also particularly popular food (Kenicer et al., 2000; Misheer et al., 2006).

The Japanese, Koreans, Chinese, British, Scottish, Philippines, Indonesians, and Hawaiians consider sea lettuces as valuable source for human food and great delicacy. Further, sea lettuces are also known for their richness in nutrition such as polysaccharides, minerals, vitamins, protein, amino acids, lipids, and fatty acids. This gives sea lettuces great potential as functional and medicinal foods.

III. NUTRITIONAL VALUE OF SEA LETTUCES

A. Polysaccharides

Sea lettuces contain large amount of polysaccharides which constitute around 38–54% of the dry matter. These include four polysaccharide family in sea lettuces; two major ones, the water soluble ulvan and insoluble cellulose, and two minor ones, xyloglucan and glucuronan (Lahaye and Robic, 2007). When faced with the human intestinal bacteria, most of these polysaccharides are not digested by human and hence can be regarded as dietary fiber. Water soluble and insoluble fibers have been associated with different biological activities and health-promoting effects. Soluble polysaccharides are primarily associated with
hypocholesterolemic and hypoglycemic effects (Panlasigui et al., 2003). Further, insoluble fiber such as cellulose are associated with excretion of bile acids, increase fecal bulk, and decrease intestinal transit time (Burtin, 2003; Moore et al., 1998).

Among polysaccharides isolated from sea lettuces, ulvan has attracted greater attention as they display several physiochemical and biological features. The name ulvan actually derived from the terms ulvin and ulvacin which refers to different fractions of Ulva lactuca water soluble polysaccharides. Presently, it is being used to refer to polysaccharides from the members of the Ulvales, mainly sea lettuces. Researchers have revealed that ulvan (Fig. 5.1) exhibited various biological activities such as anticoagulant, antiviral, antioxidant, antiallergic, anticancer, anti-inflammatory, antihyperlipidemia, etc. For example, Qi et al. (2005) have prepared different molecular weight ulvans from Ulva pertusa by sulfur trioxide/N,N-dimethylformamide (SO₃–DMF) in formamide and their antioxidant activities were investigated. The results showed that low molecular weight ulvans had a strong antioxidant activity. The rationale for this is low molecular weight of ulvan may incorporate into the cells more efficiently and donate proton effectively compared to high molecular weight one (Qi et al., 2005). Ulvan may also modulate lipid metabolism in rats and mice. A decrease of serum high-density lipoprotein cholesterol (HDL-cholesterol) and an increase of low-density lipoprotein cholesterol (LDL-cholesterol) and triglyceride are considered to be significant risk factors in cardiovascular diseases. Ulvan or ulvan derived oligosaccharides significantly lowered the level of serum total cholesterol, LDL-cholesterol, and reduced triglyceride, while they increased the levels of serum HDL-cholesterol (Pengzhan et al., 2003). In addition, Mao et al. (2006) found that the anticoagulant activity of ulvan from Ulva conglobata mainly consisted of rhamnose with variable contents of glucose and fucose, fucose, trace amounts of xylose, glucose, and mannose. Anticoagulant activity of ulvan has also been reported from Ulva lactuca (AhdEl-Baky et al., 2009). In comparison, Ulva neumatoidea extracts have higher anticoagulant activity compared to other seaweed species such as Egregia menziesii, Silvetia compressa, and Codium fragile (Guerra-Rivas et al., 2010).

FIGURE 5.1 Chemical structure of the main repeating disaccharides in Ulvan.
These ulvan biological properties open up a wide field of potential applications; food, pharmaceuticals, agricultural, cosmetic, and chemical industry. Some of those potential applications are already the subject of patents.

B. Protein and amino acids

The protein content of sea lettuces varies with the species but generally present in high amounts. For example, protein content in *Ulva reticulata* is 21.06% of the dry weight, whereas higher protein contents are recorded in *Ulva lactuca* 27.2% of the dry weight (Ortiz *et al.*, 2006; Ratana-arporn and Chirapart, 2006). These level are comparable to those found in high-protein terrestial vegetables such as soybeans, in which protein make up 40% of the dry mass (Murata and Nakazoe, 2001).

The general levels of some amino acids in sea lettuces proteins are higher than those found in terrestrial plants. Eight essential amino acids (cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine, and valine) which cannot be synthesized by our body are present in a high level in sea lettuces. The amino acid compositions of sea lettuces are presented in Table 5.1. Several sea lettuces species such as *Ulva lactuca*, *Ulva pertusa*, and *Ulva armoricana* are rich in leucine. Leucine is one of the building blocks for protein, and recent studies reported that a diet rich in the amino acid leucine might help prevent the muscle loss that typically comes with aging (Anthony *et al.*, 2000). French researchers found that a leucine supplemented diet restored a more youthful pattern of muscle-protein breakdown and synthesis to elderly rats. In addition to leucine, other amino acids which found in a high amount in sea lettuces are threonine, arginine, alanine, aspartic acid, and glutamic acid (Fleurence *et al.*, 1999; Fujiwara-Arasaki *et al.*, 1984; Mai *et al.*, 1994; Ratana-arporn and Chirapart, 2006; Wong and Cheung, 2000). The proteins from *Ulva reticulata* and *Ulva armoricana* exhibit an amino acid composition close to that of soybean protein (Fleurence *et al.*, 1999; Ratana-arporn and Chirapart, 2006). Further, *Ulva reticulata* proteins are of high quality since the essential amino acids represented almost 40% of total amino acids (Ratana-arporn and Chirapart, 2006). Bioactive lectins are found in sea lettuces (Sampaio *et al.*, 1998; Wang *et al.*, 2004). However, lectins derived from sea lettuces are relatively recent ones compared to other seaweeds. In human body, lectins are involved in numerous biological processes such as cell–cell communication, induction of apoptosis, host–pathogen interaction, cancer metastasis and differentiation, recognizing and binding carbohydrates, increase the agglutination of blood cells (erythrocytes), detection of disease related alterations of glycan synthesis, including infectious agents such as viruses, etc. (Holdt and Kraan, 2011).
C. Lipids and fatty acids

Lipids are a broad group of naturally occurring molecules which includes fat, waxes, sterols, fat-soluble vitamins, mono-, di-, and tryacylglycerol, diglycerides, phospholipids, etc. The literature has been established that, in general, the lipid content in sea lettuces is less than 4% (Ortiz et al., 2006). Total lipid contents in Ulva lactuca, Ulva reticulata, Ulva fasciata were 1.64, 0.75, 3.6 g/100 g, respectively (McDermid and Stuercke, 2003; Ratana-arpon and Chirapart, 2006; Wong and Cheung, 2000).

Further, sea lettuces show an interesting polyunsaturated fatty acid (PUFA) composition (Table 5.2). There are two major families of dietary PUFA, the (ω6) and (ω3) families. The ω6 PUFA are derived from the parent compound linoleic acid [LA; 18:2(ω6)]. They contain at least two double bonds where the first double bond is located at sixth carbons from the methyl end of the molecule (Whelan and McEntee, 2004). Meanwhile, the ω3 PUFA has the first double bond at the third carbon from the methyl
TABLE 5.2  Fatty acid profiles of some sea lettuces species

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Ulva lactuca&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ulva reticulata&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ulva fasciata&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ulva pertusa&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ulva arasakii&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ulva conglobata&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0 (decanoic acid)</td>
<td>–</td>
<td>–</td>
<td>0.78</td>
<td>0.96</td>
<td>0.29</td>
<td>0.55</td>
</tr>
<tr>
<td>C12:0 (lauric acid)</td>
<td>0.14</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C14:0 (myristic acid)</td>
<td>1.14</td>
<td>–</td>
<td>0.70</td>
<td>0.68</td>
<td>0.44</td>
<td>1.03</td>
</tr>
<tr>
<td>C14:1 (myristoleic acid)</td>
<td>–</td>
<td>–</td>
<td>1.81</td>
<td>2.07</td>
<td>0.87</td>
<td>3.35</td>
</tr>
<tr>
<td>C15:0 (pentadecanoic acid)</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0 (palmitic acid)</td>
<td>14</td>
<td>1.43</td>
<td>29.32</td>
<td>27.36</td>
<td>25.43</td>
<td>34.16</td>
</tr>
<tr>
<td>C16:1 (palmitoleic acid)</td>
<td>0.69</td>
<td>0.32</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:0 (stearic acid)</td>
<td>8.39</td>
<td>0.92</td>
<td>0.91</td>
<td>1.03</td>
<td>–</td>
<td>2.39</td>
</tr>
<tr>
<td>C18:1ω9 (oleic acid)</td>
<td>0.37</td>
<td>0.13</td>
<td>5.12</td>
<td>5.15</td>
<td>7.4</td>
<td>6.31</td>
</tr>
<tr>
<td>C18:2ω6 (linoleic acid)</td>
<td>8.31</td>
<td>0.14</td>
<td>7.87</td>
<td>8.09</td>
<td>21</td>
<td>6.81</td>
</tr>
<tr>
<td>C18:3ω3 (linolenic acid)</td>
<td>4.38</td>
<td>0.19</td>
<td>17.25</td>
<td>22.96</td>
<td>22.98</td>
<td>14.37</td>
</tr>
<tr>
<td>C18:4ω3 (stearidonic acid)</td>
<td>0.41</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C20:0 (arachidate)</td>
<td>0.19</td>
<td>0.11</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C20:1 (eicosanoate)</td>
<td>4.21</td>
<td>0.06</td>
<td>1.98</td>
<td>1.35</td>
<td>0.42</td>
<td>1.77</td>
</tr>
<tr>
<td>C20:4ω6 (arachidonic acid)</td>
<td>0.34</td>
<td>0.04</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C20:5ω3 (eicosapentanoic acid)</td>
<td>1.01</td>
<td>0.03</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C22:0 (Behanate)</td>
<td>0.27</td>
<td>0.03</td>
<td>0.80</td>
<td>0.65</td>
<td>2.87</td>
<td>2.5</td>
</tr>
<tr>
<td>C22:1 (erucate)</td>
<td>0.79</td>
<td>0.003</td>
<td>2.46</td>
<td>2.88</td>
<td>1.42</td>
<td>2.57</td>
</tr>
<tr>
<td>C22:6ω3 (docosahexaenoic acid)</td>
<td>0.8</td>
<td>0.04</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Adapted from: <sup>a</sup>Ortiz <i>et al</i> (2006), <sup>b</sup>Ratana-arpon and Chirapart (2006), <sup>c</sup>Alamsjah <i>et al</i> (2008).
terminus and contain up to six double bonds. Sea lettuces are particularly rich in ω3 fatty acids (Ortiz et al., 2006). Eicosapentanoic acid (EPA; 20:5) and docosahexanoic acid (DHA; 22:6) are the two important fatty acids of sea lettuces, along with the precursor α-linolenic acid (ALA; 18:3). Both EPA and DHA are basically derived from ALA through elongation and desaturation (Alamsjah et al., 2008; Ortiz et al., 2006; Ratana-arporn and Chirapart, 2006). The ω3 fatty acids have been demonstrated to play significant role in human body. In human body, the beneficial effect of ω3 fatty acids can be classified into two main areas. First, these fatty acids sustain normal healthy life through the reduction of blood pressure, plasma triglycerides, and cholesterol, together with increased blood coagulation time. Both EPA and DHA are important for maintenance of normal blood flow as they lower fibrinogen levels and also prevent platelet from sticking each other. Second, they alleviate certain diseases such as blood vessel disorders and inflammatory conditions. Deficiency of ω3 fatty acids causes several disorders such as restrictive growth, abnormality of the skin and hair, damage of reproductive system, and abnormal composition of serum and tissue fatty acids. The human body cannot synthesize ω3 fatty acids de novo; hence to obtain their potential health promoting effects, ω3 fatty acids should be introduced in human diet. One of the potential sources of EPA, DHA, and ALA is sea lettuces. In addition, sea lettuces also contain significant quantities of C18:4ω3 (stearidonic acid) which recently has been demonstrated to possess several biological activities similar to EPA (Whelan, 2009). Ulva lactuca are the best source of EPA and DHA among several sea lettuces species tested in several studies. Meanwhile, Ulva arasakii are a better source of palmitic acid. Sea lettuces are therefore a good source of ω3 fatty acids and also an important source of supply of ω3 fatty acids for the homeostasis and promoting human health.

D. Vitamins

Sea lettuces contain considerable amount of vitamins. These include both water- and fat-soluble vitamin such as vitamin A, B, D, and E (Table 5.3).

1. Vitamin B complex

Sea lettuces are a source of vitamins from group B (MacArtain et al., 2007; McDermid and Stuercke, 2003). For instances, Ulva lactuca contain high amount of cobalamin or vitamin B12. Vitamin B12 plays a key role in homeostasis of the brain and nervous system, and for the formation of blood (Scalabrino, 2009). Daily ingestion of 1.4 g/day of Ulva lactuca will be enough to meet the daily requirements of vitamin B12 (MacArtain et al., 2007). One of the most important vitamins B occurring in Ulva reticulata is riboflavin (vitamin B2). Vitamin B2 deficiency is often endemic in human
populations that subsist on diets poor in dairy products and meat. Vitamin B2 cannot be synthesized by mammals, and there is only limited, short-term storage capacity for this vitamin in the liver. Humans are vulnerable to develop a vitamin B2 deficiency during periods of dietary deprivation or stress, and this may lead to a variety of clinical abnormalities, such as growth retardation, anemia, skin lesions, and degenerative changes in the nervous system. Therefore, this water-soluble vitamin should be present in the diet on a daily basis (Van Herwaarden et al., 2007).

2. Vitamin C
Water-soluble vitamins such as vitamin C are present in large amount in sea lettuces. The levels of vitamin C in sea lettuces average from 500 to 3000 mg/kg of dry matter. These levels of vitamin C are comparable with that of parsley, blackcurrant, and peppers. In sea lettuces, the highest level of vitamin C were found in Ulva fasciata (22 mg/100 g) (McDermid and Stuercke, 2003). Vitamin C is of interest for many reasons. First, it strengthens the immune defense system, activates the intestinal absorption of iron, as a reversible reductant and antioxidant in the aqueous fluid and tissue compartments. Further, this vitamin is specifically required for the activity of eight human enzymes involved in collagen, hormone, amino acid, and carnitine synthesis or metabolism (Jacob and Sotoudeh, 2002).

3. Vitamin E
Burtin (2003) reported that green seaweeds contain low amount of vitamin E (Burtin, 2003). In contrast, Ortiz et al. (2006) demonstrated that Ulva lactuca showed high level of vitamin E. Daily ingestion of 109.5 g/day of

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Ulva lactuca&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Ulva reticulata&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ulva fasciata&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>6050</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B1</td>
<td>0.42</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>B2</td>
<td>0.03</td>
<td>13</td>
<td>0.1</td>
</tr>
<tr>
<td>B3</td>
<td>8</td>
<td>–</td>
<td>6.6</td>
</tr>
<tr>
<td>B9</td>
<td>0.01</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B12</td>
<td>6.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>–</td>
<td>22</td>
</tr>
<tr>
<td>D*</td>
<td>848</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E</td>
<td>13.7</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Adapted from: <sup>a</sup>Briand and Morand (1997), <sup>b</sup>MacArtain et al. (2007), <sup>c</sup>Ratan-arporn and Chirapart (2006), <sup>d</sup>McDermid and Stuercke (2003).
Ulva lactuca consumption will be enough to meet the daily requirements of vitamin E (Briand and Morand, 1997). The determined levels of vitamin E show a good nutritional complement that confirms the importance of sea lettuces in normal diets for human.

E. Minerals

Minerals are inorganic elements that retain their chemical identity in foods. It can be classified into two groups; macro- (calcium, phosphorous, potassium, sulfur, sodium, chloride, and magnesium) and trace minerals.

Sea lettuces which draw from the sea contain wealth of mineral elements (Table 5.4). Calcium, one of the most important minerals essential for human body, is accumulated in sea lettuces at a higher level compared with milk, brown rice, spinach, peanuts, and lentils (MacArtain et al., 2007). Calcium contents in Ulva lactuca, Ulva reticulata, Ulva fasciata were 32.5, 147, and 0.47 mg/100 g edible portion, respectively. Moreover, potassium and sodium are known as electrolytes because their ability to dissociate into positively and negatively charged ions when dissolved in water. Potassium is the major cation of intracellular fluid. Together with sodium, it maintains normal water balance. In addition, potassium also promotes cellular growth and maintains normal blood pressure. Potential source of potassium is Ulva reticulata, which contains 1540 mg potassium per 100 g edible portion (Ratana-arpong and Chirapart, 2006).

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Ulva lactuca&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ulva reticulata&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ulva fasciata&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>32.5</td>
<td>140</td>
<td>0.47</td>
</tr>
<tr>
<td>K</td>
<td>24.5</td>
<td>1540</td>
<td>2.87</td>
</tr>
<tr>
<td>Mg</td>
<td>46.5</td>
<td>140</td>
<td>2.19</td>
</tr>
<tr>
<td>Na</td>
<td>34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cu</td>
<td>0.03</td>
<td>0.6</td>
<td>5</td>
</tr>
<tr>
<td>Fe</td>
<td>1.53</td>
<td>174.8</td>
<td>86</td>
</tr>
<tr>
<td>I</td>
<td>0.16</td>
<td>1.124</td>
<td>–</td>
</tr>
<tr>
<td>Zn</td>
<td>0.09</td>
<td>3.3</td>
<td>9</td>
</tr>
<tr>
<td>N</td>
<td>–</td>
<td>–</td>
<td>3.62</td>
</tr>
<tr>
<td>P</td>
<td>–</td>
<td>180</td>
<td>0.22</td>
</tr>
<tr>
<td>S</td>
<td>–</td>
<td>–</td>
<td>5.24</td>
</tr>
<tr>
<td>B</td>
<td>–</td>
<td>–</td>
<td>77</td>
</tr>
<tr>
<td>Mn</td>
<td>–</td>
<td>48.1</td>
<td>12</td>
</tr>
</tbody>
</table>

Adapted from: <sup>a</sup>MacArtain et al. (2007), <sup>b</sup>Ratana-arpong and Chirapart (2006), <sup>c</sup>McDermid and Stuercke (2003).
Iodine is an important mineral in metabolic regulation and growth patterns. The recommended daily intake of iodine for adults is 150 μg per day. During pregnancy and lactation, an additional dose of 25 and 50 μg per day are recommended. Notably, the iodine deficiency is prevalent worldwide, which may correspond to the worldwide phenomena of brain damage and mental retardation. During pregnancy, infancy and childhood may lead to endemic and irreversible cretinism in infants or children. *Ulva reticulate* and *Ulva lactuca* have been described as a good source of iodine. Hence, considering the high mineral contents, sea lettuces could be used as a food supplement to meet the daily intake of essential minerals.

**IV. CONCLUSIONS**

Sea lettuces are rich in nutrients with medicinal and health-promoting effect. From a nutritional standpoint, the main properties of sea lettuces are their richness in polysaccharides, protein and amino acids, fatty acids, minerals, and vitamins. Therefore, their nutritional value makes them valuable food supplements. Further, sea lettuces may be used to fortify processed foods. Food preparations from sea lettuces worldwide may be studied to increase sea lettuce utilization. Moreover, recognition of sea lettuces as sources of diverse bioactive principles may open medicinal potential of sea lettuces and there is a great potential to be used in pharmaceuticals. Therefore, combination between culinary use and research on bioactive compounds may revitalize the use of sea lettuces in the newly health conscious consumers. Sea lettuce products could be used for food fortification, enrichment, and multipurpose applications.

**REFERENCES**


CHAPTER 6

Marine Algal Sources for Treating Bacterial Diseases

M. L. Arvinda Swamy¹

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Abstract

Microorganisms are the causative agents for various types of diseases in humans, animals, and plants. The invention of antibiotics against the bacterial diseases in the early twentieth century improved the heath conditions of the humans, but it resulted in the development of variable drug/multidrug-resistant strains which are now posing great challenge to cure the diseases. The need for searching novel bioactive compounds having potential therapeutic value resulted in exploration of oceans. Screening diverse fauna and flora in oceans opened new avenues for the development of novel therapeutic agents such as sesquiterpenes, phlorotannins, bromoditerpenes, halogenated furanones, and algal lectin which show effect on a wide range of Gram-negative and positive bacteria. Hence these bioactive compounds can be used as broad spectrum antibiotics, antibacterial, and antifouling agents.

I. INTRODUCTION

The encounter between the humans and the infectious agents is known from the ancient times. There are various types of infectious agents like bacteria, virus, and fungi which cause various types of diseases in humans, and the outcome of the disease symptoms varies from disease causing agents (Nelson and Williams, 2001; Nene, 2007). Humans have produced various types of treatments/remedies for different types of bacterial diseases from ancient times by using variety of practices like Ayurveda depending upon the availability of the natural resources in those countries (Gopal et al., 2008; Kerr and Kerr, 1999).

Irrational use of antibiotics from biological sources or by chemical synthesis for treating different bacterial diseases resulted in the development of the drug/multidrug-resistant strains, posing great challenge to cure the bacterial diseases. Considering novel mechanisms of action, drug resistance, toxicity, production, and cost--effectivity, various researchers from different parts of the globe turned their eyes on the diverse marine fauna and flora having potential therapeutic values. The cutting edge technological developments in science helped the researchers to discover the untapped antimicrobial potentials of marine algal sources in the development of novel antibiotic compounds (Hornsey and Hide, 1974; Morse et al., 1979; Troxler and Lester, 1967).

This chapter is focused on the marine algal sources for treating bacterial diseases, and emphasis would be on marine algae and their extracts/bioactive compounds treating various types of bacterial diseases.
II. MARINE ALGAL SOURCES AS THERAPEUTICS

Marine alga has rich biodiversity potential; can serve various requirements as food, natural sources, bioactive compounds for treatment/remedies for various types of bacterial diseases like tuberculosis, multidrug-resistant bacteria, viral infections like HIV, Herpes viruses, fungal infections, protozoan infection like malaria; and is also helpful against helminthiases, cancer; immunogenic, cardiovascular, neurological, and other diverse mechanisms and functions of these bioactive compounds need to be explored (Bernam et al., 2004; Chang et al., 2003; Fennell et al., 2003; Luescher-matti, 2003; Maskey et al., 2004; Mayer et al., 1999; Venkateshwar Goud et al., 2003; Zhu et al., 2004).

III. TYPES OF MARINE ALGAE USED FOR ANTIBACTERIAL SCREENING

Macroalgae are generally classified into Phaeophyta (brown algae), Rhodophyta (red algae), and chlorophyta (green algae) based on the photosynthetic pigmentation on their cellular composition.

A. Phaeophyta (brown algae)

Brown algae are the largest group of marine algal species found in oceans. The number of identified species is increasing with the large-scale screening and isolation of the novel therapeutic compounds. The exact number of species in the oceans is not known but it is expected to be several thousands, its pigmentation varies from yellow brown to dark brown and produces huge amounts of protective mucous (Ginsburg, 2003; Stegenga et al., 1997).

B. Rhodophyta (red algae)

Rhodophyta, second largest, most primitive group of macroalgae present in more diverse environments, is compared to the other types of marine algae. It produces various types of antibacterial products but the numbers of antibacterial products from these species are low when compared to the brown algae (Ginsburg, 2003; Stegenga et al., 1997).

C. Chlorophyta (green algae)

Chlorophyta (green algae) are present in lower ratios when compared to the Phaeophyta and Rhodophyta. The pigmentation of these species varies from yellowish green to dark green in color. These species are very closely
related to the terrestrial plants, and this algae exhibit few structural and chemical properties which can have an impact in the development of novel antibiotics (Ginsburg et al., 2000; Stegenga et al., 1997). Marine algae are vulnerable to the various types of biological and physiological agents, and in response to these, marine algae produce different types of bioactive secondary metabolites which provide protection from the biological and physical agents (Donia and Hamann, 2003; Haefner, 2003). Few bioactive compounds from the marine algae strongly deter the growth of the surrounding bacteria, and these active compounds have shown their effect on various human pathogens (Vairappan, 2003; König et al., 2000).

IV. BIOACTIVE COMPOUNDS OF MARINE ALGAE

A. Sesquiterpenes

Bioactive sesquiterpenes isolated from red algae species Laurencia rigida; Laurencia luzonesis yielded deschloroelatol, elatol, luzonenone, luzofuran, 3,4-epoxypalisadin, 1,2-dehydro-3,4-epoxypalisadin B, and 15-hydroxypalisadin; and a new diterpene former has shown antibacterial action on Bacillus megaterium and also possess antifungal action (König et al., 2000; Kuniyoshi et al., 2005).

B. Phlorotannins

Crude extracts, purified diverse phlorotannins (phloroglucinol, eckol, phlorofucofuroeckol A, dieckol and 8.8’-beckol) extracted from brown algae, Ecklonia kurome tested on multiresistant Staphylococcus aureus and foodborne pathogens exhibited the antibacterial activity on Gram-positive bacteria, S. aureus, B. Cereus and Gram-negative bacteria C. jejuni, E. coli, S. Enteritidis, S. typhimurium, V. parahaemolyticus (Nagayama et al., 2002). Antibacterial mechanism of action of phlorotannins is not precisely known but one study supports that it may be due to the interaction of phlorotannins with the bacterial proteins and enzymes which will result in the bactericidal action. The toxicity of phlorotannins was evaluated by the studies on the mice and it found to be safe without causing toxicity to the animals, moreover in some parts of Japan, it is consumed as food so that this can be used as food supplement or drug for the treatment (Gopal et al., 2008; Schulz et al., 1992).

C. Bromoditerpenes

Two bromoditerpenes sphaerolabdadiene-3,14-diol and bromosphaerone isolated from the marine red algae Sphaerococcus coronopifolius from the Atlantic ocean, sea coast of Morocco, exhibited antibacterial
action against Gram-positive organism *S. aureus* and antimalarial activity against the chloroquine-resistant plasmodium falciparum; the exact mechanism of bactericidal action is not discovered (Etahiri *et al.*, 2001).

Diethyl ether extracts of seaweeds *Cystoseira mediterranea, Enteromorpha linza, Ulva rigida, Gracilaria gracilis,* and *Ectocarpus siliculosus* are isolated from the Urla coast (Turkey showed effective results against all test organisms such as *Candida* sp., *Enterococcus faecalis, S. aureus, Streptococcus epidermidis, Pseudomonas aeruginosa,* and *Escherichia coli*). Fresh weights of algal extracted using the diethyl ether showed the strong broad spectrum antibiotic activity against the tested bacterial strains; moreover they have shown the more activity against the Gram positive, which was more when compared to the Gram-negative bacteria (Tuney *et al.*, 2006).

### D. Halogenated furanones

Halogenated compounds elatol extracted from the marine red algae *Laurencia majuscule* elatol has shown activity against the human pathogenic bacteria species *Staphylococcus epidermis, Klebsiella pneumoniae,* and *Salmonella,* whereas iso-obtusol shown action on *K. pneumoniae* and *Salmonella* species (Vairappan, 2003). Halogenated furanone isolated from the marine alga *Delisea pulchra* has shown broad spectrum antibiotic action against the bacterial bio film formation, quorum sensing (QS), and swarming, but the molecular mechanism is completely not elucidated. Studies on action of halogenated furanones on biosynthetic pathway AI-2, which is found in most of the Gram-positive and Gram-negative bacteria and will covalently modify the LuxS enzyme (S-ribo-sylhomocysteine lyase, EC 4.4.1.21) which produces autoinducers-2 (AI-2), thereby showing action on bacterial QS; therefore, these furanones can be used for clearing the bacterial films on the ponds and lakes (Zang *et al.*, 2009).

### E. Algal lectins

Lectins are the substances extensively distributed in the plants, animals, and marine algae. They are proteinaceous in nature having the capacity of attaching distinctive carbohydrates for producing unique biological properties like aggregation of erythrocytes, algae, yeast, and bacteria. Study conducted on the marine algal lectins belonging to eight Rhodophyta, three chlorophyta, and two phaeophyta species on four vibrio bacterial species (*V. neresis* and *V. pelagius, Eucheuma serra,* and *Galaxaura marginata*) strongly inhibited *V. vulnificus* (fish pathogen) but were inactive against the other two vibrios. Aqueous Ethanol and saline
extracts of red algae *E. serra* and *Pterocladia capillacea* have shown strong action at lowest concentrations on the *V. Vulnificus*. The strong antimicrobial action of marine algal lectins toward fish pathogenic bacteria *V. Vulnificus* can be used as the antibiotics in the aquacultures (Liao *et al.*, 2003).

**V. MARINE ALGAE AGAINST HUMAN PATHOGENIC DRUG/ MULTIDRUG-RESISTANT STRAINS**

Various workers used different types of solvents like acetone, ethyl alcohol, chloroform, ethyl, methanol, acetic acid, and benzene for the extraction of bioactive compounds from the marine algal sources; method of extraction and solvents play an important role in varying degrees of antibacterial activity against the Gram-positive and Gram-negative bacterial strains (*Rosell and Srivastava, 1987; Sastry and Rao, 1994; Zheng *et al.*, 2001). Screening 151 marine algal species for antibacterial properties on five bacterial species found that the antibiotic production of the algal species varies with the seasons and also varies within the morphological similar species. The changes in the antibiotic production of the several species are also fluctuated due to the seasonal changes (*Hornsey and Hide, 1974*). Marine seaweeds *Ulva fasciata* (green algae) and *Hypnea musciformis* (red algae) isolated from the southeast and southwest coast of India showed antibacterial activities. Green algae *U. Fasciata* exhibited broad spectrum antibiotic inhibited *B. cereus, E. coli, B. Subtilis, A. hydrophila, V. fischeri*, and shrimp pathogen *V. harveyi* at incubation temperatures 20°C and 30°C (*Selvin and Lipton, 2004*).

The methanol extracts of seaweeds isolated from Gulf of Mannar, India, exhibited differential activity on multidrug-resistance bacterial strains *E. coli, K. pneumoniae, P. aeruginosa, and S. Aureus, Padina tetra-stromatica*, of human urinary tract infections. *Stocheospermum marginatum* shown strong action against nonmultidrug-resistance bacterial strains. *Grateloupia lithophila* has shown effect on both multidrug-resistant and nonmultidrug-resistant strains, *Caulerpa sp., Gracilaria corticata*, and *Valoniopsis pachynema* exhibited weak activity (*Manikandan et al., 2011*). Methanolic extracts of 26 marine seaweeds isolated from Morocco, Mediterranean coast are tested against the three Gram-positive *E. faecalis* (ATCC-29212), *E. faecalis* (ATCC-29213) and two Gram-negative bacteria *E. coli, Klebseila, Pneumonia* all the tested seaweeds shown the activity against the bacteria. Few types of seaweed exhibited the strong activity and few have shown moderate activity. The seaweed *H. musciformis* shown the broad spectrum antibiotic activity with zone of inhibition ranged from 10 to 35 mm (*Rhimou et al.*, 2010). Study
conducted on 32 marine algal isolates from Karachi, Pakistan showed the promising results against Gram-positive and Gram-negative bacteria, human, animal, plant pathogens, and common pests. *Codium shamelii* and *Iyengaria stellata* have shown the action against the three Gram-positive bacteria; *Colpomenia sinuosa* on three Gram-negative/positive bacteria; *Cystoseira indica* on two Gram-positive/negative bacteria; *Sargassum ilicifolium* shown action against two Gram-negative bacteria. In Rhodophyta, *Botryocladia leptopoda* has shown action against two Gram-positive and three Gram-negative bacteria, *Champia compressa* has shown action only on two Gram-negative bacteria (Muhammad and Shameel, 2004). Crude methanol extracts of *Saragassum cinereum* (brown algae) antibacterial activity against the *staphylococcus aureus*, *P. aeruginosa*, *Salmonella typhi*, *Streptococcus*, and *Klebsiella* species has shown the high activity against them which indicates the potential use in medical application (Divya *et al.*, 2011).

Several species of marine brown algae *Plocamium telfairiae*, *Gelidium amansii*, *Plocamium sp.*, *P. Hamatum*, *Lessonia nigrescens Bory*, *Sargassum ringgoldianum* have shown antimycobacterial activity (Table 6.1; Collins and Franzblau, 1997; Ikekawa *et al.*, 1968; Kamimoto, 1956; König *et al.*, 1999).

**VI. MARINE ALGAE IN CONTROLLING BIOFILM/ANTIFOULING BACTERIA**

Marine algae are susceptible for various types of disease from the surrounding bacteria; in order to protect from these, they developed the defense mechanisms to combat the bacterial diseases by producing the various secondary metabolites like halogenated furanones, these secondary metabolites are generally found on the surface of the algae. *Delisea pulchra* (red algae) has shown the antifouling action (Dworjanyn and Steinberg, 1999; Givskov *et al.*, 1996). However, *Bonnemaisonia hamifera* (macroalgae) also shown the greater antifouling action against nine diverse strains from five varied bacterial groups (Nylund *et al.*, 2005; Smyrniotopoulos *et al.*, 2003). The compounds isolated from the green algae *Caulerpa prolifera* have shown the antifouling activity against antifouling bacteria and microalgae *Phaeodactylum tricornutum* (Smyrniotopoulos *et al.*, 2003). Antifouling action of the halogenated furanones can be used for treating the aquatic systems. However, the higher concentrations ranging from 1 to 50 μM are toxic to various higher organisms like rainbow trout and artemia (Defoirdt *et al.*, 2004; Rasch *et al.*, 2004, 2007). The exact mechanism of action of these algal metabolites is inexplicable (Table 6.2).
<table>
<thead>
<tr>
<th>Source</th>
<th>Bioactive compounds</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Laurencia rigida</em></td>
<td>Sesquiterpenes</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>Ecklonia Kurome</em></td>
<td>Phlorotannins</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>Sphaerococcus coronopifolius</em></td>
<td>Bromoditerpenes</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>Laurencia majuscula</em></td>
<td>Halogenated compounds</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>Ulva fasciata</em></td>
<td>Methanol extract</td>
<td>Broad spectrum</td>
</tr>
<tr>
<td><em>Cladophora glomerata</em></td>
<td>Methanol extract</td>
<td>Multidrug resistant bacteria</td>
</tr>
<tr>
<td><em>Hypnea musciformis</em></td>
<td>Methanol extract</td>
<td>Broad spectrum</td>
</tr>
<tr>
<td><em>Codium shameelii</em> (chlorophyta)</td>
<td>Methanol extract</td>
<td>Gram positive</td>
</tr>
<tr>
<td><em>Iyengaria stellata</em> (Phaeophyta)</td>
<td>Methanol extract</td>
<td>Gram positive</td>
</tr>
<tr>
<td><em>Sargassum ilicifolium</em> (brown algae)</td>
<td>Methanol extract</td>
<td>Gram negative</td>
</tr>
<tr>
<td><em>Botryocladia leptopoda</em> (Rhodophyta)</td>
<td>Methanol extract</td>
<td>Broad spectrum</td>
</tr>
<tr>
<td><em>Ecklonia Kurome</em></td>
<td>Phloroglucinol, eckol, phlorofucofuroeckol A, dieckol and 8.8'-beckol</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>Laurencia majuscula</em></td>
<td>Elatol, iso-obtusol</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>Grateloupia lithophila</em></td>
<td>Methanol extracts</td>
<td>Multidrug resistance/ nonmultidrug resistance</td>
</tr>
<tr>
<td><em>Cystoseira mediterranea, Enteromorpha linza, Ulva rigida, Gracilaria gracilis</em></td>
<td>Diethyl ether</td>
<td>Broad spectrum antibacterial activity</td>
</tr>
<tr>
<td><em>Plocamium telfairae, Gelidium amansii, and G. Capillaries</em></td>
<td>Organic extracts</td>
<td>Antimycobacterial</td>
</tr>
<tr>
<td><em>Plocamium sp., P. hamatum</em></td>
<td>Monoterpenes</td>
<td>Antimycobacterial</td>
</tr>
<tr>
<td><em>Lessonia nigrescens Bory</em></td>
<td>Phytosterol saringosterol</td>
<td>Antimycobacterial</td>
</tr>
<tr>
<td><em>Sargassum ringgoldianum</em></td>
<td>Saringosterol</td>
<td>Antimycobacterial</td>
</tr>
</tbody>
</table>
VII. ALGAL SOURCES FOR TREATING FISH BACTERIAL DISEASE

Commercial aquaculture production is badly affected by several bacterial and viral infections (Muroga, 2001). Antibiotics are frequently used for treating the bacterial diseases, which not only pose risk of developing drug resistance against these antibiotics but also transfer of the drug-resistance genes to the human pathogenic bacteria which may have huge impact on health of human beings (Guglielmetti et al., 2009; Verschuere et al., 2000).

Use of chemical disinfectants on bacterial diseases in aquaculture has shown toxic effects on environment and to higher animals (Planas and Cunha, 1999; Subasinghe et al., 2000). The search for the novel mechanisms and therapeutics from the oceans has begun to cure the bacterial diseases. QS is one of the universal mechanism by which the bacterial cells communicate with each other depending upon the presence or absence of the signaling molecules in the surrounding environment. Aquatic organisms like microalgae, macroalgae, and invertebrates have the potential mechanism to disturb the QS mechanism by degradation of signals through enzymatic or chemical inactivation against the antagonist organism, and this action varies from organism to organism. This mode of action of marine algae can be useful in controlling the pathogenic bacteria (Defoirdt et al., 2004; Schauder and Bassler, 2001; Wang et al., 2008; Waters and Bassler, 2005).

QS using the microalgae is not completely understood, but this method of curing bacterial diseases is practiced; this technique is also known as green water technique, which was observed in the freshwater species (Palmer et al., 2007). Green water technique is used for controlling the bacterial infections in the aquafarming which makes use of the addition of diverse algal species mixture to the aquafarming. The exact positive mechanism of action of these algae is not well reported but it may be due to involvement of combined factors like nutritive values, stimulatory

<table>
<thead>
<tr>
<th>Source</th>
<th>Bioactive compounds</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delisea pulchra</td>
<td>Halogenated furanones</td>
<td>Antifouling</td>
</tr>
<tr>
<td>(red algae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caulepra prolifera</td>
<td>Sesquiterpenoids</td>
<td>Antifouling</td>
</tr>
<tr>
<td>(green algae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bonnemaisonia hamifera</td>
<td>Halogenated furanones</td>
<td>Antifouling</td>
</tr>
<tr>
<td>(macroalgae)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 6.3 Antibacterial activity of marine algae against fish bacterial diseases

<table>
<thead>
<tr>
<th>Source</th>
<th>Bioactive compounds</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cladophora glomerata</em></td>
<td>Methanol extract</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>E. serra</em></td>
<td>Lectins</td>
<td>Antibacterial</td>
</tr>
<tr>
<td><em>Pterocladiad capillacea</em></td>
<td>Lectins</td>
<td>Antibacterial</td>
</tr>
</tbody>
</table>

Yuvaraj et al. (2011) studied antibacterial effects of *Cladophora glomerata* (green seaweed) of crude and purified extract on multidrug-resistant bacteria *Acinetobacter baumannii*, a human pathogen and the various fish pathogens like *Vibrio fischeri*, *V. Vulnificus*, *V. Anguillarum*, *V. parahaemolyticus*, *E. coli*, and *B. Cereus*. Second, third, and fifth fractions of *C. glomerata* exhibited good antibacterial activity against fish pathogens *V. fischeri*, *V. Vulnificus* and human pathogen *A. baumannii*, respectively. The result presumes that the long-chain hydrocarbons may act as potential bioactive substance and can be exploited in pharmaceutical preparations (Yuvaraj et al., 2011). Lectins isolated from the red algae are effective in controlling the fish pathogenic bacteria *V. Vulnificus* (Liao et al., 2003) (Table 6.3).

VIII. CONCLUSION

For thousands of years, man was dependent upon the nature for various purposes and he used nature’s ability for the treatment and remedies against different human diseases. The latest improvements in the science and technology explored the untapped potentials of marine resources. The marine algal bioactive sources from *Phaeophyta*, *Rhodophyta*, and *Chlorophyta* will provide potential drugs for treating drug/multidrug-resistant bacterial diseases of human as well as aquaculture can have profound effect on the health and economic status of nations.

ACKNOWLEDGMENTS

The author thanks Ira Bhatnagar for encouraging and providing the information for the improvement of this chapter.
REFERENCES


CHAPTER 7

Physical, Chemical, and Biological Properties of Wonder Kelp—*Laminaria*

**Se-Kwon Kim***,†,1 and **Ira Bhatnagar***,‡

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**Abstract**

*Laminaria* is a kelp that finds its place in the brown algae family. It has been an area of study for past many years, and its wonderful biological properties have always attracted medical professionals and researchers to explore more and more from this wonder kelp. The constituents of *Laminaria* include iodine, potassium, magnesium, calcium and iron. Iodine compounds, TEA-hydroiodide in particular, are great lipolytic agents as they stimulate lipase activity. Laminarins on the other hand are used as a tumor angiogenic blocker.

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This genus of the kelps is also rich in algin, a high molecular weight polysaccharide that forms viscous colloidal solutions or gels in water leading to the use of kelp derivatives as bulk laxatives. It has great applications in cosmeceutical science, as well as some antibacterial properties have also been assigned to Laminaria. A deeper insight into the physical, biological, and chemical properties of this wonder kelp would lead to further exploitation of Laminaria for medicinal and cosmeceutical purpose.

I. INTRODUCTION

Our ecosystem is unique in its biodiversity. The most complex are the underwater ecosystems with ample of creatures and species, many of which are not yet discovered. We are unaware of many biological properties that may be buried deep in the oceans. Kelps are one such species which remain deep rooted in the marine environment. There are many varieties of kelps in underwater ecosystems that vary in size, shape and color. They are a major keystone species in this ecosystem, and without them, many organisms would die. They also possess ample of nutrients and minerals which make them highly bioactive. Anticoagulants, antibiotics, antiparasites, antihypertensives, reducers of blood cholesterol, dilatory drugs and insecticides are made with the help of such properties.

Sea kelp that grows on the bottom of the sea is especially rich in nutrients that are good for the human body. Beta carotene, fiber, chlorophyll, protein, enzymes, and amino acids are found in this ancient fruit of the sea. It contains a complex of sodium, potassium, iron, calcium, magnesium, phosphorus, and other minerals. The nutrients in sea kelp make for a perfect dietary recommendation (www.articleclick.com). Scientists believe that this ancient vegetable from the sea consists of many elements that are no longer present in the terrestrial soil on which we grow our vegetables. As a matter of fact, our body resembles in composition with the water found on deep levels of the sea and we share approximately 56 components in deep sea water that also circulate through our bodies. Thus, in sea kelp, the chemical composition resembles that of human plasma and consuming it contributes to regulate our internal balance.

In this chapter, we would like to deal with the physical, chemical, and biological properties of kelp taking Laminaria as the keystone species. We will try to explain the secret of this sea kelp. We would discuss the geographical distribution, life cycle, chemical composition, as well as the array of biological activity that Laminaria displays, may it be anticancer, antioxidant, antiviral, immunomodulatory or antihypertensive.
II. PHYSICAL PROPERTIES

Physically the kelps appear to be plants. The main body consists of a holdfast resembling roots that attaches to a substratum. While they remain attached, the body of the kelp remains floating and appears to be dancing on the tunes of ocean currents. The short branches like stipes resemble a stem whereas its blades appear to be the leaves. Kelp blades reach to the available light near the surface of the ocean through floats or pneumocystis which are gas-filled compartments otherwise known as gas bladders. *Laminaria japonica* is a common species of kelp that inhabits temperate and cold waters in the northern hemisphere and temperate waters in the southern hemisphere. It is known in China as “Kun Bu” and in Japan as “Kombu” (Fig. 7.1).

Genomic information has led to increased understanding of this group, and kelps are no longer considered as plants. Although structurally similar, kelps are functionally different from plants in certain aspects such as the use of root-like holdfast which only serves as an anchor and has no role in securing nutrition. Moreover, the task of mineral absorption is accomplished with the help of blades together with photosynthesis. Further, kelps use chlorophyll a during photosynthesis which appears to be a plant like feature. However, it also uses chlorophyll c, only present in chromists. A possible explanation for the use of chlorophyll c may be its

![Generalized structure of *Laminaria* sp.](image)

*FIGURE 7.1 Generalized structure of *Laminaria* sp.*
basis of fucoxanthin, a pigment that is most efficient in utilizing the blue-green light that penetrates the ocean. And that is one good reason for the brown coloration of kelp. Presence of fucoxanthin adds to the biological properties of kelps (discussed later in this chapter) as it is medically interesting and is under investigation for anticancer and anti-inflammatory properties.

III. LIFE CYCLE OF LAMINARIA SP.

The life cycles of many of the kelps are well characterized and can be controlled by environmental factors, and some have been used for significant molecular analyses (Billot et al., 1998; Crepineau et al., 2000; Yoon et al., 2001). The order Laminariales is characterized by individuals with a heteromorphic alternation of generations, comprising two free-living life phases, a large diploid sporophyte and a microscopic haploid gametophyte generation (www.geol.utas.edu.au). Laminaria sp. consists of sori which are regions of epidermis along the length of the blade. The importance of these sori lies in the unilocular sporangia found inside them. These unilocular sporangia are in turn intermingled with sterile paraphyses that are filamentous structures found packed between sporangia or gametangia. In Laminaria sp., the unilocular meiosporangia produce 32 haploid meiospores. These meiospores produce the small filamentous gametophyte generation after settling to the substrate. The male gametophytes produce the motile, biflagellate sperm whereas the female gametophytes transform into elongated oogonia containing eggs. A single egg is released from the oogonium prior to fertilization. After fertilization, the zygote germinates to form a flat proembryo that subsequently differentiates into the mature sporophyte (Fig. 7.2).

IV. CHEMICAL PROPERTIES

Unlike plants where the main storage compound is starch, chromists store laminarin (hence the name Laminaria) in the form of chains of mannitol and glucose, and the organelles used for the storage are pyrenoids. Apart from the laminarin in pyrenoids, the cell walls of chromists contain algin, a gum used as a thickener and emulsifier in the food and cosmetics industry (www.biology-online.org). East Asian cultures have traditionally exploited kelp for food and medicinal values. On an average, 100 g of the dry kelp contains 17.1–32.0 g alginic acid, 8.46–28.48 g mannitol, 5.97–18.99 g crude protein, and 19.35–45.29 g ash, including 0.13–0.69 g iodine and 4.35–12.65 g potassium, yielding 262 kcal. Chemical analysis of the kelp shows that it may be regarded
FIGURE 7.2  Life cycle of *Laminaria* sp.
as a “health food,” especially desirable in the winter season in the north when green vegetables are comparatively scarce.

Owing to the balanced nature of the qualitative and quantitative composition of biologically active substances [amino acids, polyunsaturated fatty acid, alginate, vitamins (A, C, D, B1, B2, B3, B6, B12, E, K, PP), macronutrients and micronutrients (K, Na, Ca, Mg, I, S, Si, etc.)], *Laminaria*, the giant wonder kelp, is highly effective to ensure normal functioning of the body and aids in treating certain diseases. Minerals mainly present are water-soluble salts of potassium and sodium (chlorides, sulfates). It also possesses relatively large amounts of calcium. The cumulative effect of these substances in varied combinations provides a high therapeutic effect. Most of the medicinal properties of *Laminaria* are due to the presence of alginic acid. Content of alginic acid in algae ranges from 11 to 60%. This alginic acid is similar to pectin in its function that is present in the berries, fruits, and vegetables. Apart from the above-mentioned elements, *Laminaria* is also found to possess copper, antimony, lead, gold, chrome, etc.

The medicinal properties of *Laminaria* depend upon the content and qualitative composition of proteins and carbohydrates. Certain other properties that aid to its therapeutic use are the ability to absorb large amounts of water and increase in volume at the same time and the higher contents of various macro- and micronutrients as compared to the terrestrial plants. Also, possession of colloidal polymer contents such as agar, alginic acid, and others as well as mannitol, specific to the marine vegetation makes *Laminaria* a very potential medicinal food.

As compared to other marine species, *Laminaria* has a greater ability to extract minerals from the sea water and hence accumulate a lot of elements, for example, 9–10-fold higher magnesium content, 17-fold higher sulfur, and 13 times higher bromine. Above all, 1 kg of kelp contains as much iodine as it is dissolved in 100,000 l of seawater. On the whole, the amount of iodine in kelp is several thousand times greater than in the terrestrial flora. Also, the boron in *Laminaria* is 90 times greater than in oats and four to five times higher than in the potato and beet. It contains an average of 0.43% phosphorus, whereas in the dried potatoes and dried carrots, it is almost half.

*Laminaria* accumulates not only large amount of various micro- and macronutrients but also many vitamins. Laboratory studies show that kelp contains a number of pro-vitamin A, which corresponds to its content in common fruits: apples, plums, cherries, oranges. As far as the content of vitamin B1 is concerned, kelp is no more inferior to the dry yeast. Reports suggest that about 100 g of dried *Laminaria* contains up to 10 μg of vitamin B12. *Laminaria* is considered of great interest as food ingredient, as it contains a fairly large amount of vitamin C (100 g of dry kelp contains approximately 15–240 mg). This content stands hand in hand with other citrus fruits of terrestrial origin such as orange,
pineapple, strawberries, gooseberries, green onions, and sorrel. In addition to the above vitamins D, K, and PP (nicotinic acid), pantothenic and folic acids are also found in *Laminaria* (www.authspot.com).

**V. BIOLOGICAL PROPERTIES (MEDICINAL APPLICATIONS OF LAMINARIA)**

*Laminaria* possesses remarkable properties that make it a wonderful component of dietary supplements. Due to its amazing medicinal benefits, it has been used in traditional Chinese medicine since centuries and is listed in the Chinese pharmacopeia. In general, *Laminaria* is considered as a cold, salty herb, prescribed to cleanse heat, resolve phlegm, and soften and disperse hard accumulations (e.g., goiters). It is often used to control edema, as an expectorant and antitussive, and as a remedy for testicular pain and swelling. Despite its salty character, the herb is slightly hypotensive.

As discussed under the chemical properties, a dietary supplement, *Laminaria*, is rich in several constituents that can be very beneficial to the health. Its high iodine content proves to be a great natural boon for the thyroid gland to prevent goiter. Apart from its high calcium, potassium, magnesium, iron, and trace minerals such as manganese, copper, selenium, and zinc, it provides vitamins B1 and B2 as well as chromium which is instrumental in blood sugar control and helps to keep diabetes in check. In Europe, *Laminaria* is basically harvested for a main source of alginate in the food industry, an emulsifying and binding agent used in the production of many foods like ice cream, toothpaste, cereals, and cosmetics as well as in paints and films (www.montereybayaquarium.org).

Dietary use of *Laminaria* dates back to the days of the First World War when it was used in raw form as a feed supplement for horses. It is used as a food, principally in Asian countries, where it is valued for its flavor, mineral content, and health giving benefits. The most important components with a medicinal point of view are *Laminaria*’s polysaccharides. It contains alginates, laminarin, laminine, and fucoidan as well as a number of other polysaccharides and simple sugars.

**A. Alginate**

The alginates are binding agents that absorb toxic heavy metals and radioactive isotopes from the body by binding with them in the gastrointestinal tract when they are present in the bile. It operates as a pump, pumping out heavy metals and other toxins. It is suggested that if *L. japonica* is consumed on a regular basis for at least 4 months, levels of dangerous metals like mercury, lead, and aluminum can be significantly reduced. Alginate also prevents a person from harmful impact of
radiation emitted by TV-screens, computers, microwave ovens, and cellular phones. *Laminaria* alginate has been used with great success in treating radiation sickness in the victims of the Chernobyl, Russia disaster via this mechanism.

**B. Fucoidan**

It is a sulfated fucopolysaccharide and is the subject of extensive research for its anticancer properties. Studies have shown fucoidan to be effective in stopping the growth of tumors, inducing cancer cell apoptosis (programmed cell death) in leukemia, stomach, and colon cancer lines, and in interfering with metastasis by inhibiting interaction between tumor cells and the host tissue basement membrane. Fucoidan from *Laminaria angustata* is composed mainly of fucose/galactose/sulfate (9/1/9) (Kitamura *et al.*, 1991). Fucoidan of *L. japonica* has anti-RNA and DNA virus functions. The antivirus effects of fucoidan on infection due to poliovirus III, adenovirus III, ECHO6 virus, Coxsackie B3 virus, and Coxsackie A16 are remarkable. Fucoidan can inhibit the development of cytopathic effect and protect cultural cells from infection caused by above viruses (Li *et al.*, 1995). Lu *et al.* (2007) added a “novel mechanistic profiling” of the previously reported sulfated polymannuroguluronate, a polysaccharide with an average molecular weight of 8.0 kDa isolated from the brown alga *L. japonica*, that has been reported to be in Phase II clinical trials in China as an anti-AIDS drug candidate (Mayer *et al.*, 2011). Fucoidan prevented concanavalin A-induced liver injury by mediating the endogenous interleukin (IL)-10 production and the inhibition of proinflammatory cytokine in mice (Saito *et al.*, 2006). The dietary fiber in edible brown seaweeds (*Laminaria* *sp.*, *Sargassum fulvellum*, and *Eisenia bicyclis*) had the repressive effect against p-galactosamine (p-GalN)-induced hepatopathy, and the protective effect was caused at least in part by fucoidan (Kawano *et al.*, 2007a,b). Fucoidans from brown algae of Laminariale order have been also described as inhibitors of the complement (Zvyagintseva *et al.*, 2000).

Antitumor activity of many polysaccharides has been reported in recent years. Fucoidan from *L. japonica* is effective against sarcoma 180 (Song *et al.*, 2000). It can inhibit hepatoma QGY7703 cells into logarithmic phase in vitro, accordingly restraining the growth of tumor (Shi *et al.*, 2000). It can also restore the immune functions of immunosuppressed mice, and it acts as immunomodulator acting directly on macrophage and T lymphocyte (Wang *et al.*, 1994). It can also promote the recovery of immunologic function in irradiated rats through a mechanism associated with the arrest of lymphocyte apoptosis by fucoidan (Wu *et al.*, 2003, 2004). Japanese scientists found out that the lowest level of cancer in Japan was reported among population of Okinawa Island. They revealed
that Okinawa residents consume raw *Laminaria*, which contains great amount of fucoidan.

Lots of studies show that fucoidan presents significant antioxidant activity in experiments *in vitro*. It is an excellent natural antioxidant and has great potential for preventing free radical-mediated diseases. Fucoidan from *L. japonica* can prevent the increase of lipid peroxide in serum, liver, and spleen of diabetic mice obviously. However, no inhibition effect was found on both spontaneous lipid peroxidation of homogenates and that induced by Cys/FeSO₄ *in vitro* (Li *et al.*, 2002). This fucoidan had strong scavenging effect on superoxide radical, its effect on hydroxyl radical was weak, and it had less influence on 1,1-diphenyl-2-picryl-hydrazyl. It inhibited H₂O₂-induced hemolysis of rat erythrocytes effectively and showed significant protective effect on lipid peroxidation of liver homogenate in rat induced by FeSO₄-ascorbic acid (Zhang *et al.*, 2003).

Antioxidant activity relates to the molecular weight and sulfate content of fucoidan. Fucoidan fractions from *L. japonica* had excellent scavenging capacities on superoxide radical and hypochlorous acid, except the highly sulfated fraction L-B. In LDL oxidation system, low molecular weight fractions L-A and L-B exhibited great inhibitory effects on LDL oxidation induced by Cu²⁺; however, F-A and F-B had little inhibitory effects in this system due to their large molecular weights (Zhao *et al.*, 2005). Yoon *et al.* (2007) reported the purification of a complex and heterogeneous sulfated fucan from the brown alga *Laminaria cichorioides*. The purified polysaccharide had potent anticoagulant activity which is resulted from enhancement of thrombin inhibition by heparin cofactor II, within the same concentration range as the clinically used heparin (Yoon *et al.*, 2007).

Both molecular mass and sulfate content of fucoidan played very important roles in the effects on the azo radicals 2-2’-azobis (2-amidino-propane)dihydrochloride-induced LDL oxidation (Li *et al.*, 2006). The correlation between the sulfate content and scavenging superoxide radical ability was positive, the ratio of sulfate content/fucose was an effective indicator to antioxidant activity of the samples (Wang *et al.*, 2008).

Fucoidan is a kind of active material similar to sialic acid; it can enhance the negative charges of cell surface so as to effect the aggradation of cholesterol in blood, as a result of decreasing the cholesterol in serum. Fucoidan of *L. japonica* remarkably decreased total cholesterol, triglyceride, and LDL-C; increased HDL-C in serum of mice with hypercholesterolemia and rats with hyperlipidemia; and efficiently prevented the formation of experimental hypercholesterolemia in mice (Li *et al.*, 1999a,b, 2001). It can also remarkably reduce the contents of cholesterol and triglyceride in serum of patients with hyperlipidemia, without side effect of damaging liver and kidney (Wang and Bi, 1994). Low molecular weight sulfated fucan (average $M_w = 8000$ Da) prepared from *L. japonica* can distinctly reduce blood lipids of hyperlipidemic rats (Li *et al.*, 1999a,
b). Fucoidan oligosaccharides show good antihypertensive effects on renovascular hypertensive rats, and one of the mechanisms underlying the antihypertensive effects might be that they can inhibit the production of plasma angiotensin II (Fu et al., 2004).

The elevated urinary protein excretion and plasma creatinine due to the induction of Heymann nephritis were significantly reduced by fucoidan from *L. japonica* by oral intubation. This indicated that fucoidan has a renoprotective effect on active Heymann nephritis and is a promising therapeutic agent for nephritis (Zhang et al., 2005).

C. Laminarin

Another constituent has been found to assist with this process via a tumor angiogenesis blocking mechanism. Last but not least, *L. japonica* is great for the hair, skin, and nails, taken either internally or applied topically in masks and creams. Because of its high mineral content and polysaccharides, the seaweed helps by adding important nutrients to the skin, and by removing toxins. In its extract form, this seaweed can be easily incorporated into a range of skin care products to help give the skin a silky smoothness.

D. Cardiolam

Cardiolam is a commercial product made of *Laminaria* extract. It is a complex supplement for preventive health care with a profound therapeutic effect for those who are subjected to high blood pressure and various cardiovascular diseases. Besides, phytocompounds of this product ensure more efficient fat exchange by lowering cholesterol and sugar rates in the blood. It activates metabolic process in myocardium, improves feeding of cardiac muscle. A powerful antioxidant, it normalizes blood pressure, cholesterol, and blood sugar rates. It increases body resistibility to any physical and psychoemotional stresses (www.seabioresources.com).

VI. CONCLUDING REMARKS

As discussed in this chapter, kelps are indispensable species of deep oceans which forms the basis of an undersea ecosystem that provides food, shelter, and protection for a variety of marine organisms including plankton, sea urchins, mussels, fish, and sea otters. But owing to the danger caused to the *Laminaria* species because of environmental pollution and excessive harvesting for dietary intake, this species is under threat of extinction. Another factor effecting the growth and proliferation of *Laminaria* is global warming as it encroaches upon the cold water
habitat of the kelp. After understanding and unraveling the great bioactive potential of this medicinal kelp, we cannot afford to loose this species. The loss of Laminaria would be great loss to mankind. An analysis of the components of the algae showed that sea kelp is rich in vitamins and minerals, more than any other vegetable known by human (www.biology-online.org). Not only this, protecting this wonder kelp is further more important because of the economic potential and also because a kelp forest is one of the undersea wonders of the world. Quick measures such as that of diver quarantine have to be implemented, and efforts should be made for the proliferation of Laminaria sp. worldwide.

REFERENCES


Medicinal Effects of Phlorotannins from Marine Brown Algae

Se-Kwon Kim*,†,1 and S. W. A. Himaya*

Abstract

Brown seaweeds are popular and abundant food in East Asia and also well known for their medicinal effects due to presence of active phenolic constituents. Phlorotannins, the major phenolic group of brown algae, have extensively investigated for their vast array of bioactivities such as antioxidant, anti-inflammatory, anti-cancer, and antidiabetic. They possess promising activity in both in vitro and in vivo systems showing promising potential to further develop as therapeutic agents. In this chapter, attempts have taken to examine and categorize the reports available on active phlorotannins which have shown strong bioactivities.
I. INTRODUCTION

The philosophy of Hypocrites “let food be thy medicine and medicine be thy food” is a promising approach for prevention or minimization of the increasing incidents of chronic diseases in the world today. There are vast numbers of research going on for inventing and identifying ingredients which are to be used as medicinal foods specifically reducing the risk of specific diseases. Therefore, development and characterization of medicinal food ingredients are among hot topics in the food industry. However, FDA (Food and drug administration) has strong legislation regarding this aspect. FDA has defined medicinal foods in section 5(b) of the orphan drug act and it states as “A food which is formulated to be consumed or administered entirely under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.” In the search of medicinal foods, increasing attention has given to marine-based sources. Among them, marine algae are under the limelight, as they are intensively used as a food ingredient, mainly in East Asia. However, their value as a food found to be far more beyond than a wrap of sushi due to low content of lipids, high concentration of polysaccharides, rich in minerals, polyunsaturated fatty acids and vitamins, and presence of vast array of bioactive metabolites (Gupta and Abu-Ghannam, 2011). Intensive efforts are being made by marine scientists to identify and characterize these compounds to exploit them as medicinal ingredients. Despite having found large number of compounds from marine algae with medicinal properties, few of those compounds have shown real potency to be used as a nutraceutical or pharmaceutical. Among them, phlorotannins are the most significant group of biologically active substances that determine the pharmacological value of algae. The phlorotannin content of the brown algae (Phaeophyceae) is found to be highest over red (Rhodophyta) and green algae (Chlorophyta) (Holdt and Kraan, 2011). These brown algal phlorotannins have been extensively characterized for their potential biological activities. Hence, this chapter has focused on discussing the medicinal potential of phlorotannins isolated from marine brown algae.

II. PHLOROTANNINS

Polyphenolic secondary metabolites are a large and diverse group of chemical compounds which exist both in terrestrial and aquatic plants. Polyphenols from terrestrial plants are derived from gallic and ellagic acids, whereas the algal polyphenols are derived from polymerized
phloroglucinol units (Fig. 8.1). These algal polyphenols are termed as phlorotannins and they are biosynthesized via acetate malonate pathway (Arnold and Targett, 2002). The monomeric units are linked through aryl-aryl bonds, and diaryl ether bonds are forming different subgroups of phlorotannins (Glombitza and Pauli, 2003). Their molecular size ranges between 162 Da (phloroglucinol) and 650 kDa (Breton et al., 2011).

In brown algae (Phaeophyceae), the only group of tannins present is phlorotannins and may constitute up to 15% of the dry weight (Arnold and Targett, 2002). The phlorotannins are localized in physodes of the algae which are membrane-bound cytoplasmic vesicles, and the fusion of physodes with cell membranes results in a secretion of phlorotannins (Li et al., 2009). The highest levels of phenolic compounds in brown algae are found either in meristic or in reproductive regions of the thallus (Breton et al., 2011). The phlorotannin’s concentration exhibits seasonal variations and has reported that highest polyphenolic content can be obtained in summer (Connan et al., 2004). Many brown algal species are popular food mainly in East Asia, and the presence of phlorotannins may affect the palatability due to their astringent taste. And also medicinal values of the brown algae are also related to the presence of phlorotannins. Medicinal values of phlorotannins are related to their structure and especially to the degree of polymerization, where oligo-phenols generally considered more active than highly polymerized compounds (Toth and Pavia, 2001).

III. MEDICINAL EFFECTS OF PHLOROTANNINS

Brown algal phlorotannins have been extensively studied for their potential health benefits and reportedly they have shown promising effects against radical-mediated oxidative stress, photodamage, cancer, allergy, diabetes, inflammation, and viral and microbial infections. Having vast range of biological activities, phlorotannins are believed to be the most promising candidates to be developed as nutraceuticals and pharmaceuticals. This section is covering up the major biological activities of phlorotannins isolated from brown algae.

A. Antioxidant effects

Oxidative stress is a primary cause for development of various human chronic diseases such as cardiovascular disease, cancers, and neurodegenerative diseases. Therefore, wide range of potential metabolites has been evaluated against oxygen-induced damage and hence lowers the risk of human chronic diseases. Among them, brown algae serve as an important bioresource of antioxidative phlorotannins with significant
Phloroglucinol

Eckol

Dieckol

6,6-Dieckol

Fucodiphloroethol G

7-Phloroeckol
pharmaceutical potential. Algae as intertidal organisms require an endogenous antioxidant capacity to withstand UV irradiation and the effects of desiccation from daily tidal fluctuations (Yuan and Walsh, 2006). In brown algae, this antioxidant protection encompasses mainly by

**FIGURE 8.1** Structures of phlorotannins isolated from marine brown algae.
phlorotannins (Yan et al., 1996). Therefore, they have received the greatest attention and have been investigated extensively since they are high free radical scavengers in nature and less toxic than synthetic antioxidants such as BHA and BHT (Jung et al., 2008).

1. Free radical scavenging ability

Unregulated production of free radicals in the cellular systems is responsible in causing cellular damage by oxidizing macromolecules, DNA, proteins, and lipids in the cell. Therefore, antioxidative therapeutics have great demand to act against free radicals. In this aspect, phlorotannins serve as one of the most promising natural antioxidants. Numbers of studies have shown the radical scavenging potential of phlorotannins isolated from marine brown algae. These phlorotannins have shown significant scavenging ability toward hydroxyl, superoxide, alkyl, and DPPH radicals. As shown in Table 8.1, most of the phlorotannin compounds have shown more potent antioxidant activities than commercially available antioxidants α-tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT). And also phlorotannins are investigated for their ability in scavenging reactive oxygen species (ROS) in cellular systems. Diphlorethohydroxycarmalol and 6,6-bieckol isolated from Ishige okamurae (Zou et al., 2008); fucodiphloroethol G and dieckol isolated from Ecklonia cava (Li et al., 2009) have shown of 77.2%, 78.9%, 75.6%, and 84.3% ROS inhibition at 50 μM, respectively, in H2O2-induced RAW264.7 cells. Moreover, Kang et al. (2004) have shown strong ROS inhibitory activity of phlorotannins; eckol (IC50 4.04 μM) and phlorofucofuroeckol A (IC50 3.8 μM) isolated from Ecklonia stolonifera on rat kidney homogenates of freshly killed male Wistar rats.

2. Photoprotective ability

Ultraviolet B (UVB; 280–320 nm) radiation of the sun is highly oxidative and directly triggers photodamage of the skin cells. This UVB radiation induces the overproduction of ROS which interacts with cellular DNA, proteins, and lipids to alter their cellular functions (Heo et al., 2010). Regular intake of antioxidants would be an useful strategy to combat UVB induced photodamage. Yan et al. (1997) and Hupel et al. (2011) have shown that brown algae are more tolerant to UVB irradiation due to the presence of phlorotannins. Therefore, these phlorotannins have been isolated from algae and investigated for their possible application in reducing the photodamage of the skin. Dieckol was found to be the most potent phlorotannin to protect the cells from UVB irradiation (50 mJ cm−2). Heo et al. (2009) have found that dieckol (100 μM) isolated from E. cava increase the cell survival up to 77.1% in UVB irradiated human dermal fibroblasts (HDF). And also they have shown that the DNA damage due to UVB irradiation was also inhibited by 57.8% with
# TABLE 8.1 Radical scavenging activities of phlorotannins from brown algae

<table>
<thead>
<tr>
<th>Compound</th>
<th>Radical scavenging activity IC$_{50}$ (µM)</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydroxyl</td>
<td>Superoxide</td>
<td>Alkyl</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eckol</td>
<td>51.8</td>
<td>26.5</td>
<td>28.4</td>
</tr>
<tr>
<td>Fucodiphloroethol G</td>
<td>33.5</td>
<td>18.6</td>
<td>18.1</td>
</tr>
<tr>
<td>Phlorofucofuroeckol A</td>
<td>39.2</td>
<td>21.6</td>
<td>21.4</td>
</tr>
<tr>
<td>7-phloroeckol</td>
<td>39.6</td>
<td>21.9</td>
<td>22.7</td>
</tr>
<tr>
<td>dieckol</td>
<td>28.6</td>
<td>16.2</td>
<td>14.5</td>
</tr>
<tr>
<td>6,6′-bieckol</td>
<td>29.7</td>
<td>15.9</td>
<td>17.1</td>
</tr>
<tr>
<td>Diphlorethohydroxycarmalol</td>
<td>28.7</td>
<td>15.4</td>
<td>17.3</td>
</tr>
<tr>
<td>8,8′-bieckol</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Phlorofucofuroeckol B</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Catechin</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>BHA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>BHT</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>A-tocopherol</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not analysed.
the treatment of 50 μM dieckol. Moreover, Ko et al. (2011) have also studied on the photoprotective effect of dieckol from *E. cava* using human epithelial keratinocytes (HaCat) and have found that dieckol treatment (100 μM) on UVB irradiated cells induces the cell survival up to 88.42%. Further, they have studied this promising photoprotective effect of dieckol using zebrafish as an alternate animal model system. Their results indicate that the ROS formation in UVB irradiated zebrafish embryos was significantly inhibited by the dieckol treatment (50 μM). ROS levels were measured using image analysis and fluorescence micrographs, and dieckol has also shown a typical fluorescence micrograph of the nonirradiated zebrafish. Diphlorethohydroxycarmalol isolated from *I. okamurae* has also been studied for its photoprotective ability in UVB (50 mJ cm⁻²) irradiated HDF cells. It has shown 45.57% ROS scavenging ability and 49.33% inhibition of DNA damage at 250 μM concentrations (Heo et al., 2010).

B. Anticancer effects

1. Antiproliferative activity
Cancer is possibly one of the most dangerous diseases, and cure for it has not yet found. Therefore, prevention of cancer through good diet practices is a well-promoted chemoprevention strategy. Marine edible seaweeds are promising candidates in this regard. Phlorotannins isolated from edible marine alga *E. cava* (dioxinodehydroeckol and 1-(3′,5′-dihydroxyphenoxy)-7-(2′,4′,6-trihydroxyphenoxy)-2,4,9-trihydroxybenzo-1,4-dioxin) have shown antiproliferative effects on human breast cancer cells (MCF-7). Among them, dioxinodehydroeckol has shown stronger ability in inducing apoptosis accounting for 55% cell death at 100 μM treatment (Kong et al., 2009). Moreover, Yang et al. (2010) have also found that the phlorotannin extracts from *Laminaria japonica* show antiproliferative effects on human hepatocellular carcinoma cells, IC₅₀ 200 μg/ml (BEL-7402) and murine leukemic cells, IC₅₀ 120 μg/ml (p388).

2. Inhibition of cancer metastasis
Invasion and migration are the principal mechanisms involved in cancer mortality, leading to spread of the cancer from its originated place to another site. In this invasion process, number of proteolytic enzymes contributes to the degradation of environmental barriers, such as the extracellular matrix (ECM) and basement membrane. Matrix metalloproteinase 2 and 9 (MMP 2,9) are principal enzymes responsible for ECM degradation, which are essential in the invasive growth, metastasis, and angiogenesis of cancer. Therefore, inhibition of these enzyme expressions is a therapeutic target to prevent cancer metastasis, and phlorotannins have found to be potent inhibitors. Zhang et al. (2010) have found that 6'6'-bieckol (100 μM)
isolated from brown alga *E. cava* significantly downregulated the expression of MMP 2,9 in an *in vitro* model of activated human fibro-sarcoma cells (HT1080). And this down regulation was found to be a cause of 6’6’-bieckol-mediated blocking of NF-κB signaling which regulate the expression of MMP 2,9. Polyphenolic extracts of *E. cava* has potential inhibitory effect against MMP 2,9 expression in human lung cancer cells (A549) through modulating Akt signaling pathway (Lee et al., 2011). Similarly, *E. cava* extract consisting of 57.98 ± 0.45% of phlorotannins (500 μM) has shown potent inhibitory activity against MMP 2,9 expression in HT1080 cells. Interestingly, this inhibition was found to be more potent than the commercially available MMP inhibitory drug doxycycline (10 μg/ml). Angiogenesis is the process where new blood vessels are made to facilitate the invasion of cancers, and fucodiphloroethol G from *E. cava* has inhibited this process in an angiogenesis-induced cellular model (Li et al., 2011). These findings show that especially the edible brown alga is a promising material to be included in the diet and for development of pharmaceuticals for chemoprevention.

C. Antiallergic effects

Allergic diseases are affecting approximately one-third of the general population in the world, and due to environmental changes and food habits, the prevalence and incidents of allergies are increasing. Wealth of research has been done to find antiallergic compounds, and phlorotannins or phlorotannin extracts from edible brown algae have shown promising potential in antiallergic therapy *in vivo* and *in vitro* models. Hyaluronidase enzyme is known to play an important role in allergic reaction, and Samee et al. (2009) have found that *Sargassum tenerrimum* phlorotannin extract is a strong inhibitor of hyluronidase (IC₅₀ 21 μg/ml). It is more potent than the commercially antiallergic drug disodium cromoglycate (IC₅₀ 39 μg/ml) and almost similar to the natural inhibitor catachin (IC₅₀ 20 μg/ml). 6’6’-bieckol (Le et al., 2009), fucodiphloroethol G, and phlorofucofuroeckol A (Li et al., 2008) isolated from *E. cava* have also shown significant antiallergic activity by inhibiting histamine release by modulating the binding between IgE and FcεRI receptors which mediate the allergen release in human basophilic leukemia (KU812) and rat basophilic leukemia (RBL-2H3). Further, Sugiura et al. (2007) have found the β-hexosaminidase enzyme (equivalent to histamine) inhibitory activity of phlorofucofuroeckol-B (IC₅₀ 7.8 μM) from brown alga *Eisenia arborea* which showed strongest inhibitory activity over antiallergic drug tranilast (IC₅₀ 46.6 μM) and epicatechin gallate (IC₅₀ 22 μM). And the presence of this very strong antiallergen would be the reason for shown *in vivo* antiallergic effects in brown Norway rats fed with dried *E. arborea* powder (Sugiura et al., 2008b).
D. Anti-inflammatory effects

Chronic inflammation is a pathophysiological condition of the body associated with various disorders such as arthritis, neurodegenerative diseases, cancer, diabetes, and cardiovascular disease. Scientists are in search of natural anti-inflammatory agents and phlorotannins have also gotten much attention due to the shown promising anti-inflammatory potential. Ryu et al. (2009) have shown anti-inflammatory effects of dieckol and 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxybenzo-1,4-dioxin as therapeutics to treat arthritis. Both phlorotannins have suppressed the unregulated expression of cyclooxygenase-2 (COX-2), inducible nitric oxide (iNOS), MMP 1,3,13 in activated human osteosarcoma cells (MG63). Phloroglucinol, the monomer unit, has also exerted significant anti-inflammatory activity by inhibiting inflammatory mediators (tumor necrosis factor \( \alpha \), Interleukin 1\( \beta \), interleukin 6, nitric oxide, and prostaglandin E\( _2 \)) in lipopolysaccharide-stimulated RAW264.7 cells (Kim and Kim, 2010). Overactivation of microglial cells is leading to chronic neurodegenerative diseases due to expression of neurotoxic inflammatory mediators. Dieckol isolated from \( E. \) cava has shown potent inhibitory activity on these inflammatory mediators and their respective downstream enzymes (cytokines, iNOS, COX-2) in lipopolysaccharide activated microglial cells (BV-2) (Jung et al., 2009). Interestingly, in all these findings, the signaling mechanism of phlorotannin-mediated anti-inflammatory effect was found to be through deactivation of NF-\( \kappa B \), the transcription factor regulated the inflammatory gene expression.

E. Antidiabetic effects

Diabetes mellitus is a chronic metabolic disorder which can damage many systems in the body, such as blood vessels and nerves. It is one of the world’s most serious health concerns, developing increasingly with the dietary patterns and age. The control of blood glucose levels is very important in hyperglycemic patients, and \( \alpha \)-glucosidase inhibitors are a cost-effective means to preventing the progression of diabetes. Fucodiphloroethol G (IC\( _{50} \) 19.52 \( \mu M \)), dieckol (IC\( _{50} \) 10.79 \( \mu M \)), 6,6' -bieckol (IC\( _{50} \) 22.22 \( \mu M \)), 7-phloreckol (IC\( _{50} \) 49.49 \( \mu M \)), and phlorofucofuroeckol A (IC\( _{50} \) 19.71 \( \mu M \)) from \( E. \) cava have shown significant inhibition of \( \alpha \)-glucosidase (Lee et al., 2009). Several other studies have conducted to investigate in vivo antidiabetic effects by feeding phlorotannin extracts to diabetic mouse models. Ecklonia stolonifera extracts have shown strong inhibition of \( \alpha \)-glucosidase in noninsulin dependent diabetic mice (Iwai, 2008). Feeding with \( E. \) cava extract (Kang et al., 2010) and \( I. \) okamurae extract (Min et al., 2011) have resulted in reduction of the plasma glucose
level and improve insulin resistance in type 1 mellitus rats and C57BL/-KsJ-db/db mice, respectively. Diabetes is closely related to the diet, and incorporating these brown algae as medicinal dietary supplements would be a promising prevalence strategy of diabetes.

IV. CONCLUSIONS

Marine brown seaweeds are abundant in phlorotannins compared to other marine plants. These phlorotannins are a highly diverse group depending on their structure, and polymerizations and oligomers serve as most promising bioactive materials. Many reports have been published on strong activities of phlorotannin oligomers against oxidative stress, cancer, inflammation, allergy diabetes, and few other disorders in vitro and in vivo. Among them, *E. cava* has been studied extensively as it produces number of highly active phlorotannins. Phlorotannin derivatives are capable of modulating cellular signaling and thereby they regulate the adverse conditions of the body. Finally, it can be suggested that marine brown algal phenolic extracts or isolated phlorotannins should be developed as medicinal foods or therapeutics for human health applications.

REFERENCES


CHAPTER 9

Biological Activities and Potential Health Benefits of Fucoxanthin Derived from Marine Brown Algae

Se-Kwon Kim*†1 and Ratih Pangestuti*

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Abstract
The importance of marine algae as sources of functional ingredients has been well recognized due to their valuable health beneficial effects. Therefore, isolation and investigation of novel bioactive ingredients with biological activities from marine algae have attracted great attention. Among functional ingredients identified from marine algae, fucoxanthin has received particular interest.

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Fucoxanthin has been attributed with extraordinary potential for protecting the organism against a wide range of diseases and has considerable potential and promising applications in human health. Fucoxanthin has been reported to exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, antiobesity, and neuroprotective activities. In this chapter, the currently available scientific literatures regarding the most significant activities of fucoxanthin are summarized.

I. INTRODUCTION

Ninety percent of the world’s living biomass is found in the oceans with marine species comprising approximately half of the total global biodiversity (Kim and Wijesekara, 2010; Swing, 2003). Therefore, the wide diversity of marine organisms is being recognized as rich sources of functional materials, including polyunsaturated fatty acids (PUFA), polysaccharides, essential minerals and vitamins, enzymes, and bioactive peptides (Shahidi, 2008; Shahidi and Alasalvar, 2011; Shahidi and Janak Kamil, 2001). Among marine organisms, marine algae or sometimes referred as seaweeds have long been used in food diets as well as traditional remedies in Eastern hemisphere (Heo et al., 2009). The term marine algae, as used herein, generally refer to marine macroalgae or sometimes referred as seaweeds.

Marine algae were mainly classified into three major classes based on their pigmentation, namely brown, red, and green algae, which are referred to as Phaeophyceae, Rhodophyceae, and Chlorophyceae, respectively (Khan et al., 2010). The amount and type of pigments present is found to differ according to the algae classes. Three basic classes of pigments found in marine algae are chlorophylls, carotenoids, and phycobiliproteins. Various pigments isolated from marine algae have shown to possess several biological activities and potential health benefits. Therefore, a great attention has been arisen to isolate marine algal pigments and identify their biological activities and potential health benefit effects. Lutein and β-carotene, a family of carotenoids which isolated from Porphyra tenera displays strong suppressive effect against mutagen-induced umu C gene expression in Salmonella typhimurium (Okai et al., 1996). Phycoerythrin, one of phycobiliproteins which are the light-harvesting accessory pigments present in red algae, cyanobacteria, and cryptomonads, has been demonstrated to possess strong antioxidant activity in vitro and hepatoprotective properties in vivo (Soni et al., 2008; Yabuta et al., 2010). Pheophytin a, a chlorophyll-related compound isolated from Sargassum fulvellum, has been demonstrated to promote neurite outgrowth in PC12 cells (Ina et al., 2007). Further, recent studies have
demonstrated the correlation between a diet rich in carotenoids and diminishing risk of cardiovascular disease, cancers, ophthalmological diseases as well as photoaging (Burtin, 2003). Compared with other pigments isolated from marine algae, fucoxanthin has attracted greater attention. It has been reported that fucoxanthin provide a myriad of health benefits effect. The health benefit effects of fucoxanthin are presented in Table 9.1. This contribution provides an overview on biological activities of fucoxanthin derived from marine brown algae and their potential health beneficial and applications in food, pharmaceutical industries.

### II. PROFILES AND BIOAVAILABILITY OF FUCOXANTHIN

Fucoxanthin (Fig. 9.1) is one of the most abundant carotenoids contributing around 10% estimated total production of carotenoids in nature (Matsuno, 2001). It has a unique structure including an unusual allenic bond and a 5,6-monoepoxide in its molecule. For different brown algal strains, the compositions and profile of fucoxanthin were found to be different. Tsukui et al. reported that Sargassum horneri had a remarkably higher level of fucoxanthin content (3.7 mg/g) in comparison with other

<table>
<thead>
<tr>
<th>Health beneficial effects</th>
<th>Source</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant</td>
<td>Hijikia fusiformis, Undaria pinnatifida, Fucus serratus, Padina tetrastromatica</td>
<td>Nomura et al. (1997), Sasaki et al. (2008), Yan et al. (1999)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Undaria pinnatifida</td>
<td>Hosokawa et al. (1999), Kotake Nara et al. (2005a,b)</td>
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<td>Myagropsis myagroides</td>
<td>Heo et al. (2010)</td>
</tr>
<tr>
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<td>Undaria pinnatifida</td>
<td>Maeda et al. (2005, 2007a,b, 2008)</td>
</tr>
<tr>
<td>Antiangiogenic</td>
<td>Undaria pinnatifida</td>
<td>Sugawara et al. (2006)</td>
</tr>
<tr>
<td>Neuroprotective</td>
<td>Hijikia fusiformis</td>
<td>Okuzumi et al. (1990)</td>
</tr>
<tr>
<td>Prevent osteoporosis</td>
<td>Laminaria japonica</td>
<td>Das et al. (2010)</td>
</tr>
<tr>
<td>Photoprotective</td>
<td>Laminaria japonica</td>
<td>Heo and Jeon (2009), Shimoda et al. (2010)</td>
</tr>
</tbody>
</table>
Sargassum species tested in their study (Tsukui et al., 2009). Moreover, fucoxanthin was reported as the major carotenoid in Hizikia fusiformis (Yan et al., 1999). In addition, Kanazawa et al. reported high fucoxanthin level in two edible marine algae species, Laminaria japonica and Undaria pinnatifida (Kanazawa et al., 2008). Fucoxanthin is widely available in various species of marine brown algae; hence, more and more fucoxanthin has been investigated in recent years for applications in foods, nutraceutical pharmaceutical, and cosmeceutical industries.

In our body, fucoxanthin absorption strongly depends on a number of factors which are not entirely understood. Numerous factors can impact fucoxanthin absorption, including the amount and possibly the type of dietary lipids consumed, the stability of the matrix to which the carotenoid was bound, and additional dietary factors such as dietary fiber (Bohn, 2008). Bioavailability of fucoxanthin seems to be very low; however, there is a scientific controversy about it. Strand et al. (1998) found that fucoxanthin metabolites but not fucoxanthin were transferred to the egg yolks of laying hens fed a diet supplemented with 15% Fucus serratus (Strand et al., 1998). Hashimoto et al. (2009) reported that fucoxanthin and its metabolites show better bioavailability than astaxanthin. More recently, Asai et al. (2008) revealed that bioavailability of fucoxanthin human is very low. The mechanism underlying the poor incorporation of fucoxanthin from diets into human plasma remains to be clarified. In a serial studies, Sugawara et al. reported that esterification of fucoxanthin in human intestinal caco-2 cells and mice was mediated by enzymatic activity after intestinal absorption (Sugawara et al., 2002, 2009). The esterified fucoxanthin was likely to be incorporated into the lipid core in chylomicron and carried into a variety of tissues including the skin. In addition, by esterifying fucoxanthin into highly nonpolar products, intestinal cells might be protected from the cytotoxic effects of fucoxanthin (Sugawara et al., 2009). Fucoxanthinol was identified as a prime metabolite of fucoxanthin in mice and rats (Asai et al., 2004; Sangeetha et al., 2010). Interestingly, amarouciaxanthin A was found as a major metabolite of fucoxanthin in rat liver, suggesting that liver enzymes may play a role in hydrolyzing fucoxanthin into amarouciaxanthin A (Sangeetha et al., 2010). In contrast,
dietary fucoxanthin was accumulated in the mice heart and liver as fucoxanthinol and in adipose tissue as amarouciaxanthin A (Hashimoto et al., 2009). However, fucoxanthinol was further converted into amarouciaxanthin A by short-chain dehydrogenase or reductase in the mice liver within 24 h and rapidly transported to other tissues.

No adverse side effects of fucoxanthin were reported in the mice study. Notably, in animal studies, fucoxanthin also appeared to stimulate liver to produce docosahexaenoic acid (DHA), a type of omega-3 fatty acid, at levels comparable to fish oil supplementation. The animal experiments with fucoxanthin stimulated researchers to recommend human clinical trials. In placebo-controlled trials, a supplement containing a 5% fucoxanthin (daily dosage 10 mg) did not reveal any harmful effects (Holt, 2008). Therefore, fucoxanthin may be considered as nontoxic, nonallergenic, biocompatible, bioactive materials.

III. BIOLOGICAL ACTIVITIES OF FUCOXANTHIN

A. Antioxidant activity

Oxidation of biomolecules has been identified as a free radical-mediated process (Mendis et al., 2004). Formation of free radicals is an unavoidable consequence in aerobic organisms during the oxygen metabolism, thus believed to be involved in many pathological diseases such as cancer, diabetes mellitus, aging, and Alzheimer’s disease (AD; Johansen et al., 2005; Kong et al., 2010; Rattan, 2006; Valko et al., 2006). In addition, deterioration of some foods, development of undesirable off-flavor and potentially toxic reaction compounds in food, has been identified as a result of free radical-mediated oxidation of fatty acids and lipids. Oxidation of lipids by reactive oxygen species (ROS) such as superoxide anion and hydroxyl radicals which shorten the shelf life of foods is attracting great concern in food and pharmaceutical industry; hence, several synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) are widely used to retard the oxidation and peroxidation processes (Je et al., 2005; Kim et al., 2001; Ni et al., 2000). However, the use of these synthetic antioxidants must be under strict regulation due to potential health hazards (Park et al., 2001; Safer and Al-Nughamish, 1999). Hence, the search for natural antioxidants as safe alternatives is important in food industry (Pena-Ramos and Xiong, 2001). Recently, there is a considerable interest in the food industry as well as pharmaceutical industry for the development of antioxidants from natural sources. Among marine organism, marine algae represent one of the richest sources of natural antioxidants (Cornish and Garbary, 2010).
Yan et al. (1999) identified that fucoxanthin is the major antioxidant of *H. fusiformis* and investigated the radical scavenging activity. Although it has been previously reported by Nomura et al. that carotenoids such as zeaxanthin, β-carotene, and lutein did not show DPPH scavenging activity, Yan et al. showed that fucoxanthin has a strong radical scavenging activity (Nomura et al., 1997; Yan et al., 1999). The potential involvement of fucoxanthin in radical scavenging activity may correlate to the presence of unusual double allenic bonds at C-7' position. These findings were confirmed in a recent study carried by Sachindra et al. which isolated fucoxanthin from *U. pinnatifida* and prepared two fucoxanthin metabolites, fucoxanthinol and halocynthiaxanthin (Sachindra et al., 2007). The antioxidant activities of these three carotenoids were assessed by DPPH and hydroxyl radicals scavenging activities and singlet oxygen quenching activity. The order of scavenging activity of each carotenoid followed a pattern of fucoxanthin > fucoxanthinol > halocynthiaxanthin (Sachindra et al., 2007). The major structural differences in these three carotenoids are the presence of an allenic bond in fucoxanthin and fucoxanthinol, suggesting that the allenic bond is responsible for the higher scavenging activity of fucoxanthin and fucoxanthinol. Therefore, fucoxanthin may have great potential for use as a nutraceutical and a pharmaceutical and a substitute for synthetic antioxidants. In addition, Sasaki et al. demonstrated that fucoxanthin when added to ground chicken meat at a level of 200 mg/kg reduced the formation of secondary oxidation products including TBA reactive substances in the same level as α-tocopherol (Sasaki et al., 2008). Oral administration of fucoxanthin has been reported to improve plasma antioxidant status and meat color in broiler chicks (Sasaki et al., 2010). Moreover, fucoxanthin obtained from *Padina tetrastromatica* has shown higher potential to be used as antioxidant in rats induced by vitamin A deficiency, followed by retinol and β-carotene (Ravi Kumar et al., 2008; Sangeetha et al., 2009). However, the exact mechanism of action as to how fucoxanthin exerts antioxidative effect in rats induced by vitamin A deficiency is not yet completely understood. In addition, cytoprotective effect of fucoxanthin against ROS formation induced by H$_2$O$_2$ has been observed *in vitro* (Heo et al., 2008). The presence of two hydroxyl groups in the ring structure of fucoxanthin may correlate to the inhibition of ROS formation. Notably, several studies have indicated that the number of hydroxyl groups on the ring structure of fucoxanthin is correlated with the effects of ROS suppression.

These evidences suggest that among various naturally occurring substances in marine organisms, fucoxanthin proves to be one of the useful candidates in search for effective, nontoxic substances with potential antioxidant activity. Moreover, fucoxanthin is one of the most abundant carotenoid in the nature and could be used as a rich source of natural antioxidants with potential application in the food industry as well as cosmetic and pharmaceutical areas.
B. Anticancer activity

Cancers can be defined as diseases in which unremitting clonal expansion of somatic cells kills by invading, subverting, and eroding normal tissues (Evan and Vousden, 2001). It has been documented that apoptosis (programmed cell death) is a key process in cancer development and progression. Inactivation of apoptosis is considered to be one of the six fundamental hallmarks of cancer, and therefore, apoptosis is a major target of cancer therapy development (Brown and Attardi, 2005). Hence, developing novel molecules that promote apoptosis by targeting both the intrinsic and extrinsic apoptotic pathways may lead to the development of effective cancer therapies.

Fucoxanthin is known to be important free-radical scavengers and antioxidants for the prevention of oxidative damage, which is an important contributor in carcinogenesis. Therefore, it might be suggested that fucoxanthin has potent capacities for new anticancer product developments in the food industries as well as in pharmaceuticals as novel chemopreventing agents for cancer therapy.

Exciting research studies have been published regarding carotenoids and its anticancer qualities. Ishikawa et al. showed anti-adult T-cell leukemia effects of fucoxanthin and its deacetylated metabolite, fucoxanthinol (Ishikawa et al., 2008). The inhibitory activities of fucoxanthin and fucoxanthinol were stronger than those of β-carotene and astaxanthin. Adult T-cell leukemia is a fatal malignancy of T lymphocytes caused by human T-cell leukemia virus type 1 infection and remains incurable. Therefore, carotenoids could be potentially useful therapeutic agents for adult T-cell leukemia patients. A recent study from Japan demonstrates that anticancer activity of fucoxanthin goes way beyond its ability to induce apoptosis. Apoptosis inducing effect of fucoxanthin on human leukemia cells (HL-60) has been reported (Hosokawa et al., 1999; Kotake Nara et al., 2005b). The apoptosis induction was associated with activation of caspase-3, -8, and -9 which can be thought of as central executioner of the apoptotic pathway (Hengartner, 2000). Very recently, Ganesan et al. reported that siphonaxanthin derived from Codium fragile is a more potent growth inhibitor against HL-60 cells than fucoxanthin (Ganesan et al., 2011). The structural differences between these two carotenoids are fucoxanthin contains epoxide and an allenic bond in its structure, whereas siphonaxanthin does not contain those functional groups; however, siphonaxanthin has an additional hydroxyl group on the 19th carbon atom. Since esterified form of siphonaxanthin showed lower inhibitory effect, it is suggested that the presence of hydroxyl group is contributed to the strong inhibitory effect of siphonaxanthin (Ganesan et al., 2011). Meanwhile, antiproliferative effect and apoptosis induction by fucoxanthin in human colon cancer cells (Caco-2, HT-29, and DLD-1) were observed by
Hosokawa et al. (2004). Fucoxanthin remarkably reduced the viability of human colon cancer cell lines and treatment with fucoxanthin induced DNA fragmentation. Exposure to fucoxanthin decreased the level of apoptosis-suppressing protein (Bcl-2), which suggest that anticancer activity of fucoxanthin were mainly caused by apoptosis. Apoptosis-inducing effect of fucoxanthin in human prostate cancer cells (PC-3, DU 145, and LNCaP) has also been observed (Kotake Nara et al., 2001, 2005a). Although current knowledge of relationship between the structure and apoptosis activity of the fucoxanthin is limited, some researchers suggest that conjugated double bonds and 5,6-monoepoxide are thought to be highly susceptible to acids, alkali and oxygen lead to their prooxidant actions which might cause apoptosis induction in the several cancer cells.

Therefore, it might be suggested that these marine algae-derived NPs have potent capacities for new anticancer product developments in the pharmaceutical as well as the food industries as novel chemopreventing agents for cancer therapy.

C. Anti-inflammatory activity

The anti-inflammatory effect of fucoxanthin is mainly based on modulation of macrophages function. Macrophages are the residents of immune cells in the innate immune system which play an important role in the maintenance of homeostasis by changing their function according to the tissue. As the residence of the immune system, macrophages are a predominant source of proinflammatory mediators including nitric oxide (NO), prostaglandin E_2 (PGE_2), proinflammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β)], and ROS (Block et al., 2007). It has been consistently demonstrated that the origin of cancer was at sites of chronic inflammation, in part based on the hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance proliferation. Chronic inflammation may also play significant role in mediating neurodegenerative diseases such as Parkinson’s disease (PD), AD, multiple sclerosis (MS), and acquired immune deficiency syndrome (AIDS) dementia complex (Kim and Joh, 2006).

Secondary metabolites derived from marine algae are known to have promising anti-inflammatory activities (Abad et al., 2008). However, the scientific analysis of anti-inflammatory activity of fucoxanthin has been poorly carried out, and until now, only few studies were reported. Fucoxanthin is recently known to be a potent anti-inflammatory agent in vitro and in vivo in responses to bacterial lipopolysaccharides (LPS). Shiratori et al. reported that anti-inflammatory effect of fucoxanthin is comparable with predinisolone, a commercially available steroidal anti-inflammatory drug (Shiratori et al., 2005). More recently, Heo et al. screened inhibitory
effect of NO production from nine species of brown algae and confirmed that inhibition of NO production correlates with fucoxanthin contents (Heo et al., 2010). In addition, Heo et al. also demonstrated anti-inflammatory effect of fucoxanthin isolated from Myagropsis myagroides in LPS-stimulated RAW 264.7 cells. Fucoxanthin treatment attenuates the productions of NO and PGE2 by inhibiting inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) expressions. The release and expression levels of inflammatory cytokines (TNF-α, IL-6, and IL-1β) were attenuated by fucoxanthin in a dose-dependent fashion. The anti-inflammatory activities of fucoxanthin were due to the suppression of nuclear factor-κB (NF-κB) and the phosphorylation of mitogen-activated protein kinases (MAPKs; Kim et al., 2010). Production of proinflammatory mediators has been continuously reported in many inflammatory tissues, along with increased expression of their mRNAs and proteins. Therefore, inhibition of proinflammatory mediators by fucoxanthin suggests its potential for the treatment of inflammatory and other related diseases.

D. Antiobesity activity

Obesity may be defined as an excessive body weight in the form of fat (Kong et al., 2009). It is one of the greatest public health challenges in the first half of this century (Inoue et al., 2000). A number of studies indicated that obesity is associated with type 2 diabetes mellitus, cardiovascular disease, certain forms of cancer, and sleep-breathing disorder (Kopelman, 2000; Lee et al., 2005; Mokdad et al., 2003; Pi-Sunyer, 2002). Moreover, obesity (from teen to seniors) continues to increase in many industrialized and developing countries, which cause a worrying health trend (Kelishadi, 2007). Therefore, the necessity of discovering alternative sources of antiobesity has arisen with interesting demand for safer antiobesity agents.

A research group from Japan reported that oral treatment with fucoxanthin significantly reduced the abdominal white adipose tissue (WAT) weight of obese mice model, KKAY female mice and normal mice fed with a high-fat diet (Maeda et al., 2005, 2007a,b, 2008). Moreover, no reductions on normal mice fed with normal diet were found. Those results suggest that fucoxanthin specifically suppresses adiposity in the obese mice. WAT is the predominant type of adipose tissue and commonly called “fat” in mammals (Trayhurn and Wood, 2005). Besides its role in energy storage, WAT is now recognized as an endocrine and active secretory organ through its production of biologically active mediators termed, adipokines (Curat et al., 2006). Most studies reported that antiobesity effect of fucoxanthin was mainly mediated by the expression of uncoupling protein-1 (UCP-1) gene in visceral adipose tissues which lead to the induction of thermogenesis in adipose tissue and dissipating excess energy intake as heat to resist body
weight gain (Mercader et al., 2010; Woo et al., 2009). Recent clinical study

carried by Abidov et al. (2010) clearly showed antiobesity effect of xanthi-
gen, an antiobesity supplement which consists of fucoxanthin and pome-
granate seeds oil. In their study, they demonstrated that xanthigen
promoted weight loss, reduced body and liver fat content, and improved
liver function tests in obese nondiabetic women (Abidov et al., 2010).

Since the excessive growth of adipose tissue in obesity has been sug-
gested to result from adipocyte hypertrophy and the recruitment of new
adipocytes from precursor cells, regulation of adipogenesis also appears to
be a potential strategy for the treatment of obesity (Wang et al., 2008).
Fucoxanthin isolated from U. pinnatifida and its metabolite fucoxanthinthol
have been reported to inhibit the differentiation of 3T3-L1 preadipocytes
into adipocytes (Hayato et al., 2006). The inhibitory effect of fucoxanthin and
fucoxanthinol on adipocyte differentiation might be mediated through the
downregulation of adipogenic transcription factors, such as peroxisome
proliferator-activated receptor-γ. Structure suppressive effect on adipocyte
differentiation has been reported by Okada et al. (2008). In their study,
they used 13 naturally occurring carotenoids found in human diet. Interes-
tingly, carotenoids with keto group, epoxy group, hydroxyl carotenoid,
epoxy-hydroxy carotenoid, and keto-hydroxy carotenoid did not show
suppressive effect on adypocyte differentiation. Meanwhile, treatment with
fucoxanthin and neoxanthin showed significant suppressive effect suggest-
ing that allenic bond is crucial factor for the antiobesity effect. Moreover, the
result of those studies leads to the hypothesis that other carotenoid with an
allenic group and an additional hydroxyl group in the end may also effective
in suppressing adipocyte differentiation.

Taken together, fucoxanthin has a potential to be used in food and
pharmaceuticals in the treatment or prevention of obesity as they may act
as a regulator of lipid metabolism in fat tissues. There are numerous
advantages of fucoxanthin derived from marine algae to be used in
functional foods and pharmaceuticals, such as relatively low production
costs, low cytotoxicity, safety, and wide acceptability. Further, fucoxan-
thin derived from marine algae may be considered as a promising food
supplement, slimming supplement, and drug in the prevention and man-
agement of obesity.

E. Neuroprotective effect

Neuroprotection may be defined as a mechanism and strategy used in
order to protect neuronal cells against injury, apoptosis, dysfunction, and
degeneration in the central nervous system (CNS) by limiting neuronal
dysfunction or death after CNS injury (Tucci and Bagetta, 2008; Zarros,
2009). Many categories of natural and synthetic compounds have been
reported to possess neuroprotective activities. However, these synthetic
neuroprotective agents are believed to have certain side effects such as dry mouth, tiredness, drowsiness, anxiety or nervousness, difficulty to balance, etc. (Narang et al., 2008; Pangestuti and Kim, 2010). Hence, scientists have studied natural bioactive compounds, which can act as neuroprotective agents. Several scientific studies have provided insight into neuroprotective properties of marine algae-derived NPs (Pangestuti and Kim, 2011). Okuzumi et al. (1990) found that fucoxanthin isolated from H. fusiformis inhibited N-myc expression and cell cycle progression of GOT0 cells, a human neuroblastoma cell line. Fucoxanthin at a concentration of 10 μg/ml reduced the growth rate of GOT0 cells to 38%, but its exact mechanisms of action are not yet completely understood. Ikeda et al. (2003) recently found that wakame was able to attenuate the development of hypertension and its related diseases in stroke-prone spontaneously hypertensive rats (SHRSP). Further, they isolated fucoxanthin from wakame and showed that fucoxanthin amazingly attenuated cell damage in cortical neurons during hypoxia and oxygen reperfusion (Khodosevich and Monyer, 2010). Since ROS generation is considered to occur after hypoxia and reoxygenation, whereby free radicals damage neurons, it may be assumed that neuroprotective activity of fucoxanthin is mainly based on their scavenging activity.

Neurite outgrowths are fundamental neuronal features which play an important role in neuronal development during embryogenesis and in the adult brain. Pheophytin a and its analog, vitamin B12 derived from S. fulvellum, have been reported to promote neurite outgrowth in phaeochromocytoma (PC12) cells (Ina and Kamei, 2006; Ina et al., 2007). Neurite outgrowth promoting activity of pheophytin a has been reported to be closely related to their low molecular weight. The rationale for this is that low molecular weight pheophytin a may incorporate into the cells more efficiently and therefore promote neurite outgrowth effectively.

Based on several findings, it may be concluded that NPs are a valuable source of neuroprotective agents and could be introduced for the preparation of novel functional ingredients in functional foods and pharmaceuticals as a good approach for the treatment and or prevention of neurodegenerative disease. Further, it can be suggested that NPs are an alternative source to synthetic ingredients that can contribute in neuroprotection. Until now, neuroprotective activities of NPs have been observed in vitro. Therefore, further researches are needed in order to investigate NPs neuroprotective activities in vivo and in human subject.

F. Antiangiogenic activity

Angiogenesis refers to the process of new blood vessel formation from a preexisting vasculature that occurs under, either physiological or pathological conditions (Carmeliet, 2003). It is observed at tightly regulated
condition in normal physiology during embryogenesis, ovary cycling, and wound healing. However, in pathological conditions such as inflammatory diseases, rheumatoid arthritis, and tumor metastasis, a chronic unregulated angiogenic state often helps spreading of the diseases (Kirk et al., 2004). Hence, preventing angiogenesis under pathological conditions is a promising approach in the prevention of cancer and other angiogenic-related diseases.

Sugawara et al. showed that fucoxanthin significantly suppressed human umbilical vein endothelial cells (HUVEC) proliferation and tube formation at more than 10 μM. Fucoxanthin effectively suppressed the differentiation of endothelial progenitor cells into endothelial cells involving new blood vessel formation (Sugawara et al., 2006). Fucoxanthin and fucoxanthinol suppressed microvessel outgrowth in vivo and ex vivo angiogenesis assay using a rat aortic ring. In a recent study, Ganesan et al. demonstrated antiangiogenic effect of siponaxanthin derived from green algae, C. fragile (Ganesan et al., 2010). The antiangiogenic effects of siponaxanthin were comparable with fucoxanthin. The structure similarity between fucoxanthin and siponaxanthin is the presence of hydroxy group on the 3 and 3’ position of both compounds. Therefore, the presence of those hydroxyl groups might conceivably be a part of their antiangiogenesis effect.

G. Skin protective effect

Fucoxanthin isolated from L. japonica has been reported to suppress tyrosinase activity in UVB-irradiated guinea pig and melanogenesis in UVB-irradiated mice. Oral treatment of fucoxanthin significantly suppressed skin mRNA expression related to melanogenesis, suggesting that fucoxanthin negatively regulated melanogenesis factor at transcriptional level (Shimoda et al., 2010). Moreover, fucoxanthin has been demonstrated to possess photoprotective properties in human fibroblast cells via inhibition of DNA damage and enhance antioxidant activity (Heo and Jeon, 2009). These studies suggest that oral administration of fucoxanthin might prevent or minimize the negative effects of UV radiation such as melanin formation.

H. Other biological activities

Recently, Das et al. showed that the effects of fucoxanthin on osteoclastogenesis were investigated using cells from the macrophage cell line RAW264.7, which have the capacity to differentiate into osteoclast-like cells when stimulated by receptor activator of NF-κB ligand (Das et al., 2010). Fucoxanthin significantly suppressed the differentiation of RAW264.7. Treatment with 2.5 μM fucoxanthin also induced apoptosis
accompanied by activation of caspase-3 in osteoclast-like cells. Those in vitro studies suggest that fucoxanthin suppresses osteoclastogenesis via the inhibition of osteoclast differentiation and the induction of apoptosis in osteoclasts. Hence, dietary fucoxanthin may be useful for the prevention of bone diseases such as osteoporosis and rheumatoid arthritis, which are known to be related to bone resorption. Moreover, dietary intake of fucoxanthin and fucoxanthinol has been demonstrated to enhance the amount of DHA and arachidonic acid (AA) content in the liver of C57BL6J/6J normal male mice (Tsukui et al., 2007, 2009). However, further studies are needed to clarify the molecular mechanisms as to how fucoxanthin promotes DHA and AA synthesis in the mice liver.

IV. CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, fucoxanthin is a valuable source of bioactive compound and could be introduced for the preparation of novel functional ingredients in food and, also a good approach for the treatment or prevention of chronic diseases. Recently, much attention has been paid by the consumers toward natural bioactive compounds as functional ingredients in foods, and hence, it can be suggested that fucoxanthin is an alternative source for synthetic ingredients that can contribute to consumer’s well-being, by being a part of new functional foods. Further, the wide ranges of biological activities associated with fucoxanthin derived from marine brown algae have potential to expand its health beneficial value in food and pharmaceutical industries.

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Phlorotannins and Fucoidans from Marine Macroalgae as Matrix Metalloproteinase Inhibitory Substances and Their possible Application as Medicinal Foods

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Abstract

Metalloproteinases especially matrix metalloproteinases are a group of endopeptidases that contribute for the extracellular matrix degradation, and several tissue remodeling processes. Improper regulation of these endopeptidases could lead to several severe pathological problems that include cardiac, cartilage, and cancer-related diseases. Until now, many synthetic matrix metalloproteinase inhibitory substances (MMPIs) have been reported; however, many of them could not make to the final clinical trials.
Hence, the emphasis on screening of MMPIs from different natural resources has gained much importance and marine resources are one among them. As marine organisms have been contributing with several biologically active compounds that have profound applications in nutraceuticals, cosmeceuticals, and pharmaceuticals; in this chapter, an attempt has been made to discuss the various MMPIs from edible seaweeds, which could be considered as medicinal foods.

I. INTRODUCTION

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade the extracellular matrix, and this is considered as a general remodeling process that enables several physiological processes like wound healing, bone resorption, uterine involution, and organogenesis as well as pathologic conditions including inflammatory, vascular, and autoimmune disorders, and carcinogenesis (Egeblad and Werb, 2002; Lee and Murphy, 2004). It is reported that MMPs are involved in tumor invasion, angiogenesis, metastasis, transformation of cancer cells, signal transduction, and apoptosis (Overall and López-Otin, 2002) that makes them an ideal target for the treatment of cancer-related disorders. Normally, the phenomena of intercellular regulation and cell matrix adhesion are regulated in a controlled fashion; however, superior expressions of MMPs deregulate this event and result in various kinds of human cancers (Bourboulia and Stetler-Stevenson, 2010). Tissue inhibitors of metalloproteinase, commonly known as TIMPs, are naturally occurring inhibitors for the overexpression of MMPs and are known to prevent the proteolytic degradation. Factors like solubility and interaction of TIMPs with pro-MMPs do determine the inhibitory activity of TIMPs (Lambert et al., 2004). Several pathological responses associated with MMPs have made them as therapeutic targets for the better management of human cancers. Till now, many synthetic MMPIs have been reported. Synthetic drugs like Batimastat (BB-94) and Marimastat (BB-516) have been successful in lowering the expression of MMPs. However, improper metabolism, low oral bioavailability, poor solubility, side effects like musculoskeletal pain and inflammation, complications, and the risk of increased drug toxicity is still a big challenge (Coussens et al., 2002; Thomas and Kim, 2010). Because of these shortcomings of the synthetic MMPIs, researchers are screening natural resources for the screening of MMPIs and few research groups have been successful in reporting natural MMPIs from terrestrial organisms (Ha et al., 2004; Seo et al., 2005). However, the diversified environment in sea water enables the marine organisms with unique abilities for their survival and makes them ideal choice for the screening of biologically active compounds.
Seaweeds or marine macroalgae are considered as dietary components and also as alternative medicine in Asian countries like Japan, Korea, and China (Ali et al., 2000). In recent times, isolation and characterization of the biologically important compounds have gained lot of attention from various research groups across the world. Marine brown algae have been extensively studied for their biologically active components that majorly include polyphenolic derivatives called phlorotannins and polysaccharides like fucoidans, alginic acid, etc. In this chapter, we would be discussing the role of phlorotannins and polysaccharides from marine macroalgae as potential MMPIs.

II. MARINE ALGAL PHLOROTANNINS AS POTENTIAL MMPIs

It is a well-understood fact that marine flora harbors a wide range of biologically active compounds that are reported to have an outstanding prospective in the medicinal, nutraceutical, and cosmeceutical applications. Natural metabolites obtained from marine seaweeds prove to be abundant resources with chemical diversity, and among them, phlorotannins are studied most for their biological activities. These phlorotannins (Fig. 10.1) are derived from tannins and are composed of several phloroglucinol units linked to each other in different ways and mostly
FIGURE 10.1  (Continued)
distributed in marine brown algae (Singh and Bharate, 2006). It is suggested that formation of phlorotannins is by polymerization of phloroglucinol (1,3,5-trihydroxybenzene) monomer units and biosynthesized through the acetate–malonate pathway, also known as polyketide pathway. Usually, their molecular sizes range from 126 to 650 kDa (Ragan and Glombitza, 1986). Recent studies recommend that the polyphenolic compounds derived from marine algae have preventive properties against human diseases including cancer, coronary heart diseases, and other allergies (Shibata et al., 2003), hence suggesting the brown algae as potential medicinal food.

Based on substrate specificity, the MMPs are categorized into three major functional groups. The main three groups include interstitial collagenases that have affinities toward collagen types I, II, and III (MMP-1, MMP-8, and MMP-13); the stromelysins with specificity for laminin, fibronectin, and proteoglycans (MMP-3, MMP-10, and MMP-11); and the gelatinases that effectively cleave type IV and type V collagen (MMP-2 and MMP-9; Nelson et al., 2000). Two phlorotannins namely dieckol and 1-(3′,5′-dihydroxyphenoxyl)-7-(2′,4′,6′-trihydroxyphenoxyl) 2,4,9-trihydroxydibenzo-1,4-dioxin isolated from the methanol extract of marine brown alga, Ecklonia cava, have been reported to suppress both the protein and gene expression levels of MMP-1, MMP-3, and MMP-13 in human osteosarcoma cells (MG-63). This in vitro study also reports that these phlorotannins were able to promote osteosarcoma differentiation by increasing alkaline phosphatase (ALP) activity,
mineralization, total protein, and collagen synthesis (Ryu et al., 2009b). Similarly, dieckol and eckol isolated from Ecklonia stolonifera have inhibited the expression of MMP-1 in human dermal fibroblast (HDF) cell, in vitro (Joe et al., 2006). More precisely, this investigation suggested that these phlorotannins interfere with the expressions of NF-κB and activator protein-1 (AP-1) which in turn enhances the MMP-1 expression that leads to skin-related damages. Hence, brown algae can be recommended as foods with medicinal values that can aid for skin care.

Skin wrinkling is normally attributed by the reactive oxygen species (ROS) which is caused by the oxidative stress. ROS stimulates mitogen-activated protein kinases that phosphorylate transcription factor AP-1, which in turn results in upregulation of MMPs that contribute for the degradation of skin collagen ultimately leading to skin ageing (Fisher et al., 1996; Rittie and Fisher, 2002). The gelatinases that include MMP-2 and MMP-9 promote UV-induced skin damage. It is reported that sun-damaged skin shows significantly elevated levels of active gelatinases (MMP-2 and MMP-9) than intrinsically aged skin (Chung et al., 2001). In vitro studies on methanol extract from marine alga Corallina pilulifera (CPM) have revealed that CPM has the ability to prevent UV-induced oxidative stress and also the expressions of MMP-2 and MMP-9 in HDF cells. This clearly suggests the role of phenolic compounds from marine algae as potential MMPIs (Ryu et al., 2009a). As it is evident that unregulated expression of MMPs leads to the photoaging, many research groups are emphasizing their research goals to check the ability of marine-derived phlorotannins as potential antiphotoaging agents. Moreover, the ROS that includes hydrogen peroxide, hydroxyl radical, and superoxide anion is involved in metabolic diseases, especially chronic inflammation. In chronic inflammation, proinflammatory cytokines induce MMPs that degrade the extracellular matrix and contribute for several inflammatory disorders. In this process of screening the medicinally valuable agents from marine seaweeds, phloroglucinol, a monomer of phlorotannins, is reported to exhibit anti-inflammatory effect in addition to free radical scavenging activity. This in vitro study has revealed the MMP-2 and MMP-9 inhibitory activities in HT1080 cells, thus suggesting the potentiality of phloroglucinol as an antimetastatic compound. Moreover, the phloroglucinol has exhibited anti-inflammatory activity by expression levels of TNF-α, IL-1β, IL-6, and PGE_2 in macrophages RAW264.7 (Kim and Kim, 2010). These in vitro studies bring front the scientific proof that phloroglucinol from marine algae can be recommended as a medicinal agent to reduce the risk of metastasis and inflammation-related diseases. On the other hand, reports suggest that some other brown alga contain higher amount of phloroglucinol (Koivikko et al., 2005) and have exhibited several medicinally beneficial effects in vitro. Hence, members of brown seaweeds can be recommended as medicinal foods because of
the abundant presence of polyphenolic compounds that aids for the betterment of human health.

The species *Ecklonia* and *Eisenia* are the mostly studied brown algae for the biological activities associated with phlorotannins. The *E. cava* extract has the capability to reduce the expression of MMP-2 and MMP-9. An *in vitro* study on the effect of *E. cava* extract in HDF cells (HT1080) has revealed that the extract which was rich in phlorotannins has attenuated the expression of MMP-2 and MMP-9 expression (*Kim et al.*, 2006). Interestingly, this *in vitro* study has revealed that the phlorotannins did not exhibit any cytotoxicity on the cells and has the MMP inhibitory effect almost same to that of doxycyclin (a commercial MMP inhibitor). Similarly, phlorotannins namely fucofuroeckol-A and eckol that are derived from *Eisenia bicyclis* had shown MMP-2 and MMP-9 inhibitory activities in HT1080 cells. In this study, it has been suggested that fucofuroeckol-A and eckol exhibited a significant inhibitory activity on the NF-κB expression, thus inhibiting the expression of MMP-2 and MMP-9 via blocking the transcription of both NF-κB and AP-1 (*Lee*, 2010). As the investigations suggest that MMP-2 (Gelatinase-A) and MMP-9 (Gelatinase-B) can degrade type IV collagen of base membranes and are known to play a crucial role in cancer invasion such as oral carcinoma and other cancers (*Ikebe et al.*, 1999) and that these enzymes play a major role in cancer metastasis (*Nagase et al.*, 1998), the MMP downregulation effect of phlorotannins and polyphenolic compounds from marine brown algae stands more promising and recommending them as potential medicinal foods to combat metastasis.

The phlorotannins eckol and dieckol that were first isolated from *Ecklonia* species possess oligomeric polyphenol of phloroglucinol unit. Dieckol from marine brown alga, *E. cava*, has been reported to suppress LPS-induced production of nitric oxide (NO) and prostaglandin E₂ (PGE₂) and expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in murine BV2 microglia, thus establishing dieckol as a potent anti-inflammatory and neuroprotective agent (*Jung et al.*, 2009). A detailed account of the various biological activities including free radical scavenging activity of phlorotannins from *Ecklonia* species has been reported earlier.

### III. MARINE ALGAL FUCOIDANS AS POTENTIAL MMPIs

Marine algae are reported to produce different polysaccharides including alginates, laminarans, and fucoidans. They usually contain large proportions of L-fucose and sulfate, together with minor amounts of other sugars such as xylose, galactose, mannose, and glucuronic acid. These algal
polysaccharides have been attributed with many biological activities such as anticoagulant, antithrombotic, antitumoral, and antiviral activities. Especially fucoidans from marine algae have been reported to exhibit outstanding biological activities that aid for human health. Fucoidans (Fig. 10.2) are sulfated polysaccharides that are exclusively found in seaweeds in their cell wall. This polysaccharide ingredient is composed of polymer of α1 → 3-linked 1-fucose with sulfate groups on some of the fucose residues at the four positions (Patankar et al., 1993). Recently, fucoidan is being studied extensively due to potential antitumor, antiviral, anticomplement, and anti-inflammatory activities (Chizhov et al., 1999). Brown algae-derived fucoidan has been reported to show strong inhibition ability on UVB-induced MMP-1 expression in vitro. In an investigation by Moon et al., human skin fibroblast (HS68) cells were pretreated by various concentrations of fucoidan and then subjected to UVB irradiation (100 mJ/cm²). As it is known that UVB irradiation induces the production of MMPs by activating cellular signaling transduction pathways, which are responsible for the degradation or synthesis inhibition of collagenous extracellular matrix in connective tissues, causing skin photoaging. Their results have suggested that fucoidan from algae has successfully inhibited the expression of MMP-1 by the suppression of extracellular signal-regulated kinase (ERK). Moreover, in fucoidan-treated cells, the expression of MMP-1 mRNA has been significantly reduced (Moon et al., 2008). As brown edible algae are considered as dietary food stuff, the consumption of brown algae that are rich in fucoidan could be beneficial in reducing the risk of MMP-related diseases.

Similarly, another research group reported the MMP inhibitory effect of a 16-kDa fucoidan fraction from seaweeds on the parameters involved

![FIGURE 10.2 Chemical structure of fucoidan unit.](image-url)
MMP Inhibitors from Marine Macroalgae

in connective tissue breakdown. It was observed that this 16 kDa fucoidan was able to successfully inhibit the gelatinase A secretion and stromelysin 1 induction by interleukin-1β on dermal fibroblasts *in vitro*. In addition, *ex vivo* studies using the tissue sections of human skin have revealed that this polysaccharide was able to minimize human leukocyte elastase activity resulting in the protection of human skin elastic fiber network against the enzymatic proteolysis due to this serine proteinase (Senni et al., 2006). These findings clearly suggest the potential role of seaweed fucoidans in reducing the risk of some inflammatory pathologies that involve extracellular matrix degradation by MMPs. Usually, high molecular weight (HMW) fucoidans are known to bind to the growth factors, such as fibroblast growth factor (FGFs), and protect them from proteolysis (Belford et al., 1993). The therapeutic ability of fucoidans thought to be associated with the fact that they can release the glycosaminoglycan-bound stromal-derived factor-1 (SDF-1) from its tissue storage sites. SDF-1 mobilizes medullary progenitors which could participate in angiogenesis with vascular endothelial growth factor (VEGF) and FGF (Salvucci et al., 2002; Sellke et al., 1996). A fraction of low molecular weight (LMW) fucoidan (7 ± 2 kDa) obtained by radical depolymerization of HMW extracts from brown seaweed have been reported to promote therapeutic revascularization in a rat model of critical hindlimb ischemia (Luyt et al., 2003). Normally, MMP-9 plays an important role in both animal models of cerebral ischemia and human stroke. The expression of MMP-9 is elevated after cerebral ischemia which is involved in accelerating matrix degradation, disrupting the blood–brain barrier, increasing the infarct size, and relating to hemorrhagic transformation (Dong et al., 2009). The therapeutic ability of seaweed fucoidans would be a best option in managing the MMP-associated cerebral ischemia.

Fucoidans isolated from *Costaria costata* have been reported to possess the MMP inhibition activities. *In vitro* study using the immortalized human keratinocyte (HaCaT) cell line pretreated with *C. costata*-derived fucoidans has shown a significant decrease in the UVB-induced MMP-1 expression. Moreover, fucoidan has significantly reduced the expression MMP-1 mRNA and inhibited UVB-induced MMP-1 promoter activity by 37.3%, 53.3%, and 58.5% at 0.01, 0.1, and 1 μg/mL, respectively, compared to UVB irradiation alone (Moon et al., 2009). Fucoidan extracts from seaweed *Cladosiphon novae-caledoniae* Kylin (Mozuku) have reduced the cellular invasiveness in human fibrosarcoma HT1080 cells by suppressing the activity of MMP-2 and MMP-9. Further, it has been reported that these fucoidan extracts suppressed the expression and secretion of an angiogenesis factor, VEGF, thereby reporting the inhibitory effects on invasion and angiogenesis of tumor cells (Ye et al., 2005). Another research group has reported that fucoidan exerts its antiproliferative action. *In vitro* studies on the cultured AGS human gastric adenocarcinoma cells treated
with fucoidans suggest that fucoidan effectively inhibits the growth of AGS cells by inducing autophagy, as well as apoptosis. They have also reported that the downregulation of antiapoptotic Bcl-2 and Bcl-xL expression, loss of mitochondrial membrane potential, activation of caspases, and concomitant degradation of poly-(ADP-ribose) polymerase protein are involved in the fucoidan-induced apoptosis (Park et al., 2011). Thus, fucoidans can be of great potential in controlling the expressions of MMPs that regulate the cell proliferation and metastasis and hence can be better dietary supplements in managing cancers (Fig. 10.3).

**IV. CONCLUSIONS AND FURTHER PROSPECTS**

The recent scientific investigations have unleashed the prominent role of metalloproteinases in several human-related pathological conditions, and the drawbacks of the currently available synthetic MMPIs encourage the present day researchers to explore natural resources for effective MMPIs. Moreover, the shortcomings of synthetic MMPIs like nonspecific selectivity, improper metabolization, and undesirable side effects also demand the researchers to screen for the MMPIs from natural resources. Until now, many therapeutic compounds have been reported from terrestrial organisms. But, the oceans’ flora and fauna are exposed to harsh environment and are equipped with variety biochemical responses to evade potential threats they encounter in marine locales. For performing such unique responses, the marine organisms produce unique biologically
active components that are structurally and functionally different from terrestrial organisms. Among these, phlorotannins and fucoidans are specifically found in the marine algae. And the above discussed *in vitro*, *in vivo* reports clearly suggest the effectiveness of phlorotannins and fucoidans from brown algae in proper downregulation of MMPs and related pathological effects. Moreover, as brown algae are considered as a dietary supplement, it could be recommended that consumption of these marine brown algae could be helpful in the proper management of imbalanced MMP expressions and thus can be considered as medicinal foods. Further, until now, majority of the phlorotannins and fucoidans reported were from the members of the species *Ecklonia* and *Eisenia*. Many more brown algal members have to be screened for novel phlorotannins and polysaccharide derivatives that can be recommended as potential MMPIs.

**REFERENCES**


CHAPTER 11

Protective Effect of Polysaccharide from *Hizikia fusiformis* Against Ethanol-Induced Toxicity

Hye-Jung Hwang, In-Hye Kim, and Taek-Jeong Nam

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Abstract

Polysaccharide extracted from *Hizikia fusiformis* (Hf-PS-1) exhibited protective effects against ethanol-induced peptic injury. In *in vivo* assay, the ethanol group exhibited decrease of total glutathione (GSH) and increase of jun N-terminal kinase (JNK) phosphorylation relative to the control group, whereas levels were significantly increased and decreased, respectively, in the Hf-PS-1 group. Hf-PS-1
reduced ethanol-induced gastric injury. In *in vitro* assay, ethanol induced IEC-6 cells’ death in a dose-dependent manner. Ethanol decreased the phosphorylation of Shc and the binding of Grb2 to Shc, and Hf-PS-1 pretreatment increased them. Ethanol also induced the phosphorylation of JNK and extracellular signal-regulated kinase (ERK), whereas Hf-PS-1 pretreatment decreased JNK activation but not ERK. Co-treatment with JNK inhibitor and ethanol decreased GSH levels, indicating that JNK phosphorylation is a critical factor during ethanol-induced injury. Therefore, Hf-PS-1 may be useful to protect against ethanol-induced gastrointestinal injury.

I. INTRODUCTION

Marine algae have provided great biological diversity for sampling in the discovery phase of drug development (Munro *et al.*, 1987). Marine organisms in general have been an important source of compounds with potential antiviral and anticancer activities (Von Vaupel Klein, 1987). Certain seaweeds are not only significant sources of essential proteins, vitamins, and minerals, but several species of algae also produce or contain secondary metabolites, polysaccharides, and glycoproteins with antitumor, antiviral, or immunostimulatory activity (Sheu *et al.*, 1996; Shin *et al.*, 2006; Yamamoto *et al.*, 1987). Yuan and Walsh (2006) reported that extracts of a variety of edible seaweeds had antioxidative and antiproliferative activities, while Bae and Choi (2007) suggested that a methanol extract of the seaweed *Gloiopeltis furcata* induced G2/M arrest and inhibited cyclooxygenase-2 activity in HepG2 cells. Furthermore, Kang *et al.* (2005) and Ara *et al.* (2005) reported the biological activities of ethanol extracts from *Callophyllis japonica* and *Spatoglossum asperum*, respectively.

Gastrointestinal disorders such as gastric and peptic ulcers, inflammation of gastric mucosa, and gastritis are important causes of human morbidity in nonindustrialized countries. The pathophysiology of gastric ulcers is dependent on the balance between aggressive and protective factors in the stomach. When aggressive factors such as acid–pepsin, secretin, and mental stress predominate over protective factors like cellular regeneration, prostaglandins, and epidermal growth factors (EGFs), ulcers are likely to be present. Many pharmaceutical products have been developed for the treatment of gastrointestinal symptoms such as hemorrhages and perforations (Higham *et al.*, 2002). However, despite recent pharmaceutical advances, many pharmaceutical products are relatively expensive and associated with various medical problems. Drugs that relieve pain, heal ulcers, delay ulcer recurrences, and even cure disease have been developed, but they generally have important side effects.
Hence, in the search for effective treatments for gastrointestinal disease, many researchers have begun to investigate the natural products that are less likely to produce side effects.

Various botanical products such as _Laurus nobilis_ seeds (Afifi et al., 1997), _Anchusa strigosa_ roots (Disi et al., 1998), _Camellia sinensis_ (Maity et al., 1995), and _Picrorhiza kurroa_ (Banerjee et al., 2008) possess antiulcer properties. Borrelli and Izzo (2000) suggested that an extensive number of chemical compounds isolated from medicinal plants have such properties. _Hizikia fusiformis_ is a widely consumed brown alga in the coastal regions of Korea, China, and Japan. This alga possesses a number of potential compounds, including antioxidants (Siriwardhana et al., 2004) and anticoagulants (Kim et al., 1998). It also contains inorganic arsenic, which is carcinogenic to humans (Nakamura et al., 2008; Watanabe et al., 1979). Furthermore, the extracts of _H. fusiformis_ markedly stimulate the proliferation of human lymphocytes (Shan et al., 1999). Brown seaweeds also contain various soluble polysaccharides in the form of alginates, fucans, and laminarins (Lahaye and Kaeffer, 1997; Mabeau and Kloareg, 1987). For example, Li et al. (2006) verified the precise structure of a fucoidan obtained from the hot water extract of _H. fusiformis_. Although many studies have been conducted on this species, the health effects of dietary _H. fusiformis_ remain scientifically unclear, especially with regard to its possible protective effects.

Several investigators have described the use of medicinal plants against ulcer diseases in traditional medicine (Afifi et al., 1997; Borrelli and Izzo, 2000; Disi et al., 1998; Maity et al., 1995; Schmeda-Hirschmann and Yesilada, 2005).

Seaweeds have been widely used as a food source and in medicine; their consumption has been strongly promoted for children and pregnant women as well-balanced, harmless, and natural sources of highly bioavailable trace elements (Booth, 1964). The reason is that marine organisms have proven to be rich sources of structurally novel and biologically active natural compounds. These compounds have served as important chemical compounds for the discovery of new drugs in the treatment of various human diseases (Usami, 2009; Zhang and Kim, 2009). Yang et al. (2010) suggested that the extracts from _Laurencia okamurae_, _Garrya elliptica_, _Spiraea thunbergii_, _G. furcata_, and _H. fusiformis_ may be considered as possible candidates for anti-inflammatory agents. They revealed that extracts should inhibit the production of proinflammatory mediators such as nitric oxide, prostaglandin E2, interleukin-6, and tumor necrosis factor-α. Especially, Shan et al. (1999) determined the effect of eight seaweed extracts on human lymphocytes, among them, the activity of _H. fusiformis_ associated with polysaccharides which were extracted with ethanol. In this chapter, potential effects of polysaccharide from _H. fusiformis_, Hf-PS-1, against ethanol-induced peptic injury in rats is discussed.
II. THE EFFECT OF HF-PS-1 AGAINST ETHANOL-INDUCED GASTRIC DAMAGE IN RATS

A. Preparation and effect of Hf-PS-1 in SD rats

The abuse of ethanol is associated with detrimental effects on several bodily organs and is one of several factors causing gastrointestinal disorders. For the treatment of gastrointestinal symptoms such as ulcerative hemorrhages and perforations, many pharmaceutical products (e.g., non-steroidal anti-inflammatory drugs, NSAIDs) have been developed (Higham et al., 2002). However, NSAID group presents many important medical problems relating to their expense and side effects, despite recent pharmaceutical advances that have generally improved their therapeutic effects. Therefore, many researchers are investigating the natural materials that have pharmaceutical effects with fewer side effects. Among these natural materials, seaweed compounds, many of which are polysaccharides, have been studied for their various pharmaceutical effects and diverse biological activities. Sulfated galactans from the red marine alga Champia feldmannii show acute anti-inflammation, anticoagulation, and antinociceptive activities (Assreuy et al., 2008). Hong et al. (1997) reported that porphyran from Porphyra yezoensis decreases cholesterol levels and possesses antimicrobial and antitumor activities. Fucoidans from Ecklonia kurome have anticoagulation and antitumor properties (Nishino et al., 1991).

Brown seaweeds contain various soluble polysaccharides, including alginates, fucans, and laminarins, together with the insoluble fibers made of cellulose (Lahaye and Kaeffer, 1997; Mabeau and Kloareg, 1987). Recent studies have suggested that H. fusiformis contains a variety of biological benefits including antioxidative properties (Kim et al., 1998) and immune modulation (Okai et al., 1998). We hypothesized that the polysaccharides from H. fusiformis have some biological effects.

The powdered H. fusiformis sample consisted of 6.7 ± 0.0% moisture, 11.8 ± 0.0% protein, 1.8 ± 0.3% fat, 35.1 ± 0.1% ash, and 17.5 ± 0.0% salt. A 3.527 g of the crude polysaccharide extract, Hf-PS, which contained 0.073 g protein, was isolated. The protein level decreased to 0.011 g after pronase treatment to produce Hf-PS-1 (Fig. 11.1). The powdered Hf-PS-1 contained 7.34 ± 1.16% moisture and 76.01 ± 1.00% carbohydrate, but no detectable protein or fat (Table 11.1). The samples were fractionated by electrophoresis on agarose gels, and polysaccharide was visualized by toluidine blue staining (Fig. 11.2; upper band Hf-PS-1, lower band Hf-PS).

Ethanol is commonly used to study gastrointestinal damage (Birdane et al., 2007; Hernandez-Munoz et al., 2000). Therefore, gastrointestinal damage was induced using a simple ethanol treatment in SD rats. For the most part, no significant changes in total body and gastric weights among the three experimental groups of animals were observed.
**FIGURE 11.1** Purification procedures of Hf-PS-1 from *H. fusiformis*. 

1. **Hizikia fusiformis**
   - Washing
   - Hot air drying (50°C)
   - Homogenization
   - Extraction (100°C, 12hr)
   - Centrifugation (8,000rpm, 30min)

2. **Supernatant**
   - added ethanol
   - precipitation
   - Incubation (3hr)
   - Centrifugation (2,500rpm, 20min)

3. **Supernatant**
   - Hot air drying (50°C)
   - Homogenization
   - Hf–PS

4. **Pronase digest (37°C, 20hr)**
   - Incubation (4°C, 12hr)
   - Centrifugation (2,500rpm, 20min)

5. **Supernatant**
   - added ethanol
   - precipitation
   - Incubation (3hr)
   - Centrifugation (2,500rpm, 20min)

6. **Supernatant**
   - Hot air drying (50°C)
   - Homogenization
   - Hf–PS–1

---

The Effect of a Polysaccharides from *H. fusiformis*
In long-term experiments (3 weeks), the feed efficiency ratio decreased in ethanol-treated animals because digestion capacity, absorption capacity, and caloric efficiency declined (Ko et al., 2002), but in short-term experiments like ours, body weight changes were not observed (Kim et al., 2004; Park et al., 2005). Ethanol intake produces pathological states such as infection of the gastric mucosa, gastritis, and gastric and acute peptic ulcers (Franke et al., 2005; Hernandez-Munoz et al., 2000; Lee et al., 2000; Taylor and Rehm, 2005). The intestinal surface of stomach samples derived from rats in the ethanol-only group indicated hemorrhaging and other damage that was not evident in samples from the control group (Fig. 11.3). Comparable samples from the ethanol + Hf-PS-1 group exhibited the least signs of gastric damage.

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>Crude lipid</th>
<th>Crude protein</th>
<th>Crude ash</th>
<th>Carbohydrate&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tr>
<td><em>H. fusiformis</em></td>
<td>4.27 ± 0.12</td>
<td>1.76 ± 0.07</td>
<td>12.94 ± 3.61</td>
<td>19.18 ± 0.09</td>
<td>61.85 ± 3.56</td>
</tr>
<tr>
<td>Hf-PS-1</td>
<td>7.34 ± 1.16&lt;sup&gt;<em>&lt;/sup&gt; Trace&lt;sup&gt;</em>&lt;/sup&gt; Trace&lt;sup&gt;<em>&lt;/sup&gt; 16.65 ± 0.16&lt;sup&gt;</em>&lt;/sup&gt; 76.01 ± 1.00&lt;sup&gt;*&lt;/sup&gt;</td>
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Results are means ± SD (n = 3) on wet weight basis. Significant differences between the means were determined by an independent t-test.

* Means significant (P < 0.05).
<sup>a</sup> 100 – (moisture + crude lipid + crude protein + crude ash).

**FIGURE 11.2** Electrophoresis bands of Hf-PS-1. Hf-PS-1 was separated by 0.6% agarose gel electrophoresis and analyzed by toluidine blue staining for polysaccharide.
The epithelial cells of the gastric surface produce mucus that protects against gastric mucosal injury from ethanol, NSAIDs, and other substances (Allen et al., 1986; Hingson and Ito, 1971). Generally speaking, corrosion of the gastric mucosal membrane is not directly related to ulceration because of epithelial cell restoration of the necrotic state with mucus and fibrin (Lacy and Ito, 1984; Morris and Wallace, 1981). At the epithelial surface, mucus cannot effectively protect against harmful agents such as ethanol or aspirin (Allen et al., 1986). Ethanol induced surface epithelial cell destruction and loss of the surface mucosa layer compared to the control group, but co-treatment with Hf-PS-1 had protective effects against these damages (Fig. 11.4).

Caspases exist as inactive precursors known as procaspases. When procaspases are cleaved and activated, they induce DNA fragmentation and death receptor activation. Caspase-3 is an effector caspase that cleaves other protein substrates under apoptotic processes, and caspases 8 and 9 are the initiator caspases that cleave inactive proforms of effectors. Caspase-8 is activated in the formation of death-inducing signaling complex, and caspase-9 is activated within the apoptosome (Kaufmann and Earnshaw, 2000). Caspase cascades include death pathways and mitochondrial pathways. Caspase-9 is related to the mitochondria-dependent apoptosis and activates caspase-3. Furthermore, PARP is a primary substrate of caspase-3. In agreement with the apoptotic results described above, exposure to 40% ethanol at 80 ml/kg body weight resulted in significant activation of caspases 3, 8, and 9, and co-treatment with Hf-PS-1 inhibited this activation (Fig. 11.5). Additionally, ethanol administration produces reactive oxidants and oxidant-induced DNA fragmentation (Sehirli et al., 2008). In our experiment, ethanol treatment induced DNA fragmentation but co-treatment with Hf-PS-1 did not (Fig. 11.6).
B. Protective effect of Hf-PS-1 against ethanol-induced oxidative stress

Glutathione (GSH), a tripeptide found in many mammalian tissues, plays a major protective role as a scavenger of free radicals that combine with nonprotein thiols at the GSH reactive center to abolish free radical toxicity.
Antioxidation by GSH protects the body from many diseases and conditions such as damage by $H_2O_2$, ethanol, and numerous other insults and toxins. GSH levels decreased by 34% in the ethanol-only group relative to the control group (100%), whereas they increased to 103.9% in the ethanol + Hf-PS-1 group (Fig. 11.7).

Mitogen-activated protein kinases (MAPKs) mediate apoptosis and cell growth, and jun N-terminal kinase (JNK) in particular is activated by oxidative stress. Yang et al. (2008) suggested that JNKs/SAPKs and p38 MAPK are classic oxidative stress-activated protein kinases, and Kim et al. (2004) observed that treatment with selenite increased intracellular reactive oxygen species (ROS) levels and JNK1 phosphorylation in Chang liver cells.
Zhang et al. (2007) reported the ethanol-induced oxidative stress and further activation of the MAPKs, JNK, extracellular signal-regulated kinase (ERK), and p38 kinase. In agreement with these results and others (e.g., Villegas et al., 2006), JNK was activated in the ethanol-only treated group. Co-treatment with Hf-PS-1 and ethanol decreased JNK activation, but phospho-ERK levels were not significantly different among the three groups. These results suggested that the protective effect of Hf-PS-1 was primarily associated with the inhibition of JNK phosphorylation (Fig. 11.8).

III. EFFECTS OF HF-PS-1 AGAINST ETHANOL-INDUCED INJURY TO IEC-6 CELLS

A. Hf-PS-1 protects the ethanol-induced injury to IEC-6 cells

MTS assays of IEC-6 cells after exposure to 100–1000 μg/ml Hf-PS or Hf-PS-1 showed no cytotoxicity (Fig. 11.9). Furthermore, Hf-PS-1, but not Hf-PS, appeared to protect against ethanol-induced cytotoxicity (Fig. 11.10A). When cell viability assays were performed on cells after exposure to 0–500 μg/ml Hf-PS-1 for 3–24 h followed by exposure to 5% ethanol for 1 h, Hf-PS-1 was found to have a concentration- and time-dependent protective effect (Fig. 11.10B). Results of MTS assays showed that ethanol treatment induced the death of the IEC-6 cells and that pretreatment with Hf-PS-1 for 6–24 h counteracted this effect. Morphological studies also confirmed protection against ethanol toxicity by Hf-PS-1. Ethanol treatment induced cell shrinkage and decreased the number of viable cells (Fig. 11.11), but Hf-PS-1 pretreatment inhibited this damage. These results indicate that Hf-PS-1 pretreatment is protective against ethanol-induced cell death.
FIGURE 11.9 Effect of Hf-PS and Hf-PS-1 on cell viability. IEC-6 cells were incubated with Hf-PS or Hf-PS-1 at the indicated concentrations for 24 h. The results indicated mean ± SD in three independent experiments. Different alphabets are significant values among the group by Duncan’s multiple range test.

FIGURE 11.10 Protective effect of Hf-PS-1 on ethanol-induced damage. (A, B) Cells were incubated with SFM or Hf-PS-1 at the indicated concentrations and times and treated ethanol for 1 h. Cell viability was measured with MTS assay kit as manufacturer’s instruction. Data were represented % of control (100%). Values are the mean ± SD. Different alphabets are significant values among the group by Duncan’s multiple range test. (C) Cell morphology (400×). ‘a’ control; ‘b’ treated only ethanol; ‘c’ treated ethanol (5%, 1 h) after Hf-PS-1 (500 μg/ml) pretreatment.
B. Hf-PS-1 inhibits ethanol-induced damage by downregulating JNK

Since binding of insulin-like growth factor-I (IGF-I) to its receptor, IGF-IR, activates multiple signal transduction cascades by activating tyrosine phosphorylation, whether IGF-IR was phosphorylated in response to ethanol and Hf-PS-1 was examined. Oh et al. (2008) recently demonstrated that ethanol treatment causes IGF-IR activation by inducing the expression and secretion of IGF-1. When IGF-1 binds to IGF-IR, the latter becomes phosphorylated on tyrosine residues, with effects on Shc and MAPKs. In studying the mechanism of ethanol-induced damage, the MAPK signaling pathway was focused, which has been proposed as an important part of the mechanism (Lee et al., 2007; Zhou et al., 2005). The oxidative metabolism of ethanol elicits the production of ROS and other oxidative mediators associated with MAPK signaling. For example, ethanol-induced generation of ROS affects MAPKs in hepatocytes (Aroor and Shukla, 2004; Lee and Shukla, 2005; Lee et al., 2006; Lieber, 2000;
Venugopal et al., 2007; Wang et al., 2008; Zhang et al., 2007). Although ethanol pretreatment induced IGF-IR activation, Hf-PS-1 pretreatment enhanced this activation. In contrast, Shc phosphorylation and the association between Shc and Grb2 were decreased by ethanol treatment, but increased by Hf-PS-1 pretreatment (Fig. 11.12). Activated Shc binds the adaptor protein Grb2 in an IRS-1-independent manner, leading to the activation of the MAPK pathway. Because several investigators have suggested that ethanol affects the MAPK signaling cascade, the possible activation of ERK1/2, JNK, and p38 were investigated. Ethanol treatment induced JNK and ERK phosphorylation, and Hf-PS-1 pretreatment decreased JNK activation (Fig. 11.13), but p38 was not detected.

Moreover, GSH levels were observed to determine if Hf-PS-1 influenced the effect of ethanol metabolism on cellular redox status. Since a Western blot analysis demonstrated the activation of JNK by ethanol, the relationship between ethanol toxicity and JNK activation using the JNK inhibitor SP600125 was examined and found that ethanol treatment decreased GSH levels to 72.72% (±14.84%) of the control level, but cotreatment with ethanol and either Hf-PS-1 or SP600125 increased GSH levels to 118.18% (±10.49%) or 95.45% (±9.09%), respectively, of the control level (Fig. 11.14). These results suggest that Hf-PS-1 protects against ethanol-induced damage by inhibiting JNK phosphorylation and that JNK plays an important role in ethanol-induced toxicity.

**FIGURE 11.12** Effects of Hf-PS-1 on MAPK signaling pathway in IEC-6 cells. Cells were pretreated with Hf-PS-1 (500 μg/ml) and ethanol (5%) or with ethanol only (5%) after incubation with SFM for 12 h. Whole-cell extracts were prepared and analyzed by Western blotting using anti-phospho-ERK1/2, anti-phospho-JNK, anti-phospho p38, and anti-β-actin antibodies. This gel photograph is representative of three experiments. Values are the mean ± SD. Different alphabets are significant values among the group by Duncan’s multiple range test.
A scheme summarizing the findings in a study is presented in Fig. 11.14. Ethanol induces the phosphorylation of IGF-IR but decreases the phosphorylation of Shc and the association between Shc and Grb2. Furthermore, although ethanol induces phosphorylation of JNK and ERK, Hf-PS-1 pretreatment decreases the phosphorylation of JNK but not of

**FIGURE 11.13** Effect of Hf-PS-1 on ethanol-induced oxidative stress. (A) GSH levels were measured as described in the materials and methods. Data were represented % of control (100%). Values are the mean ± SD. Different alphabets are significant values among the group by Duncan’s multiple range test. (B) JNK phosphorylation by Hf-PS-1 or SP600125 pretreatment. Whole-cell extracts were prepared and analyzed by Western blotting using anti-phospho-JNK and anti-β-actin antibodies. This gel photograph is representative of three experiments.

**FIGURE 11.14** The proposed model of Hf-PS-1’s protective mechanism on ethanol-induced damage in IEC-6 cells.

A scheme summarizing the findings in a study is presented in Fig. 11.14. Ethanol induces the phosphorylation of IGF-IR but decreases the phosphorylation of Shc and the association between Shc and Grb2. Furthermore, although ethanol induces phosphorylation of JNK and ERK, Hf-PS-1 pretreatment decreases the phosphorylation of JNK but not of
ERK. Since the JNK inhibitor SP600125 inhibits ethanol-induced oxidative stress, the JNK activation is a critical factor in ethanol cytotoxicity. Hf-PS-1 pretreatment suppresses ethanol-induced cell death by increasing the phosphorylation of Shc and its association with Grb2 and by decreasing JNK phosphorylation. Therefore, the effect of Hf-PS-1 against ethanol-induced damage is a result of JNK downregulation. Hence, we suggest that Hf-PS-1 might provide a new, natural treatment option for ethanol-induced gastric damage in humans.

IV. CONCLUSION

Misuse of ethanol is associated with detrimental effects on several body organs and is one of several predisposing factors for gastrointestinal disorders. The pathophysiology of the gastric ulcer centers on an imbalance between aggressive and protective factors in the stomach; when aggressive factors (such as secretion of acid, pepsin, and emotional stress) have stronger effects than those of protective factors (such as cellular regeneration, prostaglandins, and EGF), gastric ulcer may occur. Many pharmaceutical products have been developed to treat gastrointestinal diseases such as ulcer hemorrhage and perforation (Higham et al., 2002). An increase in these disorders has been attributed to the increased use of NSAIDs, which have significant side effects despite the large amount of money and effort poured into their development by pharmaceutical companies. Hence, there is a need for new therapeutic compounds without side effects.

Polysaccharide extracted from *H. fusiformis* (Hf-PS-1) exhibited protective effects against ethanol-induced peptic injury and related mechanisms in rats. Experimental animals were divided into three groups: control, ethanol-only, and ethanol + Hf-PS-1. The ethanol-only group exhibited decreased levels of total GSH and increased levels of JNK phosphorylation relative to the control group, whereas levels were significantly increased and decreased, respectively, in the ethanol + Hf-PS-1 group. The ethanol-only group also exhibited increased levels of ERK 1/2 phosphorylation relative to the control group; these levels were not significantly different in the ethanol + Hf-PS-1 group. Hf-PS-1 appeared to reduce ethanol-induced gastric injury.

The signaling pathways related to the ethanol-protective effect of Hf-PS-1 in IEC-6 cells. Ethanol induced the death of IEC-6 cells in a dose-dependent manner, and pretreatment with Hf-PS-1 abrogated the ethanol toxicity. When examined whether the effect of Hf-PS-1 on ethanol cytotoxicity was associated with IGF-IR signaling pathways, involving MAPK, it was found that ethanol treatment decreased the phosphorylation of Shc and the binding of Grb2 to Shc, and Hf-PS-1 pretreatment
increased them. Ethanol treatment also induced the phosphorylation of JNK and ERK, whereas Hf-PS-1 pretreatment decreased JNK activation but not ERK activation. Using a JNK inhibitor (SP600125), GSH levels were evaluated to determine whether Hf-PS-1 pretreatment might protect against ethanol-induced gastric intestinal damage by downregulating JNK. Co-treatment with SP600125 and ethanol decreased GSH levels, indicating that JNK phosphorylation is a critical factor during ethanol-induced injury and that the effect of Hf-PS-1 occurs via JNK downregulation. Therefore, Hf-PS-1 may be useful as a biofunctional food source to protect against ethanol-induced gastrointestinal injury.

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Functional Properties of Brown Algal Sulfated Polysaccharides, Fucoidans

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

Abstract

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

I. INTRODUCTION

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

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

II. SULFATED POLYSACCHARIDES, FUCOIDANS

Polysaccharides widely exist in animals, plants, microorganisms, and algae (Yang and Zhang, 2009). They are polymeric carbohydrate structures, usually composed of various monosaccharides linked with different glucosidic bonds. Depending on the structure, polysaccharides can have distinct
functional properties from their building blocks. Sulfated polysaccharides are among the most abundant and broadly studied polysaccharides from nonanimal origin (Pereira et al., 2002). They are widespread in nature. Seaweeds are abundant source of sulfated polysaccharides with various biological activities. Therefore, sulfated polysaccharides are of special interest. Most naturally occurring sulfated polysaccharides are complex mixtures of molecules showing wide variations in their structure as well as their activities (Alban et al., 2002).

Fucoidan (Fig. 12.1) is a sulfated polysaccharide mainly found in the cell-wall matrix of various brown seaweed species (Kim et al., 2010a; Teruya et al., 2007). It contains substantial percentages of l-fucose and sulfate ester groups (Jiang et al., 2010; Li et al., 2008; Matou et al., 2002). Fucose is a hexose deoxy sugar with the chemical formula C₆H₁₂O₅ and is the fundamental subunit of the fucoidan polysaccharide. For the past decade, fucoidan has been extensively studied due to its numerous biological activities. Recently, researches for new drugs have raised interest in fucoidans. In the past few years, several fucoidans’ structures have been isolated and many aspects of their biological activity have been elucidated (Li et al., 2008).

III. PURIFICATION OF FUCOIDAN FROM BROWN SEAWEEDS

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

![Chemical structure of fucoidans.](image-url)
the isolation of active components from plant materials. In addition, extraction is influenced by the chemical nature of the components, the extraction method employed, and the presence of interfering substances (Chirinos et al., 2007).

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

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

IV. BIOLOGICAL ACTIVITIES OF FUCOIDANS

Fucose-containing sulfated polysaccharides from brown seaweeds might exhibit interesting biological properties (Matsuhiro et al., 1996). The profound functional properties of the sulfated polysaccharides are probably due to the presence of sulfate groups in varying amounts. In addition,
positions of the sulfated groups along the macromolecular backbone also play a vital role in their biological activities. Among the sulfated polysaccharides, fucoidans found in seaweeds are well known to have numerous biological activities (Fig. 12.2) and the potent biological properties of fucoidans seem to be determined by their high degree of sulfation, fine structure, and molecular weight (Jiang et al., 2010; Zvyagintseva et al., 2003). However, the composition of algal fucans varies according to several factors such as species, extraction procedure, season of harvest, and climatic conditions (Dietrich et al., 1995; Grauffel et al., 1989). Thus, each newly isolated and described fucan is a unique compound with unique structural features, consequently having the potential of being used as novel pharmaceuticals (Silva et al., 2005). Table 12.1 provides a summary of biological activities of fucose-rich sulfated polysaccharides and fucoidan isolated from various brown seaweeds.

A. Anticoagulant and antithrombotic activity

Anticoagulants are substances that prevent coagulation; that is, they stop blood from clotting (Desai, 2004). Therefore, they are a group of pharmaceuticals that can be used in vivo as a medication for thrombotic disorders. Heparin, a highly sulfated polysaccharide present in mammalian tissues, is one of the commonly used drugs of the choice in prevention of thromboembolic disorders (Lee et al., 2008b). However, there are some well-documented problems related to its clinical application (Alban et al., 2002). Therefore recently, alternative drugs for heparin are in high demand due to its bad and long-term side effects (Athukorala et al., 2005).
Over the years, isolation and purification of natural sulfated polysaccharides responsible for anticoagulant activity from different seaweed species had been reported (De Zoisa et al., 2008). The ability of sulfated polysaccharides to interfere with biological systems has a longstanding record, as illustrated with heparin (Huynh et al., 2001). In addition, anticoagulant and antithrombotic activities are among the most widely studied properties of sulfated polysaccharides.

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

<table>
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<tr>
<th>Seaweed species</th>
<th>Biological activity</th>
<th>Reference</th>
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<tr>
<td><em>E. cava</em></td>
<td>Anticoagulant (<em>in vitro</em>)</td>
<td>Athukorala et al. (2006)</td>
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<td><em>E. cava</em></td>
<td>Antithrombotic</td>
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<td><em>F. evanescens</em></td>
<td>Anticoagulant</td>
<td>Kuznetsova et al. (2003)</td>
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<td><em>E. cava</em></td>
<td>Anticoagulant (<em>in vivo</em>)</td>
<td>Wijesinghe et al. (2011)</td>
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<td><em>P. gymnospora</em></td>
<td>Anticoagulant</td>
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<td><em>A. nodosum</em></td>
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<td>Anticoagulant</td>
<td>Soeda et al. (1992)</td>
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<td><em>S. fulvellum</em></td>
<td>Anticoagulant</td>
<td>De Zoisa et al. (2008)</td>
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<td><em>Laminaria cichorioides</em></td>
<td>Anticoagulant</td>
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<td><em>U. pinnatifida</em></td>
<td>Antitumor</td>
<td>Synytsya et al. (2010)</td>
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<td><em>E. cava</em></td>
<td>Antiproliferation</td>
<td>Athukorala et al. (2009)</td>
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<td><em>F. evanescens</em></td>
<td>Antitumor and antitumorigenic</td>
<td>Alekseyenko et al. (2007)</td>
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<td><em>L. guryanovae</em></td>
<td>Anticancer</td>
<td>Lee et al. (2008a)</td>
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<td><em>F. vesiculosa</em></td>
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<td><em>L. japonica</em></td>
<td>Anti-inflammatory</td>
<td>Li et al. (2011)</td>
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<td><em>E. cava</em></td>
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<td><em>A. nodosum</em></td>
<td>Angiogenesis</td>
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<td><em>U. pinnatifida</em></td>
<td>Antiviral</td>
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including activated partial thromboplastin time, thrombin time, and prothrombin time. According to their results, the pure compound showed almost similar anticoagulant activity to that of heparin. Further study demonstrated that fucose-containing sulfated polysaccharide isolated from *E. cava* strongly inhibits the activities of coagulation factors via interaction with antithrombin III in both the extrinsic and the common coagulation pathways (Jung *et al.*, 2007). Possible anticoagulation mechanism and molecular interaction of fucoidan isolated from the brown seaweed *E. cava* with blood coagulation factors are shown in Fig. 12.3. Fucoidans enhance ATIII-mediated coagulation factor inhibition in coagulation pathways. This contributes to its high anticoagulant activity. Wijesinghe *et al.* (2011) demonstrated *in vivo* anticoagulant activity of isolated fucose-rich sulfated polysaccharide obtained from *E. cava*. Anticoagulant and antithrombin activities of over sulfated fucans having different sulfate contents were reported (Nishino and Nagumo, 1992). There results showed that heparin cofactor II-mediated antithrombin activity of the over sulfated fucans also increased significantly with increase in sulfate content. In addition, it was reported that the major antithrombin activity by fucoidan was mediated by heparin cofactor II (Qui *et al.*, 2006). Another previous study reported the partial characterization and anticoagulant activity of a heterofucan from the brown seaweed, *Padina gymnospora* (Silva *et al.*, 2005). Further, they have reported that 3-O-sulfation at C-3 of 4-α-L-fucose-1→ units was responsible for the anticoagulant activity of fucoidan from the particular seaweed species.

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

**FIGURE 12.3** Possible anticoagulation mechanism of fucoidan from brown seaweed *Ecklonia cava*.
report, fermentation could offer a tool to increase the bioactive potentials. Therefore, the report facilitates further screening and large-scale production of the bioactive molecules from fermented marine seaweed in the future.

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

B. Antiproliferative/antitumor/anticancer activity

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

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

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

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

**FIGURE 12.4** Possible immunomodulatory effects of brown algal fucoidan by activated splenocytes via JNK, NF-κB, and NFAT signal pathways.
fucoidan in vitro. Their data suggest that arabinogalactan and fucoidan are activators of lymphocytes and macrophages. This property may contribute to their effectiveness in the immunoprevention of cancer. Yang et al. (2008) reported the effects of fucoidan on maturation process and activation of human monocyte-derived dendritic cells. Their results suggest that dendritic cells appear to be a potential target for the immunomodulatory capacity of fucoidan. Therefore, fucoidan may be used on dendritic cells-based vaccines for cancer immunotherapy.

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

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

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

D. Anti-inflammatory activity

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

Potent effect of the fucose-containing sulfated polysaccharide from E. cava on anti-inflammatory activity in LPS-stimulated RAW 264.7 cells was successfully investigated (Kang et al., 2011). According to their results, isolated sulfated polysaccharide-containing fucose, dose-dependently inhibited the LPS-induced iNOS and COX-2 gene expression, as well as the subsequent production of NO and PGE2 by LPS in RAW 264.7 macrophages. Resent in vivo study revealed that the administration of fucoidan, isolated from brown seaweed Laminaria japonica, could regulate the inflammation response via HMGB1 and NF-κB inactivation in I/R-induced myocardial damage on rats (Li et al., 2011).
Connective tissue destruction during inflammatory diseases, such as chronic wound, chronic leg ulcers, or rheumatoid arthritis, is the result of continuous supply of inflammatory cells and exacerbated production of inflammatory cytokines and matrix proteinases (Senni et al., 2006). According to the Senni et al. (2006), fucoidan from Ascophyllum nodosum is a potent modulator of connective tissue proteolysis. Further, the authors suggested that fucoidan could be used for treating some inflammatory pathology in which uncontrolled extracellular matrix degradation takes place.

E. Other biological activities of fucoidan

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

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

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

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

V. POSSIBLE INDUSTRIAL APPLICATIONS OF FUCOIDANS

Over the years, there are significant developments in the fields of pharmaceutical, nutraceutical, cosmeceutical, and functional food. There is a growing interest among producers and the public in those areas that may provide health benefits beyond basic nutrition. This fact has brought great interest for searching new functional ingredients that can contribute to develop new opportunities in the relevant applications. Therefore, today,
in the modern market, there is an increasing number of novel products available with functional ingredients from different natural sources. Discussed biological properties of sulfated polysaccharides fucoidans might give a clear evidence of its potential uses in medical and food industry.

VI. CONCLUDING REMARKS

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

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CHAPTER 13

Polysaccharides from Capsosiphon fulvescens Stimulate the Growth of Gastrointestinal Cells

Hye-Jung Hwang, In-Hye Kim, and Taek-Jeong Nam

Abstract

Capsosiphon fulvescens is a green alga that is abundant along the southwest coast of South Korea. Although it is consumed for its purported health-enhancing properties, particularly as a treatment for stomach disorders and hangovers, the health effects of dietary C. fulvescens remain unclear. Polysaccharides extracted from C. fulvescens (Cf-PS) are investigated for their effects on the proliferation of rat small intestinal epithelial IEC-6 cells. Cf-PS stimulated IEC-6 cell proliferation in a dose-dependent manner. Further, Cf-PS treatment induced the translocation of β-catenin, an effector of the Wnt signaling pathway, from the cytosol to the nucleus and increased the expression of cyclinD1 and c-myc. Cf-PS also induced ERK1/2 phosphorylation, which is activated by mitogenic and proliferative stimuli such as growth factors, but the phosphorylation of JNK and p38 was not enhanced. Therefore, this chapter discusses the effect of Cf-PS on the growth of gastrointestinal cells.
I. INTRODUCTION

*Capsosiphon fulvescens* is a green alga that is rich in vitamins A and C and minerals such as iron, potassium, and iodine. Hence, it has great potential as a human food and it is indeed consumed in Korea. Moreover, the alga is often used to treat stomach disorders and hangovers and shows immunostimulatory, anticancer, and antiatherosclerotic effects. However, in contrast to other sea vegetables, there are few reports about the potential benefits of *C. fulvescens*. For example, Cho *et al.* (2010) observed that the EtOH extract of *C. fulvescens* has the antioxidant effect. They suggested that the antioxidant activities were correlated with total phenolic and flavonoid contents. The water-soluble polysaccharide of *C. fulvescens* (SPS-CF) has also immunostimulating activity (Na *et al.*, 2010). That is, they determined that SPS-CF stimulates the release of inflammatory cytokines, TNF-α, IL-6, i-NOS, COX-2, and PGE2. Moreover, Sheu *et al.* (1996) and Yamamoto *et al.* (1987) reported that oral consumption of several seaweeds significantly decreased the incidence of carcinogenesis in vivo. Alekseyenko *et al.* (2007) suggested that polysaccharide from *Fucus evanescens* has antitumor and antimetastatic activity in C57Bl/6 mice transplanted Lewis lung adenocarcinoma. Downregulating tissue factor expression, Grateloupia longifolia polysaccharide inhibits angiogenesis in HMEC-1 endothelial cells (Zhang *et al.*, 2006). A study of Porphyra yezoensis polysaccharide (Guo *et al.*, 2007) has shown that it has protective effect against carbon tetrachloride-induced hepatotoxicity in mice. Jung *et al.* (2008) have investigated the effect of the extract (CFE: *C. fulvescens* extract) on the liver tissue and fecal cholesterol content in rats. CFE diet induced the increase of bile acid, dietary fiber, and cholesterol excretion in feces and the improvement of lipid metabolism in Sprague Dawley rats. The extract consisted of 68.32% dietary fiber and they suggested that the effect of CFE were due to the fiber. Cho *et al.* (2010) observed that the EtOH extract of *C. fulvescens* has the antioxidant effect. They suggested that the antioxidant activities were correlated with total phenolic and flavonoid content. In this chapter, we demonstrated that polysaccharides extracted from *C. fulvescens* (Cf-PS) stimulated the growth of rat small intestine epithelial IEC-6 cells.

Cell proliferation depends on intracellular signal transduction, mediated by receptors such as enzyme-linked receptors (e.g., tyrosine kinase) and protein degradation dependent receptors (e.g., secretory Wnt protein). The Wnt signaling pathway plays an important role in a number of development processes, including β-catenin stabilization and translocation to the nucleus, where it associates with TCF/LEF family transcription factors, resulting in activation of specific target genes such as cyclinD1 and c-myc. In the intestine, the canonical Wnt signaling cascade plays a crucial role in driving proliferation of epithelial cells (Greogrieff *et al.*, 2005). The signaling pathway also plays an important
role in a number of developmental processes, including body axis formation, development of the central nervous system, axial specification in limb development, and mouse mammary gland development (Akiyama, 2000). Other signaling pathways related to cell proliferation include the growth factor signaling pathways. The EGF signaling pathway is activated in a variety of tissues of epithelial, mesenchymal, and neuronal origin, where it plays fundamental roles in development, proliferation, and differentiation (Olayioye et al., 2000). The MAPK signaling pathway is a major signaling cascade downstream of activated epidermal growth factor receptor (EGFR) receptors involved in regulation of cellular proliferation and differentiation. Once activated, MAPK can translocate to the nucleus, where it presumably regulates expression of different transcription factors (Garrington and Johnson, 1999; Velarde et al., 1999).

Further, the MAPK signaling pathway may be related to the Wnt signaling pathway. For example, Givenni et al. (2003) reported that the Wnt signaling pathway activates the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway in mouse mammary epithelial cells via EGFR transactivation. In this study, we extracted polysaccharides from C. fulvescens and evaluated how they affect the growth of IEC-6 cells, using the Wnt and MAPK signaling pathways, which were activated by cell growth stimuli and promote cell proliferation.

II. EFFECTS OF CF-PS ON PROLIFERATION OF IEC-6 CELLS

Many sea vegetables have high levels of nutrients and other potentially beneficial components that may be useful for the treatment of various diseases. In particular, the antitumor activities of sea vegetables have been widely studied. For example, an algal lectin from Galaxaura marginata exhibits antibacterial activity (Liao et al., 2003), and palmitic acid isolated from the marine alga Colpomenia sinuosa has antitumor activity (Heiba et al., 1997). Marine algae also contain a large amount of polysaccharides such as alginate, fucoidan, carageenan, and agarose.

Active polysaccharides were extracted from C. fulvescens and subjected to agarose gel electrophoresis (Fig. 13.1) using two-band detection. Cf-PS has sulfate and 3,6-anhydrogalactose content of 28.7% and 18.6%, respectively. In addition, Cf-PS contains monosaccharides xylose (85%) and mannose (15%). Although there are no similar experiments replacing Cf-PS with each individual constituent, Cf-PS was evaluated to check whether it may stimulate the proliferation of normal intestinal cells. Cf-PS induced proliferation of IEC-6 cells, as determined by the MTS assay (Fig. 13.2A). After exposure to 0–1000 μg/ml Cf-PS for 24 h, relative cell numbers increased in a concentration-dependent manner because exposure to 500 μg/ml of Cf-PS for 24 h was sufficient to stimulate
growth. Cell morphology results showed that cells treated with Cf-PS appeared to increase in number compared to untreated cells (Fig. 13.2B). Further, expression of PCNA increased in treated cells (Fig. 13.2C), confirming that Cf-PS induced growth of IEC-6 cells.

III. EFFECTS OF CF-PS ON WNT SIGNALING COMPONENTS

Promotion of cell proliferation and division depends on intracellular signaling pathways such as the Wnt signal transduction pathway. In the absence of Wnt signaling, β-catenin is bound to the GSK3β/Auxin/APC complex in the cytosol; GSK3β phosphorylates β-catenin and consequently induces degradation of β-catenin through the ubiquitin proteosome pathway (Akiyama, 2000). Receptors for Wnt proteins are members of the frizzled family of transmembrane proteins, and the Wnt signal is converted to a cytoplasmic protein called disheveled (Dvl). Upon activation by the Wnt signal, GSK3β activity is inhibited by Dvl (Akiyama, 2000). This causes β-catenin to accumulate after it is translocated from the cytosol to the nucleus. In turn, β-catenin associates with TCF/LEF transcription factors and alters the expression of the Wnt signaling target genes cyclinD1 and c-myc. We believe that Cf-PS-induced cell proliferation was involved in the Wnt signaling pathway.

To identify the mechanism of Cf-PS-induced proliferation in IEC-6 cells, the effects of Cf-PS were evaluated on canonical Wnt signaling pathway proteins. Further, time-course experiment was conducted to assess the effect of Cf-PS on cyclinD1 and c-myc expression, which plays major roles in the cell cycle and in the proliferation of eukaryotic cells (He et al., 1998; Sun and Jin, 2008; Tetsuo and McCormick, 1999; van de Wetering et al., 2002; Willert et al., 2002; Yamaguchi et al., 2004).
FIGURE 13.2 Effect of Cf-PS on the IEC-6 cell proliferation. (A) Effect of Cf-PS treatment on cell proliferation was assayed by MTS assay. (B) Microscopy analysis of the cells (200×). (C) Effect of Cf-PS on PCNA protein expression. One representative gel from three separate experiments is shown.
Expression of cyclinD1 increased 6 and 24 h but decreased 12 h after Cf-PS exposure. In contrast, expression of c-myc increased at every time point after Cf-PS exposure (Fig. 13.3). The decreased expression of cyclinD1 at 12 h was likely an experimental error. Expression of Wnt signaling target genes is needed to transfer β-catenin from the cytosol to the nucleus. To confirm translocation of β-catenin from the cytosol to the nucleus, cytosolic-enriched fractions of Cf-PS-treated or untreated cells were used. Cytosolic β-catenin protein significantly decreased in the presence of Cf-PS as early as 12 h after exposure, and this reached a maximum by 24 h after exposure. In contrast, nuclear β-catenin increased only 24 h after Cf-PS exposure. There were no changes of nuclear β-catenin 12 h after exposure, but this may have been due to experimental error. These demonstrate that Cf-PS induced β-catenin translocation from the cytosol to the nucleus, which may increase cyclinD1 and c-myc expression. c-myc plays a major regulatory role in the cell cycle and the growth of eukaryotic cells (McMahon and Monroe, 1992; Wang et al., 1993). The c-myc gene codes for a nuclear protein that functions as a transcription factor controlling cell division, differentiation, and apoptosis (Marcu et al., 1992; Varmus, 1984). Moreover, c-myc modulates cell proliferation and governs cell cycle progression in the intestinal mucosa (Yamaguchi et al., 2004). D-type cyclins are cell cycle regulators, among which cyclinD1 is a major regulator of the progression of cells into the proliferative stage of the cell cycle. CyclinD1 has a positive effect on cell cycle progression (Koseoglu et al., 2009). Cf-PS-induced expression of cyclinD1 and c-myc may play a critical role in the stimulation of normal intestinal epithelial cell proliferation.
IV. CF-PS INDUCES ACTIVATION OF ERK1/2 IN IEC-6 CELLS

Stimulation of cell proliferation and division also depends on multisignaling pathways by tyrosine kinases, including MAPK and phosphatidylinositol 3-kinase (PI3K) (Khandwala et al., 2000; LeRoith et al., 1995; Rother and Accili, 2000). The MAPK family in mammalian cells includes ERK1/2, the JNK, and p38 MAPK (Paruchuri et al., 2002). To further investigate the mechanism of Cf-PS-induced proliferation, MAPK proteins were used to examine whether Cf-PS activates the EGFR signaling pathway in IEC-6 cells. In the absence of Cf-PS, JNK and p38 were phosphorylated in a time-dependent manner. However, Cf-PS treatment inhibited the activation of JNK and p38 in a time-dependent manner (Fig. 13.4). Further, ERK1/2 phosphorylation increased after treatment with Cf-PS as early as 0 min and reached a maximum 5 min after EGF exposure. JNK and p38 are involved in the cell death pathway and oxidative stress. Therefore, Cf-PS-induced cell proliferation appears to be related to the activation of MAPK (ERK1/2, p38, JNK), especially ERK1/2. Therefore, cell proliferation was analyzed after inhibition of ERK1/2 using U0126 to examine the role of ERK1/2 activation during the Cf-PS effect. As shown in Fig. 13.5, exposure to U0126 with Cf-PS inhibited cell proliferation, but viability was higher than that of untreated cells. These results indicate that ERK1/2 plays a role in the proliferation of IEC-6 cells.

Wnt peptides transactivate EGFR, presumably by increasing the availability of EGFR ligands, which in turn leads to strong stimulation of the MAPK pathway (Givenni et al., 2003). Transactivation of EGFR has a specific biological effect: stimulation of cyclinD1 expression (Givenni et al., 2003). Therefore, there might be cross talk between the MAPK

![FIGURE 13.4 Cf-PS induces activation of ERK1/2. MAPK protein expression was measured by Western blotting. One representative gel from three separate experiments is shown.](Image)
signaling pathway and the Wnt signaling pathway during Cf-PS-induced proliferation of IEC-6 cells. To investigate this hypothesis, the activation of ERK1/2 was inhibited using U0126 and the effects of this inhibition on the Wnt signaling pathway was investigated.

Inhibition of ERK1/2 led to a decrease in the level of cytosolic β-catenin (Fig. 13.6). This decrease was also observed when cells were treated with Cf-PS or U0126 only (Fig. 13.3), but to a greater degree after U0126 treatment (Fig. 13.6). Further, β-catenin in the nucleus, which increased when treated with Cf-PS alone (Fig. 13.3), also decreased in the presence of U0126 (Fig. 13.6). These results suggest that translocation of β-catenin from the cytosol to the nucleus was inhibited, and the resulting accumulation of β-catenin in the cytosol was also inhibited by inhibition of ERK1/2. Moreover, we have made a hypothesis model of the cross talk between Wnt signaling and MAPK signaling pathways and given in Fig.13.7.

**FIGURE 13.5** ERK1/2 phosphorylation affects Cf-PS-induced cell proliferation. (A) MTS assay was used to assess ERK1/2 activation on Cf-PS-induced cell proliferation. (B) Inhibition of phospho-ERK1/2 level by U0126. One representative gel from three separate experiments is shown.
V. CONCLUSION

Seaweeds have recently received a great deal of attention from scientific researchers. A number of investigators have found that these traditional sources of food not only provide nutritional benefits but also help to fight against diseases and contribute to the maintenance of good health. Certain types of seaweeds contain significant amounts of essential protein, vitamins, and minerals. Moreover, various polysaccharides from seaweeds have diverse biological activities, including effects on the immune system and cancer. Although *C. fulvescens*, a green alga, is consumed for its purported health-enhancing properties, particularly as a treatment for stomach disorders and hangovers, the health effects of dietary *C. fulvescens* remain unclear. There are also a few studies on the *C. fulvescens*.

In this chapter, stimulated effect of Cf-PS on IEC-6 cell growth was discussed. Many pharmaceutical products have been developed for cancer treatment. However, despite recent pharmaceutical advances, these
products that relieve pain, heal tumors, and delay malignancy are relatively expensive and are associated with various side effects. Hence, in the search for effective treatments for cancer, many researchers have begun to investigate natural products that are less likely to produce side effects. Among natural foods, seaweeds have received much research attention in past decades. In this study, Cf-PS stimulated IEC-6 cell proliferation in a dose-dependent manner. Further, Cf-PS treatment induced the translocation of β-catenin, an effector of the Wnt signaling pathway, from the cytosol to the nucleus and increased the expression of cyclinD1 and c-myc. Cf-PS also induced ERK1/2 phosphorylation, which is activated by mitogenic and proliferative stimuli such as growth factors, but the phosphorylation of JNK and p38 was not enhanced. Hence, this chapter confirmed that Cf-PS regulates proliferation via stimulating the nuclear translocation of β-catenin and ERK1/2 activation in intestinal epithelial cells.
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Potential Beneficial Effects of Marine Algal Sterols on Human Health

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Abstract

The importance of bioactive derivatives as functional ingredients has been well recognized due to their valuable health beneficial effects. Therefore, isolation and characterization of novel functional ingredients with biological activities from marine algae have gained much attention. Sterols are important structural component of cell membranes. It has been reported that plant sterols exhibit various beneficial biological activities such as hypercholesterolemic, antioxidant, anticancer, antidiabetic, antihypertensive, anti-inflammatory, antifungal, and antibacterial activities. Marine algae...
with a great diversity can be a very interesting natural resource of sterols. This chapter focuses on biological activities of marine algae derived sterols with potential health beneficial applications in functional foods and pharmaceuticals.

I. INTRODUCTION

Sterols are an important family of lipids, present in the majority of eukaryotic cells and they are categorized to the steroids group, which also contain the same fused four-ring core structure and have different biological roles as hormones and signaling molecules. In addition, sterols have a hydroxyl group at the 3-position of the A-ring. This hydroxyl group is the polar group in the structure; the rest of aliphatic chain is nonpolar. Sterols are essential components of the membranes of all eukaryotic organisms, controlling membrane fluidity and permeability. Sterols are highly diverse in nature. Their composition depends on the environment and on specificity of the organism. Because of different routes of synthesis, sterols from plants, fungi, and animals show marked differences. Sterols are synthesized from the common precursor squalene. A cytochrome P450 enzyme belonging to CYP51 class catalyzes the synthesized process. P450 enzyme used intermediated substrate lanosterol for synthesize sterol in animals, fungi, and stramenopiles. Moreover, plant sterols are synthesized from intermediate substrate, obtusifoliol (Fahy et al., 2005; Gaulin et al., 2010; Kamenarska et al., 2006).

Plant sterols have been shown to inhibit uptake of both dietary and endogenous produced (biliary) cholesterol from the intestine. Clinical studies have demonstrated that sterols had ability in lowering “bad” low-density lipoprotein cholesterol (LDL-C) levels. In addition, plant sterols did not have effect on “healthy” high-density lipoprotein cholesterol (HDL-C) and triacylglycerol levels. Moreover, clinical studies have showed that plant sterols could reduce the risk of heart disease by prevention and reduction of hypercholesterolemia. Several studies have indicated that phytosterols may have health-promoting effects such as anticancer activity. Studies on cellular base models showed that plant sterols were toxic to breast cancer, colon cancer, and prostate cancer cells. Additionally, plan sterols have been suggested that they have anti-inflammatory, antibacterial and antifungal activities. Further, long-term studies on animal models and humans did not show toxicity effect of plant sterols (Moreau et al., 2002).

Sterols also can be found in marine algae. It has been reported that brown algae (Phaeophyceae) contain mainly fucosterol and fucosterol derivatives, red algae (Rhodophyceae) contain mainly cholesterol and cholesterol derivatives, and green algae (Chlorophyceae) contain mainly
ergosterol and 24-ethylcholesterol (Sánchez-Machado et al., 2004). Hence, the search for natural bioactive sterols as safe alternatives from marine algae is important in the food industry. This chapter focuses on potential benefits of marine algae-derived sterols on human health.

II. POTENTIAL HEALTH BENEFITS OF STEROLS FROM MARINE ALGAE

A. Antibacterial activity

Tuberculosis is the second commonest cause of death worldwide. Thirty-two percent of the world’s population is infected with *Mycobacterium tuberculosis*, the main cause of tuberculosis. Most forms of active tuberculosis can be treated with 6 months of medication. Unfortunately, outbreaks of multidrug resistant tuberculosis have been occurring since 1990s. Natural products form one avenue in the search for new antituberculosis agents. Recently, marine algae have become targets for screening in search of novel compounds of potential medical value. It has been reported that saringosterol had been isolated from brown algae *Sargassum ringgoldianum* and had antitubercular activity (Ikekawa et al., 1968). Its minimum inhibition concentration (MIC) against *M. tuberculosis* H₃₇Rv was determined as 0.25 µg/ml, which is the lowest value found for plant-derived natural products, compare to the tuberculosis drug rifampicin that was determined as 0.25 µg/ml of MIC in same assay. In addition, low concentration of saringosterol showed no toxicity against in the monkey kidney epithelial (Vero) cells. Sargosterol showed half maximal inhibitory concentration (IC₅₀) of RS mg/ml on Vero cells. The sargosterol isolated from *Lessonia nigrescens* are both 1:1 mixture of 24S and 24R epimers. Individual isomers were separated by normal-phase high-performance liquid chromatography (HPLC). In the antitubercular assay, the 24R isomer was found eight times more active against *M. tuberculosis* H₃₇Rv with MIC of 0.125 µg/ml than that of the 24S isomer which had a MIC of 1 µg/ml. Saringosterol could be considered as an excellent lead compound due to its activity, specificity, and low toxicity (Wächter et al., 2001). Recently, 15 algae extracts were screened antibacterial activity and it was found that extracts from *Isochrysis galbana* inhibited multidrug resistant (MDR) *M. tuberculosis*. Screening on seven isolated MDR *M. tuberculosis*, which resisted more than three antibacterial drugs, extract from *I. galbana* showed the MIC of 50 µg/ml compare to those of the tuberculosis drugs rifampicin (40 µg/ml), amikacin (700 µg/ml), streptomycin (4 µg/ml), p-amino salicylic acid (2.5 µg/ml), and isoniazid (0.2 µg/ml). Thirteen unsaturated sterols with three major sterols such as 24-oxocholesterol acetate, ergost-5-en-3 β-ol, and cholest-5-24-1,3-(acetoxy)-3β-ol have been purified (Prakash et al., 2010).
This finding indicated the presences of sterols may have effect on MDR M. tuberculosis. Therefore, marine algal sterols have potential in the development of urgently needed tuberculosis drugs.

B. Antioxidant activity

The deterioration of some foods has been identified due to oxidation of lipids or rancidity and formation of undesirable secondary lipid peroxidation products. Lipid oxidation by reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals, and H₂O₂ also causes a decrease in nutritional value of lipid foods and affects their safety and appearance. In the food and the pharmaceutical industries, many synthetic commercial antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) have been used to retard the oxidation and the peroxidation processes. However, the use of these synthetic antioxidants must be under strict regulation due to potential health hazards (Hettiarachchy et al., 1996; Park et al., 2001). Hence, the search for natural antioxidants as safe alternatives from natural resources such as marine algae is important in the food industry. Fortunately, it was reported that fucosterol isolated from marine algae Pelvetia siliquosa had antioxidant activity. Rats were treated with fucosterol at dose of 30 mg/kg/day for 7 days, prior the administration of carbon tetrachloride (CCl₄). Fucosterol causes a significant elevation of free radical scavenging enzyme activities such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-px). Increase in the catalase activity with respect to CCl₄ treatment indicated that fucosterol could play an important role in scavenging hydrogen peroxide. Elevation of SOD activity indicated that fucosterol could help in cellular defense mechanism by preventing cell membrane oxidation. In addition, the increase in glutathione peroxidase activity indicated that fucosterol also helped in the restoration of vital molecules such as cytochrome, glutathione. The results showed that sterol have antioxidant activity on rat model (Lee et al., 2003). Hence, fucosterol can be used as potential antioxidants in the food industry.

C. Anticancer activity

Some of the marine algae and their secondary metabolites have shown promising anticancer activities, and hence, they are important sources to manufacture novel anticancer drugs. Sheu et al. (1997) had shown that sterols from brown alga Turbinaria ornata have cytotoxicity effect on P-388 (mouse lymphocytic leukemia) cells, KB (human mouth epidermoid carcinoma) cells, and HT-29 (human colon carcinoma) cells. Oxygenated fucosterol from Turbinaria conoides also has cytotoxic effect on cancer
cells (Sheu et al., 1999). Moreover, new sterols from Sargassum carpophyl-lum have shown cytotoxic effect on human promyelocytic leukemia HL-60 cells (Tang et al., 2002). It has also been reported that oxysterol from red alga Jania rubens showed an ID50 value of 0.5 µg/ml toward KB cells (Ktari et al., 2000). Recently, two sterols glycoside isolated from red alga Peyssonnelia sp. displayed moderate activity toward human cancer cell lines (Lin et al., 2010). These compounds displayed significant cytotoxicity toward human breast cancer MDA-MB-468 cells and human lung cancer cell line A549 with IC50 = 0.71 and 0.86 µM, respectively, which were often more resistant to cytotoxins than those of other common cancer cells that inhibited with IC50 = 0.93 and 0.97 µM. Collectively sterols from marine algae have a promising potential to be used as valuable chemopreventive agents in cancer therapy.

D. Antidiabetic activity

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels. Diabetes without proper treatments can cause many complications. Acute complications include hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, and retinal damage. Hence, antidiabetic agents are urgently required. It has been reported that fucosterol from P. siliquosa have antidiabetic activity. Fucosterol at a dose of 100 and 300 mg/kg reduced hyperglycemic effect by 25–33% in epinephrine-induced diabetes mouse model. Moreover, fucosterol at a dose of 100 and 300 mg/kg was shown to decrease the glycogen degradation of mouse liver by 23–29%. Hence, it was suggested that fucosterol from marine alga P. siliquosa has potential in development of antidiabetic agent (Lee et al., 2004).

E. Antihypertensive and antihypercholesterolemic activities

Angiotensin-I-converting enzyme (ACE) plays an important physiological role in regulation of blood pressure by converting angiotensin I to angiotensin II, a potent vasoconstrictor. Further, ACE is implicated in cell oxidative stress, augmenting the generation of ROS and peroxynitrite and also in thrombosis, during which induces platelet activation, aggregation, and adhesion (McFarlane et al., 2003). Inhibition of ACE is considered to be a useful therapeutic approach in the treatment of hypertension. Therefore, in the development of drugs to control high blood pressure, ACE inhibition has become an important activity. Many studies have been attempted in the synthesis of ACE inhibitors such as captopril, enalapril, alacepril, and lisinopril, which are currently used in the treatment of hypertension and heart failure in human. However, these synthetic drugs
are believed to have certain side effects such as cough, taste disturbances, skin rashes, or angioneurotic edema and all of which might be intrinsically linked to synthetic ACE inhibitors. The search for natural ACE inhibitors as alternatives to synthetic drugs is of great interest to prevent several side effects (Atkinson and Robertson, 1979). Fucosterol was reported as safety agent on animal models. The modulation of ACE levels was studied using fucosterol in cultured bovine carotid endothelial cells. Dexamethasone was treated to elevate the levels of ACE in the cells. After adding fucosterol to the culture medium, the activity of ACE in endothelial cells has decreased; however, fucosterol did not directly inhibit ACE activity. It has been found that fucosterol lowers the ACE levels in endothelial cells by inhibiting the synthesis of glucocorticoid receptors involved in the regulation of ACE levels (Hagiwara et al., 1986).

LDL-C is called “bad” cholesterol because elevated level of LDL cholesterol is associated with an increased risk of coronary heart disease. LDL lipoprotein deposits cholesterol on the artery walls, causing the formation of a hard, thick substance called cholesterol plaque. Over the time, cholesterol plaque causes thickening of the artery walls and narrowing of the arteries, a process called atherosclerosis. In contrast, HDL cholesterol is called the “good cholesterol” because it prevents atherosclerosis by extracting cholesterol from the artery walls and disposing them through the liver. The highest HDL-C level gives the greater capacity to remove cholesterol and prevent dangerous blockages from developing in blood vessels. HDL-C helps to keep blood vessels widened (dilated), thereby promoting better blood flow. HDL-C also reduces blood vessel injury through its antioxidant and anti-inflammatory functions, among other effects (Toth, 2005). Plant sterols have been reported as agents that can reduce the risk of heart disease by lowering LDL-C levels. Therefore, it suggested that marine algal sterols could be used to prevent cardiovascular diseases. According to Plaza et al. (2008), sterols from several edible marine algae, such as Himanthalia elongate, Undaria pinnatifida, Phorphyra spp., Chondus crispus, Cystoseira spp., Ulva spp., have potential effect on reducing the total and LDL-C level. In addition, 4-methylsterols from Cryptothecodinium cohnii had no effect on any serum or liver lipid parameter. However, the percentage of serum HDL-C level was increased by 25%. Addition of bile salt to a cholesterol containing diet raises the serum and the liver cholesterol levels. Rats fed with cholesterol diet and cholesterol plus bile salt have shown significant increase in total serum cholesterol and decreased HDL-C level. Moreover, cholesterol-bile salt plus 4-methylsterols significantly raised the amount of HDL-C and triglyceride levels, but no other serum or liver lipid parameters were affected (Kritchevsky et al., 1999). These effects have shown the potential application of fucosterols in the prevention of risk of cardiovascular diseases.
III. CONCLUSIONS

Marine algae-derived functional ingredients play a vital role in human health and nutrition. Increasing knowledge on marine algal bioactive compounds has raised the demand for novel functional food ingredients and pharmaceuticals. Hence, sterols from marine algae can be used as functional ingredients to reduce chronic diseases in human body. However, there are few studies on marine algal sterols compared with those of plant sterols. Therefore, the future studies on marine sterols will lead more beneficial sterols bioactive compounds. Collectively, the sterols, which derived from marine algae, have potential to expand its health beneficial value not only in the food industry but also in the pharmaceutical industry.

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Laminaria japonica as a Food for the Prevention of Obesity and Diabetes

Miyuki Shirosaki and Tomoyuki Koyama

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Abstract

Various seaweeds have traditionally been used as flavoring materials, food additives, and foodstuffs in many countries, especially those in Asia. The seaweed Laminaria japonica (LJ) is popular as “kombu” in Japanese cuisine. Laminaria sp. is one of the most important marine medicinal foodstuffs, as its biological functions have been widely investigated in both in vitro and in vivo experiments. This chapter introduces recent reports on the ability of Laminaria to prevent obesity and diabetes, and some approaches for effectively using the bioactivities found in Laminaria. The inhibitory effects of Laminaria sp. on triglyceride absorption were investigated in triglyceride-loaded mice and in mice with high-fat-diet-induced obesity. Shaved Laminaria, known as “tororokombu,” showed more effective activities in...
these experiments. The active component was considered to be algic acid in the water-soluble fraction. On the other hand, the antihyperglycemic effects of a hot water extract of immature Laminaria were investigated in carbohydrate-loaded mice and in in vitro experiments using Caco-2 cells. The potential usefulness of Laminaria sp. as marine medicinal foods may be increased through the use of different processing methods and/or growth stages. These reports suggest that LJ may be useful for preventing lifestyle-related diseases.

I. INTRODUCTION

Many types of seaweed are commonly consumed as food worldwide. Seaweeds have been established as healthy food materials that are rich in minerals and dietary fibers. Especially, Laminaria japonica (LJ) “kombu,” Undaria pinnatifida “wakame,” Gelidium crinale “tengusa,” and Cladosiphon okamuranus “mozuku” have traditionally been consumed in Japan. In addition, seaweeds have been harvested or cultivated, especially in Asia, as a source of alginate, agar, and carrageenan, that is, gelatinous substances, for various industrial applications. Laminaria sp. contain soluble fibers such as alginate and fucoidan, as well as fat-soluble components such as fucoxanthin and fucosterol (Mizuno et al., 2009; Stevan et al., 2001; Zhang et al., 2008), in addition to being particularly rich in minerals such as magnesium, iodine, calcium, iron, and zinc.

Recently, seaweeds have been attracting attention as healthy foods that contain beneficial components, which may be useful for the prevention and treatment of lifestyle-related diseases. Thus far, extracts of Laminaria sp. have been reported to exhibit anticancer (Reddy et al., 1985; Yamamoto and Maruyama, 1985), antioxidative (Reddy et al., 1984), antiviral (Makarenkova et al., 2010), antiatherogenic (Matanjun et al., 2010), immunostimulatory (Jeong et al., 2006; Oomizu et al., 2006), and anti-inflammatory (Shiratori et al., 2005) effects. The modern tendency to consume nutritionally rich diets coupled with irrational dietary habits has contributed to the growth of lifestyle-related diseases such as obesity and diabetes.

In this chapter, the antiobesity and the antidiabetic effects of Laminaria sp. are described. The latest research on the biological activity found in LJ is introduced.

II. ANTIOBESITY EFFECTS

A. Obesity

Obesity is an abnormal condition in which excessive triglycerides (TGs) accumulate in the adipose tissue. Obesity has reached epidemic proportions globally, and the World Health Organization estimates that there are
more than 1 billion overweight adults, of whom at least 300 million are obese. Economic growth and the modernization, urbanization, and globalization of food markets are some of the elements that have contributed to the obesity epidemic. Obesity is the most important risk factor for lifestyle-related diseases such as hypertension, type 2 diabetes, and hyperlipidemia (Golay and Ybarra, 2005; Matsuzawa et al., 1995). In particular, obesity causes an imbalance in the level of adipocytokines secreted by adipocytes, such as leptin, adiponectin, resistin, and plasminogen activator inhibitor-1, due to the excessive accumulation of visceral fat and can cause metabolic syndrome (Matsuzawa, 2006; You et al., 2005).

The plasma TG level is also related to cardiovascular disease (Hokanson and Austin, 1996), and a high postprandial TG level is a risk factor for atherosclerotic disease. Patients with hypertriglyceridemia tend to have prolonged postprandial hypertriglyceridemia after a high-fat diet (HFD) (Patsch et al., 1992). An excess postprandial hypertriglyceridemia response indicates poor TG clearance from the bloodstream and is often associated with atherosclerosis, insulin resistance, and obesity (Gott, 1998). Thus, the prevention of postprandial hypertriglyceridemia is very important for a healthy life.

B. Antiobesity effects of seaweeds

Over the past few decades, several studies have focused on fucoxanthin contained in seaweed. Fucoxanthin, a characteristic carotenoid of brown algae, has a unique structure that includes an unusual allenic bond and 5,6-monoepoxide. Wakame (Ul. pinnatifida), an edible seaweed, is rich in fucoxanthin.

Maeda et al. (2005) reported that fucoxanthin has an antiobesity effect by modifying uncoupling protein 1 (UCP1) expression in white adipose tissue (WAT) in KKAY mice, an animal model of type 2 diabetes with obesity. When fucoxanthin is orally administered to mice, it is metabolized to fucoxanthinol, which is further converted into amarouciaxanthin A (Asai et al., 2004; Sugawara et al., 2002). Fucoxanthin and its metabolite fucoxanthinol have been shown to reduce the expression of peroxisome proliferator-activated receptor (PPAR) γ in 3T3-L1 preadipocytes, which in turn inhibits differentiation to mature adipocytes (Maeda et al., 2006), suggesting that fucoxanthin inhibits adipocyte maturation and stimulates UCP1 expression in WAT.

In addition, Maeda et al. (2009) reported that fucoxanthin-rich wakame lipids (WLs) had antiobesity and antidiabetic effects on HFD-induced obesity in mice. The increased expression of monocyte chemoattractant protein-1 (MCP-1) mRNA in HF mice was normalized by ingestion of WL with a HFD. Moreover, the HF-WL diet may ameliorate alterations in lipid metabolism and insulin resistance induced by a HFD by promoting

Laminaria japonica as a Preventive Food
the expression of β3-adrenergic receptor (Adrb3) mRNA in WAT and glucose transporter 4 (GLUT4) mRNA in skeletal muscle tissues. These results suggest that there is a biochemical and nutritional basis for the application of fucoxanthin-rich WLs for the treatment of obesity and diabetes-related disorders.

The effects of fucoxanthin-rich seaweed extract (Fx-SEE) on body weight gain and lipid metabolism in HF-fed C57BL/6J mice were investigated by Jeon et al. (2010). They demonstrated that Fx-SEE affects the plasma and hepatic lipid profile, fecal lipids, and body fat mass, and alters hepatic cholesterol metabolism, FA synthesis, and lipid absorption in mice.

The antiobesity and antidiabetic effects of some allenic compounds including fucoxanthin were reported by Miyashita et al. (2011). These compounds improved insulin resistance and decreased blood glucose levels through the regulation of cytokine secretions from WAT by inducing UCP1. The key structures of these activities were thought to be an allenic bond and two hydroxyl groups.

Some reports have focused on the alginate contained in seaweed. Sodium alginate from the brown seaweed Laminaria digitata (LD) is currently marketed as a weight-loss supplement, but its effects on gastric motor functions and satiation are unknown. Odunsi et al. (2010) clinically investigated the effects of 10 days of treatment with alginate or placebo on gastric function, satiation, appetite, and gut hormones associated with satiety in overweight or obese adults. They found that treatment with alginate for 10 days had no effect on any of the above parameters. These results suggested that the daily continuous intake of alginates may be required to prevent obesity.

“Tororokombu” (TK) is a traditional Japanese food that is made by shaving Laminaria (kombu) very thinly. Miyata et al. (2009) first investigated the effects of NSK (non-shaved kombu) and TK on the absorption of TGs in the intestine by an oil-loading test in female SD rats (7 weeks old). One feature of this study is the improved efficiency of dissolution of the active component. SD rats were first divided into three groups: distilled water-treated, NSK-treated, and TK-treated groups. Next, corn oil (5 ml/kg) was administered orally. The elevation of the serum TG level in the NSK- and TK-treated groups was significantly lower than that in the normal rat group.

Next, the antiobesity effects of NSK and TK were investigated by a long-term experiment on obese female ddY mice (4 weeks old) induced by a HFD for 63 days. Mice were divided into four groups: ND (normal diet), HFD, HFD-NSK (HFD containing 3% NSK), and HFD-TK (HFD containing 3% TK) groups. The body weights on the 63rd day after treatment started and the serum TC levels in both the HFD-NSK and the HFD-TK groups were significantly lower than those in the HFD
group. The parauterus adipose tissue weight, and hepatic TG, serum TG, and TC levels in the HFD-TK group were significantly less than those in the HFD-NSK group. The fecal TG and TC levels in the HFD-TK group were significantly higher than those in the HFD group, and fecal TG in the HFD-TK group was significantly higher than that in the HFD-NSK group. Consequently, it was demonstrated that TK consumption reduced the accumulation of visceral fat caused by HFD, and this effect of TK was more powerful than that of NSK, due to TG and cholesterol excretion in the feces. This report concluded that alginate may be one of the active components in Laminaria sp. In previous reports, alginate has been reported to have hypoglycemic and cholesterol-lowering effects by acting as a viscous soluble dietary fiber (Kimura et al., 1996; Pasquier et al., 1996; Paxman et al., 2008).

The inhibitory activities of NSK and TK against lipase were examined. The inhibitory activities of TK were greater than those of NSK, as shown in Fig. 15.1. In addition, TK had significantly higher alginate content than NSK, as shown in Fig. 15.2. The effects of extracted alginate with weak alkaline solution on lipase activity are shown in Fig. 15.3. Based on these results, alginate was thought to contribute to the inhibition of lipase. A lipase inhibitor should inhibit TG absorption and have an antiobesity effect in vivo (Miyata et al., 2009).

![FIGURE 15.1](image.png)

**FIGURE 15.1** Effect of non-shaved kombu (NSK) and tororokombu (TK) on pancreatic lipase activity in vitro. Pancreatic lipase (from porcine) activity was measured using a Lipase Kit S according to the manufacturer’s protocol. Data are presented as the mean ± SE (n = 5). *p < 0.05, ***p < 0.005 versus control. #p < 0.05 NSK versus TK.
FIGURE 15.2 Alginate content eluted into weak alkaline solution from non-shaved kombu (NSK) and tororokombu (TK). The uronic acid content was analyzed using total components eluted from NSK and TK into phosphate-buffered saline (pH 8.0) for the determination of alginate by the carbazole method. Data are presented as the mean ± SE (n = 3). *** p < 0.005 versus NSK.

FIGURE 15.3 Effect of weak alkaline-soluble fraction (AS) from non-shaved kombu (NSK) and tororokombu (TK) on pancreatic lipase activity in vitro. Lipase activity was measured using a Lipase Kit S according to the manufacturer’s protocol. Data are presented as the mean ± SE (n = 5). * p < 0.05, *** p < 0.005 versus control, ### p < 0.005 NSK AS versus TK AS.
These results suggest that TK, but not NSK, had an antiobesity effect. The shaving process alters the amount of active component that dissolves to cause physiological effects. The active component in the crude extract of *Laminaria* sp. can be considered to be an alginate.

### III. ANTIDIABETIC EFFECTS

#### A. Diabetes

Diabetes mellitus is a group of metabolic diseases in which the patient has a high glucose level, either because the pancreas does not produce enough insulin (insulin secretory failure) or because the body does not respond to insulin that is produced (insulin resistance). The number of diabetes cases has been increasing worldwide in recent years. Most cases of diabetes mellitus can be classified into two types: type 1 and type 2. Type 1 diabetes is mainly related to genetic factors. On the other hand, type 2 diabetes is related to environmental factors including lack of exercise, high-calorie diet, obesity, stress, aging, and so on. Persistent hyperglycemia can lead to various complications including diabetic neuropathy, diabetic retinopathy, and diabetic nephropathy. Diabetes is a lifestyle-related disease that not only impairs the quality of life but also poses a threat to life.

To prevent or treat diabetes, stabilization of the blood glucose level is known to be effective. In general, to control the blood glucose level, it is important to reduce postprandial hyperglycemia primarily by inhibiting carbohydrate-digestive enzymes and/or delaying glucose absorption in the small intestine. Medications that inhibit α-glucosidase have been used clinically to treat diabetic patients (Krause *et al.*, 1982; Nakamura, 2005; Sels *et al.*, 1999).

#### B. Antidiabetic effects of seaweeds

Over the past few decades, several studies have focused on seaweeds as a source of potential bioactive materials. Vaugelade *et al.* (2000) investigated the possible effects of algal polysaccharides on postprandial blood glucose and insulin responses in pig. Three seaweed fibers of different viscosities, extracted from *Palmaria palmata* (PP), *Eucheuma cottonii* (EC), or LD, were compared with purified cellulose (CEL). The addition of LD to the diet resulted in a dramatically reduced glucose absorption balance, accompanied by a higher amount of starch left in the small intestine. This study demonstrated that highly viscous alginates could affect the intestinal absorption of glucose and the insulin response.
Related studies on the bioactivity of LJ have also been reported. Jin et al. (2004) investigated the preventive effects of LJ aqueous extract (LJE) on alterations in the activity of hepatic xanthine oxidase and oxidative stress in streptozotocin-induced experimental diabetes. Pretreatment with LJE at 100 mg/kg orally for 5 days significantly reduced blood glucose levels and hepatic lipid peroxidation in diabetic rats due to the antioxidant activity of the extract.

The rhizoid of LJ is widely used in Chinese medicine as a treatment for diabetes. Bu et al. (2010) focused on the \( \alpha \)-glucosidase inhibitor in the LJ rhizoid. This compound was determined to be butyl-isobutyl-phthalate (BIP) by spectral analysis. BIP exhibited significant concentration-dependent, noncompetitive inhibitory activity against \( \alpha \)-glucosidase in vitro, with an IC\(_{50}\) of 38 \( \mu \)m. The ethyl acetate fraction (EAF) and purified BIP had a significant hypoglycemic effect in streptozotocin-induced diabetic mice in vivo. These results conclude that BIP could be considered an \( \alpha \)-glucosidase inhibitor and may become an important agent for diabetes therapy.

During spring and summer, when LJ is cultivated in the waters surrounding Japan, much of the immature material is culled for density adjustment and disposed of as waste. This immature Laminaria may be a new abundant source of marine medicinal foods for preventing lifestyle-related diseases. The effects of LJE on the postprandial blood glucose level were examined in carbohydrate-loaded mice, as shown in Fig. 15.4.

![Fig. 15.4 Effects of LJE on postprandial elevation of the blood glucose level in carbohydrate-loaded mice. Carbohydrate (1000 mg/kg each) and sample solution were intubated to mice (1 ml/30 g BW). Sample solution contained distilled water only (control: closed circles), 640 mg/kg of mature LJ (mature LJ: open squares), or 640 mg/kg of immature LJ (immature LJ: open triangles). Data are expressed as the average ± SE (n = 4). ** p < 0.01, *** p < 0.005 versus control.](image-url)
Mice were divided into three groups: distilled water-treated, immature LJ-treated, and mature LJ-treated groups. Next, carbohydrates (1000 mg/kg) were administered orally. When immature LJ (640 mg/kg) was administered orally simultaneously with starch (A), maltose (B), or glucose (C), the blood glucose levels at 30 min after administration were significantly suppressed ($p < 0.005$). LJE also had an antihyperglycemic effect in glucose-loaded mice and requires no enzymatic digestion for its absorption. Thus, the inhibitory action toward glucose absorption was evaluated by the measurement of remaining glucose in the small intestine. As shown in Fig. 15.5, the amount of remaining glucose in the immature LJ group was significantly higher than that in the control group. These results suggest that immature LJE may inhibit the absorption of glucose into the small intestinal mucosa.

In addition, to analyze the mechanism of the inhibition of glucose absorption in the small intestine, the effect of immature LJE on glucose intake was estimated using Caco-2 cells and particularly by examining glucose transmission in monolayer Caco-2 cells. Caco-2 cells are derived from a colon carcinoma and represent a very common cell culture model for human enterocytes. Glucose was taken into Caco-2 cells and transported to a lower layer by glucose transporters in Caco-2 cells (Fig. 15.6).

To measure the level of D-[6-3H] glucose intake in Caco-2 cells, tritium-labeled glucose and immature LJE were added to a culture medium. Phloridzin is an inhibitor of sodium-dependent glucose transporters. Glucose intake was significantly suppressed in phloridzin-treated Caco-2 cells, but not in those treated with immature LJE (Fig. 15.7).

![Figure 15.5](image-url)

**Figure 15.5** Effects of LJE on intestinal glucose absorption in mice. Data are expressed as the average ± SE ($n = 6$). *$p < 0.05$ versus control.
FIGURE 15.6 Scheme for the glucose transmission test of immature LJE in Caco-2 cells. Caco-2 cells were precultured for 2 weeks to cover the membrane filter as an experimental model for intestinal epithelia in vitro. Completion of the cell monolayer was detected by TEER measurement (Millicell-ERS). The transport of glucose from the upper cavity (apical site) to the lower cavity (basal site) through glucose transporters was evaluated by measuring both glucose concentrations for constant periods.

FIGURE 15.7 Effect of immature LJE on D-[6-3H] glucose intake in Caco-2 cells. To measure the amount of glucose intake in Caco-2 cells, tritium-labeled glucose and immature LJE were added to the culture medium. Phloridzin inhibits sodium-dependent glucose transporters. Data are expressed as the average ± SE (n = 4). ***p < 0.005 versus control.
On the other hand, LJ suppressed glucose transmission from the upper layer to the lower layer in a concentrate-dependent manner, as shown in Fig. 15.8. Caco-2 cells express sodium-dependent transporter 1 (SGLT1) in microvillus membrane and glucose transporter 2 (GLUT2) in the whole cell. This transporter operates via the glucose concentration in the small intestine. Mainly, SGLT1 or GLUT2 transports glucose from the gut lumen to cells and GLUT2 transports glucose from the apical side to the basolateral side. These results suggest that immature LJE did not affect glucose intake in Caco-2 cells but significantly suppressed the transmission of glucose in monolayer membranes by inhibiting GLUT2.

The results of the oral carbohydrate tolerance test and the long-term administration test suggest that immature LJE suppresses the postprandial blood glucose level. These results suggest that immature LJE can be used as a food for the prevention of diabetes. The active component in LJE remains to be elucidated.
IV. CONCLUSIONS

The popular edible seaweed LJ exhibited antiobesity and antidiabetic effects, as described above. In recent studies, the shaving process has been shown to alter the amount of active component that dissolves to cause physiological effects. The potential usefulness of *Laminaria* sp. as a marine medicinal food may be increased through the use of different processing methods and/or different growth stages. Such studies could provide new possibilities for the use of this seaweed. Therefore, these products of marine origin may be promising candidates for preventing obesity and diabetes and may continue to be one of the best marine medicinal foods for maintaining our good health in the future.

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Anticancer Compounds from Marine Macroalgae and Their Application as Medicinal Foods

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Abstract

Cancer is one of the most challenging medical conditions that need a proper therapeutic approach for its proper management with fewer side effects. Until now, many of the phytochemicals from terrestrial origin have been assessed for their anticancer ability and few of them are in clinical trials too. However, marine environment also has been a greatest resource that harbors taxonomically diverse and a variety of life forms and serves as store house for several biologically beneficial metabolites. Hitherto, many metabolites have been isolated from marine biomasses that have exhibited excellent biological activities, especially as anticancer agents. In particular, marine macroalgae which are considered as dietary...
constituents in Pacific Asian region have become chief resources for their unparalleled and unique metabolites like sulfated polysaccharides (SPs), phlorotannins, and their ability in reducing the risk of cancer and its related diseases. In this chapter, we have discussed the anticancer activities of marine algae-derived SPs, phlorotannins, and carotenoids and the possibilities of marine algae as potential medicinal foods in the management of cancer.

I. INTRODUCTION

Cancer is a dreadful pathological condition that remains one of the high-ranking causes of death in the world though there are considerable therapeutic approaches for its management. It is considered as the second life-taking disease which is preceded by heart diseases. Moreover, it is estimated that by 2020 cancer would be accounting for more than 10.3 million deaths per year if more secure and effective therapeutic approaches are not devised to cure cancer (Schumacher et al., 2011). Though there is an appreciable advancement in chemotherapy and therapeutic medicines for the management of cancer, the search for novel and safe anticancer treatments continues. Due to the shortcomings like high toxicity and unwanted side effects by the synthetic drugs, the natural products derived from medicinal plants have gained significance in the treatment of cancer. The National Cancer Institute (NCI) of the USA has screened about 1,14,000 extracts from an estimated 35,000 plant samples against a number of tumor systems. Of the 92 anticancer drugs commercially available prior to 1983 in the USA and approved worldwide between 1983 and 1994, approximately 62% can be related to natural origin (Cragg et al., 1997). According to the WHO, 80% of the world’s population, primarily those of developing countries, rely on plant-derived medicines for the health care. Natural products and their derivatives represent more than 50% of all the drugs in clinical use of the world. Higher plants contribute not less than 25% of the total. Almost 60% of drugs approved for cancer treatment are of natural origin. Fruits and vegetables are the principal sources of vitamins C, B, E, carotenoids, and fibers, and these contribute to the apparent cancer-protective effects of the foods. Moreover, the increased dietary intake of natural antioxidants can reduce the risk of lifestyle diseases like heart diseases, and cancer mortality, and helps for a longer life expectancy (Gurib-Fakim, 2006; Halliwell, 2007; Rios et al., 2009).

For many years, research has essentially focused on plants and terrestrial microorganisms, mainly because these specimens are easily available and folk traditions have described beneficial effects from their use. Recently, there is an increase in the screening of potential drug candidates.
from plant origin which include anticancer compounds like vinblastine and vincristine (*Catharanthus roseus*), epipodophyllotoxin, an isomer of podophyllotoxin (*Podophyllum peltatum* roots), paclitaxel (*Taxus baccata, Taxus brevifolia, Taxus canadensis*), camptothecin (*Camptotheca acuminata*), homoharringtonine (*Cephalotaxus harringtonia* var. *drupacea*), elliptinium (*Bleekeria vitensis*), flavopiridol (*Dysoxylum binectariferum*), and ipomeanol (*Ipomoea batatas*). However, based on the fact of the lack of natural defense systems, the metabolites produced by the marine organisms to evade potential threats have gained much preference in the field of science for potential medicinal compounds. It is well understood that these metabolites help the marine organisms to sustain and cope up with the harsh and vulnerable conditions that marine environment offers them. As a matter of fact, this silent world, in other words, marine environment, is many folds richer in its biodiversity that makes marine organisms and their metabolites unique. Efforts to exploit this biodiversity through the identification of new chemical compounds have only begun: approximately 22,000 natural products of marine origin have been discovered so far, whereas 131,000 terrestrial natural products exist (Blunt *et al.*, 2011).

Based on the above facts, in recent times, the isolation and characterization of the biologically active components from marine algae have gained attention from various research groups across the world. Among marine algae, brown algal species such as *Ecklonia cava, Eisenia arborea, Ecklonia stolinifera,* and *Eisenia bicyclis* have been studied for their potential biological activities. Majority of the investigations on the metabolites derived from brown algae have revealed their potentiality as antioxidant, anti-inflammatory, antidiabetic, antitumor, antihypertensive, and antiallergic, and in hyaluronidase enzyme inhibition and matrix metalloproteinases (MMPs) inhibition. The marine algae have been studied for biologically active components that include sulfated polysaccharides (SPs), phlorotannins, carotenoids, sterols, etc. In this chapter, we have made an attempt to throw light on the potential anticancer activities of marine algal metabolites.

II. POTENTIAL ANTICANCER AGENTS FROM MARINE MACROALGAE

A. Sulfated polysaccharides

In the field of pharmacology, the biologically active polysaccharides (PSs) that are derived from natural sources have attained a special place for the development of drug lead molecules. Marine macroalgae contain a significant amount of soluble PSs and have potential function as dietary fiber. Specially, brown marine algae are known to produce functional PSs such as alginites and fucoidans. Seaweed-derived PSs have been
reported to exhibit biological activities like anticoagulant, anti-inflammatory, antiviral, and antitumoral activities (Costa et al., 2010). Among the various PSs found in seaweeds, fucoidans, carrageenan, and ulvan (Fig. 16.1) have been studied for their potential role in controlling cancer.

1. Fucoidans
Fucoidan, an ingredient of marine algae, is composed of a polymer of α1 → 3-linked l-fucose, with sulfate groups on some of the fucose residues at four positions (Patankar et al., 1993). Out of the many biologically effective activities of fucoidans, in vitro and in vivo studies have reported that marine algae fucoidans possess antitumor, anticancer, antimetastatic, and fibrinolytic properties in mice (Religa et al., 2000). In one of the in vitro studies, fucoidan isolated from Laminaria guryanovae has effectively inhibited the neoplastic cell transformation by inhibiting the phosphorylation of epidermal growth factor receptor (EGFR) in mouse epidermal JB6 Cl41 cells (Lee et al., 2008). As it is evident that EGFR, one of the receptor tyrosine kinases, plays a pivotal role in regulating cell proliferation, differentiation, and transformation and is also considered as a target for the treatment of cancers, the inhibitory activity of fucoidan suggests that marine edible algae could possibly reduce the risk of cancers when considered as dietary supplements. In Asian countries such as China, Japan, and Korea, seaweeds are considered as dietary supplements and also are reported to have been used for boosting up the immune system. Mekabu (sporophyll of Undaria pinnatifida), a dietary alga, is reported to
exert antitumor activity and enhance the immune response. In vivo studies of effect of fucoidans isolated from Mekabu are reported to reduce the tumor growth. The experimental mice (T cell receptor transgenic (DO11.10—Tg)) were fed with a diet containing 1% Mekabu fucoidan, and it was reported that mice that were fed with the Mekabu fucoidan had a reduction on tumors by 65.4%. This in vivo study also reports that Mekabu fucoidan mediates tumor destruction through T-helper (Th1) cell and natural killer (NK) cell responses (Maruyama et al., 2006). These findings clearly suggest the efficacy of marine algal fucoidans for the possible application in the treatment of tumors and cancers.

The antitumor and antimetastatic activities of fucoidan from Fucus evanescens have been studied in vivo in C57Bl/6 mice with transplanted Lewis lung adenocarcinoma. It was observed that single and repeated administration of fucoidan in dose of 10 mg/kg has exhibited moderate antitumor and antimetastatic effects and potentiated the antimetastatic, but not antitumor, activities of cyclophosphamide (Li et al., 2008).

2. Carrageenans
The linear form of SPs and carrageenans extracted from red algae has been reported to have many applications in the food industry as well as in the medicinal industry. Carrageenans, a family of SPs isolated from marine red algae, are widely used as food additives, such as emulsifiers, stabilizers, or thickeners (Campo et al., 2009). Out of the various forms of carrageenans from red algae, λ-carrageenan is a sulfated galactan isolated from some red algae and has been reported to have many kinds of biological activities. One of the research groups has isolated λ-carrageenan with different molecular weights 650, 240, 140, 15, and 9.3 kDa from a Chinese red algae Chondrus ocellatus to study their tumor-inhibiting activities. Their research investigation on λ-carrageenan-treated mice of transplanted S180 and H22 tumor has shown considerable antitumor and immunomodulation activities in different degrees. It is although reported that molecular weight of this PS has notable antitumor and immunomodulation activities by means of activating the immunocompetence of the body (Zhou et al., 2004). The antioxidant and antitumor activities of marine algal extracts are becoming popular in current days’ research. Hot-water-soluble PS of the marine alga Capsosiphon fulvescens have been reported to exhibit inhibition activities on cultured human cancer cells in a dose-dependent manner in vitro. The detailed mechanistic studies at molecular level have revealed that the cancer inhibitory effect of these hot-water-soluble PSs was in correlation with an increase in caspase-3 activation and a decrease in Bcl-2 expression, thus inducing apoptosis by inhibiting IGF-IR signaling and the PI3K/Akt pathway (Kwon and Nam, 2007). The carrageenan’s low cytotoxic effects and their immunomodulation and antitumor activities should be considered and can be
recommended for the anticancer therapies that can give a breakthrough for the proper management of cancer-related therapies.

3. Ulvans
Ulvans are structural acid PSs present in cell wall of green algae (Ulva and Enteromorpha). They are highly sulfated and essentially composed of rhamnose 3-sulfate, xylose, xylose 2-sulfate, glucuronic acid, and iduronic acid units. Ulvan displays several physiochemical and biological features of potential interest for food, pharmaceutical, agricultural, and chemical applications (Lahaye and Robic, 2007). Formation of free radicals due to the oxidative stress is thought to be a major contributor for the formation of cancer cells in the human body. Several research groups have suggested that low molecular weight SPs have shown potent antioxidant activity than high molecular weight SPs. For instance, different molecular weight ulvans from Ulva pertusua (Chlorophyceae) were investigated for H₂O₂ degradation and their antioxidant activities. Their results showed that low molecular weight ulvans have a strong antioxidant activity. The rationale for this is low molecular weight SPs may incorporate into the cells more efficiently and donate proton effectively compared to high molecular weight SPs (Qi et al., 2005). This antioxidation ability of the ulvans can be exploited for the preparation of medicinal compounds that can control the progress of cancer. On the other hand, in vitro and in vivo antitumor properties of an SP isolated from the marine algae Champia feldmannii (Cf-PLS) have revealed that inhibition rates of sarcoma 180 tumor development were 48.62% and 48.16% at the doses of 10 and 25 mg/kg, respectively, which clearly suggest the efficacy of marine algal PSs as potential antitumor agents (Lins et al., 2009). Importantly, SPs from marine algae are known to be important free-radical scavengers and antioxidants for the prevention of oxidative damage, which is an important contributor in carcinogenesis. Therefore, it might be suggested that these seaweed-derived SPs have potent capacities for new anticancer product developments in the pharmaceutical as well as in the food industries as novel chemo-preventing agents for cancer therapy (Wijesekara et al., 2011). Moreover, the marine macroalgae are considered as dietary constituents and are rich in SPs like fucoidans, carrageenan, and ulvan and thus can be recommended as medicinal foods to reduce the incidence of cancers.

III. PHLOROTANNINS

It is an undeniable fact that sea algae possess enormous biologically important and beneficial ingredients that aid for the betterment of human health. Recently, the marine algal species have been widely investigated for their bioactive compounds like PSs, pyropheophytin,
tripeptides, and phlorotannins in particularly *E. cava* and *E. bicyclis* (Kousaka et al., 2003). Especially, polyglucinol derivatives called phlorotannins from marine macroalgae (confined to brown algae) have gained a lot of importance in the fields of food, medicinal, and cosmeceutical industries. These phlorotannins are suggested to be formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) monomer units and biosynthesized through the acetate–malonate pathway, also known as polyketide pathway. The phlorotannins are highly hydrophilic components with a wide range of molecular sizes ranging between 126 and 650 kDa. Phlorotannins are tannin derivatives composed of several phloroglucinol units linked to each other in different ways and mostly isolated from brown algae (Ragan and Glombitza, 1986; Singh and Bharate, 2006). Dioxinodehydroeckol (Fig. 16.2) isolated from *E. cava* has exhibited a remarkable antiproliferative effect on human breast cancer cells (MCF-7). Scientific investigation suggests that dioxinodehydroeckol’s potential antiproliferative activity might be associated with the induction of apoptosis through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) family and NF-κB-dependent pathway (Kong et al., 2009).

Phlorotannin extract (PE) derived from brown algae *Laminaria japonica* Aresch (*L. japonica*) has shown considerable antiproliferative activity in the human hepatocellular carcinoma cell line (BEL-7402) and on murine leukemic cell line (P388) in a dose-dependent manner. Microscopic observation have revealed that the morphologic features of tumor cells treated with PE and 5-fluorouracil (a commercial chemotherapy drug) are markedly different from the normal control group, suggesting the antiproliferative effect of PE (Yang et al., 2010). In pretumor bearing mouse, the dietary feeding (0.1% and 0.5%) of brown algae polyphenols significantly reduced tumor multiplicity (45% and 56%) and tumor volume (54% and 65%), and topical administration (3 and 6 mg) significantly decreased tumor multiplicity (60% and 46%) and tumor volume (66% and 57%), respectively. It is believed that brown algal polyphenols inhibit the

![Figure 16.2](image)

**FIGURE 16.2** Dioxinodehydroeckol from *E. cava* which exhibits anticancer properties.
cyclooxygenase-2 activity and cell proliferation, hence preventing the
tumor progression (Hwang et al., 2006). It has been reported that the total
polyphenolic content in the algae is the key for their antiproliferative ability.
These scientific reports clearly suggest the importance and the ability of
phlorotannins in reducing the risk of cancer and its related diseases. Thus
recommending marine algae as ideal resources for novel and effective
anticancer compounds and their predominant role as medicinal foods.

IV. CAROTENOIDs

Carotenoids are organic pigments that have tetraterpinoid structures and
are commonly found in the photosynthetic organisms that include macro-
algae. It is believed that consumption of vegetables that are rich in carote-
noids and other active components could reduce the risk of malignancies in
human colon, lung, and breast cancers (Block et al., 1992; Le Marchand et al.,
1993; Zhang et al., 1999). Among the carotenoids, fucoxanthin and astax-
anthin (AX) (Fig. 16.3) have been known for their antioxidation activities
that might help to control the progress of cancers. Fucoxanthin, a major
carotenoid in brown sea algae, has recently been demonstrated by us to
inhibit the proliferation of colon cancer cells, and this effect was associated
with growth arrest. The molecular mechanisms of fucoxanthin against the
hepatic cancer using the human hepatocarcinoma HepG2 cell line (HepG2)
were evaluated. This investigation has led to confirm that fucoxanthin
reduced the viability of HepG2 cells accompanied with the induction of

![Carotenoids Diagram]

**FIGURE 16.3** Marine-derived carotenoids, (A) astaxanthin and (B) fucoxanthin.
cell cycle arrest during the G\textsubscript{0}/G\textsubscript{1} phase at 25 μM (Das et al., 2008). The apoptosis-inducing effects of fucoxanthin from brown alga *U. pinnatifida* have been studied *in vitro* on human leukemic HL-60 cells. On treatment of HL-60 cells with fucoidan, the DNA content that was fragmented which is thought to be an enrichment factor has increased with the increase in the concentration of fucoxanthin, thus suggesting the apoptosis-inducing ability of fucoxanthin in HL-60 cells (Hosokawa et al., 1999).

AX, a member of carotenoid which is found abundantly in seaweeds, also has been reported to exhibit several biological activities. The chemopreventive effects of two xanthophylls, AX and canthaxanthin (CX), on urinary bladder carcinogenesis induced by N-butyl-N(4-hydroxybutyl) nitrosamine (OH-BBN) was investigated in male ICR mice. In this investigation, it was understood that, in particular, AX administration after OH-BBN exposure significantly reduced the incidence of bladder cancer (transitional cell carcinoma) ($P > 0.003$). Moreover, preneoplasms and neoplasms induced by OH-BBN, and the antiproliferative potential, were greater for AX than CX. These results indicate that AX is a possible chemopreventive agent for bladder carcinogenesis and such an effect of AX may be partly due to suppression of cell proliferation (Tanaka et al., 1994). Stress may play an important role in the incidence of several types of cancer. Studies have indicated that psychological stress enhances the initiation and progression of cancer in humans and animals (Bergsma, 1994). The inhibition of the NK cells plays a major role in the antitumor effector activity and the inhibition of cancer metastases. One of the research groups has evaluated the effect of fucoidan in stress-induced mice with inhibited NK cells. The stress also caused a significant increase in the lipid peroxidation of liver tissue. ASX (100 mg/kg/day, p.o., 4 days) improved the immunological dysfunction induced by restraint stress. Daily oral administration of ASX (1 mg/kg/day, p.o., 14 days) markedly attenuated the promotion of hepatic metastasis induced by restraint stress. These results suggested that AX improves antitumor immune responses by inhibiting lipid peroxidation induced by stress (Kurihara et al., 2002). Considering these above specified research observations, marine algae that are dietary constituents could be recommended as medicinal foods because of the abundant occurrence of the secondary metabolites that act as immunomodulators for combating cancer-related diseases.

**V. CONCLUSIONS AND FURTHER PROSPECTS**

Marine organisms have been serving the mankind with outstanding metabolites that have remarkable and effective activities that aid for the betterment of the human health. Marine environment offers highly challenging circumstances that enable the marine organisms to synthesize
unique and effective metabolites to thrive in such harsh situations. Hence, marine organisms and their metabolites are considered as exceptional resources in the fields of food, medicinal, and pharmaceutical prospects. Moreover, since ages marine organisms like fish, algae crustaceans have been serving the human kind as food resources. Scientific investigations have already proven the effectiveness of the marine algae as functional foods that could cure various kinds of human diseases. The antioxidant and, in particular, the anticancer abilities of the sea algae derived SPs, phloroglucinol derivatives, and carotenoids have already made clear about their possible candidature as leads for the designing of anticancer medicines. Dietary intake of these algae could definitely offer humans an outstanding immunomodulation that could enhance the chances to inhibit cancers and related diseases, and hence marine macroalgae could be recommended as medicinal foods. These phytochemicals possibly activate macrophages, induce apoptosis, and prevent oxidative damage of DNA, thereby controlling carcinogenesis. However, in spite of vast resources enriched with chemicals, the marine floras are largely unexplored for anticancer lead compounds and research strategy should be focused on screening of more sea algae as potential resources for the phytochemicals that could cure this deadliest disease, human cancer. And with the available sophisticated approaches, the research should proceed for the clinical trials to establish these marine algal metabolites as potential anticancer compounds.

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Marine Algae: Natural Product Source for Gastrointestinal Cancer Treatment

Se-Kwon Kim*†,1 and Mustafa Zafer Karagozlu*

Abstract

Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. In order to survive in a highly competitive environment, freshwater or marine algae have to develop defense strategies that result in a tremendous diversity of compounds from different metabolic pathways. Recently, their importance as a source of novel bioactive substances is growing rapidly and many reports have been published about isolated compounds from algae with biological activities. Many researchers reported anticancer activity of the compounds isolated from marine algae. Gastrointestinal tract cancer is one of the most frequent death causes of cancer in men and women. Especially stomach cancer and colon cancer are the second and third common cancer type in the world after lung cancer. Hence investigation of bioactive compounds against gastrointestinal cancer cells has recently become an important field for researchers.
I. BACKGROUND

Around 70% of the earth’s surface is covered by oceans and seas, and the organisms that learn to survive in this 70% has potential for biomass production. Algae survive in a large scale in the earth: in the sea, rivers, and lakes, on the land, or on other organisms. To survive in a competitive environment, freshwater and marine algae have developed defense strategies that result in a significant level of structural-chemical diversity, from different metabolic pathways (Barros et al., 2005; Puglisi et al., 2004). They are simple chlorophyll containing organism. They are accepted as simple because comparison of land plants and algae shows that algae do not have tissues which are organized into distinct organs. Algae are divided into two major groups: unicellular microalgae (Microphytes) and multicellular macroalgae (seaweed). The macroalgae can be identified in three major groups as green algae (Chlorophyta), brown algae (Phaeophyta), and red algae (Rhodophyta) (El Gamal, 2010).

The researches on algae for industrial and pharmaceutical purposes have revealed important chemical prototypes for the discovery of new agents, new sophisticated physical techniques, and new compounds with biomedical application. Besides, algae are promising organisms for providing both novel biologically active substances and essential compounds for human nutrition (Burja et al., 2001; Mayer and Hamann, 2004). On the other hand, toxins produced by freshwater and marine algae represent an increasing hazard to water supplies, reservoirs, recreational beaches, as well as seafood contamination (Gehringer, 2004).

The nutritional value of marine algae has long been recognized in the Eastern culture; on the other hand, in the Western culture, the use of sea vegetables as nutrient is limited. Especially in the Far East countries like Japan, Korea, or China, sea vegetable has been preferred because the features of them such as containing vitamins and minerals. In many countries, the food industries consume a wide range of algae, which are well known to have high contents of fiber, minerals, vitamins, and different antioxidants. In the past few decades, the emphasis has moved from wild harvests to farming and controlled cultivation to produce valuable new products on a large scale (Nagaoka et al., 2000).

Although pharmaceutical research on the algae products is very active, antigastrointestinal tract cancer activity from marine source studies have been few and mainly concerned with saccharides rather than the peptides or secondary metabolites. Even if there are a few saccharides that show antigastrointestinal tract cancer activity have been extracted from marine algae, they are still promising to be a rich source for human being to fight against cancer.
II. GASTROINTESTINAL TRACT CANCER

Gastrointestinal tract cancer is the malignant condition of the digestive tract, and it is the major health problem worldwide. Prognosis of the gastrointestinal tract cancer is grim against patients. Gastrointestinal cancer is often reported to result in metastatic conditions. Surgery is a curative option in 50% of colorectal cancers, but is less effective in gastric cancer, where the overall 5-year survival rate is less than 10%. Gastrointestinal cancer is divided into two main groups, depending on the organs which are affected from cancer. First group is called upper gastrointestinal tract cancer (Hasegawa et al., 2003). This group includes esophagus and stomach cancer. Second group is lower gastrointestinal tract cancer and this group includes small intestine, appendix, colon/rectum (colorectal), and anus cancer.

III. GASTRIC CANCER

Gastric or stomach cancer is the second most frequent death cause of cancer, after lung cancer, around the world. Almost two-thirds of cases occur in Eastern Europe, South America, and Asia with 42% in China alone. In the United States, in 2009, an estimated 21,130 new cases of gastric cancer were diagnosed and were associated with 10,620 deaths (Jemal et al., 2009), and it is one of the most common cancers in Europe ranking fifth after lung, prostate, colorectal, and bladder cancers in men and breast, colorectal, lung, and cancer of the corpus uteri in women. Sex-dependent ratio is (the male-to-female ratio in incidence rates) about 1.6:1 (Boyle and Ferlay, 2005).

There are geographic and ethnic differences in gastric cancer incidence in the world and in its trends for each population with time. The incidence patterns observed among immigrants change according to where they live. These factors indicate the close association of gastric cancer with modifiable factors such as diet. Substantial evidence from ecological, case–control, and cohort studies strongly suggest that the risk of cancer increases with a high intake of various traditional salt-preserved foods as well as salt per se and that this risk could be decreased with a high intake of fruit and vegetables (Kono and Hirohata, 1996). Also, there is some evidence that the intake of green tea and vitamin C is associated with the risk of gastric cancer. A recent report of a joint World Health Organization (WHO) and Food and Agriculture Organization (FAO) Expert Consultation concluded that salt-preserved food and salt probably increase the risk of gastric cancer, whereas fruit and vegetables probably decrease the risk (Petersen, 2003). Genetic factors are also important for the risk.
of gastric cancer. Approximately 10% of cases show a genetic component. Other established nondietary factors include cigarette smoking (Anton-Culver et al., 1993) and infection with the Helicobacter pylori. Although H. pylori lives in between the mucosal and the epithelial cells of the human stomach without adverse consequences, the presence of H. pylori is associated with an increased risk of gastric adenocarcinoma (Linz et al., 2007).

The potential of the marine algae has been the driving force for the researchers to focus on the benefits of algae (Barros et al., 2005; Puglisi et al., 2004). The prevention of gastric cancer therefore represents one of the most important aspects of any cancer control strategy in around the world. Hence, radical scavenging compounds such as polysaccharides from seaweeds can be used indirectly to reduce cancer formation in human body.

Porphyran is sulfated polysaccharides from marine algae. Kwon and Nam purified polysaccharides from Porphyra haitanesis and evaluated its anticancer activity on AGS human adenocarcinoma cell (Kwon and Nam, 2006). It has been known that specific IGF-IR inhibition with neutralizing antibody, antagonistic peptide, or selective kinase inhibitor has activity against diverse tumor cell types and is one of the causes of antiproliferative/proapoptotic molecular induction (Li et al., 2004; Saxena and Moorthy, 2007). In the study, the effect of IGF-I on porphyran-treated AGS cells was determined, and the result they declared that porphyran-induced apoptosis is involved in the IGF-IR-mediated signaling pathway in AGS gastric cancer cells. Moreover a porphyran purified from Porphyra yezoensis confirmed its apoptotic activity on AGS human adenocarcinoma cell line (Kwon and Nam, 2007). Porphyran that was isolated from red alga P. yezoensis also shows the apoptotic activity on AGS cell line. Further, Kwon and Nam declared that porphyran from different marine algae showed same apoptotic activity on AGS cell line by following mitochondrial pathway (Kwon and Nam, 2007).

Hwang et al. declared that polysaccharide extracted from Capsosiphon fulvescens inhibited alcohol-induced cell death and reduced the expressions of cyclooxygenase-2 (COX-2) and the inducible form of nitric oxide (iNOS), proteins related to ulcers (Hwang et al., 2008). They proved that polysaccharide from marine algae also can be used as cancer protection agent.

Moreover, fucoidan is a fucan sulfate occurring in brown marine algae. Shibata et al. studied the inhibitory effect of Cladosiphon fucoidan on H. pylori adhesion to human stomach (Shibata et al., 1999). Their researches proved that fucoidan inhibited bacterial binding to human gastric cell. It was also shown that this fucoidan blocks both Leb- and sulfatide-mediated attachment of H. pylori to gastric cells.

Hiroe et al. reported that glycoprotein which extracted from brown alga “Laminaria japonica induces apoptosis on AGS adenocarcinoma...
cells.” They declared that glycoprotein extracted from L. japonica inhibited AGS cell growth by following multiple apoptotic (extinct and instinct) pathways. Treatment of glycolipid caused some changes in the Fas receptor pathway and the mitochondrial pathway (Go et al., 2010).

Besides, acetone + methylene chloride and methanol extract of Carpospeltis affinis, Sargassum tortile, Sargassum horneri, Sargassum fulvellum, Colpomenia sinuosa, Sargassum yezoense, and Sargassum hemiphyllum inhibit the AGS cells’ growth (Choi et al., 2006). Besides methanol extract of marine algae L. japonica, Porphyra tenera, Gelidium amansii, and Ecklonia cava has inhibitory activity on AGS cell growth (Choi et al., 2006). Their mechanism to inhibitions has not been clarified yet.

IV. COLORECTAL CANCER

The colon is a muscular organ and the last part of the digestive system in human, and the rectum is the final portion of the colon. Colorectal cancer is the third most common type worldwide (Boyle and Ferlay, 2005; Parkin, 2004) after lung and stomach cancer. Among them colon cancer is more frequent than rectal cancer. Especially in developed countries, the ratio of colon to rectum cancer cases can increase up to 2:1 or more. However, in nonindustrialized countries, rates are almost similar. On the other hand, comparison of the incidence rate of colon cancer in developed and underdeveloped countries shows that colorectal cancer is more common in industrialized countries. Also 50% of the colorectal cancer death has been seen in developed countries (Tyczynski et al., 2003). It remains relatively uncommon in Africa and much of Asia. And rates of this cancer increase with industrialization and urbanization. It has been much more common in high income countries, but also now increasing in middle- and low-income countries too. It remains relatively higher in North America, Europe, and Australia rather than South America, Asia, and Africa (Parkin, 2004).

The development of colorectal cancer in humans involves genetic and environmental factors. A major environmental factor appears to be diet. Even if it has not been proved strongly, too much intake of red meat, processed meat, and alcohol drinks increases the risk of colorectal cancer (Chao et al., 2005). The evidences are stronger for colon cancer than for rectum cancer. On the other hand, dietary calcium and vitamin D are inversely related to the incidence of colon cancer (Kwak and Chung, 2006). Another environmental risk factor for colorectal cancer is smoking. Smoking has consistently been positively associated with large colorectal adenomas (Giovannucci, 2001). There is strong evidence to suggest that alcohol and smoking have a greater relative effect together than alone. Another risk factor for colorectal cancer is genetic factors. Family history
is important for the risk of colorectal cancer like most of the cancer case. Especially the patients who are affected in early ages have family history in colorectal cancer (Strate and Syngal, 2005).

Natural products have yielded numerous effective anticancer drugs. In particular, many studies over the past few decades have focused on the benefits of algae. Algae are mainly composed of carbohydrates, proteins, and other minor components. They are used as food especially in Asian culture. This traditional food source appears to maintain good health by providing nutritional benefits and thus helping to combat diseases. Further, many compound extracted from seaweeds exhibit diverse biological activities, including effects on colorectal cancer.

Hiroe et al. reported that glycoprotein which extracted from brown alga “L. japonica” induces apoptosis on HT-29 human colon cancer cells. They declared that glycoprotein that extracted from L. japonica inhibited growth in a dose- and time-dependent manner (Go et al., 2010). Also, the growth inhibition by glycolipid is associated with multiple apoptotic (extinct and instinct) pathways. Treatment of glycolipid caused some changes in the Fas signaling pathway and mitochondrial pathway. The Fas signaling pathway is a major apoptosis-related extinct signaling pathway. In this pathway mechanism, the Fas receptor signaling pathway is initiated by binding of the ligand on the cell surface, which then forms the DISC and activates caspase-8. Activation of caspase-8 initiates a cascade of caspases and leads to apoptotic cell death. Moreover, they also observed decreased levels of Bcl-2 expression and increased levels of Bad expression after L. japonica glycoprotein treatment. Bcl-2 and Bad are members of Bcl apoptotic protein family, and they play a vital role in mitochondrial apoptotic pathway (Go et al., 2010).

Hosokawa et al. reported that fucoxanthin extracted from another brown algae Undaria pinnatifida induce apoptosis on Caco-2, HT-29, and DLD-1 cell lines. They declared “Fucoxanthin reduced the viability of human colon cancer cell lines Caco-2, HT-29 and DLD-1 although the sensitivity for fucoxanthin among the three was different.” Fucoxanthin decreased the level of the apoptosis-suppressing protein, Bcl-2. This indicates that the downregulation of Bcl-2 protein may contribute to fucoxanthin-induced apoptosis. It has a unique structure including an unusual allelic bond and 5,6-monoepoxide in its molecule. This structure may also give fucoxanthin an ability to regulate the redox signals and then facilitate the progression of apoptosis through Bcl-2 protein suppression and the caspase-dependent and -independent pathway (Hosokawa et al., 2004).

Recently, only extract of brown algae shows activity against colon cancer cells. Mei et al. have published a study on that. In their study, they have collected brown algae, Lethariella zahlbruckneri, and extracted with methane and acetone. They declared that both extracts showed dose- and time-dependent antiproliferative activity on HT-29 cells (Ren et al.,
2009). Further, the apoptotic activities of extracts on HT-29 cells were evaluated. Finally, the study showed that acetone extract induced apoptosis via caspase-dependent and caspase-independent pathways (Ren et al., 2009).

Besides, many active compounds have been characterized by researchers: sulfur-containing polybromoindoles were isolated from red alga, Laurenda brongniartii (El Gamal, 2010); aromatic sesquiterpenes, dimeric sesquiterpene of the cyclolaurane-type, sesquiterpene alcohol of bisabolene type were isolated from the organic extracts of Laurencia microcladia (Kladi et al., 2007); terpenoid was isolated from tropical brown algae, Stylolpodium zonale (Dorta et al., 2002); furoplocamiod, perfuroplocamiod, pirene, and tetrachlorinated cyclohexane from the red alga, Plocumium carttilagineum (Argandoña et al., 2002); tetrahydro-β-carboline from the red alga, Callophycus oppositifolius (Ovenden et al., 2011) which were active against HT-29 and SW480 cells.

V. CONCLUSIONS

While microalgae is the last ring of the food chain in all aquatic system, recently studies have provided that potential bioactive metabolites from macroalgae or sea vegetable can play a vital role in human health and nutrition. The designing of new functional foods and pharmaceuticals from marine algae makes them one of the most valuable marine sources. Many bioactive compounds have been purified from marine algae, but until now, most of the antigastrointestinal cancer activities of marine-derived extracts or compounds have not been observed in vitro. Therefore, further researches are needed in order to investigate their activity in human subjects.

REFERENCES


Anticoagulant Effect of Marine Algae

Se-Kwon Kim*†,† and Isuru Wijesekara*

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

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

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

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

Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly, and researchers have revealed that marine algal originated compounds exhibit various biological activities with potential anticoagulant effect (Wijesekara and Kim, 2010; Wijesekara et al., 2010, 2011).
Sulfated polysaccharides (SPs) and phlorotannins from marine algae have been shown potent anticoagulant effect, and according to most of the studies, SPs are the main bioactive for this anticoagulant effect. The anticoagulant activity of the SPs and phlorotannins from marine algae has been determined by prolongation of activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) assays. Most of the studies reported that the anticoagulant activity of marine SPs is based on APTT and TT pathways. The prolongation of APTT suggests inhibition of the intrinsic factors and is the measure of the intrinsic pathway-dependent clotting time. The TT revealed the inhibition of thrombin activity or fibrin polymerization as thrombin inhibition-dependent clotting time. PT is the extrinsic pathway-dependent clotting time. This chapter focuses on anticoagulant agents derived from marine algae and presents an overview of their anticoagulant effect.

II. ANTICOAGULANT AGENTS IN MARINE ALGAE

A. Sulfated polysaccharides

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

B. Phlorotannins

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

**FIGURE 18.1** Anticoagulant SPs from marine algae (A) fucoidan, (B) carrageenan, and (C) ulvan.
FIGURE 18.2 Phlorotannin derivatives from marine brown algae.
III. ANTICOAGULANT ACTIVITY OF MARINE ALGAE

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

<table>
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<th>Marine algae with anticoagulant SPs</th>
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<td><em>Chlorophyta</em></td>
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<tr>
<td><em>Monostroma latissimum</em></td>
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<td><em>Monostroma nitidum</em></td>
<td>Maeda et al. (1991)</td>
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<td><em>Ulva conglobata</em></td>
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<td><em>Codium fragile</em></td>
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<td><em>Codium pugniformis</em></td>
<td>Matsubara et al. (2000)</td>
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<td><em>Codium cylindricum</em></td>
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<td><em>Phaeophyta</em></td>
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<td><em>Ecklonia cava</em></td>
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<td><em>Ecklonia kurom</em></td>
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<td><em>Laminaria japonica</em></td>
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<td><em>Ascophyllum nodosum</em></td>
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<td><em>Lessonia vadosa</em></td>
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<td><em>Rhodophyta</em></td>
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<td><em>Porphyra haitanensis</em></td>
<td>Zhang et al. (2010)</td>
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<td><em>Nothogenia fastigiata</em></td>
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the anticoagulant-active SPs from *Codium fragile* subspecies *atlanticum* (Chlorophyta) contain xyloarabinogalactans. A sulfated galactan with anticoagulant activity has also been reported from *Codium cylindricum* (Matsubara *et al.*, 2001). In addition, Maeda *et al.* (1991) have revealed that the anticoagulant SPs from *Monostroma nitidum* (Chlorophyceae) yielded a sixfold higher activity than that of heparin. In comparison, marine brown algae extracts exhibit higher anticoagulant activity than red and green algae extracts (Chevolot *et al.*, 1999; Patankar *et al.*, 1993).

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

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

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

**IV. CONCLUSIONS**

Recent studies have provided evidence that marine algal derived SPs and phlorotannins play a vital role in human health and nutrition. Further, seaweed processing by-products with bioactive SPs and phlorotannins can be easily utilized for producing functional ingredients. The possibilities
of designing new functional foods and pharmaceuticals to support reducing blood clotting or regulating the coagulant-related chronic malfunctions are promising. Therefore, it can be suggested that due to valuable biological functions with health beneficial effects, marine algae have much potential as active ingredients for preparation of nutraceutical and pharmaceutical products. Until now, most of researches of marine algal anticoagulant effect have been observed in vitro or in mouse model systems. Therefore, further research studies are needed in order to investigate their activity in human subjects.

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Potential Application of Marine Algae as Antiviral Agents in Medicinal Foods

Se-Kwon Kim,*†,1 Thanh-Sang Vo,* and Dai-Hung Ngo*

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Abstract

Viral diseases, caused by pathogenic virus infections, are still the leading cause of death in humans worldwide. Although many antiviral agents have been developed and are used for treatment of infectious diseases, emergence of drug resistance, side effects, and the necessity for extensive clinical use are the main reasons for failure of antiviral therapy. Therefore, the development of new antiviral agents with diverse kinds of antiviral actions is required. The search for new antiviral agents focuses on not only synthetic compounds but also natural products such as plants, insects, animal organs, and their components. Recently, a great deal of interest has been expressed regarding marine algae as potential antiviral agents. This contribution focuses on antiherpes virus therapeutic agents...
I. INTRODUCTION

Herpes is a family of viruses of which herpes simplex virus 1 and herpes simplex virus 2 (HSV-1 and HSV-2) are the most serious human pathogens. HSV-1 is more frequently associated with oral–facial infections and encephalitis, whereas HSV-2 usually causes genital infections and can be transmitted from infected mothers to neonates. Both viruses establish long-term latent infections in sensory neurons and lesions at or near point of entry into the body (Whitley and Roizman, 2001). HSV infections are among the most common diseases of humans, with an estimated 60–95% of the adult population being infected by at least one of them (Brady and Bernstein, 2004). Effective antiherpes drugs, such as acyclovir, valacyclovir, penciclovir, famciclovir, trifluridine, cidofovir, and vidarabine, are available for treatment. However, the prolonged therapies with the available antiherpes drugs have resulted in some undesirable effects and also induced the emergence of drug-resistant strains (Morfin and Thouvenot, 2003). For this reason, the search for new types of antiherpes virus agents with high efficacy on resistant mutant viral strains is urgently needed.

The marine environment, which represents approximately half of the global biodiversity, contains a rich source of structurally diverse and biologically active metabolites. Specially, products from marine algae show many interesting activities, such as anticancer, antidiabetic, antifungal, anticoagulant, anti-inflammatory, and other pharmacological activities (El Gamal, 2010). In relation to antiviral properties, marine algae are believed to be able to provide novel leads against pathogenic viruses that are evolving and developing resistance to existing pharmaceuticals (Vo and Kim, 2010). Thus, marine algae are regarded as a promising source for the production of therapeutic drugs against viral diseases. This chapter focuses on antiherpes virus therapeutic agents derived from marine algae and their potential medical application as novel functional ingredients in antiherpes virus therapy.

II. POTENTIAL ANTIHERPES VIRUS AGENTS FROM MARINE ALGAE

A. Red macroalgae

The importance of red macroalgae as a source of novel anti-HSV agents has been recognized and reported by many researchers. According to Serkedjieva (2000), the water extract of Polysiphonia denudate exhibited
selective inhibition on the reproduction of HSV-1 and HSV-2 at their effective concentration 50% (EC\textsubscript{50}) range of 8.7–47.7 mg/ml. The inhibition affected adsorption as well as intracellular stages of viral replication. Likewise, methanol extract of \textit{Symphyocladia latiuscula} and its fractions was effective against acyclovir and phosphonoacetic acid-resistant HSV-1 (AP\textsuperscript{r} HSV-1), thymidine kinase deficient HSV-1 (TK HSV-1), and wild-type HSV-1 \textit{in vitro} without cytotoxicity (Park \textit{et al.}, 2005). Specially, the major component of CH\textsubscript{2}Cl\textsubscript{2}-soluble fraction, 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB), inhibited wild-type HSV-1, as well as (AP\textsuperscript{r} HSV-1) and (TK HSV-1) with their inhibitory concentration 50% (IC\textsubscript{50}) values of 5.48, 4.81, and 23.3 \(\mu g/ml\), respectively. Moreover, the oral administrations of TDB significantly delayed the development of skin lesions and suppressed virus yields in HSV-1-infected mice. In another study, Persian Gulf \textit{Gracilaria salicornia} was elucidated for its capability against HSV-2 (Zandi \textit{et al.}, 2007). The antiviral activity of water extract from \textit{G. salicornia} displayed not only before attachment and entry of virus to the Vero cells but also on post attachment stages of virus replication. Accordingly, the extracts of marine red macroalgae can be a rich source of potential antiviral components. Interestingly, it has known that marine red macroalgae contain significant quantities of sulfated polysaccharides that may be responsible for anti-HSV properties (Damonte \textit{et al.}, 2004).

Indeed, numerous sulfated polysaccharides from red macroalgae have been determined to possess significant inhibition on herpes virus. Xylomannan, a sulphated polysaccharide from \textit{Nothogenia fastigiata}, was found to inhibit efficiently the replication of HSV-1 and HSV-2 under various experimental conditions (Damonte \textit{et al.}, 1994; Pujol \textit{et al.}, 1995). Further, the xylomannan sulfate of \textit{Scinaia hatei} exhibited potent antiviral activity against reference strains, syncytial formation, and TK acyclovir-resistant strains of HSV-1 and HSV-2 at IC\textsubscript{50} range of 0.5–4.6 \(\mu g/ml\) (Mandal \textit{et al.}, 2008). Additionally, the sulfated xylomannan from \textit{Sebdenia polydactyla} was identified to have a stronger inhibition than the known sulfated xylomannan with IC\textsubscript{50} range of 0.35–2.8 \(\mu g/ml\) (Ghosh \textit{et al.}, 2009). The appreciable inhibition produced by sulfated xylomannan was similar to that of standard antiherpetic polysulfates, such as heparin and dextran sulfate. Notably, the inhibitions of \textit{in vitro} HSV replication by these xylomannans were observed at concentrations, which did not have any effect on cell viability. These sulfated xylomannans represent a potential candidate for further clinical studies.

Similar to xylomannan, galactans were found to be highly selective antiviral substances against the replication of the different strains of herpes viruses. Remarkably, \textit{Gymnogongrus griffithsiae} and \textit{Cryptonemia crenulata} have known to represent an interesting source of galactans with selective and potent antiviral action against reference strains,
Syncytial variants, and acyclovir-resistant strains of HSV-1 and HSV-2 at IC\textsubscript{50} values in the range of 0.5–5.6 µg/ml (Talarico et al., 2004). The active galactans that extracted from \textit{C. crenulata} exhibited a more effective antiviral action higher than the reference compounds heparin and dextran sulfate. Further, the crude galactan of \textit{C. crenulata} showed a significant protective effect \textit{in vivo} against HSV-2 vaginal infection in a murine model, suggesting the potential use of this low-cost product, easy to obtain in large quantities, for prophylaxis of virus infection. On the other hand, the strong antitherpetic activity of galactan sulfate obtained from \textit{Grateloupia indica} has been shown in recent study (Chattopadhyay et al., 2007). The isolated galactan exhibited potent anti-HSV effect on reference strains, syncytial variants, and TK ACV-resistant strains at low value of IC\textsubscript{50} (0.12–1.06 µg/ml), mainly affecting virus adsorption to the host cells.

The natural carrageenans isolated from the red seaweed have recently identified as potent and selective inhibitors of HSV-1 and HSV-2. Carrageenans isolated from \textit{Meristiella gelidium} were found to be among the most potent sulfated polysaccharides obtained from red seaweeds according to their inhibitory activity against herpes virus. The most active fraction obtained from \textit{M. gelidium} showed a selectivity index against HSV-2 of 25,000, a value high enough to regard this carrageenan as a potential agent to be evaluated for the treatment of genital HSV-2 infection (De S-F-Tischer et al., 2006). Additionally, the inhibition of \textit{in vivo} HSV by carrageenan has been investigated in model of murine infection (Carlucci et al., 2004; Pujol et al., 2006). Among them, the \(\lambda\)-carrageenan extracted from the red seaweed \textit{Gigartina skottsbergii} revealed 100% protection against HSV-2 mortality and replication in a very strict model of murine infection at a high dose of virus. Further, virus or neutralizing antibodies against HSV-2 was not detected in serum of \(\lambda\)-carrageenan-treated animals until 3 weeks after infection. These evidences warrant the availability of \(\lambda\)-carrageenan to protect the whole infectable surface of the mouse vagina.

**B. Brown macroalgae**

Besides red macroalgae, brown macroalgae also provide useful additional therapy for treating several enveloped viruses infections. Fucoidans, a complex sulfated polysaccharide found mainly in brown macroalgae, have been reported in many papers for their anti-HSV activities. Feldman and colleagues isolated fucoidan fractions (Ee, Ec, and Ea) from \textit{Leathesia difformis} and determined their selective antiviral abilities against HSV-1 and HSV-2 (Feldman et al., 1999). Fucoidan Ea was shown to be the most active agent, with IC\textsubscript{50} value in the range of 0.5–1.9 µg/ml. Continually, fucoidans were found in different marine brown macroalgae due to their
anti-HSV property, including *Adenocystis utricularis*, *Sargassum horneri*, *Cystoseira indica*, *Stoechospermum marginatum*, and *Sargassum tenerrimum* (Adhikari et al., 2006; Mandal et al., 2007; Ponce et al., 2003; Preeprame et al., 2001; Sinha et al., 2010). Noticeably, *Undaria pinnatifida*, the most commonly eaten brown seaweed in Japan, contains sulphated polyanions and other components with appreciable anti-HSV effect. Galactofucan, the major component of an aqueous extract of *U. pinnatifida*, was evaluated for antiviral activity against 32 clinical strains of HSV, including 12 ACV-resistant strains (4 HSV-1 and 8 HSV-2) and 20 ACV-susceptible strains (10 HSV-1 and 10 HSV-2). The median IC$_{50}$ of galactofucan for the 14 strains of HSV-1 and 18 strains of HSV-2 was 32 and 0.5 μg/ml, respectively. The mode of action of the galactofucan was shown due to the inhibition of viral binding and entry into the host cell (Thompson and Dragar, 2004). In addition, a fucoidan from sporophyll of *U. pinnatifida* (Mekabu) was examined for its antiviral activity. The IC$_{50}$ values for HSV-1 and HSV-2 were 2.5 and 2.6, respectively, under conditions in which the fucoidan was added at the same time as viral infection (Lee et al., 2004a). In the *in vivo* conditions, ingestion of fucoidan from *U. pinnatifida* was associated with increased healing rates in patients with active infections (Cooper et al., 2002). Moreover, oral administration of the fucoidan from *U. pinnatifida* could protect mice from infection with HSV-1 as judged from the survival rate and lesion scores (Hayashi et al., 2008). Substantially, natural killer and cytotoxic T lymphocytes activity in HSV-1-infected mice was enhanced by oral administration of the fucoidan. The production of neutralizing antibodies in the mice inoculated with HSV-1 was significantly promoted during the oral administration of the fucoidan for 3 weeks. According to these results, fucoidan from *U. pinnatifida* was suggested as a topical microbicide for the prevention of transmission of HSV through direct inhibition of viral replication and stimulation of both innate and adaptive immune defense functions.

In recent years, anti-HSV activity of brown macroalgae was known due to their diterpene and glycolipid components. Two compounds of diterpenes, 8,10,18-trihydroxy-2,6-dolabelladiene (1) and (6R)-6-hydroxydichotoma-4,14-diene-1,17-dial (2), were isolated from *Dictyota pfaffii* and *Dictyota menstrualis* (Abrantes et al., 2009). It was observed that compounds 1 and 2 inhibited HSV-1 replication in a dose-dependent manner at EC$_{50}$ values of 5.10 and 5.90 μM, respectively. Similarly, the dolastane diterpenes 4-hydroxy-9,14-dihydroxydolasta-1(15),7-diene and 4,7,14-trihydroxydolasta 1(15),8-diene isolated from *Canistrocarpus cervicornis* also exposed the suppressive effect on replication of HSV-1 (Vallim et al., 2010). In an investigation of El-Baroty et al. (2011), they revealed that glycolipid achieved from brown alga *Dilophys fasciola* possessed noticeable effect against HSV-1. At concentration range of 25–100 μg/ml, glycolipid of *D. fasciola* caused remarkably inhibition % of HSV-1 with various
degrees (78.5–100%). The IC₅₀ value was 10 μg/ml, compared to that 55 μg/ml for acyclovir. A suggestion for active mechanism of glycolipid might involve in the binding of the virus glycoprotein to algal glycolipid and cause an irreversible denaturation that blocks the viral infectivity. In general, many of natural lipid classes have been shown to have high virucidal activity and are being developed as microbicidal ingredient in drug formulas to kill viruses (Hilmarsson et al., 2006).

C. Green macroalgae

As expected, green macroalgae also emerged as novel antiviral agents. Herein, Lee et al. (2004b) have estimated anti-HSV-1 activity of natural sulfated polysaccharides from 10 green macroalgae (Enteromorpha compressa, Monostroma nitidum, Caulerpa brachypus, Caulerpa okamurai, Caulerpa scapelliformis, Chaetomorpha crassa, Chaetomorpha spiralis, Codium adhaerens, Codium fragile, and Codium latum). Except for one from E. compressa, other sulfated polysaccharides displayed strong anti-HSV-1 activities with IC₅₀ range of 0.38–8.5 μg/ml, while having low cytotoxicities with 50% inhibitory concentrations of >2900 μg/ml. In the delineation of the drug-sensitive phase, the polysaccharides of SX4 and SP4 from C. brachypus and SP11 from C. latum showed potent anti-HSV-1 activities with IC₅₀ values of 6.0, 7.5, and 6.9 μg/ml, respectively, even when added to the medium 8 h postinfection. Subsequently, a polysaccharide from M. nitidum, rhamnan sulfate, was found to be effective against HSV-2 via blockade of virus adsorption and penetration steps onto host cell surface (Lee et al., 2010). Thus, it was indicated that some sulfated polysaccharides from green macroalgae not only inhibited the early stages of HSV replication, such as virus binding to and penetration into host cells, but also interfered with late steps of virus replication. Likewise, sulfated polysaccharide fraction isolated from the hot water extract of the green alga Caulerpa racemosa was regarded as a selective inhibitor of reference strains and TK⁻ acyclovir-resistant strains of HSV-1 and HSV-2 in Vero cells, with EC₅₀ values in the range of 2.2–4.2 μg/ml (Ghosh et al., 2004).

D. Microalgae

In fact, microalgae are attracting enormous attention, and the topics have been discussed by a number of researchers. Microalgae have been recognized to provide chemical and pharmacological novelty and diversity, and they are considered as the actual producers of some highly bioactive compounds found in marine resources (Shimizu, 1996). Among the different compounds with highly biological activities, microalgae present attraction due to antiviral profile. According to Hayashi and coworkers,
the water extract of *Spirulina platensis* was shown to inhibit the replication *in vitro* of HSV-1 in HeLa cells within the concentration range of 0.08–50 mg/ml (Hayashi *et al.*, 1993). Addition of the *S. platensis* extract (1 mg/ml) at 3 h before infection causes blockade of virus-specific protein synthesis at 50% effective inhibition dose (ED$_{50}$) value of 0.173 mg/ml without affecting host cell protein synthesis. Moreover, it was observed that food containing the *S. platensis* extract effectively prolonged the survival time of infected hamsters at doses of 100 and 500 mg/kg per day. Subsequently, Hayashi *et al.* (1996a,b) isolated from *S. platensis* a novel sulfated polysaccharide, calcium spirulan (Ca-SP), which inhibits the replication *in vitro* of several enveloped viruses including HSV-1, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1 virus. The anti-HSV-1 activity of Ca-SP was determined to be fivefold higher than that of dextran sulfate (Hayashi *et al.*, 1996a,b). While *S. platensis* was efficient for anti-HSV-1, *Spirulina maxima* exposed inhibitory activity against HSV-2. A hot water extract of *S. maxima* showed appreciable suppression HSV-2 infection at the initial events of adsorption and penetration (ED$_{50}$, 0.069 mg/ml; Hernández-Corona *et al.*, 2002). Otherwise, blue-green alga *Aphanothece sacrum* and red microalga *Porphyridium* sp. exhibited significant inhibitions in both *in vitro* and *in vivo* model. Specially, cyanovirin-N, a protein produced from blue-green alga *Nostoc ellipsosporum*, was demonstrated to block HSV-1 entry into cells and inhibited membrane fusion mediated by HSV glycoproteins (Tiwari *et al.*, 2009). This compound was suggested as a stronger potential for antiviral therapy against HSV-1.

### III. CONCLUSION

The infections with HSV can lead to a variety of skin and mucosal diseases. Despite intensive research, no prophylactic HSV vaccine has proven to be effective because the viruses establish latency and reactivations occur in the presence of humoral and cell-mediated immunity. The suggested options for treatment of HSV infections include oral acyclovir, valacyclovir, and famciclovir. However, the further discovery of new drugs as well as the adaptation of current drugs is very necessary for the war against viral infection and drug-resistant viruses. As expected, a large number of anti-HSV components from marine algae have been identified based on the specific assay system or screening approach. The extensive studies of marine algae with anti-HSV activity will contribute to the development of novel antiviral agents. Thus, it is believed that the marine algae play a vital role in the pharmaceutical industry to develop novel drugs against herpes simplex virus.
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Anti-HIV Activity of Extracts and Compounds from Marine Algae

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Abstract
In recent years, elucidation of novel bioactive substances from different marine organisms is gaining importance rapidly not only from the research and publications but also from controlled clinical studies of natural product-derived substances. They offer important leads for the development of antiviral drugs against viral infections caused by human immunodeficiency virus type 1 (HIV-1). Regarding this issue, numerous anti-HIV-1 therapeutic agents from marine resources have been reported for their potential medicine/medical application as novel functional ingredients in anti-HIV therapy. In detail, marine macroalgae have attracted much of attention as a reliable source for potential anti-HIV compounds. Up to date, several types of compounds such as tannins, polysaccharides, lectins, and derivatives have been isolated, identified, and reported to possess significant anti-HIV-1 activity.
I. BACKGROUND

High amounts of secondary metabolites produced by microorganisms, plants, and marine organisms have been of much attention as bioactive substances for disease treatment (Lam, 2007). Among these diseases, acquired immunodeficiency syndrome (AIDS) stands as one of the most important diseases worldwide with about 33.2 million people infected by human immunodeficiency virus type-1 (HIV-1; Unaids Global Report, 2010). HIV is an enveloped retrovirus that belongs to the lentivirus family. The structure of HIV is relatively complex with each virus expressing 160 kDa glycoproteins composed of gp120 and gp41. The gp41 molecule is a transmembrane glycoprotein that crosses the membrane of the viral envelope. There is a noncovalent interaction between gp120 and gp41. The entry of HIV into the host cell requires the interaction of viral envelope glycoprotein, gp120, with the CD4 glycoprotein and a chemokine receptor on the host cell surface (Kwong et al., 1998). The viral envelope is composed of the host cell membrane and naturally contains some host cell membrane proteins, including class I and class II major histocompatibility complex molecules which were previously located on host-cell membrane. Within the envelope, viral core (nucleocapsid) that includes a layer of protein called p17, and an inner layer of protein called p24, is located. The HIV genome is composed of two identical single-stranded RNA (+) and some proteins are attached to the genome such as two molecules of reverse transcriptase (RT), a protease, and an integrase (Reeves et al., 2000).

The knowledge of HIV replication is important for the understanding of its pathogenesis and the development of therapeutic agents. Following entry of HIV into cells and getting into contact with the appropriate receptors and coreceptors, formation of the viral double-stranded DNA genome is followed by the integration of proviral-DNA into the host cell genome, creating a provirus. Attachment of the virus occurs upon the binding of the gp120 on the viral envelope to the CD4+ host cell. Receptors on the host cells could be either one of the following major chemokine receptors: CXCR4 or CCR5. CXCR4 is found on naive T-cells, whereas CCR5 is located on monocytes, macrophages, and activated or memory subset of T-cells. Once in the cytoplasm, the viral RNA is converted to DNA by the action of a viral RNA-dependent DNA polymerase activity and a virus-specified ribonuclease H activity found in the HIV-1 RT enzyme. Following several stages of integration through integrase enzyme activity, new virus particles are started to be synthesized by host cell genome. Forming new virus particles are followed by trimming by HIV protease inside the capsid. This process produces the functioning proteins such as RT, integrase, and protease enzymes. The newly released virions now have the capacity to infect new target cells, starting a new replication cycle as fully mature viruses.
HIV-1 is identified as the causative agent of AIDS, and up to now, there are significant advances in rational drug design and highly active compounds can be synthesized (De Clercq, 2009). However, HIV drug resistance, side effects, and the need for long-term antiviral treatment urge the inevitable development of new anti-HIV agents, targets, and therapies (D’Aquila et al., 2002; Worm et al., 2010). In this regard, naturally occurring products are still known as the richest source of bioactive compounds. Natural products promote excessive availability for discovering anti-HIV treatment, while they contain a massive amount of potential, being a consistent source for successful drug discovery. There are quite big amounts of marine-based natural products, which are reported to have bioactivities such as antifungal and antimicrobial effects. In addition, several natural products from marine organisms have been reported to express bioactivity against prevalent diseases such as cancer, diabetes, and obesity (Mayer et al., 2011). So far, numerous compounds isolated from natural resources have been reported to exhibit significant anti-HIV activity and were able to inhibit HIV-1 activity in almost every stage of the viral life cycle (Jiang et al., 2010). Several compounds of plant origin such as alkaloids, coumarins, carbohydrates, flavonoids, lignans, phenolics, quinines, phospholipids, terpenes, and tannins have been elucidated and reported to possess inhibitory activity against various targets during the viral life cycle of HIV. Considering that the marine species comprise more than half of the total biodiversity of the earth, the sea holds considerably high potential of lead compounds for novel drugs. On this matter, among the terrestrial organisms for compound isolation, the marine organisms present rich resources of diverse compounds with various crucial antiviral activities.

II. COMPOUNDS FROM MARINE ALGAE WITH ANTI-HIV ACTIVITY

A. Phlorotannins

Tannins are naturally occurring water-soluble polyphenolic compounds, and it has been reported that they show their anti-HIV activity by inhibiting polymerase and ribonuclease activities of HIV-1 RT. Phlorotannins are tannin derivatives which contain several phloroglucinol units linked to each other in different ways and formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) monomer units and biosynthesis through the acetate–malonate pathway. So far, phlorotannins mostly have been isolated from red and brown alga. Numerous bioactivities of phlorotannins have been reported up to date such as antioxidant, anti-inflammatory, antibacterial, and anti-MMP activities (Kim et al., 2006; Nagayama et al., 2002).
For the first time, seaweeds extracts have been tested for their anti-HIV-1 activity in terms of inhibiting RT, protease, and integrase of HIV-1 (Ahn et al., 2004). Following the mentioned research, two phlorotannins from brown alga Ecklonia cava Kjellman have been isolated and reported to inhibit the HIV-1 protease and RT. These phlorotannins, 8,8'-bieckol and 8,4'''-dieckol, which are dimers of eckol, isolated from E. cava, inhibited the RT and protease activity efficiently. In case of inhibition of HIV-1 RT, 8,8'-bieckol which has a biaryl linkage showed a 10-fold higher activity than that of 8,4'''-dieckol which has a diphenyl ether linkage with the IC₅₀ values of 0.5 and 5.3 µM, respectively. Hence the significant RT inhibitory activity of 8,8'-bieckol was favorable against its protease inhibition and comparable to the positive control nevirapine which has an IC₅₀ value of 0.28 µM. In the light of recent reports, 8,8'-bieckol might be employed as a drug candidate for development of new generation therapeutic agents against HIV. In addition to these results, in another report, 6,6'-bieckol from E. cava reduced the cytopathic effects of HIV-1 including HIV-1-induced syncytia formation and viral p24 antigen levels, as well as inhibited RT and HIV-1 entry activity (Artan et al., 2008). It has been strongly suggested that 6,6'-bieckol is a safe tannin derivative exhibiting a relatively lower cytotoxicity in comparison to other polyphenols and significant anti-HIV-1 activity. In detail, 6,6'-bieckol protected 96% of the HIV-1 infected cells from infection-induced lytic effects and inhibited the syncytia formation up to 88% with an EC₅₀ value of 1.72 µM. Moreover, 6,6'-bieckol inhibited the RT activity and p24 production with IC₅₀ values of 1.07 and 1.26 µM, respectively. The performed studies also clearly showed that addition of 6,6'-bieckol successfully prevented the HIV-1 entry dependent on the inhibition of production of specific proteins such as p55 and p41. These results were strengthening by coculture assays which again show clear inhibitory effect against HIV-1 infection in vitro. This important anti-HIV activity in various stages of viral cycle including viral entry and RT activity, however, excluding a sufficient protease inhibition, promotes 6,6'-bieckol as a significant lead for further drug design on the way to a full inhibition of HIV-1 activity.

Besides the brown alga E. cava, another phlorotannin, diphlorethohydroxycarmalol has been isolated from Ishige okamurae Yendo (Ahn et al., 2006). This phlorotannin was assayed for its inhibitory activity against HIV-1 RT, integrase, and protease while any study for its HIV-1 activity in vitro has not been carried out. Diphlorethohydroxycarmalol inhibited the RT and protease activity with IC₅₀ values of 9.1 and 25.2 µM, respectively; however, it failed to show any efficiency against HIV-1 protease. Therefore, this phlorotannin also can be regarded as an important compound for anti-HIV drug designing, or nutraceutical and pharmaceutical industries.
B. Polysaccharides

Biological effects of polysaccharides have been observed by several researches. Activities such as anticoagulant, anti-inflammatory, antitumor, and antiviral stand as the main bioactivities of polysaccharides from different natural sources. In this respect, marine algae are abundant sources of different type of plant-originated bioactive polysaccharides. The chemical structure, amount and bioactivity of these polysaccharides vary according to marine algae species and divisions such as Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae). In recent studies, numerous saccharides isolated from marine algae have attracted quite attention in the fields of biochemistry and pharmacology due to their efficiency as anti-HIV-1, antiadhesive, anticoagulant, anticancer, and anti-inflammatory agents (Schaeffer and Krylov, 2000; Wijesekara et al., 2010). Moreover, polysaccharides have attracted much of attention as antiviral compounds since the inhibitory activities of algal polysaccharides against mumps and influenza virus were firstly reported (Gerber et al., 1958). Further, a comparative study has been reported on the inhibition of herpes simplex virus and other viruses by polysaccharide fractions from the extracts of 10 red algae (Ehreshmann et al., 1977). It is proposed that polysaccharides are quite efficient in disrupting the viral peptide attachments which are supposed to be highly preserved in the drug-resistance mutation process. Therefore, polysaccharides are directed to affect these peptides as potential anti-HIV targets.

Fucans are sulfated polysaccharides of high molecular weight which can be found widely in various brown algae species. They have fucose as their main repeating unit; however, they can include other sugars as well such as glucose, mannose, galactose, and uronic acid. Several fucans from the seaweed species Dictyota mertensii, Lobophora variegata, Spatoglossum Schroederi, and Fucus vesiculosus were reported to successfully inhibit the activity of HIV RT (Queiroz et al., 2008). An isolated galactofucan which is mainly formed by galactose-linked fucose and with lower sulfate content from L. variegata inhibited the 94% HIV-1 RT activity at a concentration of 1.0 \( \mu \)g/mL. Another isolated fucan with a higher sulfate content and containing mostly fucose units exerted a high inhibitory effect on RT as well. Same fucan from two different algae, S. Schroederi and D. mertensii, showed similar inhibition ratios which are 99.03% and 99.30%, respectively, at 1.0 mg/mL concentration. However, a higher sulfate containing fucan from S. Schroederi with the units of galactose and fucose could only show a 53.90% inhibitory against the RT activity at the same concentration. As a part of this comparative approach, a homofucan containing only sulfated fucose units have been isolated and surprisingly exhibited a strong RT inhibitory effect. At a concentration of 0.5 mg/mL, this fucan
inhibited 98.10% of the RT activity with poly(rA)-oligo(dT) nucleotides. In order to possess more information regarding the structure–activity relationship of fucans, chemical modifications were carried out. Fucans which were modified by carboxyreduction and desulfation showed approximately fourfold lower inhibitory activities for RT under same conditions. Detailed comparison regarding the chemical structure and the inhibitory activity, RT inhibition of fucans are suggested to be dependent on both the ionic changes and the sugar rings that act to spatially orientate the charges in chemical configuration and recognizes the enzyme, therefore defining the specificity of the enzyme–compound binding.

In addition, a recent study has exhibited that galactofucan fractions from the brown algae *Adenocystis utricularis* showed *in vitro* anti-HIV-1 activity (Trinchero *et al*., 2009). Two fractions among five had strong inhibitory effects on HIV-1 replication with IC₅₀ values of 0.6 and 0.9 μg/mL, respectively. Moreover, these fractions showed their activity against both wild-type and drug-resistant viral strains. Researchers suggest that this inhibitory effect of galactofucan fractions is due to blocking of viral infection and early steps of replication rather than inactivating the viral particle production.

The glucuronogalactan from red algae *Schizymenia dubyi* was also reported to exhibit anti-HIV activity (Bourgougnon *et al*., 1996). Glucuronogalactan from *S. dubyi* successfully protected the MT4 cells from the cytopathic effects of HIV-1 infection by means of reducing the syncytia formation almost to 1% of untreated infected control at a concentration of 5 μg/mL. At same concentration, HIV-1 RT activity was also inhibited significantly without any cytotoxic effect. *In vitro* studies on glucuronogalactan suggest that the disturbing virus–host cell linkage and inhibiting early steps of HIV infection can be regarded as the possible action mechanism of this polysaccharide.

In addition to *S. dubyi*, Grateloupia filicina and Grateloupia longifolia are also sources for two sulfated galactans (Wang *et al*., 2007), GFP and GLP, respectively, which have been shown to possess anti-HIV activity *in vitro*. The sulfate contents of the GFP and GLP were confirmed as 25.7% and 18.5%, respectively. The sulfated galactan GFP has sulfate ester groups at carbon 2 and at carbon 2 and 6 for GLP. The anti-HIV activities of these polysaccharides have been tested on a primary isolate of HIV-1 and human peripheral blood mononuclear cells, and both GFP and GLP showed strong anti-HIV activity with low cytotoxicity. EC₅₀ values for the HIV-1 infection protective effect of GFP and GLP addition were calculated as 0.010 and 0.003 μM, respectively.

Moreover, a variety of potential bioactive polysaccharides are also isolated from brown algae where some of them exhibit anti-HIV activity with different mechanism of action. Sulfated polymannurogluronate
SPMG is a novel member of polysaccharides extracted from brown algae (Meiyu et al., 2003; Miao et al., 2005). It has 8 kDa average molecular weight and is rich in 1,4-linked β-D mannuronate with 1.5 sulfated and 1.0 carboxyl groups per sugar residue. Studies suggested a possible linkage between SPMG and gp120 of HIV-1. It has been reported that binding of SPMG to gp120 alone or gp120-CD4 complex occurred through V3 loop region of the protein. SPMG showed partial suppression of gp120 binding to CD4 when treated prior to infection. However, SPMG addition to preinfected cells did not show any significant suppression of virus–host cell linkage. Therefore, it is suggested that SPMG either shares common binding sites on gp120 with CD4 or masks the docking sites of gp120 for CD4.

As stated in recent studies, the various advantages of natural-based antiviral drugs, such as relatively low-production costs, broad range of activity, low cytotoxicity, safety, and novel modes of action suggest that polysaccharides from marine algae are promising drug candidates and lead for novel drug design in the near future.

C. Lectins

Lectins are carbohydrate-binding proteins which are found in a variety of species ranging from prokaryotes to corals, algae, fungi, plants, invertebrates, and vertebrates. Due to their specific carbohydrate-binding properties, they are highly involved in crucial biological processes such as host–pathogen interaction, cell–cell communication, induction of intracellular signaling cascades, and cell targeting. In this regard, lectins are showed to have potential to block the binding between HIV-1 and host cells, preventing the viral infection and dissemination. Evidently, HIV-1 envelope glycoprotein gp120 is extensively glycosylated with numerous N-linked glycosylation sites. Glycans which are seated in these glycosylation sites are rich in mannose and can easily serve as ligands for lectins. The importance of gp120 in viral infection of the target cell makes this protein suitable target for anti-HIV treatment or prophylaxis. In this manner, there are several types of lectins which were isolated from marine sources with potential anti-HIV activity (Sato and Hori, 2009).

Griffithsin is a novel lectin isolated from the red algae Griffithsia sp. with a molecular weight of 12.7 kDa. This 121 amino acid protein is reported to display promising anti-HIV activity (Mori et al., 2005). Griffithsin is completely novel with no cysteine residues and does not have any homology to any of the proteins or translated nucleotide sequences. Studies showed that Griffithsin potently prevented the T-lymphoblastic cells from the cytopathic effects of both laboratory strains and clinical primary isolates of HIV-1. It was also exhibited to be active against both T-cell tropic and macrophage-tropic strains of HIV-1 at concentrations as
low as 0.043 μM. More importantly, Griffithsin blocked cell–cell fusion between chronically infected and uninfected cells at subnanomolar concentrations without any cytotoxic effect. In connection with predicted mode of action, this lectin disturbed the binding of CD4 host cell membrane receptor to gp120 in a glycosylation-dependent manner and prevented HIV-1 infection. On the other hand, gp120-Griffithsin bond was inhibited by higher glucose and mannose but not by galactose, xylose, or sialic acid-containing glycoproteins. In a structure–activity relationship perspective, the linker sequences of Griffithsin, Gly-Gly-Ser-Gly-Gly-Gly is credited to its unusually potent activity.

In a recent study, a high-mannose-binding lectin (BCA) is isolated from green alga *Boodlea coacta* with potent antiviral activity against HIV-1 and influenza viruses (Sato et al., 2011). Carbohydrate-binding specificity determination of BCA evidently showed that this lectin has strong specificity for α1-2 linked mannose at nonreducing terminal. The potent anti-HIV-1 activity of BCA was easily predicted from carbohydrate-binding propensity and similarity with formerly reported antiviral lectins. Studies showed that BCA inhibited the HIV-1 infection with EC50 value of 8.2 nM. In addition, surface plasmon resonance analysis reported a high affinity between BCA and the HIV envelope glycoprotein gp120 with an association constant of 3.71 × 10^8 M⁻¹.

D. Other

As a part of wide screening of seaweed extracts, 47 marine macroalgae extracts were tested for their ability to inhibit HIV-1 RT and integrase (Ahn et al., 2002). Results clearly showed that one of four Chlorophyta, eight of 17 Phaeophyta, and six of 26 Rhodophyta species showed inhibitory activity against HIV-1 RT. In addition, among these 47 algae extracts, five species (*E. cava*, *I. okamurae*, *Sargassum confusum*, *Sargassum hemiphylum*, *Sargassum ringgoldianum*) were able to inhibit the activity of HIV-1 integrase. Moreover, in vitro studies confirmed that extracts of *Bossiella sp.* and *Chondria crassicaulis* successfully prevented the MT4 cells from HIV-1 induced cytopathic effects in case of noncytotoxic concentrations. Following these screening results, a carmalol derivative, diphlorethohydroxycarmalol, was isolated from brown alga, *I. okamurae* (Ahn et al., 2006), as mentioned earlier which urges the further isolation processes for elucidation of active compounds of these extracts.

Further, two diterpenes, named (6R)-6-hydroxydichotoma-3,14-diene-1,17-dial (DT1) and (6R)-6-acetoxydichotoma-3,14-diene-1,17-dial (DT2), were isolated from the brown alga *Dictyota menstrualis* (Pereira et al., 2004). It has been reported that DT1 and DT2 inhibited the virus replication with EC50 values of 40 and 70 μM, respectively, in addition to HIV-1 RT inhibitory activity with IC50 values of 10 and 35 μM. Another study
reported a dolabellane diterpene, 8,10,18-trihydroxy-2,6-dolabelladiene, from brown alga *Dictyota pfaffii* with a HIV-1 infection inhibitory effect with an EC$_{50}$ of 8.4 and 1.7 µM in peripheral blood mononuclear cells and macrophages, respectively. Moreover, this diterpene inhibited the HIV-1 RT activity with an IC$_{50}$ of 16.5 µM (Barbosa *et al.*, 2004).

III. CONCLUSION

Recent studies have evidently proved that marine-derived anti-HIV agents may play a vital role against HIV. There are promising results in the way to discover or design new drug candidates and pharmaceuticals to support reducing or regulation HIV-1 infection and related complications. Moreover, these results promote agents from marine macroalgae with beneficial health effects and potential anti-HIV activity on the road to novel highly effective HIV-1 treatment. Further studies including human subjects will reveal the true anti-HIV potential of marine algae-derived compounds in near future.

REFERENCES


CHAPTER 21

Antiallergic Benefit of Marine Algae in Medicinal Foods

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Abstract

The prevalence of allergic diseases such as asthma, atopic dermatitis, and allergic rhinitis has increased during the past two decades and contributed a great deal to morbidity and an appreciable mortality in the world. Until now, few novel efficacious drugs have been discovered to treat, control, or even cure these disorders with a low adverse-effect profile. Meanwhile, glucocorticoids are still the mainstay for the treatment of allergic disease. Therefore, it is essential to isolate novel antiallergic therapeutics from natural resources. Recently, marine algae have received much attention as they are a valuable source of chemically diverse bioactive compounds with numerous health benefit effects.
This contribution focuses on antiallergic agents derived from marine algae and presents an overview of their potential application in medicinal foods for the treatment of allergic disorders.

I. INTRODUCTION

Allergy, also referred to atopy, is caused by an exaggerated reaction of the immune system to harmless environmental substances, such as animal dander, house dust mites, foods, pollen, insects, and chemical agents (Milián and Díaz, 2004). The prevalence, severity, and complexity of these allergic diseases in the population are rapidly rising and considerably adding to the burden of health-care costs. Therefore, the knowledge about the pathophysiology of allergic diseases has increased, offering new opportunities for therapeutic intervention. Substantially, allergic reactions or type I hypersensitivity reactions are induced upon binding of allergen to the IgE, which is tethered to the high affinity IgE receptor on the surface of mast cells and basophils. Following the aggregation of cell surface receptors is a cascade of intracellular events, including the increase of intracellular Ca\(^{2+}\) level; the release of preformed inflammatory mediators from secretory granules such as histamine and β-hexosaminidase; the synthesis and release of the newly synthesized mediators such as lipid mediators and cytokines. These mediators cause allergic inflammatory responses with airway constriction, mucous production, and recruitment of inflammatory cells (Galli et al., 2008). According to this mechanism, the control of the downstream signaling molecules is especially important for the regulation of type I allergic reaction; thus allergic diseases may be managed. The current drugs that are used to treat allergies, such as antihistamines or corticosteroids, ameliorate symptoms but do not stop progression. There are also concerns regarding the side effects from chronic use of current drugs, particularly by children. Thus, the search for potential drug candidates containing higher antiallergy activity is increasing in the pharmaceutical industry. In this regard, natural bioactive compounds and their derivatives are great sources for the development of new generation antiallergic therapeutics which are more effective with fewer side effects.

The world’s oceans, covering more than 70% of the earth’s surface, represent an enormous resource for the discovery of potential therapeutic agents. During the past decades, numerous novel compounds have been isolated from marine organisms and many of these substances possess interesting biological activities. Notably, marine algae have been known as a promising group to provide not only novel biologically active substances but also essential compounds for human nutrition (El Gamal, 2010). In recent years, biological activities, nutritional value, and potential
health benefits of marine algae have been intensively investigated and reviewed. This contribution, however, focuses specifically on the antiallergic effects of marine algae and emphasizes their potential application as pharmaceutical candidates to prevent allergic disorders.

II. MARINE ALGAE AS THERAPEUTIC INHIBITORS AGAINST ALLERGIC DISORDERS

A. Marine macroalgae as crude materials with antiallergic activities

Although marine algae have been believed to be safe and efficient agents for antiallergic treatment, they have not been as extensively studied as terrestrial plants. Recently, a number of marine macroalgae have been studied for their capability against allergic reactions (Kimiya et al., 2008; Sugiura et al., 2006b). Among them, Petalonia binghamiae, Eisenia arborea, and Sargassum thunbergii were found to be effective inhibitors of histamine and β-hexosaminidase release from mast cells. Moreover, Sargassum hemiphyllum and Carpopeltis affinis, which are used in Korean folk medicine for the therapeutic treatment of various allergic diseases, have been determined to suppress atopic allergic reaction by attenuating the release of histamine, β-hexosaminidase, IL-8, and TNF-α from the activated mast cells (Na et al., 2005a,b). Notably, brown alga Ecklonia cava has been identified as a suppressor of FcεRI, a high-affinity receptor for IgE on the cell surface of mast cell and basophils (Shim et al., 2009b). The methanol extract of E. cava exhibited inhibitory effect on degranulation of KU812F cells due to reducing the cell surface expression of FcεRI and blocking the binding of IgE with its receptor. In another sense, the administration of ethanol extract of E. cava and Laurencia undulate caused a significant suppression of all asthmatic reactions induced by ovalbumin (OVA) in a mouse asthma model (Jung et al., 2009; Kim et al., 2008). The rats were fed with E. arborea, and this resulted in the decrease of IgE and histamine level in the serum, the reduction of Th2 cytokines release, and enhancement of Th1 cytokine expression from the spleen and mesenteric lymph nodes (Sugiura et al., 2008a). The intraperitoneal administration of Sargassum tenerrimum, Sargassum cervicorne, and Sargassum graminifolium in turn induced the inhibition of both passive cutaneous anaphylaxis (PCA) and active cutaneous anaphylaxis (ACA) in mice triggered by OVA and shrimp allergen (Samee et al., 2009). Herein, the extract of S. tenerrimum exhibited the most active suppression of PCA and ACA, which is comparable to antiallergic drug disodiumcromoglycate. According to these results, algae extracts could be useful crude materials for the treatment of allergic diseases.
B. Potential antiallergic compounds derived from marine macroalgae

1. Phlorotannins
Brown algae have been recognized as a rich source of phlorotannins, which are formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) monomer units and biosynthesized through the acetate–malonate pathway. Notably, phlorotannins exhibited versatile beneficial bioactivities such as antioxidant, anticancer, antidiabetic, antihuman immunodeficiency virus, matrix metalloproteinase enzyme inhibition, and antihypertensive (Vo and Kim, 2010). In relation to antiallergic properties, many phlorotannins from brown algae were considered as potential natural inhibitors of allergic reactions. Phlorotannins of fucodiphloroethol G, eckol, dieckol, 6,6'-bieckol, and phlorofucofuroeckol A (PFF-A) purified from *E. cava* were evidenced to be efficient against A23187- or FcεRI-mediated histamine release from KU812 and RBL-2H3 cells. The inhibitory mechanism was known due to the blockade of these compounds on binding activity between IgE and FcεRI (Le et al., 2009; Li et al., 2008). Similarly, phlorotannins of dioxinodehydroeckol (DHE) and PFF-A obtained from *Ecklonia stolonifera* showed a suppressive effect on cell surface expression of FcεRI, and total cellular protein and mRNA levels of the FcεRI α chain in KU812 cells (Shim et al., 2009a). Further, both of these compounds exerted inhibitory effects against the elevation of intracellular calcium level and histamine release from anti-FcεRI α chain antibody (CRA-1)-stimulated cells. On the other hand, several phlorotannins of eckol, dieckol, 6,6'-bieckol, 6,8'-bieckol, 8,8'-bieckol, PFF-A, and PFF-B from *Eisenia bicyclis* and *E. arborea* have been recognized as strong inhibitors of hyaluronidase, phospholipase A2, cyclooxygenase, and lipoxygenases (Shibata et al., 2002, 2003; Sugiura et al., 2008b, 2009), which correlated to suppression of eicosanoid synthesis and release (leukotriene and prostaglandin) from RBL cells (Sugiura et al., 2009). Among these phlorotannins, PFF-B exposed the strongest activity against histamine and β-hexosaminidase release from RBL cells with IC<sub>50</sub> value of 7.8 µM (Sugiura et al., 2006a, 2007). Accordingly, these bioactive phloroglucinol derivatives may be promising candidates for the design of novel inhibitors of FcεRI-mediated allergic reaction and enzymes in allergic inflammation.

2. Polysaccharides
Marine algae are the most important source of polysaccharides and the chemical structure of the polymers varies according to the alga species. In recent years, various polysaccharides isolated from marine algae have been used in the fields of food, cosmetic, and pharmacology due to their beneficial biological activities, such as antivirus, anticoagulant,
anticancer, and anti-inflammation (Vo and Kim, 2010). A role of polysaccharides from marine macroalgae as antiallergic agents has been suggested. Alginic acid, a naturally occurring hydrophilic colloidal polysaccharide obtained from the several species of brown seaweeds, exhibited inhibitory effects on hyaluronidase activity and histamine release from mast cells (Asada et al., 1997). Further, the antiallergic activities of alginic acid have also been found due to its suppressive effects on the activity and expression of histidine decarboxylase, the production of IL-1β and TNF-α, protein level of nuclear factor (NF)-κB/Rel A in the nucleus, luciferase activity, and DNA-binding activity in PMA plus A23187-stimulated HMC-1 cells (Jeong et al., 2006). Noticeably, alginic acid oligosaccharide (ALGO), a lyase lysate of alginic acid, was able to reduce IgE production in the serum of mice immunized with beta-lactoglobulin (Uno et al., 2006; Yoshida et al., 2004). Moreover, antigen-induced Th2 development was blocked by ALGO treatment via enhancing the production of IFN-γ and IL-12 and downregulating IL-4 production in splenocytes of mice (Yoshida et al., 2004).

In addition, porphyran, a sulphated polysaccharide isolated from red seaweeds, has been recognized to be effective against different allergic responses. According to Ishihara et al. (2005), porphyran of red algae Porphyra tenera and Porphyra yezoensis were capable to inhibit the contact hypersensitivity reaction induced by 2,4,6-trinitrochlorobenzene through decreasing the serum levels of IgE and IFN-γ in Balb/c mice. Meanwhile, fucoidan from Undaria pinnatifida reduced the concentrations of both IL-4 and IL-13 in bronchoalveolar lavage fluid and inhibited the increase of antigen-specific IgE in OVA-induced mouse airway hypersensitivity (Maruyama et al., 2005). In the recent study, Yanase et al. (2009) have reported that the peritoneal injection of fucoidan inhibited the increase of plasma IgE via suppressing a number of IgE-expressing and IgE-secreting B cells from OVA-sensitized mice (Yanase et al., 2009). On the other hand, the inhibitory effect of fucoidan on IgE production was proved due to preventing Cε germline transcription and NF-κB p52 translocation in B cells (Oomizu et al., 2006). However, the effect of fucoidan was not observed if B cells were prestimulated with IL-4 and anti-CD40 antibody before the administration of fucoidan. These observations suggested that fucoidan may not prevent a further increase of IgE in patients who have already developed allergic diseases and high levels of serum IgE. Conversely, Iwamoto et al. (2010) have determined that fucoidan effectively reduced IgE production in both peripheral blood mononuclear cells from atopic dermatitis patients and healthy donors (Iwamoto et al., 2010). These findings indicated that fucoidan suppresses IgE induction by inhibiting immunoglobulin class switching to IgE in human B cells, even after the onset of atopic dermatitis.
C. Marine microalgae and their antiallergic properties

Microalgae are considered as the actual producers of some highly bioactive macromolecules in marine resources, including carotenoids, long-chain polyunsaturated fatty acids, proteins, chlorophylls, vitamins, and unique pigments (Kay, 1991). Thus, they have been used as additives in a variety of human foods and animal feeds. Ingestion of various edible microalgae not only supplies protein and other nutrients but also modulates both adaptive and innate aspects of immunity (Price et al., 2002). Indeed, *Spirulina* was determined to decrease IgE antibody level, and increased IgG1 and IgA antibody production in the serum of the mice immunized with crude shrimp extract as an antigen (Hayashi et al., 1998). In a clinical trial, *Spirulina* consumption resulted in the significant amelioration in symptoms and physical findings of allergic rhinitis patients compared with placebo (Cingi et al., 2008). The clinical effect of *Spirulina* on allergic rhinitis was determined due to inhibiting the production of IL-4 and thus may suppress the differentiation of Th2 cells (Mao et al., 2005). In addition, it has been documented that *Spirulina* had a great inhibition on allergic reaction via suppressing anaphylactic shock, PCA, and serum histamine levels in rats activated by compound 48/80 or anti-DNP IgE. Also, the *in vitro* experiment revealed that *Spirulina* inhibited histamine release and TNF-α production from rat peritoneal mast cells (Kim et al., 1998; Yang et al., 1997). As a result, *Spirulina* can be a rich source of potential anti-allergic components. Indeed, phycocyanin, a bilin protein of *Spirulina*, has been revealed to be an inhibitor of allergic responses (Remirez et al., 2002). Moreover, phycocyanin has been demonstrated to enhance biological defense activity against infectious diseases through sustaining functions of the mucosal immune system and reduce allergic inflammation by the suppression of antigen-specific IgE antibody (Nemoto-Kawamura et al., 2004).

Besides *Spirulina*, several other microalgae appeared as promising new candidates for antiallergic agents. *Porphyridium purpureum* and *Dunaliella salina* displayed their appreciable inhibition on the activation of hyaluronidase with IC$_{50}$ values of 180 and 150 μg/ml, respectively, which were almost the same as that of disodium cromoglycate (IC$_{50}$ = 140 μg/ml) (Fujitani et al., 2001). Further, oral administration of hot water extract of *Chlorella vulgaris* (CVE) in mice suppressed the production of IgE against casein antigen accompanied by increasing mRNA expression of Th1 cytokines, including IFN-γ and IL-12 (Hasegawa et al., 1999). Likewise, *Chlorella pyrenoidosa* was found to inhibit the production of IL-5 and IgE-dependent cytokine GM-CSF from mast cells. *In vivo*, mice treated with *C. pyrenoidosa* during OVA sensitization process significantly reduced eosinophil and neutrophil infiltration in the airways (Kralovec et al., 2005). Collectively, the above studies suggested a potential
beneficial role of microalgae against allergic responses and thus they may be used as functional food or medicinal food ingredient to prevent and treat allergic diseases.

### III. CONCLUSION

Recent studies have provided evidence that marine algae play a vital role in human health and nutrition. Specially, marine algae possess the immune-modulating effects due to suppressing Th2 development and enhancing the immunological function toward Th1 activity. Simultaneously, marine algae are effective inhibitors of the immediate-type allergic reactions via inhibiting degranulation, cytokine production, and FcεRI expression in mast cells and basophils. Hence, marine algae can be used as functional food ingredients to prevent and reduce allergic reactions in human body. Collectively, the wide range of biological activities associated with the antiallergic ingredients which derived from marine algae has the potential to expand its health beneficial value not only in the food industry but also in the pharmaceutical and cosmeceutical industries.

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Marine Macro- and Microalgae as Potential Agents for the Prevention of Asthma: Hyperresponsiveness and Inflammatory Subjects

Mahinda Senevirathne* and Se-Kwon Kim*†,1

Abstract
Asthma is a variable disease and various factors are affected to increase the asthmatic symptoms and level of asthma control. It is believed that the cause for this disease is a combination of genetic and environmental factors. Numerous medications are available at present to treat this disease but it has been failed to control number of incidences successfully. Hence, recently many researchers have paid their interest to identify potential drugs from marine-based resources such as marine algae. In vitro and in vivo
experiments have been conducted with extracts or compounds from algae and found that they showed significant activities against asthma. Accordingly, many marine macro- and microalgae have been reported to have potential to ameliorate the effect of asthma. However, detailed studies are needed in relation to identify the molecular mechanism of this disease to apply those marine resources against asthma effectively. In this chapter, an attempt has been taken to discuss the potential antiasthmatic activity of marine macro- and microalgae.

I. INTRODUCTION

Asthma is a common chronic disorder of the airways that is characterized by reversible airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation (NHLBI, 2007). The symptoms of this disease are wheezing, coughing, chest tightness, and short breathing. This interaction can be highly variable among patients and within patients over time. The interactions of these features determine the severity and the clinical manifestation of asthma. It is believed that the cause for this disease is the combination of genetic and environmental factors (Martinez, 2007). Asthma is clinically categorized depending on the frequency of the symptoms, forced expiratory volume in 1 s, and peak expiratory flow rate (Yawn, 2008). Further, asthma is classified as atopic or nonatopic based on whether symptoms are occurred allergens (atopic) or not (nonatopic) (Kumar et al., 2010). Hundreds of thousands of people are suffering from asthma around the world. There is no age barrier for this and even school children are suffering at their very young ages. There are a number of chemopreventive methods available to control this problem but still there are new drugs needed to alleviate this disease as day by day patients are increasing.

Recently, huge interest has been paid to find natural drugs from marine-based resources to combat this menace. Both marine macroalgae and microalgae have been evaluated against asthma to find out a potential preventive drug. Many macroalgal species have reported to have significant activity in vitro and in vivo experiments. Ecklonia cava is an edible brown alga that widely distributes on the southern coast in Korea and Japan and widely evaluated against numerous activities including asthma. It has been reported that Ecklonia species have numerous biological and pharmaceutical activities, including antioxidative and anti-inflammatory activities (Senevirathne et al., 2006; Shin et al., 2006), antimutagenic activity (Han et al., 2000; Lee et al., 1998), bactericidal activities (Nagayama et al., 2002), and inhibitory effects on HIV-1 reverse transcriptase, protease, and tyrosinase (Ahn et al., 2004; Kang et al., 2004).
Recently, it was shown that *E. cava* extract could block the release of histamine from anti-DNP IgE-sensitized rat basophile leukemia cells, RBL-2H3 cells, and it was suggested that *E. cava* extract may contain beneficial elements for human health and may be useful as a functional food (Sugiura *et al.*, 2006).

**II. FACTORS AFFECTING ASTHMA**

Several factors are affecting asthma including environmental and individual factors. There is seasonal variability in asthma; in autumn, it is increased, while in summer, it is minimum in the United States (Silverman *et al.*, 2003). The reasons for these variations include increased exposure to allergens such as pollen, house dust mites, and mould spores among younger generation and in adults it may be increased influenza or other respiratory tract infections (Riccioni *et al.*, 2001). Moreover, cigarette smoke is one of the most common asthmatic reasons. Both indoor and outdoor air pollutants may also increase asthma. Inhalation of toxic vapors from industrial fumes, bleach sulfur, or smoke from fire or tobacco may also affect asthma (Just *et al.*, 2002).

Several individual factors may also influence the variability of asthma. Poor inhaler technique for both metered-dose and dry powder inhalers is often observed in asthma patients and may be the cause for an increased risk of death. Further, personnel characteristics may also be the cause for the changes in lung functions. In healthy individuals, lung function exhibits a circadian rhythm. However, this will be changed in the individuals those who have nocturnal asthma. Moreover, obesity may also be a risk factor for asthma. Further, women may experience premenstrual and perimenstrual worsening of asthma symptoms. Asthma symptoms may be also triggered by exercise.

**III. CHARACTERISTICS OF ASTHMA**

The most important functional abnormality in asthma is increased resistance to airflow. This is the basis of most striking clinical manifestation of asthma, including breathlessness and wheezing. The mechanisms of increased airflow resistance include (1) decreased physical dimensions of the airways as a consequence of bronchoconstriction, (2) luminal narrowing due to airway wall edema, and (3) luminal obstruction resulting from hypersecretion of mucus (McFadden, 1998). Those changes induced by the various inflammatory mediators released by mast cells as part of hypersensitivity reactions are reversible. Another functional abnormality
in asthma is a heightened responsiveness of the airways to stimuli that might normally be expected to provoke airway narrowing.

Inflammation of the airway is the peculiar pathological abnormality in asthma (Hegele and Hogg, 1996). Inflammatory responses are associated with the accumulation of numerous cells including lymphocytes, macrophages, and plasma cells in the lamina propria (Laitinen et al., 1993). Further, more of those cells are found in the adventitial connective tissues of outer wall of smaller airways (Haley et al., 1998). Moreover, the airway epithelium is infiltrated by the numerous leukocytes; eosinophils are prominent among them.

Another characteristic feature in asthma is airway wall remodeling. This refers to a variety of structural changes in the airway wall. Most prominent is the accumulation of a band of type III and type V collagens (Hoshino et al., 1998) and other matrix components in the subepithelial region of the airway wall. Airway wall thickening and shortening of airway smooth muscle may occur and these may cause airway resistance.

IV. BRIEF MECHANISM ABOUT ASTHMA

FcεRI is located on the surfaces of basophils and mast cells, which act as effector cells in IgE-mediated immune responses (Kinet, 1990). FcεRI is composed of four subunits: one β-chain, one α-chain, and two disulfide-linked γ-chains. Most of the α-chain extends into the extracellular region, where it binds directly and with high-affinity to the Fc portion of IgE antibodies; thus, the α-chain is the most important component of the FcεRI molecule (Hakimi et al., 1990). The cross-linking of FcεRI with allergen-IgE complexes causes the release of inflammatory mediators such as histamine, leukotrienes, and prostaglandins from activated basophils and mast cells, which contributes to the allergic responses in asthma, atopic dermatitis, allergic rhinitis, and food allergies (Drombrowicz et al., 1993; Gauchat et al., 1993; Metzer, 1991; Yanagihara et al., 1997).

The progression of airway inflammations involves several types of cells such as CD4+, Th2 cells, eosinophils, and mast cells (Wills-Karp, 1999). The immunopathogenic role of Th2 cells is determined by the roles of their products, such as IL-4, IL-5, and IL-13 in the recruitment and activation of the primary effector cells of the allergic response, eosinophils, and mast cells. Activation of these cells results in the release of many inflammatory mediators that seem to induce AHR individually or coordinately (Drazen et al., 1996; Galli, 1997), although the precise molecular mechanisms predisposing to the development of AHR in asthmatics are largely unknown.

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V. EXISTING REMEDIES AND DISADVANTAGES/FAILURES

Since the identification of asthma, many remedies have been implemented for curing it. However, most methods have failed to control this problem successfully; they are able to control the symptoms up to some extent. Presently, a number of treatments are used to control asthma. Glucocorticoids are effective for the treatment of allergic inflammation in asthma and result in reduced airway hyperresponsiveness which is thought to be due to reduced inflammation (Djukanovic et al., 1992). Moreover, it has been reported that long-term use of corticosteroids does not eliminate the airway hyperresponsiveness (Van Essen-Zandvliet et al., 1992). Therefore, this suggests that there are limitations to the use of these remedies in airway remodeling changes. However, there are some evidences that inhaled corticosteroids may stop or reverse the structural changes in airway (Hoshino, 2004). Another drug used for the treatment of asthma is β-agonists. In many instances, it is used together with corticosteroids and shows synergistic effects. A leukotriene receptor antagonist has been successful to prevent the smooth muscle thickening around the airway in an investigation in an animal model of asthma (Wang et al., 1993). Further, leukotriene receptor antagonist has also been shown to inhibit other aspects of airway remodeling as a result of suppression of cell infiltration into the airway wall (Henderson et al., 2002). Mast cell tryptase inhibitors may have potential in addressing not only bronchoconstriction but also airway remodeling, in particular, fibrosis (Cairns, 2005).

VI. MACROALGAE AS POTENTIAL ANTIASTHMATIC AGENTS

Algae extracts and compounds are well known for variety of biological activities including antiasthmatic activity. In addition, some algal species are used in Asian menus from long time. In a study, Kim et al. (2008) have shown that extract from *E. cava* suppresses the cytokine signaling in an animal model. The treatment of animals with *E. cava* extracts significantly reduced the concentration of Th2 (IL-4 and IL-5) cytokine in the airway. Hence, in their studies, they have proved that *E. cava* extract reduced the airway inflammation and hyperresponsiveness via inhibition of SOCS-3 protein expression. Shim et al. (2009) have also shown that extracts from *E. cava* inhibited the expression of FcεRI in human basophilic KU812F cells. Moreover, active compounds that are responsible for some specific activity have been isolated from *E. cava*. Compounds isolated from *E. cava* including phloroglucinol, dieckol, 6,6'-bieckol, and 1-(3''',5'''-dihydroxyphenoxy)-7-(2''',4''',6-trihydroxyphenoxy)-
2,4,9-trihydroxydibenzo-1,4-dioxin were assessed by histamine release by human basophilic and rat basophilic leukemia cells and showed increased activities against them (Le et al., 2009). Eisenia arborea, edible brown seaweed, has been used as a folk medicine for allergic disease for centuries by Koreans and Japanese. In a study, several bioactive phlorotannin compounds have been isolated from E. arborea and evaluated for antiallergic activities (Sugiura et al., 2007). Phlorotannin compounds inhibited β-hexosaminidase released from basophilic leukemia (RBL-2H3) cells in their study. Moreover, the compounds showed higher activity (IC$_{50}$ = 7.8 µM) than that of Tranilast (IC$_{50}$ = 46.6 µM), a pharmaceutical agent used for the treatment of inflammation disorders including asthma, allergic rhinitis, and atopic dermatitis in Japan and Korea. Results revealed that they are potential agents to prevent the effect through various modes of actions. Further, preliminary investigation with the dried powder from E. arborea possessed antiallergic effects on Brown Norway rats, a type of allergic model animals (Sugiura et al., 2007). The amount of histamine in the blood of Brown Norway rats fed with a diet containing 5% of powdered E. arborea was significantly lower (144.9 nM) than that of the animals fed with a diet without E. arborea (311.8 nM).

The red algal genus Laurencia is known to produce a wide array of natural products exhibiting number of biological activities, due to produce natural bioactive materials of polysaccharides, polyphenols, terpenes, and the other halogenated secondary metabolites. The protective effect of red algae, Laurencia undulate, has been evaluated against OVA-induced murine allergic airway reactions (Jung et al., 2009). Porphyra dentata, edible red seaweed, has long been used as folk medicine for the treatment of inflammatory diseases including, bronchitis, hypersensitivity, and lymphadentis. The macrophage-based assays revealed that the crude extract and phenolic compounds present in P. dentata significantly inhibit inflammatory mediators such as NO, iNOS, and NF-κB (Kazłowskaa et al., 2010).

VII. MICROALGAE AS POTENTIAL ANTIASTHMATIC AGENTS

Blue-green algae known as cyanobacteria have shown antioxidant, neuroprotective, cytoprotective, antiviral, antifungal, antibacterial, and anti-inflammatory activities. Edible blue-green algae, such as Spirulina and Aphanizomenon flos-aquae, are currently marketed as dietary supplements with various health claims for immune function, inflammation, heart disease, and general well-being. Nostoc commune, a blue-green edible microalga, has been used as a food delicacy or herbal medicine in Asian, African, and South American countries for centuries (Cao, 1998).
It has been used as potential source to treat various diseases in Chinese medicine and has been suggested that it can be used to treat a variety of diseases including inflammation, night blindness, digestion, and burns (Qui et al., 2002). Depending on the variety of usages of this important microalga, Park et al. (2008) have shown that the lipid extract from *N. commune* var. *sphaeroids* represses the expression of several genes involved in the proinflammatory responses to inflammatory stimuli. Fatty acid mixture significantly reduced RNA abundance of TNF-α and COX-2. Further, DNA binding activity was also evaluated as NF-κB is the major regulator of proinflammatory gene expression and revealed that DNA binding activity of NF-κB significantly reduced by the treatment with *N. commune* lipid extract. Evaluation of dialyzed *Chlorella pyrenoidosa* extract (DCPE) on mast cell mediator release *in vitro* and overalbumin-induced airway inflammation *in vivo* revealed that *in vitro* treatment of mouse bone marrow-derived mast cells with DCPE for 18 h significantly inhibited antigen (trinitrophenyl-BSA)-induced IL-5 production (Kralovec et al., 2005). *In vivo*, treatment of mice with DCPE during ovalbumin sensitization and stimulation process significantly reduced eosinophil and neutrophil infiltration in the airways. Further, unicellular *Chlorella pyrenoidosa* is used as a food supplement (Kay, 1991). It is a valuable source of nutrients and low in cellulose and exhibits a remarkable diversity of physiological and biochemical properties. Further, the beneficial effects of *Chlorella* preparations include the enhancement of immune functions and control of ulcerative colitis and hypertension (Merchant and Andre, 2001; Merchant et al., 2002).

**VIII. CONCLUSIONS**

This review regarding the antiasthma activity of micro- and macroalgae provides an insight about asthma using the information published up-to-date. It is very clear that macroalgae provide a variety of biological activities against numerous diseases and have been reported as potential sources to be used in human-related dietary supplement production. Further, various bioactive compounds have been isolated and identified in marine microalgae. Hence, marine micro- and macroalgae can be developed as health promoting nutritional foods for the prevention of anti-inflammatory-related diseases. However, more deep studies are needed to be done regarding the microalgae to identify their molecular basis of the mechanisms, and clinical trials should be recommended before using these valuable agents.
REFERENCES


Impact of Marine Micro- and Macroalgal Consumption on Photoprotection

Ramjee Pallela* and Se-Kwon Kim*‡,†

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Abstract

The enormousness of species diversity of oceans leads to the isolation and development of health- and beauty-enhancing components from various marine organisms. The significance of these marine-derived compounds or substances has been scientifically well implied for various biological and biomedical parameters. One such important parameter is photoprotectivity, which is the major concern nowadays because of the depletion in ozone layer and the possible high risk of UV irradiation to humans. The marine macro- and microalgae and their food products, knowingly and unknowingly, have been used since hundreds of years. These foods possess tremendous implications in defending the highly hazardous UV radiation, thereby facilitating photoprotection to humans. In addition, based on the recent studies, many of the UV-protecting algal species is directed for the use as medicinally

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valuable foods and food ingredients. This chapter describes certain micro- and macroalgal species along with their photoprotective importance.

I. INTRODUCTION

Marine environment possesses a diverse cluster of plants and animals of biomedical, pharmacological, and nutritional importance. The exploration and exploitation of marine micro- and macroorganisms toward various health benefits are increasing day by day via the fundamental and biotechnological approaches. One of such organisms of marine habitat are the algae, which have been proved to possess tremendous health benefits from ancient times. In fact, the terms “seaweeds” or “sea-vegetables” are used interchangeably for the large and visible macroalgae, whereas microalgae have been recognized as the microscopic producers of some highly bioactive compounds of chemical and pharmacological diversity. Moreover, microalgae are considered as the actual producers of biomedical substances found in marine resources.

Algae can provide sustainable and affordable food and fuel, as well as ecological and novel solutions. Any food, fibers, or materials that can be achieved from land-based crops can be made from algae because land plants evolved from algae 500 million years ago (Edwards, 2010). Seaweeds are majorly consumed in the oriental countries, such as Japan, China, and Korea, and nowadays, in the United States and Europe (NAAS, 2003). The earliest recorded use of seaweeds dates back to 2700 BC in “Chinese Herbs” compiled by the Emperor Shen Nung. Humans are dependent on micro- and macroalgae, which have been utilized for hundreds of years as food, fodder, medicine, cosmetics, etc., and hence, consumption of algae is recommended for best health and medicinal prospects worldwide. A market-based survey at Arizona State University found that nearly 70% of medicinal and cosmetic products commonly depend on algal components. Most people do not consume algae directly as food but enjoy the products made from algal components that include algal flour in lieu of wheat, corn, or soy flour; algal oils that are healthier and less fattening than corn oil; and algal nutrients such as ω-3 fatty acids, etc. Several species of macroalgae, belonging to Rhodophyceae (red), Phaeophyceae (brown), and Chlorophyceae (green algae), are also used as foods and food ingredients/components in Asian, Pacific, Canadian, and Icelandic traditions (Yuan, 2008).

Photoprotection is a group of mechanisms that nature has developed to minimize the damages that an organism encounters, when exposed to UV radiation (UVR). These mechanisms can be controlled or organized by certain organic and inorganic compounds or substances (e.g., melanin)
produced by different terrestrial and aquatic sources. A number of photo-protective compounds such as scytonemins (exclusively in cyanobacteria), mycosporines (in fungi), mycosporine-like amino acids (MAAs; in cyanobacteria, algae, and animals), phenyl propanoids and flavonoids (in higher plants), melanins (in humans and other animals and even some bacteria), and several other UV-absorbing substances of unknown chemical structures from different organisms have been developed to counteract the photodamage. Besides the nutritional value, many algal species that are commonly exposed to elevated solar radiation synthesize and accumulate high concentrations of UV-absorbing compounds (Rastogi et al., 2010). This principle of photoprotection has been taken into consideration by the researchers to develop and isolate various photoprotecting and antiaging compounds/formulations from marine algae, and basing the fundamentals of photoprotective consequences by these macro- and microalgal species, certain species of algae are well recommended as various foods and nutritional supplements. Although majority of research is pending with regard to the identification of certain algal species of photoprotective importance, ancient people have used a variety of these organisms in their regular diet and noticed the medicinal value of them; for example, previous reports show that seaweeds have been a part of the Japanese diet since 300 BC. It was reported earlier that more than 10 million algal species presumed to exist all over the world and around 90% of all their bioactive and therapeutic compounds remain to be discovered (Edwards, 2010; Singleton, 2011).

In spite of possessing photoprotecting principle, algae are believed to have iodine, which is responsible for low rates of goiters in areas where algae are consumed frequently. In addition, the Memorial Sloan Kettering Cancer Center (MSKCC) notes that the protein and vitamin combination in algae may help decrease fatigue.

II. UV-INDUCED PHOTODAMAGE AND PHOTOPROTECTION

UVR is the main etiological agent for most of the skin cancer incidence and a key factor responsible for photoaging and photodamage (González et al., 2008). UV spectrum reaching earth’s surface has been classified as UVB (290–320 nm) and UVA (320–400 nm). UVB causes acute sunburn, DNA mutation, or even cancer by its absorbance in the epidermis, whereas the longer wavelengths of UVA region can penetrate much deeper into the skin.

Continuous exposure to UV irradiation (both UVA and UVB) leads to skin cancer and other photoaging complications, which are typically mediated by the reactive oxygen species (ROS), generated in the oxidative pathways (Dummermuth et al., 2003; Pallela et al., 2010). Normal skin cells
generate ROS such as superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) as a result of normal metabolism in minute concentrations. Both O$_2^-$ and H$_2$O$_2$ may be converted to the highly reactive hydroxyl radical (OH$^-$) by iron (Fe$^{2+}$)-catalyzed Haber–Weiss and Fenton reactions. Similarly, reactive nitrogen species (RNS) are generated as a result of sequential reactions that begin with nitric oxide synthase (NOS)-mediated conversion of arginine to citrulline. In this reaction, NO is generated, which reacts with O$_2^-$ to produce peroxynitrite (ONOO$^-$). Similarly, ROS and RNS can be formed as a result of exposure to environmental agents including chemicals (xenobiotics) and solar UVA and UVB. Many xenobiotics are converted to toxic quinones by the family of functionally related enzymes known as cytochrome P450 (CYP). These quinones are redox-sensitive agents and are reversibly reduced to semihydroquinones/hydroquinones, which generate O$_2^-$.

Both UVA and UVB produce similar free radicals and/or singlet oxygen (1$^1$O$_2$) either directly following interaction with cellular components or in the presence of chemical agents known as photosensitizers. These photoactive chemicals while in their lowest energy or ground state absorb incident radiation (including UVA/UVB), within their absorption spectrum. The energy of the absorbed photon creates an excited state molecule, which is highly unstable under ambient conditions. In returning to the ground state, excited species transfer energy to adjacent intracellular chemical moieties particularly molecular oxygen (O$_2$) and thereby convert it into ROS. These ROS interact with lipid-rich plasma membranes and initiate a reaction known as lipid peroxidation. Numerous intracellular enzymes serve to degrade these reactive species. Some of these enzymes are specific such as SODs, which dismute O$_2^-$ to H$_2$O$_2$, whereas others have overlapping substrate affinities such as catalase and glutathione peroxidases, both of which can degrade H$_2$O$_2$ to water and O$_2$ but glutathione peroxidases also degrade organic peroxides to relatively nontoxic alcoholic species. These enzymes also require GSH during the course of peroxide degradation and convert GSH into its oxidized form, which is recycled by the enzyme glutathione reductase. Similarly, toxic quinones are converted to relatively less toxic hydroquinones by quinone reductases (QR).

As a result of UVA/UVB-mediated ROS generation during the pathogenesis of various skin diseases, a number of signaling pathways are activated. ROS drive activation of MAPKs, the most important of which are ERK, JNK, and p38 kinases. ERK and JNK are important in recruiting c-Fos and c-Jun to the nucleus where they activate the transcription factor AP-1, whereas activation of p38 and inhibitory kappa kinases (IKK) is important in the transcriptional activation of NF-$\kappa$B. Both of these factors are important in regulating the diverse array of genes, which play key roles in the pathogenesis of inflammation (such as iNOS, COX-2) and in regulation of cell cycle, proliferation, and apoptosis (cyclin D1, Bcl2, Bclx, IAP, p21, p53, etc.).
Hence, photoprotection is a critical and crucial concern to avoid these undesired effects. The increase in solar UVR on the earth’s surface due to the deterioration in the stratospheric ozone layer has increased the interest for searching natural, photoprotective compounds from various sources (Rastogi et al., 2010). Stratospheric ozone depletion leads to an increase in the short wavelengths of UVR and in consequence to an increase in H$_2$O$_2$ formation in surface waters. In such cases, algae undergo oxidative stress, where UVR induces the formation of H$_2$O$_2$ by photoactivation of dissolved organic material (DOM), photochemical degradation, and liberation of exited electrons, which initiate reduction of molecular oxygen (Dummermuth et al., 2003). Hence, photoprotective processes prevent or minimize generation of oxidizing molecules, scavenge ROS efficiently, and repair damage that inevitably occurs (Niyogi, 1999). Micronutrients such as carotenoids, tocopherols, ascorbates, flavonoids, or $\omega$-3 fatty acids can act as UV absorbers or antioxidants, or can modulate signaling pathways elicited upon UV exposure. In addition, protection depends on the topical applications as well as the algal consumption, which constitute many of the UV absorbers as mentioned above. Hence, algae are the richest source for these photoprotecting constituents and can be recommended as highly nutritional supplements, which can bring resilience to human body.

III. PHOTOPROTECTIVE ROLE OF VARIOUS ALGAL FOODS

Knowingly or unknowingly, algae are well consumed by most of the Asian countries, which is the gateway to understand the potentiality of the algal foods. As human beings are health as well as beauty cautious, several nutritional supplements containing algae have entered the food markets. Below are such microalgal and macroalgal foods that are very important in terms of health and photoprotection. As per a published article on Web, pleasant taste, minerals, and vitamin and bioactive compounds are just some of the reasons for the applications of seaweed in the kitchen, stall, and health products.

A. Microalgal foods

Microalgae have been used in various foods because of their ubiquitous availability in oceans and also due to their tremendous medicinal values. Earlier, as many as 206 strains of 152 marine microalgal species with UVA- and UVB-absorbing compounds were identified (Jeffrey et al., 1999). *Arthrospira platensis* or *Spirulina platensis* (commonly called as nutraceutical spirulina) is a blue-green microalgae with a long history as a food source in East Africa and precolonial Mexico (Wikipedia).
Spirulina is high in protein and other nutrients, finding use as a food supplement and for malnutrition. Previous studies of the photoprotection of various pigments in *S. platensis* were carried out in the presence and the absence of biopterin-a-glucoside to evaluate the photostability of these photosynthetic pigments in the photosynthetic vesicles that undergo UV irradiation (Noguchi et al., 1999).

Another popular microalga, *Chlorella*, has similar nutritional values as that of spirulina and is majorly popular in Japan. It is also used as a nutritional supplement with possible effects on human metabolic rate, and few researchers reported that *Chlorella* can reduce mercury levels in humans via the chelation of mercury to the cell wall of the organism.

The abundance of diatoms in the oceans makes them an important food source for many of the macroorganisms. These microscopic organisms tend to live in diverse climates and are able to attain resistance against aberrant conditions. One such important stress condition is the photostress caused by the extra radiation that enters the planet earth. The xanthophyll cycle pigments and the content of \( \alpha \)-tocopherol in *Phaeodactylum tricornutum* were previously studied to get molecular information about the physiological reasons of light-stress resistance (Müller and Wilhelm, 1997). According to these studies, it was clearly understood that under natural conditions when the light is fluctuating between optimal, sub-, and supraoptimal intensities, the photostress resistance is much higher than under conditions of the absence of light stress. These principles of photoacclimation, photoadaptation, and photoinhibition/photoproduction direct these microorganisms as potential sources for antiphotoaging foods.

**B. Macroalgal foods**

Several species of algae are raised for food; for example, purple laver (*Porphyra umbilicalis*) is the most widely domesticated marine algae. It is used as nori and gim in most of the Asian countries. In addition to the Asian countries, in Wales, it is used as a traditional food called laver bread, and in Ireland, it is collected and made into a jelly by stewing or boiling. Preparation also can involve frying or heating the fronds with a little water and beating with a fork to produce a pinkish jelly. Harvesting also occurs along the west coast of North America and in Hawaii and New Zealand. Dulse (*Palmaria palmata*) is a red species sold in Ireland and Atlantic Canada. It is eaten raw, fresh, dried, or cooked like spinach.

Macroalgal extracts possess various proteins and peptides which play an important role in the formulation of new collagen as well as increased skin hydration. In addition, marine red algae increases skin hydration significantly because of the enzymes and minerals present in the algae which are prominent in Hawaiian Islands. French seaweeds are other
important algae that play effective role in photoprotection and this principle is used even to formulate certain shaving gels (Somersets).

In addition, extracts and oils from algae, for example, ω-3 and ω-6 fatty acids, are also used as additives in various food products. Sargassum species are an important group of seaweeds with photoprotecting principle. These algae have many phlorotannins that involve in various biological activities including photoprotection.

It has been well demonstrated previously that seaweeds and seaweed extracts can help to protect skin from UVR (Bulteau et al., 2006; Ryu et al., 2009). Methanolic extracts of Corallina pilulifera (CPM) possess high phenolic content, which reduces the expression of UV-induced MMP-2 and -9 in human dermal fibroblasts, which is an indication of photoprotection to use this algae as food. Phlorotannins from Ecklonia cava are gaining tremendous interest for endeavoring novel photoprotecting mechanisms in various skin oriented in vitro and in vivo experiments (Kim et al., 2006). These kelps are highly recommended to use as food sources by many Asian countries, and in fact, few of the beauty products have already entered the cosmetic markets, with strong photoprotecting and antiphotoaging principle. For example, Edicos, Fibroboost (contains Seanol-F), are naturally occurring substances that act as antioxidative as well as anti-inflammatory material from seaweeds. Along with other species of this genus, E. cava has been used as a traditional food from ancient times toward various health benefits and other biomedical applications (Wijesekara et al., 2010, 2011).

The most active natural UV-absorbing substances are the MAAs that are produced by algae and possess tremendous implications for being used as medicinal foods. A human study showed that a cream containing MAAs from the red alga P. umbilicalis efficiently protects the skin against UVA exposure on a typical working day (Schmid et al., 2003).

In addition, blue-green algae contain all essential amino acids as well as most of the nonessential amino acids, making these foods a complete protein supplement in regular diets.

Irish moss (Chondrus crispus) is a good source of carrageenan, which is used as a stiffening agent in instant puddings, sauces, and dairy products such as ice cream. C. crispus can be directed to be a UVB-protecting food source because of the presence of carrageenan; especially κ-carrageenan oligosaccharides with molecular weight of about 3 kDa (κ-ca3000) are proven to be the strong antioxidants when they are tagged with some peptides (Heo and Jeon, 2008).

Lettuce species like Ulva lactuca have been used in Scotland and other provinces where it is added to soups and salads. Similar species, Ulva thalli, contain only two cell layers; however, it can withstand the higher levels of both visible and UVR (Figueroa et al., 2009). This is achieved with the involvement of UVB in the protection from UV damage, which has
already been reported in another macroalgae, *Dictyota dichotoma*, and in the blue-green algae, *Ulva pertusa* (Flores-Moya *et al*., 1999; Han and Han, 2005).

The functional significance of phlorotannins from the spores of Arctic Laminariales as UVR screens has been presented before (Roleda *et al*., 2006). *Alaria esculenta* and *Laminaria digitata* showed strong absorption in the UV waveband, characteristic of several phlorotannins. *A. esculenta* is commonly known as dabberlocks or badderlocks, eaten either fresh or cooked in Greenland, Iceland, Scotland, and Ireland.

### IV. CONCLUSIONS

Marine algae hold immense potential to be utilized as photoprotective elements. Proper utilization of this resource would pave a pay for future detonation in the cosmeceutics and medicinal food market. Advanced biotechnological approaches need to be implied to ocean sciences to explore the vast prospective of these photoprotective agents. Strategies need to be formulated for the appropriate usage of the marine algal photoprotective compounds as an answer to the photodamage caused by UVA/UVB or even the xenobiotics.

### REFERENCES


Seaweed Proteins and Amino Acids as Nutraceuticals

Monika Černá

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Abstract
Seaweeds demonstrate original and interesting nutritional characteristics. Protein concentration ranges from 5% to 47% of dry basic. Its value depends particularly on species and the environmental conditions. Seaweed protein is a source of all amino acids, especially glycine, alanine, arginine, proline, glutamic, and aspartic acids. In algae, essential amino acids (EAAs) represent almost a half of total amino acids and their protein profile is close to the profile of egg protein. In case of non-EAAs, all three groups (green, brown, and red seaweeds) contain the similar amount. Red seaweed seems to be a good source of protein because its value reaches 47%.
The issue of protein malnutrition supports the trend to find a new and cheap alternative source of protein. Algae could play an important role in the above-mentioned challenge because of relatively high content of nitrogen compounds. Algae may be used in the industry as a source of ingredients with high nutritional quality.

I. CRITERIA AND SIGNIFICANCE OF DIETARY PROTEIN IN HUMAN

A. Role of amino acids and protein in the human body

Proteins are an essential component of the diet needed for the survival of animals and human (Friedman, 1996) and a major source of carbon, which is contained in about 16%. Proteins may be divided into fibrous and globular proteins according to the shape of their tertiary structures (Zimmermann, 2003). Proteins could also be grouped into seven categories with reference to their functions: enzyme catalysis, defense, transport, support, motion, regulation, and storage proteins (Losos et al., 2008). Proteins play a very important role in all processes in the human body. These processes are facilitated by enzymes, which are actually specialized proteins increasing the reaction rate without its changes. Deficiency of enzymes causes a slower reaction. Enzymes are a globular type of proteins and they are absolutely different in the composition (Marshall, 2005). Variety of globular proteins has a unique ability to transport substances across cell membranes; others use their shape to recognize foreign microbes and cancer cells. Proteins also have regulatory roles within the cell—turning on and shutting off genes during genesis. Moreover, proteins also receive information and act as cell-surface receptors. However, fibrous proteins play a structural role. These fibers include collagen in skin, keratin in hair, fibrin in blood clots, ligaments, tendons, and bonds. They are the most abundant type of proteins in a vertebrate body (Losos et al., 2008). Proteins are needed to initiate every biochemical process in the body, and they provide invaluable source of energy (Marshall, 2005). In cells, proteins are continually being made and resolved in a process known as a protein turnover. The supply of amino acids (amino acid pool) is derived from either food or body proteins collected in the cell and circulating blood and stands ready to be incorporated in proteins and other compounds or use for energy.

B. Human amino acid and protein requirements

Recommended dietary allowance of good-quality protein is 0.83 g/kg of body weight per day with the protein digestibility-corrected amino acid score value of 1.0 (WHO, 2002). Acceptable macronutrient distribution
range for protein is 10–35% of energy for adults (Food and Nutrition Board, 2005). If the body synthesizes more proteins than it degrades and intakes, nitrogen status becomes positive, but protein gained over the need is degraded and stored as the body fat. If the body degrades more proteins than it synthesizes and loses, nitrogen status becomes negative. This nitrogen status is found in the body of people who are starving or suffering from other stresses such as burns, injuries, infections, etc. (Whitney and Rolfes, 2008). There is a need to considerate not only the total intake of protein but also the quality of protein. The protein quality is discussed in terms of biological value (or net protein utilization used in animal growth studies) (FAO/WHO, 1991). Amino acid composition and protein digestibility are the main factors influencing protein quality. Proteins are linear polymers of 20 different amino acids (essential and nonessential). To prevent protein degradation, dietary protein has to provide at least nine essential amino acids (EAAs), other amino groups, and energy for the synthesis of the rest. Generally, food of animal origin provides high-quality proteins (except from gelatin). Plant proteins have more amino acids patterns and tend to be limiting in one or more EAAs. Protein digestibility/bioavailability (comprises digestibility, chemical integrity, etc.) depends on many factors (Kies, 1981). The first is the source of proteins. Digestibility of most animal and plant proteins ranges between 90–99% and 70–90%, respectively (Gropper et al., 2008; Whitney and Rolfes, 2008). The influence of other food components should be considered. For example, dietary fiber might reduce the available energy by 2–3% at a moderate level and by the additional 2–3% in vegetable diets (FAO/WHO, 1985). It also depends on an accepter of proteins. Different animal species have different digestive enzyme systems and other physiological/biochemical differences in their gastrointestinal systems. Physiological and psychological stress may cause profound changes in the activity of gastrointestinal tract as well (Kies, 1981).

According to WHO (2002), protein requirement could be defined as “the lowest level of dietary protein intake that will balance the losses of nitrogen from the body and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health.” Protein and amino acid requirements are differentiated into the safe individual intake and safe population intake (this value is greater than the safe individual intake in most cases). The requirements are suggested for health and disease condition of all age groups (eventually sexes) and women during pregnancy and lactation as well (Tables 24.1 and 24.2). The recommendations for developing countries are also stated. They were based as a sum of the total protein content of the diet (total
nitrogen \times 6.25) and the available protein in the diet, which is calculated as total protein \times protein digestibility-corrected amino acid score value (digestibility factor \times amino acid score) (WHO, 2002). Nutritional value of proteins has been evaluated by comparing their amino acid composition with the reference protein according to WHO (2002) or with egg or soya protein used as a pattern.

There are several additional methods available for determining protein quality of food and evaluating protein adequacy of the diet. Nitrogen balance studies assess intake of dietary nitrogen and also measurement and summation of nitrogen losses from the body. Chemical score (amino acid score) involves determination of amino acids composition (with using amino acid analyzer or high-performance liquid chromatography techniques) of a test protein (reference or egg pattern). Biological value of protein shows how much nitrogen is retained in the body for the maintenance and growth rather than it is absorbed. Other methods possible to use to assess the protein quality are protein efficiency ratio or net protein utilization (Gropper et al., 2008).

### TABLE 24.1 Proteins and amino acid requirements (WHO, 2002)

<table>
<thead>
<tr>
<th>Age (years)/average weight (kg)</th>
<th>Protein intake (g/day)</th>
<th>Lysine</th>
<th>Sulfur amino acids</th>
<th>Threonine</th>
<th>Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/9.8</td>
<td>11.6</td>
<td>45.0</td>
<td>22.0</td>
<td>23.0</td>
<td>6.4</td>
</tr>
<tr>
<td>5/19.7</td>
<td>17.1</td>
<td>35.0</td>
<td>18.0</td>
<td>18.0</td>
<td>4.8</td>
</tr>
<tr>
<td>12/45.6</td>
<td>40.5</td>
<td>35.0</td>
<td>17.0</td>
<td>18.0</td>
<td>4.8</td>
</tr>
<tr>
<td>16 (boy)/66.5</td>
<td>57.9</td>
<td>33.0</td>
<td>16.0</td>
<td>17.0</td>
<td>4.5</td>
</tr>
<tr>
<td>16 (girl)/56.4</td>
<td>47.4</td>
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<td></td>
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<td></td>
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<tr>
<td>&gt;18/70.0</td>
<td>58.0</td>
<td>30.0</td>
<td>15.0</td>
<td>15.0</td>
<td>4.0</td>
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### TABLE 24.2 Proteins requirements (WHO, 2002)

<table>
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<tr>
<th>Prenatancy</th>
<th>Extra protein intake (g/kg)</th>
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<tbody>
<tr>
<td>1 trimester</td>
<td>1</td>
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<tr>
<td>2 trimester</td>
<td>10</td>
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<tr>
<td>3 trimester</td>
<td>31</td>
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<table>
<thead>
<tr>
<th>Lactation</th>
<th>Extra protein intake (g/kg)</th>
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<tr>
<td>&lt;6 months</td>
<td>19</td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>13</td>
</tr>
</tbody>
</table>
C. The role of protein in human health

Proteins, unlike fatty acids and carbohydrates, are not stored in the body, but they are deaminated followed by the oxidation of carbon skeleton through the pathways of glucose or fat metabolism, or they are stored in the form of glycogen or fat. It depends on the specific amino acid and the energy balance at the time. Nitrogen waste is excreted in urine as either urea or ammonia. Considering the body need of proteins for sustaining the essential physiological functions, diet low in protein could be deficient in many important vitamins and minerals in comparison to protein-rich diet. During fasting and starvation, muscle provides a source of amino acids. The balance between protein synthesis and resolution is closely regulated (Becker and Smith, 2006). FAO/WHO (De Onís et al., 1993) defines malnutrition as “the cellular imbalance between the supply of nutrients and energy and the body’s demand for them to ensure growth, maintenance, and specific functions.” In the world, especially in developing countries, protein-energy malnutrition is one of the most common health problems in both children and adults (Stephenson et al., 2000). Protein-energy malnutrition plays a fundamental role in developing countries due to poverty, minimal medical care, endemic infections, and poor sanitary conditions. This manifests particularly in children, who are offered little food or food of inadequate quality for their needs. Studies in Nigeria found that protein-energy malnutrition has been the second most frequent cause of death of children less than 5 years (Nnakwe, 1996). More than 70% of children with protein-energy malnutrition live in Asia, 26% in Africa, and 4% in Latin America and the Caribbean (WHO, 2000). However, Grover and Ee (2009) mentioned that protein-energy malnutrition also occurred in developed countries.

Protein deficiency has negative effects on all organs. It has been proved that it has adverse effects on the brain, immune system, and kidney function (Food and Nutrition Board, 2005). Feoli et al. (2006) reported that protein malnutrition increased oxidative damage of lipids and proteins gained from rat brain areas. This malnutrition may be the indication of important mechanism of changes in the brain development. Low protein diet could cause genetic damage as well. Frequency of chromosomal aberrations in peripheral blood lymphocytes was nearly seven times higher in malnourished infants than in eutrophic children (Padula et al., 2009). However, results of recent study show that the effect of protein and energy intake on plasma insulin-like growth factor I (IGF-I) is not temporary, and that long-term protein (0.73 g/kg body weight/day, 9% of energy) and calorie (1989 kcal/day) restriction may cause chronic decrease of plasma IGF-I concentrations independent on the body fat mass. These data suggest that a lower protein and particularly calorie intake may have some additional protective effects against some types of cancer (Fontana et al., 2006).
Epidemiologic studies point out that the modern lifestyle of developed countries with high calorie and protein consumption, low physical activity, and obesity increases the risk of cancer (Calle and Kaaks, 2004). It has been reported that general diet in Western Europe and the United States contains 1.5–2 times higher amounts of protein. High protein intakes may increase urinary calcium excretion, although the effect on calcium balance is controversial, as amino acids increase the efficiency of intestinal absorption. High protein intake is connected with the increasing urinary nitrogen excretion, vasopressin plasma levels, creatinine clearance, glomerular filtration rate, kidney hypertrophy, renal hemodynamics, and eicosanoid production in renal tubules (Bankir and Kriz, 1995). Moreover, the relationship between high protein intake and risk of renal cell cancer (Chow et al., 1994) or prostate cancer (Vlajinac et al., 1997) has been discussed. Other health effects of high protein intake, such as diabetic nephropathy, have not been clarified yet.

D. Bioavailability, digestion, and absorption of amino acids

Bioavailability of proteins (or amino acids) is a popularized term for an important factor of determining protein quality. It is usually considered as a level, up to which amino acids or small peptides from a sample protein consumed by a living organism are finally transported into the body. Therefore, it includes digestibility and absorption mechanisms. Some showings of bioavailability of food proteins should be given by the results of valid in vitro digestibility methods (Kies, 1981). Normally, protein digestion starts in the stomach by the activity of HCl, which denatures quaternary, tertiary, and secondary structures of proteins and causes the activation of pepsinogen to pepsin. Final products of gastric protein digestion include especially large polypeptides, oligopeptides, and free amino acids. Protein digestion is completed by intestinal enzymes in the lumen of the small intestine (Gropper et al., 2008). Amino acids are absorbed along the entire small intestine, most of them particularly in the proximal small intestine. Amino acids are transported by specific carriers.

Transport of amino acids across the apical membrane is not only via sodium-dependent symporters but also due to the proton-motive force and the gradient of other amino acids providing to absorb amino acids from the lumen efficiently. In the basolateral membrane, antiporters work together with facilitators to release amino acids without depleting cells of valuable nutrients. Individual amino acids are mostly transported by more than one transporter, affording a backup capacity for the absorption during the mutational inactivation of a transport system (Bröer, 2008).

Briefly, neutral and anionic amino acids are transferred by Na\(^+\) symporters during the secondary active transport from the lumen into
mucosal cells and then they are transported into the blood with carriers or by diffuse. If Na\(^+\) was absent, no accumulation was observed. There is recent evidence that methionine transport is made separately of other neutral amino acids transport. Cationic amino acids (arginine, lysine, and ornithine) are partly taken up into the enterocytes by Na\(^+\)-independent mechanisms, as the membrane potential is a driving force for their uptake. Anionic amino acids are already resolved in the mucosal cells. They also have their own (Na\(^+\) and K\(^+\) dependent) carrier systems, and finally neutral amino acids apply several different transporters (Despopoulos and Silbernagl, 2009; Preston et al., 1974). In addition, diacidic amino acids (glutamic and aspartic acids) are largely transamminated to alanine during the absorption. Dipeptides and tripeptides are usually absorbed more rapidly than free amino acids. Inside enterocytes, peptides are hydrolyzed, and amino acids are released together with those absorbed by amino acid transporters. A number of specific amino acid absorption disorders are congenital and combined with similar defects of renal tubular reabsorption. A lot of inborn disorders influence amino acid transport in epithelial cells, such as cystinuria, lysinuric protein intolerance, Hartnup disorder, iminoglycinuria, dicarboxylic aminoaciduria, and some other less well-described disturbances of amino acid transport (Bröer, 2008).

II. PROTEIN AND AMINO ACIDS IN SEAWEEDS

Seaweeds have been used as human food, particularly in China, Japan, and the Republic of Korea for several centuries. Recently, seaweeds have appeared in the cuisine of North America, South America, and Europe as well (FAO, 2003). Because of their low content of energy but high concentration of dietary fibers, minerals, and vitamins, they seem to be a good source of healthy food (Ito and Hori, 1989). Algae provide a significant amount of nitrogen compounds, namely, amino acids and proteins as well (Darcy-Vrillon, 1993; Fleurence, 1999a; Oohusa, 1993).

A. Factors influencing the amino acid and protein content

Very common seaweeds, which are used as human food, are species of red algae Porphyra (nori), brown algae Laminaria (kombu), and Undaria (wakame) (FAO, 2002). USDA (2010) shows that the raw seaweed Undaria spp. contains approximately 3.0% of proteins and, with regard to 80% moisture content, it has about 15.2% of protein of dry matter. Admittedly, protein values range from 5% to 47% according to the species, environmental conditions, habitats, maturity, and applied methods used for protein and amino acid determination (Ito and Hori, 1989).
Seaweed proteins contain all amino acids, which significantly depend on the seasonal period (Fleurence, 1999a; Galland-Irmouli et al., 1999). Generally, the highest protein value has been found during the period of winter–early spring and the lowest during summer–early autumn (Galland-Irmouli et al., 1999). According to Denis et al. (2010), in red algae Grateloupia turuturu, the maximal concentration of protein was observed from January to April and the lowest amount of protein from July to August. The minimum of protein value in summer could be connected with the destruction of phycobiliproteins (Galland-Irmouli et al., 1999). In contrary to the other compounds (ash or dietary fiber), proteins were put through large changes within the year. Moreover, it was reported that different protein levels depend on the specific areas, too. For example, Yaich et al. (2011) mentions that Ultrica lactuca, which come from the littoral area of Tunisia, contains almost by 50% higher amount of protein than the same species from Philippines. From the data gained by Renaud and Luong-Van (2006), it is evident that the highest concentration of protein was found in red algae collected in summer (4.8–12.8%), while it was significantly lower during winter.

Protein concentration varied especially within the same species among populations (McDermid and Stuercke, 2003). Generally, the protein amount of brown seaweeds is low. It has been reported that it is lower than 15% (Fleurence, 1999a). On the contrary, red seaweeds contain a high quantity of protein, and this value is often comparable with the amount of other foods such as soybean or eggs. There were established some differences in the protein value between red and green seaweeds. In agreement with Qasim (1991), in brown seaweeds, there was determined the interval of 21–28%, in green 14–26%, and in red seaweeds 11–24%. Roslin (2003) found a minimum of protein at the level of 1.5% in green algae. It was observed that red alga Gracilaria changii contains a relatively high amount of protein, approximately 34% of dry weight (Norziah and Ching, 2000), which is comparable with the value of protein in green peas (USDA, 2010). Nevertheless, protein quantity is very changeable in dependence on different species, for example, red alga Corallina officinalis provided a very low protein content, approximately 7% (Marsham et al., 2007). Generally, the protein content decreases in the order of the seaweed group: red > green > brown. In Tables 24.3 and 24.4, there are presented protein content of some seaweed demonstrating their variability.

Many researchers have assessed protein content by measuring nitrogen content and multiplying it by different conversion factors. In the case of seaweed, the nitrogen-to-protein factor ranges from 3.75 to 5.72 (Lorenc¸o et al., 2002); therefore, the traditional value might overestimate the protein content. However, nitrogen in seaweeds is a component of many types of molecules in addition to protein, such as DNA, ATP, etc. High amount of polysaccharides could limit the accessibility of protein.
### TABLE 24.3  Protein content (% dry matter) of red seaweed

<table>
<thead>
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<th>Red seaweed</th>
<th>Protein</th>
<th>Methods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahnfeltiopsis concinna</td>
<td>5.7</td>
<td>Lowry</td>
<td>McDermid and Stuercke (2003)</td>
</tr>
<tr>
<td>Amansia multifida</td>
<td>25.6</td>
<td>Kjeldahl</td>
<td>Ramos et al. (2000)</td>
</tr>
<tr>
<td>Asparagopsis taxiformis</td>
<td>6.1</td>
<td>Lowry</td>
<td>McDermid and Stuercke (2003)</td>
</tr>
<tr>
<td>Bryothamnion seaforthii</td>
<td>17.3</td>
<td>Kjeldahl</td>
<td>Ramos et al. (2000)</td>
</tr>
<tr>
<td>Bryothamnion triquertrum</td>
<td>11.8</td>
<td>Kjeldahl</td>
<td>Ramos et al. (2000)</td>
</tr>
<tr>
<td>Corallina officinalis</td>
<td>6.9</td>
<td>Kjeldahl</td>
<td>Marsham et al. (2007)</td>
</tr>
<tr>
<td>Corallina officinalis</td>
<td>2.3</td>
<td>Kjeldahl</td>
<td>Ramos et al. (2000)</td>
</tr>
<tr>
<td>Digenea simplex</td>
<td>15.6</td>
<td>Kjeldahl</td>
<td>Ramos et al. (2000)</td>
</tr>
<tr>
<td>Enantioclada duperreyi</td>
<td>19.5</td>
<td>Kjeldahl</td>
<td>Ramos et al. (2000)</td>
</tr>
<tr>
<td>Eucheuma cottonii</td>
<td>9.8</td>
<td>Kjeldahl</td>
<td>Matanjun et al. (2009)</td>
</tr>
<tr>
<td>Eucheuma denticulatum</td>
<td>4.9</td>
<td>Lowry</td>
<td>McDermid and Stuercke (2003)</td>
</tr>
<tr>
<td>Gelidiella acerosa</td>
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<td>Biuret</td>
<td>Manivannan et al. (2009)</td>
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<tr>
<td>Gracilaria birdiae</td>
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<td>Bradford</td>
<td>Gressler et al. (2010)</td>
</tr>
<tr>
<td>Gracilaria domingensis</td>
<td>6.2</td>
<td>Bradford</td>
<td>Gressler et al. (2010)</td>
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<td>Gracilaria folifera</td>
<td>7.0</td>
<td>Biuret</td>
<td>Manivannan et al. (2008)</td>
</tr>
<tr>
<td>Gracilaria changgi</td>
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<td>Kjeldahl</td>
<td>Norziah and Ching (2000)</td>
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<td>Ramos et al. (2000)</td>
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<td>Denis et al. (2010)</td>
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<td>Hypnea japonica</td>
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<td>Laurencia filiformis</td>
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Kjeldahl method based on the nitrogen-to-protein factor 6.25.
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<th></th>
<th>Protein</th>
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<td>Cladophora glomerata</td>
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<td>Akköz et al. (2011)</td>
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<td><strong>Brown seaweed</strong></td>
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TABLE 24.4 (continued)

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<th>Protein Content</th>
<th>Methods</th>
<th>References</th>
</tr>
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<td>Sargassum echinocarpum</td>
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<td>Kjeldahl</td>
<td>Ramos et al. (2000)</td>
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<td>Sargassum obtusifolium</td>
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<td>Sargassum tenerimum</td>
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<td>Sargassum vulgare</td>
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<td>Undaria pinnatifida</td>
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<td>Dawczynski et al. (2007)</td>
</tr>
</tbody>
</table>

Kjeldahl method based on the nitrogen-to-protein factor 6.25.

(Fleurence, 1999b). For example, McDermid and Stuercke (2003) used Lowry’s method (Lowry et al., 1951), which is specific for protein. Another method used for the estimation of protein is Biuret method (Manivannan et al., 2008). Gressler et al. (2011) reported that the value of soluble protein obtained by Bradford’s method (with bovine serum albumin as a standard) was quite similar to that determined by the method based on the nitrogen-to-protein factor 4.43.

B. Amino acid composition of seaweeds

All amino acids are presented in seaweeds (Matanjun et al., 2009). With respect to total EAAs in the FAO/WHO (1991) pattern, seaweeds (especially red and green) seem to be able to contribute to adequate levels of total EAA (Wong and Cheung, 2000). On the contrary, Matanjun et al. (2009) reported higher amounts of amino acid (AA) in green seaweeds than in red and brown. Despite this, many papers indicated that EAAs in red algae represented almost half of total AAs and it meant the ratio of EAA to AA about 0.4–0.5. The ratio of EAA to nonessential amino acids (NEAAs) was about 0.7–0.9 (Gressler et al., 2010; Norziah and Ching, 2000). These data agreed with the results of Galland-Irmouli et al. (1999) who reported that proteins of Palmaria palmata contained 26–50% of EAAs of AA, and its protein profile was close to the profile of egg protein. Wong and Cheung (2000) found EAA on the level of 42–48% in red and green seaweeds. Methionine and cysteine were detected in a high amount in red seaweeds than in green and brown (Qasim, 1991), but the value showed low amounts in red algae, less than 0.3% and 0.1%, respectively (Gressler et al., 2011). The highest EAA was phenylalanine in species belonging to three groups: red, green, and brown algae (Matanjun et al., 2009). Glycine,
alanine, arginine, proline, glutamic, and aspartic acids composed together a large part of the AAs fraction, whereas AAs tyrosine, methionine, and cysteine occurred in a lower amount (Gressler et al., 2010; Norziah and Ching, 2000) on contrary to the results of Qasim (1991). However, Ortiz et al. (2006) concluded that brown alga Durvillaea antartica stood out of a high level of histidine and valine. Seaweeds have the amounts of glutamic and aspartic acids in a range from 15% to 44%.

In the case of NEAAs, glutamic and aspartic acid constituted a predominant quantity of AA. In brown seaweeds, it represented 20–44% (Fleurence, 1999a; Munda, 1977; Wong and Cheung, 2000), in green seaweeds 26–32% (Fleurence et al., 1995), and in red seaweeds 14–19% (Fujiwara-Arasaki et al., 1984), respectively. Glutamic acid (or a salt form) is intricately involved in sustaining proper function of the brain and its mental activity. Aspartic acid, in a form of energy, helps initialize two of the body’s most important pathways (Krebs and urea cycles) (Braverman et al., 2003). But it is needed to consider that no author analyzed all AAs, namely, tryptophan and cysteine, consequential in differences in a sum of AAs (Gressler et al., 2011). In general, all three groups, green, brown, and red seaweeds, contain the similar amount of NEAA (Matanjun et al., 2009). Seaweeds contain also nonprotein nitrogenous materials, such as free AAs, chlorophyll, nitrate and nitrite nitrogen, ammonium ions, and nucleic acids. Only free AAs are probably in a connection with typical flavors and taste, especially glutamic and aspartic acid (Yaich et al., 2011), and also glycine and alanine (Norziah and Ching, 2000). Therefore, it could be assumed that this amount is not significant because of a very comparable value of the crude protein and AA content (Qasim, 1991).

Many methods with different analysis condition are used for the determination of AAs. These methods conclude two steps: hydrolysis of substrate, and chromatographic separation and detection of residues. Hydrolysis is the most critical part which is affected by several factors such as temperature, time, hydrolysis agent, and additives (Fountoulakis and Lahm, 1998). Weiss et al. (1998) focused on the effects of the hydrolysis method on the AA content and composition of protein with a conclusion that the conventional hydrolysis delivered more accurate data in comparison with the microwave radiation-induced hydrolysis.

C. Nutritional evaluation

Protein quality is evaluated by different methods with various patterns of standard; hence it is very difficult to compare the published data. Chosen standard, which is used for the computation of these factors, strongly affects the limiting AA. Rama et al. (1964) identified the highly significant correlation with the biological value of 11 protein samples from 15.
In spite of this fact, in most studies, amino acid score (AAS), index of essential amino acid, and egg protein have been applied. Seaweeds have usually low AAS (about 20–67%); nevertheless, it is common similarly to other plants (Matanjun et al., 2009). Matanjun et al. (2009) showed that Sargassum polycystum had AAS higher (67%) than soybeans (47%) or casein (58%) and then this value could be compared with beef (69%).

As limiting acids depending on the species and standard protein, lysine was indicated (Matanjun et al., 2009, Mišurcová et al., 2010; Norziah and Ching, 2000; Ortiz et al., 2006; Ramos et al., 2000), also leucine and isoleucine (Ortiz et al., 2006), and further as the second limiting amino acid, it was methionine (Ramos et al., 2000).

In comparison to the standard protein, seaweed proteins (as well as other plant proteins) are not full-valued proteins because of low amounts of some amino acids. Nevertheless, the presence of all EAAs in the considerable quantities indicates that seaweed proteins are nutritionally superior to the terrestrial plant proteins (Qasim, 1991). In Tables 24.3 and 24.4, there are presented protein contents of some seaweed demonstrating their variability.

In vitro protein digestibility of seaweed proteins is influenced by the species, seasonal period, and content of antinutritional factors such as phenolic and polysaccharides (Fleurence, 1999a; Mabeau and Fleurence, 1993). Activity of proteolytic enzymes may be reduced due to the reaction of amino acids with oxidized phenolic compound (Wong and Cheung, 2001). Differences between the prediction of protein quality (based on amino acid content/amino acid requirement ratios) and the actual protein quality (based on the performance in living organisms) seem to be a reason of variations in the utilization of the amino acids comprised in different proteins.

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Medicinal Effects of Peptides from Marine Microalgae

Se-Kwon Kim*†,1 and Kyong-Hwa Kang†

Abstract

Nowadays, there are numerous commercial applications of microalgae, and they have been used to enhance the nutritional value of food and animal feed owing to their chemical composition. They are cultivated as a source of highly nutritional and valuable source. Recently, microalgae have been reported to use as a potent source for food additive, nutraceutical, or pharmaceuticals. According to the criteria of nutritional quality and cost, variety of marine organisms has been investigated for their suitability to be applied in the production of protein hydrolysates in functional foods. Recently, a great deal of interest has been expressed regarding marine-derived...
bioactive peptides because of their numerous health benefits. In addition, many studies have been reported that marine bioactive peptides can be used as functional foods, nutraceuticals, or pharmaceuticals due to their therapeutic potential in the treatment or prevention of various diseases. Hence, in this chapter, we discussed the importance of marine microalgae in relation to their medicinal value.

I. INTRODUCTION

Marine microalgae utilization by indigenous populations has occurred for centuries. However, the cultivation of microalgae is only a few decades old. Marine microalgae have been reported as valuable new sources with pharmacologically active compounds. Nowadays, there are numerous commercial applications of microalgae. For example, (1) microalgae can be used to enhance the nutritional value of food and animal feed owing to their chemical composition, (2) they play a crucial role in aquaculture, and (3) they can be incorporated into cosmetics (Borowitzka, 1995). Moreover, they are cultivated as a source of highly valuable molecules. However, their metabolites have not been studied extensively because of difficulties in the isolation and cultivation of microalgae (Brown et al., 1997). Microalgae perform a major part of primary production, being responsible for 46% of global productivity and supporting food webs in water from ponds to oceans (Kim et al., 2001). Dozens of microalgal species are produced commercially for single-cell proteins, polysaccharides, healthy food materials such as polyunsaturated fatty acids, and vitamins in the pharmaceutical and food industries (Spolaore et al., 2006).

Microalgae for human nutrition are nowadays marketed in different forms such as tablets, capsules, and liquids. They can also be incorporated into pastas, foods, candy bars or gums, and beverages. Owing to their diverse chemical properties, they can act as a nutritional supplement or represent a source of natural food colorants (Spolaore et al., 2006). The commercial applications are dominated by three strains: Arthrospira, Chlorella, and Dunaliella salina. Arthrospira (Fig. 25.1) is used in human nutrition because of its high protein content and excellent nutritive value. In addition, these microalgae have various possible health-promoting effects. Chlorella can also be used as a food additive owing to the taste and flavor-adjusting actions of its coloring agent. D. salina is exploited for its β-carotene content that can reach 14% of dry weight (Chisti, 2007). For human consumption, nutrition and health, the world’s largest producer of this strain, offers Dunaliella powder as an ingredient of dietary supplements and functional foods. Also, microalgae are required for larval nutrition during a brief period, either for direct consumption in the case
of mollusks and peneid shrimp or indirectly as food for the live prey fed to small fish larvae. The most frequently used species are Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema, and Thalassiosira (Brown and Jeffrey, 1995).

Recently, microalgae have been gained to more attention as nutraceutical and healthy food additive in markets. Extract of Chlorella sp. and Spirulina sp. is proposed to use with noodles, bread, green tea, beer, and candy (Liang et al., 2004). Protein content of Navicula incerta was found to be higher than lipid and carbohydrates (Kang et al., 2011). The percentage of carbohydrates in N. incerta was similar to that in Skeletonema costatum (4.9%), and the lipid content (6.54%) was very low compared with that in

FIGURE 25.1 The commercial applications of microalgal species.
Nitzschia closterium, Cylindrotheca fusiformis, and S. castatum (18–20%). However, the protein content (50.10%) of this species was very high compared with that of N. closterium and C. fusiformis (16–38%) (Brown and Jeffreyi, 1995; Kang et al., 2011). Some microalgae with high protein content are used very commonly as can be seen in Table 25.1. Many microalgal species have similar amino acid compositions and were rich in the essential amino acids (Brown, 1991).

II. POTENTIAL FUNCTIONAL PEPTIDES FROM MICROALGAE

A. Bioactive peptides from microalgae

Bioactive peptides released by enzymatic proteolysis of various proteins that act as potential physiological modulators of metabolism during intestinal digestion have been reported in recent reports. These peptides usually contain 3–20 amino acid residues, and their activity depends on their amino acid composition and sequence (Pihlanto-Leppala, 2001). Based on their structural, compositional, and sequential properties, they may exhibit different kinds of bioactivities such as antioxidative (Jung et al., 2005; Kim et al., 2001), antihypertensive (Suetsuna et al., 2004), and immunomodulatory effects (Chen et al., 1995; Tsuruki et al., 2003).

Arthospira, Chlorella, and D. salina were used in human nutrition diets because of their high protein content and their excellent nutritive value. In addition, this microalga has various possible health-promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal Lactobacillus, and suppression of elevated serum glucose level. A significant amount of

<table>
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<tr>
<th>TABLE 25.1</th>
<th>General composition of different human food sources and microalgae (% of dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commodity</td>
<td>Protein</td>
</tr>
<tr>
<td>Milk</td>
<td>26</td>
</tr>
<tr>
<td>Rice</td>
<td>8</td>
</tr>
<tr>
<td>Meat</td>
<td>43</td>
</tr>
<tr>
<td>Baker’s yeast</td>
<td>39</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>51–58</td>
</tr>
<tr>
<td>Dunaliella salina</td>
<td>50–57</td>
</tr>
<tr>
<td>Porphyridium cruentum</td>
<td>28–39</td>
</tr>
<tr>
<td>Spirulina maxima</td>
<td>50–60</td>
</tr>
<tr>
<td>Navicula incerta</td>
<td>45–50</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>40–48</td>
</tr>
</tbody>
</table>
Arthrospira production is realized in China and India (Kato and Suzuki, 1971; Morris et al., 2007). Therefore, new interest has been developed to search natural and safe bioactive peptides from natural sources. Furthermore, antioxidant peptides have been isolated from hydrolysates of various proteinaceous food materials and recently the possible roles of food-derived bioactive peptides in reducing the risk of diseases have been reported (Kim and Wijesekara, 2010). In addition, two peptides were identified from the enzyme hydrolysis of *N. incerta*. Table 25.2 shows some microalgae-derived peptides.

### B. Antioxidant activity of the peptides from microalgae

Many synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate have been used to retard the oxidation process; however, the use of synthetic antioxidants are under strict regulation due to potential health hazards (Park et al., 2001). The search for natural antioxidants as alternatives is therefore of great interest among researchers.

Recently, bioactive peptides from enzymatic hydrolysis of various food proteins such as soy protein, casein, whey protein, gelatin, and wheat gluten have been shown to possess antioxidative activity (Elias et al., 2008). However, antioxidative peptides from marine food sources are gaining more attention as new antioxidative alternatives in the past few years (Mendis et al., 2005; Qian et al., 2007, 2008; Rajapakse et al., 2005). *Chlorella vulgaris* is a popular edible microalga in Japan and its safety is well established (Suetsuna and Chen, 2001). The commercial applications of microalgae as nutritional supplements, natural dyes, and skin care products are reported (Spolaore et al., 2006), but there are no studies reporting the antioxidative activity of microalgae protein-derived peptides.

Antioxidants have also been demonstrated to be effective in reducing the risk of carcinogenesis partially due to their antioxidative activity.

**TABLE 25.2** Some bioactive peptides from microalgae

<table>
<thead>
<tr>
<th>Amino acid sequence of the peptide</th>
<th>Marine microalga</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile-Val-Val-Glu, Ala-Phe-Leu, Phe-Ala-Leu, Ala-Glu-Leu, and Val-Val-Pro-Pro-Ala</td>
<td><em>Chlorella vulgaris</em></td>
</tr>
<tr>
<td>Ile-Ala-Glu, Phe-Ala-Leu, Ala-Glu-Leu, Ile-Ala-Pro-Gly, and Val-Ala-Phe Pro-Gly-Trp-Asn-Gln-Trp-Phe-Leu, Val-Glu-Val-Leu-Pro-Pro-Ala-Glu-Leu</td>
<td><em>Spirulina platensis</em></td>
</tr>
<tr>
<td></td>
<td><em>Navicula incerta</em></td>
</tr>
</tbody>
</table>
The peptide developed in the study had excellent antioxidant properties and might also be employed as an adjuvant to the conventional therapeutic modalities for gastric cancer potency. There are numerous reports on bioactive compounds in microalgae. Many studies have been done to evaluate the antioxidative potency of peptides from microalgae using different assays in vitro (Sheih et al., 2009; Suetsuna and Chen, 2001) and have shown significant activities. Therefore, the peptides from microalgae protein have the potential to be used as a good dietary supplement for the prevention of oxidative stress-related diseases such as atherosclerosis, coronary heart disease, and cancer.

C. Antihypertensive activity of peptides from microalgae

The angiotensin I-converting enzyme (ACE) participates in regulating blood pressure in the renin–angiotensin system. The inhibitors such as captopril (Suetsuna and Chen, 2001) and enalapril (Sawayama et al., 1990) have been used as antihypertensive drugs. The ACE-inhibitory activity of various source have studied, and it was found that some ACE-inhibitory peptides were produced by enzymatic digestion of various marine food proteins, including tuna muscle (Kohama et al., 1991; Ondetti, 1977), sardine muscle (Suetsuna et al., 1991), dried bonito (Yokoyama et al., 1992), dried-salted fish (Astawan et al., 1995), fish sauce (Okamoto et al., 1995), and fish water-soluble protein (Wako et al., 1999).

A lot of human and animal studies have revealed that ingestion of Chlorella results in decreased blood pressure, but the mechanism is still unknown. Murakami et al. (1987) and Miyakoshi et al. (1980) suggested that Chlorella decreases the blood pressure by regulating the renin–angiotensin system. Moreover, Inoue et al. (1995) also reported that ingestion of Chlorella decreases blood pressure in humans.

D. Anticancer activities of the peptide from microalgae

A variety of compounds such as flavonoids, phenolic acids, and carotenoids, derived from natural sources, have been shown to be beneficial for the inhibition of cancer. The mechanisms which suppress tumor genesis often involve inhibition of tumor cell-mediated protease activity (Yang et al., 2001), attenuation of tumor angiogenesis (Kandaswami et al., 2005), promotion of cell cycle arrest (Moosavi et al., 2005), induction of apoptosis (Lee et al., 2004), and immunostimulation (Tzianabos, 2000).

Anticancer peptides have attracted concern recently due to their characteristics features such as multifunction, high sensitivity, and stability. There are number of publications on anticancer peptides from food protein, such as fish sauce (Lee et al., 2004), soy protein (Kim et al., 2000),
mollusk protein (Leng et al., 2005), milk protein, and beef protein (Jang et al., 2008), but few studies have been reported about microalgae protein as a source for anticancer peptides. However, recent studies suggest that the microalgae-derived peptides could be potentially useful adjuncts in the treatment of gastric cancer (Sheih et al., 2010). Hence, it will be potential protein source in future for industrial production of functional peptides.

E. Protective effects of peptide from microalgae on the liver

Alcohol is mostly metabolized in the liver and excessive alcohol use can lead to acute and chronic liver diseases including hepatitis, liver cirrhosis, fatty liver, and liver cancer (Dey and Cederbaum, 2006). Further, chronic alcohol abuse has become a major health problem that causes liver and pancreatic diseases and is known to impair hepatic alcohol dehydrogenase, myocardial infarction, pancreatitis, and disorders of the immune, endocrine, and reproductive systems (Lima et al., 2006). Liver is the primary organ for metabolism, disposition, and toxicity of ingested ethanol. There is considerable interest in the role of oxidative stress and ethanol generation of reactive oxygen species (ROS) in the mechanism by which ethanol is hepatotoxic. The ethanol exposure can be extremely toxic to tissues due to heightened oxidative stress and it induces the production of ROS (Albano, 2006). Further, several systems likely contribute to the ability of ethanol to induce a state of oxidative stress. However, ethanol can also be metabolized by catalase and more selectively by cytochrome P-450 2E1 (CYP2E1) (Wu and Cederbaum, 1999). Induction of CYP2E1 by alcohol is proposed as a mechanism augmenting the formation of reactive paracetamol metabolites and its hepatotoxicity (Dilger et al., 1997).

Recently, a great deal of interest has been expressed regarding marine-derived bioactive peptides because of their numerous health beneficial effects. It has been reported the bioactivities from enzymatic hydrolysis of N. incerta (Kang et al., 2011), but there are few publications on antihapatocyte peptides. Therefore, in our recent study, we investigated the protective effect of the peptides (Pro-Gly-Trp-Asn-Gln-Trp-Phe-Leu, Val-Glu-Val-Leu-Pro-Pro-Ala-Glu-Leu) from microalgae, N. incerta, on the liver disease (Table 25.2). Addition of peptides from N. incerta hydrolysate clearly reduced the amount of procollagen which is an important indicator for hepatic fibrolysis (Fig. 25.2). Moreover, many studies have reported that marine bioactive peptides can be used as antihypertensive, antioxidative, anticoagulant, and antimicrobial components in functional foods, nutraceuticals, or pharmaceuticals due to their therapeutic potential in the treatment or prevention of diseases.
III. CONCLUSIONS

Microalgae were studied as a potent source for food additive, nutraceutical, or pharmaceuticals. In fact, 30% of the current world algal production is used for animal feed where over 50% of the current world production of *Arthrospira* is used as feed supplement. It also needs to be of the correct size and shape to be ingested and to have high nutritional qualities and a digestible cell wall to make nutrients available. Protein content is a major factor determining the nutritional value of microalgae.
Recent studies have provided evidence that marine-derived bioactive peptides including the compound derived from microalgae play a vital role in human health and nutrition. In recent study, we have proved that two peptides isolated from *N. incerta* showed hepatoprotective activity. These evidences suggest that due to valuable biological functions with health beneficial effects, marine microalgae-derived bioactive peptides have potential as active ingredients for preparation of various functional foods or nutraceutical and pharmaceutical products.

**REFERENCES**


Seaweed as a Source of Novel Nutraceuticals: Sulfated Polysaccharides and Peptides

A. Jiménez-Escrig, E. Gómez-Ordóñez, and P. Rupérez

Abstract

Seaweeds and seaweed-derived products are underexploited marine bioresources and a source of natural ingredients for functional foods. Nutritional studies on seaweeds indicate that brown and red...
seaweeds possess a good nutritional quality and could be used as an alternative source of dietary fiber, protein, and minerals. Moreover, bioactive sulfated polysaccharides are the main components of soluble fiber in seaweeds and also bioactive peptides can be prepared from seaweed protein. This chapter gives an overview of the main biological properties of sulfated polysaccharides and peptides from brown and red seaweeds. Recent studies have provided evidence that sulfated polysaccharides from seaweeds can play a vital role in human health and nutrition. Besides, peptides derived from algal protein are most promising as antihypertensive agents. Further research work, especially in vivo studies, are needed in order to gain a better knowledge of the relation structure–function by which bioactive compounds from seaweeds exert their bioactivity.

I. INTRODUCTION

A. Seaweeds as an underexploited bioresource

Seaweeds have been used as a food in Asian countries, especially in China, Japan, and Korea, since ancient times (Chapman and Chapman, 1980; Indegaard and Minsaas, 1991; Nisizawa et al., 1987). In Western European countries, seaweeds are mainly used in the pharmaceutical, food, and cosmetics industry as a source of hydrocolloids (Indegaard and Ostgaard, 1991; Juanes and Borja, 1991). Around 16 million tons of seaweeds (fresh weight basis) and other marine plants are annually produced or collected with an estimated value of 5575 million euros (FAO, 2007) worldwide; at the same time, seaweeds are currently considered as an underexploited natural resource (Cardozo et al., 2007; Khan et al., 2009). Moreover, seaweeds are a potential source of new biologically active substances and essential nutrients for human nutrition (MacArtain et al., 2007; Smit, 2004). Therefore, systematic studies on nutrition and health protection of specific marine algae consumed in Europe (Denis et al., 2010) and other countries are currently developed to provide the consumer with nutritional recommendations on a scientific base. These studies will also contribute to the economic exploitation of seaweeds.

B. Nutritional assessment of seaweeds

Brown and red seaweeds possess a good nutritional value and can be an alternative source of proteins, minerals, and vitamins (Jiménez-Escrig and Cambrodón, 1999; Plaza et al., 2008; Rupérez and Saura-Calixto, 2001). Oil content is generally low but contains a great amount of essential fatty acids (Gómez-Ordóñez et al., 2010; Rupérez and Saura-Calixto, 2001; Sánchez-Machado et al., 2004).
Biochemical and nutritional aspects of seaweed proteins have been reported. Enzymatic degradation of algal fibers could be attempted to improve protein digestibility (Fleurence, 1999) and also to prepare bioactive peptides. A great deal of interest has been developed nowadays to isolate antihypertensive bioactive peptides, which act as angiotensin-converting enzyme (ACE) inhibitors because of their numerous health beneficial effects (Wijesekara and Kim, 2010).

Minerals are attributed to different ions associated with the charged polysaccharides of seaweeds. Seaweeds contain sulfate, representing different percentages of the ashes (Gómez-Ordóñez et al., 2010; Rupérez and Saura-Calixto, 2001). Sulfate anion is derived from homo- or heteropolysaccharides in brown algae or from galactans in red ones. Sulfate seems to be a typical component of marine algal polysaccharides, related to high salt concentration in the environment and with specific functions in ionic regulation. Such sulfated mucilages are not found in land plants. Mineral bioavailability depends on the linkage type between polysaccharide and mineral and also on polysaccharide digestibility (Gómez-Ordóñez et al., 2010). Typically, there is a strong positive correlation between sulfate content and biological activity of polysaccharides from seaweeds (Jiao et al., 2011).

Besides, seaweeds are considered an excellent source of dietary fiber with a high proportion of soluble to total dietary fiber (Gómez-Ordóñez et al., 2010; Jiménez-Escrig and Sánchez-Muniz, 2000; Rupérez and Saura-Calixto, 2001). Dietary fiber in seaweeds is mainly composed of indigestible sulfated polysaccharides (Gómez-Ordóñez et al., 2010; Rupérez et al., 2002), which are resistant to human digestive enzymes (Rupérez and Toledano, 2003). Several storage and structural polysaccharides commonly found in brown and red seaweeds are laminaran, alginate, fucan, carrageenan, and agar (Gómez-Ordóñez et al., 2010; Rupérez et al., 2002). Alginates from brown seaweeds are traditionally used as hydrocolloids, while fucans are most interesting because of their biological activity (Riouxf et al., 2007). Fucans from brown seaweeds are by-products in the preparation of alginites for the food and cosmetic industries (Boisson-Vidal et al., 1995). Different biological activities and potential health benefits of sulfated polysaccharides derived from marine algae have been reviewed recently (Jiao et al., 2011; Wijesekara et al., 2011).

Seaweeds have to survive in a highly competitive environment subjected to light fluctuation, oxygen exposure, dehydration process, etc.; therefore, they develop defense strategies in different metabolic pathways. Thus marine organisms are rich sources of structurally diverse bioactive minor compounds such as carotenoids, polyphenols, minerals, vitamins, and fatty acids (Cardozo et al., 2007). Besides, they possess other major compounds such as complex carbohydrates and protein, from which bioactive sulfated polysaccharides and peptides can be isolated.
II. SEAWEEDS AS A SOURCE OF BIOACTIVE SULFATED POLYSACCHARIDES

Sulfated polysaccharides play storage and structural roles in seaweeds and may exhibit many interesting biological properties. As mentioned above, seaweeds are the main source of sulfated polysaccharides in vegetables; thus different amounts of sulfated heteropolysaccharides can be found in green seaweeds (Chlorophyta), while other sulfated polysaccharides such as laminaran, alginate, and fucan are present in brown seaweeds (Phaeophyta) and sulfated galactans such as agar and carrageenan appear in red seaweeds (Rhodophyta) (Costa et al., 2010).

Several studies have demonstrated that composition—sulfated polysaccharide and other nutrients—and biological properties of seaweed could depend on ripening stage or environmental factors such as geographical localization, seasonal variation, nutritional quality of sea water, and other postharvest factors such as seaweed drying or extraction procedures for phycocolloid preparation (Rioux et al., 2007).

A. Preparation of sulfated polysaccharides from seaweeds

They can be sequentially extracted based on their different solubility. For example, the extraction procedure in the brown seaweed *Fucus vesiculosus* includes water, acid, and alkali treatments (Rupérez et al., 2002). Thus, laminarans are water soluble, but their solubility depends on branching level: the higher the branching degree, the higher the solubility. Fucans are extracted with diluted hydrochloric acid, while alginates are extracted with alkali. Alginates form insoluble precipitates of alginic acid at low pH, but they are stable in solution between pH 6 and 9. The acid- and alkali-insoluble material from *F. vesiculosus* contains residual polysaccharides plus cellulose.

For red seaweeds, the solubility of sulfated galactans is dependent on temperature. Thus, highly charged sulfated galactans are soluble in aqueous solution at 20 °C, while those less modified such as agar in Nori (Porphyra spp.) are soluble at 60–80 °C. A neutral galactan from agar, agarose, is soluble at acidic pH. Finally, in most red and brown edible seaweeds, cellulose is the main polysaccharide of the acid- and alkali-insoluble fraction (Rupérez and Toledano, 2003).

B. Biological activity of sulfated polysaccharides from seaweeds

Bioactivity of sulfated polysaccharides seems to be due to a complex interaction of structural features including sulfation level, distribution of sulfate groups along the polysaccharide backbone, molecular weight,
sugar residue composition, and stereochemistry (Jiao et al., 2011). Although research studies dealing with the chemical structure of seaweed polysaccharides have been reported (Deniaud et al., 2003; Lahaye and Robic, 2007; Lahaye et al., 2003; Lechat et al., 2000), relationship between macromolecular structure and biological activity is not clearly established (Jiao et al., 2011).

1. **In vitro studies**

Relevant pharmacological properties of algal sulfated polysaccharides, such as anticoagulant, antioxidant, antiviral, anticancer, and immunomodulating activities, have been reviewed recently (Jiao et al., 2011; Wijesekara et al., 2011). Besides, other less well known biological properties have been described for sulfated polysaccharide, namely, antimicrobial, antiproliferative, anti-inflammatory (Wijesekara et al., 2011), liver protection (Charles and Huang, 2009), effect on glucose (Hoebler et al., 2000; Vaugelade et al., 2000) and lipid metabolism (Amano et al., 2005; Bocanegra et al., 2006; Hoebler et al., 2000; Huang, 2010), and prebiotic effect (Devillé et al., 2007).

**Anticoagulant.** The anticoagulant capacity of sulfated polysaccharides from seaweeds has been the most studied property in an attempt to find an algal substitute for heparin. For example, the anticoagulant activity of fucans was shown to depend on their sugar composition, molecular weight, extent of sulfation, and distribution of sulfate groups in the polysaccharide repeating units (Jiao et al., 2011; Pereira et al., 1999). Marine sulfated polysaccharides other than fucans have also been shown to possess anticoagulant and antithrombotic capacity. Thus, the sulfated galactofucan from a brown seaweed lacks significant anticoagulation activity, making it an ideal candidate as an antithrombotic agent (Rocha et al., 2005). Results suggest that algal sulfated polysaccharides could be an alternative to heparin because they present a promising potential to be used as natural anticoagulant agents in the pharmaceutical industry (Wijesekara et al., 2011). Moreover, the development of antithrombotic algal polysaccharides would avoid the potential for contamination with prions or viruses (Jiao et al., 2011) of commercial heparins, currently obtained from pig and bovine intestine.

**Antioxidant.** Sulfated polysaccharides not only function as dietary fiber, but they also contribute to the antioxidant activity of seaweeds. It has been demonstrated that they exhibit potential antioxidant activity *in vitro* and several of them derived from brown seaweeds, such as fucoidan, laminaran, and alginic acid, have been shown as potent antioxidants (Rocha De Souza et al., 2007; Rupérez et al., 2002; Wang et al., 2008, 2010).

The presence of sulfate groups seems to make feasible the interaction between polysaccharide and target centers of cationic proteins (Mulloy,
Another factor which could specifically modulate the antioxidant activity of sulfated polysaccharide is molecular size (positively at lower size) and the presence of nonsulfated sugar units at polysaccharide terminals (negatively) (Silva et al., 2005). This fact suggests a stereospecificity in anticoagulant activity and not just a quantitative presence of sulfate in the molecule (Costa et al., 2010).

Likewise, the relationship between sulfated polysaccharides and antioxidant capacity in vitro has been shown for red seaweed extracts (Chandini et al., 2008; Rocha De Souza et al., 2007) from Indian seaweeds. Also, the biological activity of sulfated polysaccharides from tropical seaweeds collected in Brazil has been evidenced previously (Costa et al., 2010).

1. In vivo studies in animals and cell model

The detoxifying effect of different seaweeds in a Wistar rat model indicates that the presence of sulfated polysaccharides is crucial in the liver protecting effect of macroalgae (Costa et al., 2010). Other studies have evidenced the protective effect of seaweeds against liver toxicity induced by galactosamine in a rat model, concluding that this protecting effect is partly mediated by fucoidan, a sulfated polysaccharide from the brown seaweed Laminaria (Kawano et al., 2007).

Sulfated polysaccharides from seaweeds are evidenced as protectors of the antioxidant status in a stressed induced rat model (Veena et al., 2007). Besides, the protecting effect of aqueous and organic extracts from brown and green seaweeds against induced oxidation has been studied in cell models (Gunji et al., 2007). Therefore, sulfated polysaccharides from edible seaweeds potentially could be used as natural antioxidants by the food industry (Rupérez et al., 2002).

The influence of seaweed intake on glucose metabolism has been shown in a pig animal model (Amano et al., 2005; Hoebler et al., 2000; Vaugelade et al., 2000). Other studies deal with the effect of edible seaweeds (Kombu (Laminaria spp.) and Nori) and fucoidan from Laminaria japonica on lipid metabolism in a hypercholesterolemic rat model (Amano et al., 2005; Bocanegra et al., 2006; Hoebler et al., 2000) and prebiotic effect (Devillé et al., 2007). Prebiotic effect of Laminaria polysaccharide has been shown in the gut metabolism through its effects on mucosal composition, intestinal pH, and short chain fatty acids production (Devillé et al., 2007).

III. EDIBLE SEAWEEDS AS POTENTIAL SOURCES OF BIOACTIVE PEPTIDES

Biologically active peptides are food-derived peptides that can exhibit diverse activities, including opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic,
and blood pressure-lowering actions (Erdmann et al., 2008). Bioactive peptides have been detected in different animal and vegetable protein sources, milk peptides being by far the most commonly known source (Jiménez-Escrig et al., 2010; Pihlanto et al., 2008).

Heart diseases, such as arteriosclerosis, coronary heart disease, stroke, peripheral arterial disease, and heart failure, may be caused by hypertension or blood pressure greater than 140 mmHg systolic and/or 90 mmHg diastolic pressures (Lo and Li-Chan, 2005). The ACE (dipeptidyl carboxypeptidase, EC 3.4.15.1) performs an important physiological function in the pathogenesis of cardiovascular and renal diseases through blood pressure regulation. In the renin–angiotensin system, ACE catalyzes the conversion of the inactive decapeptide angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to the potent vasoconstrictor, the octapeptide angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), by hydrolytic removal of the histidyl leucine group from the C-terminal (Ondetti and Cushman, 1982). Further, ACE is implicated in cell oxidative stress, through the generation of reactive oxygen/nitrogen species (Jung et al., 2006).

Certain biologically active peptides may act as ACE-inhibitory peptides and thus may prevent hypertension and its pathological consequences. ACE-inhibitory peptides from foods are less active than synthetic drugs such as captopril; however, their significance lies in the fact that they meet the need for naturalness and safety (Wu and Ding, 2002).

Edible seaweeds have been considered over the past few decades as promising organisms for providing both novel biologically active substances and essential compounds for human nutrition (MacArtain et al., 2007). However, to date, scarce work of the potential ACE-inhibitory compounds such as biopeptides (Sato et al., 2002a,b; Suetsuna and Nakano, 2000) or phlorotannins (Jung et al., 2006) on seaweeds has been done.

A. In vitro and in vivo evaluation of antihypertensive activities: different approaches

The isolation of protein from seaweeds is a difficult task due to the link between polysaccharides and protein within the seaweed matrix. It is described that the extraction of proteins from the tissues of laminarialean algae (Nagai et al., 2008) or Saccharina japonica (Kim et al., 2011) is difficult due to high levels of nonprotein interfering compounds, mainly viscous polysaccharides. As a consequence, isoelectric point (Ma et al., 1996) or ammonium sulfate saturation (Hernandez-Mireles and Rito-Palomares, 2006) or trichloro acetic acid (Barbarino and Lourenço, 2005) approaches, which are commonly used for protein precipitation, are not completely useful for seaweeds. Thus, to solve this task, different approaches are proposed such as proteolytic treatment of the whole seaweeds followed by filtration and dialysis (Suetsuna and Nakano, 2000) or treatment
of seaweed matrix with alginate lyase S to obtain an enriched-protein precipitate which is recovered by centrifugation (Sato et al., 2002a,b).

It is described the identification of ACE-inhibitory peptides derived from Undaria pinnatifida (Wakame), and hypotensive action of orally administered peptides on spontaneously hypertensive rats (SHRs). These studies are based on the previous evidence that dietary ingestion of whole Wakame, one of the most widely eaten brown seaweeds in Japan, has been shown to decrease blood pressure in humans. Specifically, the systolic blood pressure (SBP) of patients decreased significantly after daily oral administration of 3.3 g of dried Wakame after 4 weeks (Nakano et al., 1999). In the work of Suetsuna and Nakano (2000), Wakame powder was digested using pepsin. Then, the filtrate of enzymatic digestion is dialyzed, the outer solution is applied sequentially to a Dowex 50W column H\(^+\) form, and peptides were eluted with ammonium solution. After concentration under vacuum, the residue was fractionated on a SP-Sephadex C-25 column and a peptide power was obtained. The fractions having a molecular weight of 300–1000 kDa were collected and concentrated to dryness. The total yield of the peptide powder from 23.6 g of seaweed powder was 3.7 g. The peptides on the most ACE-inhibitory potent fraction were purified further by HPLC with an ODS-5 column. Although approximately 100 peaks were detected by this chromatography, potent inhibitory peptides were obtained in four peaks. Afterward, using protein sequencing, primary structures of the individual peptides were identified. The amino acid sequences of the peptides were Ala-Ile-Tyr-Lys, Tyr-Lys-Tyr-Tyr, Lys-Phe-Tyr-Gly, and Tyr-Asn-Lys-Leu. All of the active peptides had a tyrosine and lysine residue in the structure. Apart from this research, some peptides with potent ACE-inhibitory activity in vitro or intravenously are inactive in oral administration. Thus, hypotensive activity of each tetrapeptide are evaluated by measuring the SBP on SHR after oral administration of chemically synthesized tetrapeptides [50 mg/kg of body weight (BW)] (Suetsuna and Nakano, 2000). SBP did not change in control rats during the study period (6 h). Captopril (10 mg/kg BW) lowered SBP significantly. A single dose (50 mg/kg BW) of the tetrapeptides significantly reduced SBP in SHR. This work firstly isolated the bioactive peptide and then evaluated the activity of each synthesized peptide in a rat model.

The ACE-inhibitory and antihypertensive activities of Wakame hydrolysates have been investigated in another study, with a different research design (Sato et al., 2002a,b). To obtain an isolated protein residue, Wakame was treated with alginate lyase S at 45 °C for 18 h and an enriched protein precipitate (46.3% dry matter) was recovered by centrifugation. Then Wakame was hydrolyzed using 17 kinds of proteases at different pH and temperature conditions, and ultrafiltered hydrolysates were tested for the inhibitory activity of the ACE. Among the proteases used in this study,
Wakame hydrolysates of pepsin, protease S and N Amano, and proleather FG-F were able to produce potent ACE inhibitors in vitro. The yield of the different enzymes used ranged from 115 to 239 mg as the weight of the solid contents obtained from 1 g of dried Wakame. In a second step, in order to evaluate the antihypertensive activity in vivo of hydrolysates produced by the four selected proteases, single oral administrations of hydrolysates were given to SHR \((n = 6)\) at dosages of 100 and 1000 mg protein/kg BW. All the Wakame hydrolysates used in this test decreased the SBP in SHR, especially hydrolysates from protease S Amano or proleather FG-F. Digestion stability was evaluated by the change in IC\(_{50}\) values of hydrolysates before and after treatment with gastrointestinal proteases (pepsin, trypsin, and chymotrypsin) to simulate in vivo resistance to digestion. In addition, a long-term feeding of hydrolysates was assayed on SHR. Seven-week-old SHR were fed a diet containing 0%, 0.01%, 0.1%, and 1.0% of the protease S Amano hydrolysate for 10 weeks. The SBP in the Wakame hydrolysate group tended to be lower than in the control group. Summarizing, there is no correlation between the in vitro and in vivo studies. These results indicated that in vivo experiments—single oral administration test and long-term feeding test—are important for the final evaluation of the antihypertensive effects of peptides. Among 17 proteolytic enzymes tested in vitro, it has been found that hypertension in SHR was suppressed by the Wakame protease S Amano hydrolysates.

Moreover, a study of isolation of potential antihypertensive agents (fucosterol and polyphenols) has been derived from seaweeds: Phaeophyta (Ecklonia stolonifera, E. cava, Pelvetia siliquosa, Hizikia fusiforme, and U. pinnatifida), Rhodophyta (Gigartina tenella, Gelidium amansii, Chondria crassicaulis, and Porphyra tenera), and Chlorophyta (Capsosiphon fulvescens) (Jung et al., 2006). The study includes the crude extracts of selected edible Korean seaweeds which were screened for ACE-inhibitory activity. Seaweed bioactive constituents are extracted with ethanol followed by partitioning with organic solvents: \(n\)-hexane, dichloromethane, ethyl acetate, and \(n\)-butanol. Then the fractions extracted were chromatographed over a silica gel column yielding different subfractions and evaluated. In the case of the extract containing phloroglucinol, purification over an RP-18 column is used. Among the tested seaweeds, the ethanol extracts at a concentration of 163.93 µg/mL of E. stolonifera and E. cava appeared to be the most active, with inhibition of 64.86 ± 0.58% and 166.67 ± 4.20%, respectively. With the notable exception of H. fusiforme, the other brown algae P. siliquosa and U. pinnatifida also exhibited favorable ACE-inhibitory activity, between 46% and 53%. Among the red algae tested, only G. amansii exhibited significant ACE-inhibitory effects, with an inhibition of 58.11 ± 1.73%. Column chromatography of the \(n\)-hexane and ethyl acetate fractions led to the isolation of fucosterol and six phlorotannins, as phloroglucinol, and its oligomers eckstolonol, eckol, phlorofucofuroeckol A (a pentamer), dieckol (a hexamer), and triphlorethol A (a trimer) from the Ecklonia and
Eisenia species of brown algae. The ACE-inhibitory properties of phlorofucofuroeckol A, dieckol, and eckol ranked high, with IC$_{50}$ values of 12.74 ± 0.15, 34.25 ± 3.56, and 70.82 ± 0.25 μM, respectively. Summarizing, other bioactive compounds, besides peptides, may be responsible for the antihypertensive capacity of seaweeds.

IV. CONCLUSION

The addition to traditional foods of edible seaweeds or seaweed-derived ingredients such as bioactive sulfated polysaccharides or peptides can be considered as a good strategy in order to increase the offer of the functional food market. To date, scarce work of the potential ACE-inhibitory compounds such as biopeptides has been done on seaweeds. The required isolation of protein is a difficult task due to the strong link between polysaccharides and protein in the seaweed matrix, and thus different extraction approaches have been proposed. Regarding antihypertensive effects of peptides, there is no correlation between in vitro and in vivo studies. Therefore, further research work on edible seaweeds through the systematic study of their sulfated polysaccharides, biopeptides, and related biological properties in vitro and especially in vivo will make possible a better knowledge of their potential benefit on human health and will contribute at the same time to their use as natural ingredients for the preparation of novel nutraceuticals.

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CHAPTER 27

Seaweed Lipids as Nutraceuticals

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Abstract
Seaweeds are known as low-energy food. Despite low lipid content, ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) introduce a significant part of seaweed lipids. PUFAs are the important components of all cell membranes and precursors of eicosanoids that are essential bioregulators of many cellular processes. PUFAs effectively reduce the risk of cardiovascular diseases, cancer, osteoporosis, and diabetes. Because of the frequent usage of seaweeds in Asia and their increasing utilization as food also in other parts of the world, seaweeds could contribute to the improvement of a low level of ω-3 PUFAs, especially in the Western diet. The major

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commercial sources of \( \omega-3 \) PUFAs are fish, but their wide usage as food additives is limited for the typical fishy smell, unpleasant taste, and oxidative nonstability. Nevertheless, growing requirements of healthy functional foods have led to produce PUFAs as nutraceuticals in controlled batch culture of marine microalgae, especially *Thraustochytrium* and *Schizochytrium* strains.

I. INTRODUCTION

Lipids belong to fundamental nutrients for human. Their components are fatty acids (FAs), which could be classified to saturated (SFAs—without double bonds), monosaturated (MUFAs—with one double bond), and polyunsaturated FAs (PUFAs—with two or up to six double bonds). Humans are able to synthesize both SFAs and MUFAs. Nevertheless, PUFAs with the first bond on the third or sixth carbon atom are essential because of the inability to be synthesized by the human body. Thus, they have to be obtained from the diet. Their main sources are chloroplasts of higher plants and fat of water organisms.

Nowadays, essential FAs (EFAs) are considered to be functional food and nutraceuticals with many health benefits including the potential of reducing the risk of cardiovascular diseases (CVD), cancer, osteoporosis, and diabetes. CVD have been believed to be the main cause of death in most Western countries. Coronary heart disease (CHD) is closely connected with a progress of atherosclerosis evoked by interactions between plasma lipids, lipoproteins, monocytes, platelets, endothelium, and smooth muscle of arterial walls, which results in narrowing of coronary arteries. Thus, the composition of dietary lipids is an important factor of genesis of hearth diseases together with the quality and alluviation of arterial walls. This could lead to thrombosis and finally to coronary infarctions.

Dietary pattern has been modified throughout the human evolution. The origin composition of a hunter-gathered diet with a lower intake of total fats has been altered by a higher intake of total lipids with a high representation of saturated and trans-FAs, which are detrimental for health. Contemporary Western human diet is noted for a low content of \( \omega-3 \) EFAs that results in an imbalance of \( \omega-3 \) and \( \omega-6 \) EFAs and in a progress of various pathophysiologies.

II. HEALTH IMPORTANCE OF PUFA

Dispensability of PUFAs for human has already been known for many decades. Oversized dietary intake of most SFAs and trans-FAs is harmful for health due to increasing the risk of CVD. The human body cannot synthesize PUFAs with the first double bond on the C3 and C6 from the
methyl-end. These FAs are EFAs, and their level in the human body depends on their intake from the diet. EFAs form two biologically important groups which are ω-3 and ω-6 EFAs according to the location of their first double bond from the methyl-end of FAs. They are also called long chains ω-3 and ω-6 PUFAs (LCPUFAs). However, some recent studies have concluded that humans of every age could transform α-linolenic acid (ALA, 18:3, ω-3) to docosahexaenoic acid (DHA, 22:6, ω-3) but only in the insufficient concentration (Brenna, 2002; Brenna et al., 2009; Burdge and Calder, 2005; Burdge and Wootton, 2002).

In Fig. 27.1, there are shown the metabolic transformations of ω-3 and ω-6 PUFAs and their important derivates such as prostaglandins (PG), thromboxanes (TX), and leukotrienes (LK).

It is generally known that primary precursors of ω-3 and ω-6 EFAs are ALA and linoleic acid (LA), respectively. Both are formed by the gradual desaturation of oleic acid in the endoplasmic reticulum and chloroplasts of plantae. Because of the absence of Δ12 and Δ15 desaturases required for the synthesis of ALA from stearic acid (18:0), humans cannot synthesize ALA. It has to be obtained from the diet.

LCPUFAs are formed by series of reactions that are catalyzed by desaturases and elongases. Further, conversion of dietary ALA (18:3, ω-3) into EPA (20:5, ω-3) is limited because ALA and LA (18:2, ω-6) compete for common desaturation and elongation enzymes. The affinity of Δ6 desaturase for ALA is greater than for LA (Burdge and Calder, 2005). It was proved that the relationship between ALA and LA is very important for the maintenance of their homeostasis. But the amount of ALA and LA in the diet is significant for ALA conversion to EPA (20:5, ω-3) and DHA and not for their ratio (Goyens et al., 2006).

Polyunsaturated ω-3 LCPUFAs have significant roles in many biochemical pathways which result in different health promotion activities. Generally, LCPUFAs show cardioprotective effect that results from their considerable antiatherogenic, antithrombotic, anti-inflammatory, antiarrhythmic, hypolipidemic effect and other health benefits, which are based on complex influence of concentrations of lipoproteins, fluidity of biological membranes, function of membraned enzymes and receptors, modulation of eicosanoids production, blood pressure regulation, and finally on the metabolism of minerals (Flachs et al., 2005; Hu et al., 2001; Kinsella et al., 1990; Tvrzická et al., 2009; Weiss et al., 2005).

PUFAs of ω-3 series have many pleiothropic metabolic effects as ligands of peroxisome proliferator-activated receptors (PPAR-α). It is assumed that the activation of PPAR-α results in decrease of lipogenesis and secretion of a very low density lipoprotein (VLDL), further in growth of lipoprotein lipase activity and decrease of apolipoprotein C-III concentration, and on increased reverse transport of cholesterol (Corton and Anderson, 2000; Olivieri et al., 2003; Tvrzická et al., 2009).
FIGURE 27.1 The metabolic transformation of ω-3 and ω-6 PUFAs and their derivates.
EPA and DHA are fundamental EPAs from ω-3 series of LCPUFAs. DHA is the main structural component of cell membranes, at high level in brain tissue and retina. DHA is formed from EPA by peroxisomal β-oxidation (Burdge and Calder, 2005). EPA and DPA (22:5, ω-3) can also be synthesized from DHA via β-oxidation in peroxisomes by catalytic activity of probably Δ-4 enoyl CoA reductase and Δ-2 enoyl CoA isomerase (Gronn et al., 1991).

The principal LCPUFA of ω-6 series is arachidonic acid (AA; 20:4) acting as a precursor for eicosanoids synthesized from LA. LCPUFAs of ω-6 series have been considered as activators of PPAR-γ. Their metabolic effects include increased synthesis of cholesterol, increased activity of LDL receptors, increased activity of cholesterol 7α-hydroxylase (Cyp 7A1), and decreased conversion of VLDL to LDL. As ligands of PPAR-γ, ω-6 PUFAs may improve insulin sensitivity, change fat distribution, and affect adipocyte differentiation (Chiang et al., 2001; Corton and Anderson, 2000).

Biological activities of individual EFAs might be derived from the course of their interactions. Their major derivates are eicosanoids, signaling molecules having important functions in many regulation systems and performing as messengers in the central nervous systems (Hertting and Seregi, 1989; Leslie and Watkins, 1985).

Eicosanoids are divided into four following classes: PG, TX, prostacyclins, and LK. Further within each class, there are two or three series of eicosanoids. Eicosanoids derived from ω-3 and ω-6 FAs have antagonistic effects. Their amount depends on the composition of dietary FAs influenced by the competition with AA and EPA FAs as substrates for cyclooxygenases and 5-lipoxygenases (Kinsella et al., 1990; Simopoulos, 2002a,b).

PG are oxygenated, unsaturated cyclic FAs responsible for the processes of many hormone-like actions. Arachidonic acid ω-6 PUFA is converted to an unstable intermediate hydroxyl-endoperoxide prostaglandin H₂ which is subsequently converted to PGE₂ by the enzymatic activity of cyclooxygenase-2 (COX-2). PGE₂ as proinflammatory eicosanoids have been related to carcinogenesis of breast and prostate, as well as cancer initiation (Kobayashi et al., 2006; Terry et al., 2003). EPA and DHA from marine oils inhibit COX-2 and suppress the production of PGE₂. It has been proved that EPA and DHA also inhibit lipoxygenases contributing to synthesis of hydroxyeicosatetraenoic acids and LK. 12-Hydroxyeicosatetraenoic acid has been connected with the suppression of apoptosis, stimulation of angiogenesis, and further with stimulation of tumor cell adhesion (Rose, 1996).

A. Significance of PUFAs in human diet

Lipids represent one of the main sources of energy for human metabolic processes. Lipid consumption in most Western countries is relatively high with the contribution of approximately 40% of total calories (Narayan
et al., 2006), despite the nutritious recommendation that 25% of energy should be covered by lipids (Sugano and Hirahara, 2000). Qualities of lipids are derived from their FAs composition which is various according to their sources. In general, vegetable oils from terrestrial plants are composed from SFAs and unsaturated FAs (UNFAs) with the chains formed by 16- and 18-carbon molecules, whereas the representation of individual FAs depends on plant species. Nevertheless, oils origined from marine organisms consist typically of UNFAs with the abundant amount of EPA and DHA, especially (Hu et al., 2001).

The absolute amount of lipids in the diet is not the main promoter of CVD. The important factor is relative concentration and distribution of dietary FAs with proved effects on lowering a risk of CVD (Cordain et al., 2002). Relative concentrations and distribution of dietary EFAs are different among various nationalities because of diverse dietary patterns. In Fig. 27.2, there are demonstrated trends of the total male and female mortality (CHD, CVD) in different countries in comparison with the distinct dietary intake of LCPUFAs. There is an evident dependency of the highest mortality in the countries with the lowest intakes of dietary n-3 LCPUFAs. The graphs have been constructed from data of several studies (Astorg et al., 2004; Hibbeln et al., 2006; Kris-Etherton et al., 2000; Meyer et al., 2003; Miyake et al., 2010).

Typical Western diet with oversized intake of ω-6 PUFAs (LA-rich oils from vegetable sources) leads to overproduction of proinflammatory ω-6 PG and cytokines, which could be suppressed by higher intake of ω-3 PUFAs from fish oils. Simopoulos (2002a,b) reported that high intake of ALA (about 15 g/day) would suppress human protein interleukin (IL-1) and tumor necrosis factor.

Many studies have been conducted on marine fish oil consumption and relation to risk of breast or prostate cancer. The inhibition of eicosanoids production from ω-6 PUFAs by higher consumption of fish oil with high levels of ω-3 PUFAs, which is a common feature of lowering a cancer risk, was reported (Bagga et al., 1997; Terry et al., 2004).

Eicosanoids derived from AA are biologically active in small quantities. Their large amounts lead to the formation of thrombi and atheromas, and to the development of allergic and inflammatory disorders (Simopoulos, 2002a,b). In general, ω-6 PUFAs have been associated with the enhancement of the promotional phase of mammary carcinogenesis (Rose, 1997).

However, contradictory results of studies on the effect of ω-3 and ω-6 PUFAs have been reported. It has been shown that AA also inhibits the growth of A549 human lung adenocarcinoma cells, even though DHA has been more effective than AA (Trombetta et al., 2007).

Differences between cis- and trans-configuration of PUFAs and the implication of their dietary intake on the human health have also been
<table>
<thead>
<tr>
<th>Country</th>
<th>Total Mortality/100,000 Population</th>
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<tr>
<td>USA</td>
<td>CHD M</td>
</tr>
<tr>
<td>Australia</td>
<td>CHD M</td>
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<tr>
<td>France</td>
<td>CHD M</td>
</tr>
<tr>
<td>Japan</td>
<td>CHD M</td>
</tr>
</tbody>
</table>

**FIGURE 27.2** Trends of total man and female mortality (CHD, CVD) in some countries with dietary intake of LCPUFAs.
reported. Trans isomers of monounsaturated octadecenoic acid (C18:1) were found as the most common trans-FAs in the diet of many European countries (Hulshof et al., 1999).

Recommended intakes of ω-3 LCPUFAs were often discussed in the scientific quarters and varied in different countries because of dissimilar dietary intake of ω-3 LCPUFAs. Approximate estimation of the consumption of ω-3 LCPUFAs is 0.1–0.5 g/day in Europe, 0.1–0.2 g/day in the United States, while in Japan, it is higher up to 2 g/day (Gómez Candela et al., 2011) due to higher consumption of fish.

B. The ω-6/ω-3 ratio as health-promoting factor

The significance of the ω-6/ω-3 ratio has also been discussed many times in research papers within the context of evolutionary aspects of the human diet. The origin ratio of ω-6/ω-3 was 1. Nowadays, the new lifestyle with the alteration of dietary pattern has caused the change of dietary intake of lipids, especially the distribution of ω-3 and ω-6 PUFAs in Western countries. At the beginning of the twentieth century, the consumption of vegetable oils and fats has risen. These oils and fats are responsible for an excessive dietary level of ω-6 PUFAs and a lowering concentration of ω-3 PUFAs in the Western diet (Cordain et al., 2005). Further contributor of altered composition of received FAs is the oversized consumption of margarine and shortening produced from refined vegetable oils by hydrogenation process resulting in the production of trans-isomers of FAs (Hu et al., 2001). The modification of present dietary pattern has led to higher intake of ω-6 PUFAs and that has caused an increase of the ω-6/ω-3 ratio up to 20–30:1 (Gómez Candela et al., 2011). The relationship between low ω-6/ω-3 ratio and rare occurrence of CHD in Inuits has already been described (Bjerregaard et al., 2003). The significance of the balance of the ω-6/ω-3 ratio is based on the fact that mammalian cells cannot convert ω-6 to ω-3 FAs due to the absence of the converting enzyme, omega-3 desaturase (Simopoulos, 2006). However, the significance of this ratio has been challenged on behalf of separate recommendations for ALA, marine ω-3 PUFAs, and LA (de Deckere et al., 1998).

III. LIPIDS AND PUFAS IN SEAWEED

Primary product of ω-3 FAs in trophic chain for fish is a product of marine microorganisms and algae. Fish and fish oil are considered as the main source of PUFAs, especially DHA (C22:6, ω-3) and AA (C20:4, ω-6). However, fish do not synthesize these EFAs, and their high level in fish oil results from the diet composed of marine zooplankton fed on phytoplankton (Yap and Chen, 2001; Yongmanitchai and Ward, 1993).
A. The variability of seaweed lipid composition

Lipid content and FA composition of seaweed are very changeable in dependence on different environmental conditions. It was reported that different levels of light and salinity could have positive or negative effect on the content and distribution of FAs across all groups of seaweeds (Floreto and Teshima, 1998). The lipid metabolism in algae is also influenced by different season period. In brown seaweed *Costaria costata*, the higher content of total lipids was established in May, whereas the abundant amount of storage lipids (triacylglycerols—TAGs) was observed in July; ω-3 PUFAs were prevailing in April, but the level of ω-6 PUFAs was similar in spring and summer (Gerasimenko et al., 2010). According to Kim et al. (1996), seasonable changes in the connection with lipids and FAs were observed also in brown seaweed *Fucus serratus*. While the highest contents of total lipids and storage lipids (TAG) were in summer, the lowest contents of both total lipids and TAG were established in spring. However, Honya et al. (1994) concluded that in another brown seaweed *Laminaria japonica* was the lowest content of total lipids and SFAs in midsummer, whereas the highest amount of ω-6 PUFAs was established during summer and ω-3 PUFAs content culminated during the cold months. These variabilities of lipid composition could be connected with the level of nitrogen in the seawater which is also a factor affecting the lipid contents in seaweeds. Nitrogen deficiency has been reported to cause the poor algal biomass production and lower lipid content. Nitrogen supplementation has led to increased amount of lipids in red seaweed *Palmaria palmata* (Mishra et al., 1993).

Further variation of lipid metabolism has been observed as the dependence on the environmental pollution by heavy metals (Cu, Cd, and Pb), herbicides, and also in the consequence of manganese deficiency in a cultivation medium for photosynthetic algae (Constantopoulos, 1970).

Finally, differences of lipid composition, in general, between wild and cultured strains of various plants and seaweeds were reported (Saito et al., 2010; Simopoulos, 2002a,b, 2004). In contrast to that, Mishra et al. (1993) observed no difference in the lipid contents of wild and cultured strains of *P. palmata*, but wild strains of *P. palmata* contained lower amount of nonpolar lipid fractions.

B. The lipid composition of seaweeds

Seaweeds are known as low-energy food. Lipid content in commonly used seaweeds does not obviously exceed 5% of dry matter. Nevertheless, the main part of lipids is formed by wide range of FAs. In Tables 27.1 and 27.2, lipid contents and FA profiles of some commercially used seaweeds from several studies are shown (Dawczynski et al., 2007; Kamlangdee and
<table>
<thead>
<tr>
<th>FAs</th>
<th>Laminaria sp.</th>
<th>Laminaria japonica</th>
<th>Laminaria japonica</th>
<th>Undaria pinnatifida</th>
<th>Undaria pinnatifida</th>
<th>Undaria pinnatifida</th>
<th>Hizikia fusiformis</th>
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<tr>
<td>a Dawczynski et al. (2007).</td>
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<td>c Mišurová, Ambrožová, and Samek (unpublished datas).</td>
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- Dawczynski et al. (2007).
- Kumari et al. (2010).
- Kamlangdee and Fan (2003).
Fan, 2003; Khotimchenko, 1998; Kumari et al., 2010; Mišurcová, Ambrožová, and Samek, unpublished data; Sánchez-Machado et al., 2004).

From these tables could be concluded that lipid content and FAs-composition are very changeable between different groups of seaweeds, and even within the same species. The content of total lipids ranged from 0.4% to 4.5% of dry seaweed matter. It is evident that palmitic acid was the most abundant SFA in all genera seaweeds.

Red seaweeds predominantly contained polyunsaturated 20C-PUFAs—eicosapentaenoic acid (EPA; \( \omega-3, \ C\ 20:5 \)) and AA (\( \omega-6, \ C\ 20:4 \)). Further, PUFAs with 18 and 20 carbon molecules were the most abundant in selected brown seaweeds. Finally, 18C-PUFAs were predominantly determined in the chosen green seaweeds. For comparison, FAs-composition of brown microalga Schizochytrium sp. as the significant source of DHA is also introduced in Table 27.2.

C. Distribution of PUFAs in seaweed lipids

Various FA compositions have been established in different groups of seaweed lipids. In algal lipids, there are presented two classes of polar and nonpolar lipids. TAGs have a storage function and form the main part of seaweed lipids. However, the group of polar lipids (phospholipids, glycolipids, and sulpholipids) is the main structural part of all cell membranes, which have the crucial function for the living processes (Gerasimenko et al., 2010; Mishra et al., 1993). Generally, acylglycerides were found better for human utilization like esters due to their easy incorporation into the plasma (Linko and Hayakawa, 1996).

As far as PUFAs distribution in seaweed lipids is concerned, it has been reported that majority of PUFAs has been distributed in TAGs (Gerasimenko et al., 2010; Mishra et al., 1993; Saito et al., 2010). According to the same pattern as FA composition of total seaweed lipids, the abundant amount of SFAs palmitic acid was obviously observed in all parts of seaweed lipids. However, in red seaweed P. palmata, the highest amount of palmitic acid was in polar lipids, while in the TAGs, there was the lowest concentration of it. The abundant PUFA in all lipid parts was EPA; nevertheless, it was in the highest concentration in the TAGs (Mishra et al., 1993).

IV. THE LIPID COMPOSITION OF MARINE MICROALGAE

Some marine microalgae from the kingdom Chromista and Protozoa were observed as a rich natural source of PUFAs. Thraustochytriaceae is the significant family of Chromista in which have been included generas Schizochytrium and Thraustochytrium (Yokoyama and Honda, 2007) with
the ability to form a high level of DHA, particularly (Lewis et al., 1999). It was published that \( \Delta^4 \) desaturase in marine heterokont brown algae *Thraustochytrium* sp. is responsible for the direct conversion of DPA (22:5, \( n-3 \)) to DHA (22:6, \( n-3 \)) in contrast to fish and mammals (Qiu et al., 2001). It was also proved that \( \Delta^4 \) desaturase gene (Fad4) from *Thraustochytrium* sp. could be transfected into human lymphocytes. This fact is significant for its utilization for perspective increase of human DHA and also for the treatment of patients with the Zellweger syndrome, which causes metabolic defect in DHA synthesis (Martinez et al., 2010). It was observed that *Thraustochytrium* sp. could accumulate more than 50% of its lipids in the form of DHA (Ward and Singh, 2005) and *Schizochytrium* sp. 35.6% of total lipids in the form of DHA (Yaguchi et al., 1997). *Cryptothecodinium cohnii*, red marine microalga from the kingdom Protozoa, was identified also as a good natural source of DHA that produces no other PUFAs. DHA forms 99.2% of total lipids in this algae (Mendes et al., 2007, 2009).

Different distribution of FAs in various parts of microalgal lipids as well as in seaweed lipids was established. According to Fan et al. (2007), marine microalga of strain *Schizochytrium mangrovei* contained 68% of total lipids of dry cell weight and 93% of FAs were distributed in TAGs. Interestingly, a higher amount of PUFAs was determined in polar lipids, fundamental components of cell membranes, with the high degree of unsaturation of the fatty acyl groups responsible for their normal functions. Lipids of *S. mangrovei* were primarily composed of palmitic FA and DHA, 50.3% and 29.7% of total lipids, respectively. With respect to DHA distribution, it was the major PUFA in all lipid classes. The highest amount of DHA was primarily contained in TAGs, which reached 93.6% of the total DHA.

*Thraustochytrids* strains could be used for the commercial production of DHA for an infant formula (Ward and Singh, 2005). Currently, the production of DHA by marine microalgae is the subject of intensive research because of the fact that microalgal oil has the advantage of presenting neither an unpleasant odor nor a high amount of cholesterol and contains squalene and phytosterols, which have additional benefits to human health (Rubio-Rodríguez et al., 2010).

V. CONCLUSION

Nowadays, process of finding new sources of \( \omega-3 \) PUFAs has been continuing. Their major commercial sources are fish and fish fat. EPA and DHA produced from fish oil are used as nutraceuticals and functional ingredients in industrial foods. However, their wide usage as food additives is limited for the typical fishy smell, disagreeable taste, and
finally for the oxidative nonstability. Further, chemical forms of PUFAs are important for their better utilization by human.

Nevertheless, the growing requirements of healthy functional foods and escalation of the environment pollution on the worldwide basis have led to produce PUFAs in controlled batch culture of marine microalgae, especially of *Thraustochytrium* and *Schizochytrium* strains. Besides marine microalgae, seaweeds seem to be an interesting natural source of ω-3 PUFAs, thanks to their better utilization.

Seaweed lipids are presented in a very small amount that does not exceed 5% of dry seaweed matter. Despite this low lipid content in seaweeds, ω-3 and ω-6 PUFAs represent the significant part of seaweed lipids. LCPUFAs of ω-3 and ω-6 series are precursors of eicosanoids which are important bioregulators of many cellular processes. Lipids composition of seaweed is very changeable due to the adaptation mechanism improving their tolerance to the environmental conditions.

In spite of health benefits of ω-3 PUFAs for human, the unfavorable effects within the context of their easy auto-oxidation should not be ignored. This fact means the incorporation of expensive steps during purifying process in the industrial production of PUFAs.

Seaweed could be potentially used in the production of low fat foods due to their high level of important PUFAs. Functional food products enriched with ω-3 LCPUFAs are widely spread nowadays. The utilization of marine microalgae to produce high value lipids is suggested as the alternative sources of PUFAs. The utilization of algal enzymes in order to produce EPA and DHA from modified crops seems to be also the perspective interest.

**REFERENCES**


Seaweeds are a good source of some water-soluble (B1, B2, B12, C) and fat-soluble (β-carotene with vitamin A activity, vitamin E) vitamins. To ensure that the adequate intake of all vitamins is received in the diet, people (especially people on special diet, strict vegetarians, and vegans) can consume foods enriched with vitamins, for example, in the form of functional foods with vitamins as nutraceuticals, extracted from natural sources such as seaweeds. Seaweed vitamins are important not only due to their biochemical functions and antioxidant activity but also due to other health benefits such as decreasing of blood pressure (vitamin C), prevention of cardiovascular diseases (β-carotene), or reducing the risk of cancer (vitamins E and C, carotenoids).
I. INTRODUCTION

Vitamins are organic essential compounds needed in the human body in trace amounts for different chemical and physiological processes. Vitamins are commonly classified into two groups according to their solubility: water-soluble vitamins (members of the vitamin B group and vitamin C) and fat-soluble vitamins (vitamin A and its provitamins—carotenoids with vitamin A activity, vitamins E, D, and K).

Even though vitamins are required only in very small quantities, to ensure that the adequate intake of vitamins in the diet is received, people can consume foods enriched with vitamins, for example, in the form of functional foods with vitamins as nutraceuticals. In addition, certain vitamins extracted from natural sources such as seaweeds have antioxidant activity and other health benefits such as decreasing of blood pressure, prevention of cardiovascular diseases, or reducing the risk of cancer.

II. IMPORTANCE OF VITAMINS FOR HUMANS

As mentioned above, vitamins are essential substances which cannot be synthesized by humans or only in limited quantities; therefore, they should be obtained from human diet. Vitamin deficiency can be caused not only by insufficient intake from foodstuffs but also because of increased requirement by certain group of people (people on special diet, smokers), poor absorption, or inadequate utilization.

Seaweeds are generally a good source of some B group vitamins (B₁, B₂, B₁₂). Other vitamins of B-complex are present too, but only in low or trace amounts (niacin, B₆, biotin, folates). Certain seaweeds contain great quantities of vitamins with antioxidant activity, vitamins C and E, and the provitamin forms of vitamin A, carotenoids.

A. Function of vitamins in human body

The hydrosoluble vitamins are needed as enzyme cofactors. The vitamin can have one or a few very specific roles or much more extensive roles. Several B group vitamins serve as coenzymes for enzymes with function in the catabolism of foodstuffs to produce energy for the body. Some of them are fundamental even for their antioxidant activity and other health benefits. Thus low levels of some B group vitamins (B₂, B₆, B₁₂) can result in reduced levels of DNA methylation and therefore in some kinds of cancer (Hernandez et al., 2003).

The following vitamins are presented in seaweeds in great amounts—thiamin, riboflavin, cobalamin, and ascorbic acid. Thiamin (vitamin B₁)
has a key role in the intermediary carbon metabolism and is essential for several enzymes such as pyruvate dehydrogenase, pyruvate decarboxylase, and transketolase. Riboflavin (vitamin B_2) is used ubiquitously throughout the cell. Cobalamin (vitamin B_12) is required for the activity of cobalamin-dependent biosynthetic enzymes: methionine synthase and methylmalonyl CoA mutase. Interestingly, although vitamin B_12 is not found in vascular plants, it is abundant in algae. As only prokaryotes have the ability to synthesize cobalamin, all of the vitamin B_12 found in algae must have originally been produced by bacteria (Croft et al., 2006). Ascorbic acid (vitamin C) is fundamental antioxidant in the ascorbate–glutathione pathway. Moreover, it protects enzymes that have prosthetic transition metal ions and is a cofactor of enzymes such as violaxanthin deepoxidase, ascorbate oxidase, and ascorbate peroxidase.

Fat-soluble vitamins, which are present in seaweeds, are vitamin E and provitamins A. Vitamin E (tocopherols and tocotrienols) is important liposoluble antioxidant which is conclusive for the prevention of oxidation of polyunsaturated fatty acids absorbed from the diet. Tocopherols block the production of reactive oxygen species formed during oxidation and help inhibit the low-density lipoprotein (LDL) oxidation as namely oxidatively modified LDL are considered to play a vital role in the development of atherosclerosis (Steinberg, 1991). Vitamin A, retinal, as visual pigments’ chromophore, is important in the vision process. Besides the epithelial tissue maintenance and prevention of its keratinization, vitamin A also presents important systematic functions in the growth and reproductive efficiency (Ribeiro et al., 2011). However, plant food such as algae does not contain intrinsic vitamin A, but its provitamins, carotenoids, linear polyenes with a cyclic structure which possesses a β-ring. The most abundant carotenoid with provitamin function in seaweed is β-carotene. It can be cleaved by a β-carotene-15,15'-dioxygenase, resulting in the formation of retinal (DellaPenna and Pogson, 2006; Ribeiro et al., 2011).

B. Vitamin requirements for humans

The recommended daily allowances (RDA) values are based on our knowledge of the minimal requirement for maximal protection for each vitamin-dependent function. Because requirements are not known exactly, these data can differ according to country experts and nutrition organization. Recommendations for some vitamins intake or RDA have been established without taking into consideration their need for enhancing body function and preventing some diseases. Therefore, the intake of vitamins which prevent certain diseases due to its strong antioxidant activity (e.g., prevention of cardiovascular disease and cancer) should be higher than that of RDA values (Weber et al., 1997).
The presented ranges of RDA and median RDA values, from data collected from up to 30 countries, recalculated Brubacher (1989).

RDA for vitamins B₁ and B₂ are 0.5–2.2 and 0.8–3.2 mg/day, respectively, with the median of 1.2 and 1.6 mg/day. The minimum daily requirement for vitamin B₁₂ is thought to be in the range of \( \approx 0.1 \mu g \), the amount of 0.2–0.25 \( \mu g \) absorbed from food is generally adequate, and 1 \( \mu g \) daily would treat people with no stores of vitamin B₁₂, for example, those with vegan diet (Herbert, 1988). According to Brubacher data (1989), RDA value for vitamin B₁₂ is 1–5 \( \mu g \)/day, the median 2 \( \mu g \)/day. The high value of vitamin B₁₂ is presented in red seaweed *Porphyra* sp. (nori)—33.8 \( \mu g/100 \text{ g dw} \) (Miyamoto *et al.*, 2009). Therefore, a consumption of only 1.5 g of this seaweed would complement the RDA of this vitamin. In result, seaweed can provide an alternative source of this vitamin for strict vegetarians and vegans. However, the bioavailability of different forms of vitamin B₁₂ in seaweeds should be discussed as some introduced results are contradictory. RDA value of vitamin C is 15–100 mg/day, the median 60 mg/day of ascorbic acid.

Concerning the fat-soluble vitamins, the range of RDA value of vitamin E is 5–50 mg of \( \alpha \)-tocopherol equivalent per day, the median 10 mg/day. The RDA value of vitamin A is 360–1650 \( \mu g \)/day of retinol equivalent (RE) with the median of 800 RE/day. As mentioned before, seaweed does not contain intrinsic vitamin A, but its provitamins, with \( \beta \)-carotene as the most abundant. The relevant dose of \( \beta \)-carotene intake is about 15 mg/day (Krinsky, 1998). The vitamin A activity (\( \mu g \) RE/100 g) is recalculated from determined content of \( \beta \)-carotene.

### III. VITAMIN COMPOSITION IN SEAWEED

There is only few published data concerning vitamin content and bioavailability of vitamin forms contained in edible seaweeds. Generally, seaweeds contain both water- and fat-soluble vitamins, B-complex vitamins, vitamin C, provitamin A, and vitamin E, but some of them only in relatively low content.

#### A. Factors influencing vitamin content in seaweed

The composition and therefore the vitamin profile of seaweeds vary and are affected by algal species, algal grown stage, geographic area and salinity, season of the year, availability of light, and temperature of sea water (Mabeau and Fleurence, 1993; Norziah and Ching, 2000).

The content of some vitamins in seaweeds, for example, vitamin B₁₂ (Yamada *et al.*, 1996a), varies greatly among samples of the same species. In many cases, light is an important regulator of vitamin biosynthesis;
thus plants growing in bright light have higher ascorbate content (Smith et al., 2007). Moreover, algae growing in the littoral zone or on the surface tend to have higher level of vitamin C than algae which is harvested from depth from 9 to 18 m (Norris et al., 1937). Further, other environmental parameters, such as concentration of certain compounds in the sea, can play the important role for vitamin occurrence in algae (Smith et al., 2007).

The importance of vitamins to plants themselves is often overlooked, but they play the essential roles in a plant metabolism too (Smith et al., 2007). Some algal species require different combinations of certain vitamins such as vitamins B_{12} and B_{1}. Because the concentration of these vitamins in the natural environment is quite low, their absorption is insufficient (Croft et al., 2006). According to Yamada et al. (1996a), red algae *Porphyra tenera* can take up the free (not protein-bounded) form of vitamin B_{12} from the incubation medium by concentration- and temperature-dependent processes. The amount of uptake increases with the time of incubation.

Loss of vitamins can be induced by storage conditions such as the influence of light and oxygen. Moreover, there is a negative influence on a vitamin content caused by technological processing such as drying (sun-, oven-, freeze-drying) and sterilization, and culinary processes such as cooking, roasting, or baking, which could decline vitamin content due to water extrusion and high temperature during these procedures. This was observed, for example, in instable ascorbic acid (Norris et al., 1937).

According to the vitamin determination by Hernández-Carmona et al. (2009), there are significant differences of certain vitamins content caused by seasonal variations, for example, in *Eisenia arborea*. It was observed that the highest content of some vitamins (A, B_{1}, B_{2}, and partly also vitamin C) was in spring opposite to the lowest spring value of vitamin E. Moreover, season was observed as the factor of carotenes content affection also in *Palmaria*. The highest content of carotenes was found in summer and the lowest in winter (Løvstad Holdt and Kraan, 2010).

**B. Bioavailability and absorption of vitamins**

Although certain macroalgae are rich in vitamins, the bioavailability of these compounds has not been studied sufficiently yet, so there is not enough data to clarify this problem for all vitamins satisfyingly. Bioavailability is influenced by several factors as follows: characteristics of the food source, location of the vitamin in the plant source, particle size, the presence of other influencing dietary components and interactions with other dietary factors, the type and extent of processing (Rock et al., 1998).

The bioavailability of vitamins is primarily related to solubility of each vitamin, which cohere with their intestinal absorption and therefore with their uptake by tissues as well. Bioavailability and absorption of
some fat-soluble vitamins depend on whether they are consumed together with foodstuffs containing lipids or not. As being fat-soluble, these vitamins follow the same intestinal absorption path as dietary fat. Further, it is also important which of vitamin form is present in food. Therefore, for example, significant contributions to vitamin E activity are α- and γ-tocopherols (Weber et al., 1997).

Although only little is known about the origin of vitamin B₁₂ in the seaweeds, the bioavailability of algal vitamin B₁₂ is quite contradictory. Generally, edible algae contain large amount of vitamin B₁₂; some of them (e.g., Porphyra sp.) comprise a substantial amount of the form bioavailable to mammals. However, van den Berg et al. (1991) mentioned that at least part of the cobalamins may be analogues not bioavailable for humans as pseudo-B₁₂. These compounds have adenosyl moiety instead of 5,6-dimethylbenzimidazole of the B₁₂ molecule (Yamada et al., 1996b). Miyamoto et al. (2009) reports that dried purple laver (Porphyra sp.) could be well digested only under pH 2.0 conditions. Further, digestion rate of B₁₂ would be estimated to be 50% in persons with normal gastric function.

It also appears that vitamins which are bound to fibers or some other carbohydrates in foods are not as available as the vitamins taken in the pure form. This is shown in seaweeds such as hijiki, which has a high percentage binding (42.7–45.6%) for thiamin. In contrast, kombu and susabi-nori showed the lowest binding for this vitamin (Suzuki et al., 1996).

C. Vitamin composition of seaweed

It is said that 100 g of seaweed provides more than the daily requirements of vitamin A, B₂, B₁₂, and two-thirds of the vitamin C requirement (Ortiz et al., 2006). Most of the red seaweeds (Palmaria, Porphyra) contain large amounts of provitamin A and significant quantities of vitamins B₁, B₂, and B₁₂, which are also present in green seaweeds. The vitamin content of brown seaweeds (Undaria, Laminaria) appears to be less remarkable, but brown seaweeds have high content of vitamin C (Mabeau and Fleurence, 1993). Some seaweed (such as Porphyra) can supply adequate amount of vitamin B₁₂ in vegans.

The following summary is concerned with the most abundant seaweed vitamins which can sufficiently contribute to daily vitamin requirements, such as water-soluble vitamins B₁, B₂, B₁₂, and C and fat-soluble provitamins A (β-carotene) and E. Because some results are given for fresh samples, some for dry ones, there is quite disputable to compare the data presented thereby.

Vitamin B₁ and B₂ are present in sufficient amount especially in brown and red marine algae. The highest amount of both vitamins was detected in wakame and kombu—0.3 and 0.24 mg B₁/100 g dw; 1.35 and 0.85 mg
B$_2$/100 g dw, respectively (Kolb et al., 2004). Lower levels of these vitamins are present in arame (0.06–0.12 and 0.65–0.92 mg/100 g dw, respectively), Caulerpa lentillifera and Ulva reticulata (Hernández-Carmona et al., 2009; Ratana-Arporn and Chirapart, 2006). French Institut de Phytonutrition (FIP) describes much higher content of these vitamins, for example, in wakame—5 mg B$_1$/100 g dw or 11.7 mg B$_2$/100 g dw (MacArtain et al., 2007).

The intake of vitamin B$_{12}$ in strict vegetarian and vegan diet is usually quite low. Therefore, this vitamin can easily become deficient. Lower levels of vitamin B$_{12}$ in a diet may result in reduced levels of DNA methylation or elevated levels of homocysteine which is a risk factor for cardiovascular diseases (Hernandez et al., 2003). A particularly rich dietary source of the vitamin is seaweed, foods enriched with them or seaweed extracts, which are good alternate source of vitamin B$_{12}$ for vegans. Thus consumption of some seaweed (nori) may keep vegans from suffering B$_{12}$ deficiency (Suzuki, 1995). The highest content of vitamin B$_{12}$ in seaweed is presented in red Porphyra sp. (nori)—133.8 µg B$_{12}$/100 g dw, in the form active for human (Miyamoto et al., 2009). Other results of B$_{12}$ content in this algae are present in range 12.02–68.8 µg/100 g dw (Takenaka et al., 2003; van den Berg et al., 1988; Watanabe et al., 1999, 2000). Further marine algae contain much less of B$_{12}$; high content is found in green laver Enteromorpha sp., following by dulse, and low levels in Ulva sp., wakame, kombu, and hijiki (MacArtain et al., 2007; Watanabe et al., 1999; Yamada et al., 1996b).

Vitamin C is present especially in brown and green seaweeds, less in red algae. The highest levels of vitamin C were discovered in Enteromorpha flexuosa and Ulva fasciata (300 and 220 mg/100 g dw, respectively) (McDermid and Stuercke, 2003). FIP states that the highest level of vitamin C is found in wakame (184.75 mg/100 g dw), red laver, and sea lettuce (MacArtain et al., 2007). Chan et al. (1997) determined the high content of vitamin C in freeze-dried algae Sargassum hemiphyllum—153.8 mg/100 g dw, smaller amount in oven- and sun-dried seaweed. Other seaweeds contain much less of vitamin C; high content is found in red algae Kappaphycus alvarezii (107.1 mg/100 g dw) and low levels in arame, ogonori, Sargassum polycystum, Eucheuma cottonii, U. reticulata, and C. lentillifera (Fayaz et al., 2005; Hernández-Carmona et al., 2009; Hong et al., 2007; Matanjun et al., 2009; Norziah and Ching, 2000; Ratana-Arporn and Chirapart, 2006).

Despite the low lipid content in seaweed, their fat contains high level of vitamin E. Generally, brown seaweeds contain more $\alpha$-tocopherol (also $\beta$- and $\gamma$-tocopherols) than red and green algae which contain only $\alpha$-tocopherol. The highest amount of vitamin E was detected in kelp Macrocystis pyrifera, 132.77 mg/100 g fat ($\alpha$-tocopherol), with total tocol content of 145.72 mg/100 g fat, and in Ulva lactuca with $\gamma$-tocopherol value
96.35 mg/100 g fat (Ortiz et al., 2006, 2009), so compared to traditional plant oils, the fat of these seaweeds contain a high level of tocols. Moderate levels were found in Durvillaea antarctica (Ortiz et al., 2006). Low content of tocols was determined in Gracilaria chilensis, followed by S. polycystum, C. lentillifera, E. cottonii, and E. arborea (Hernández-Carmona et al., 2009; Matanjun et al., 2009; Ortiz et al., 2009). FIP states that the highest level of vitamin E is in wakame and dulse (MacArtain et al., 2007).

Plant food such as algae does not contain intrinsic vitamin A, but its provitamins such as β-carotene. The high values of β-carotene with vitamin A activity were found in red seaweeds Gracilaria changgi, K. alvarezzi, and in brown algae kombu (5.2, 5.26, and 2.99 mg/100 g dw, respectively, recalculated to 865, 865, and 481 RE/100 g dw) (Fayaz et al., 2005; Kolb et al., 2004; Norziah and Ching, 2000). Moderate levels of vitamin A were determined in wakame, arame, sea grapes, and sea lettuce (Hernández-Carmona et al., 2009; Ratana-Arporn and Chirapart, 2006).

D. Antioxidant activity of some vitamins contained in seaweed

Among the compounds found in seaweeds, those with antioxidant activity have an excellent potential for application in food industry and also in cosmetics and pharmacology industry, and for consumer interest too. Due to the presence of these compounds, marine algae may also have other health beneficial effects and therefore they could be used as nutraceuticals or in functional foodstuffs.

When seaweeds are exposed to a combination of light and high oxygen concentrations, the formation of free radicals and other oxidative reagents is induced. The absence of structural damage in the structural components suggests that seaweeds are able to generate the necessary compounds to protect themselves against oxidation (Jiménez-Escrig et al., 2001). Therefore, marine algae can be considered the important source of antioxidants. According to Tsuchihashi et al. (1995), the antioxidant potency is determined by several factors such as intrinsic chemical reactivity of antioxidant toward radical, site of generation and reactivity of the radicals, site of antioxidant, concentration and mobility of the antioxidant at the microenvironment, stability and fate of antioxidant-derived radical, and interaction with other antioxidants.

The compounds which are responsible for antioxidant activity in seaweed include vitamin E (α-tocopherol), carotenoids (β-carotene), and vitamin C (ascorbic acid), and partially vitamin B1 and niacin.

Oxidative forms, which arise in foodstuffs, are responsible for various free-radical-induced diseases such as cardiovascular diseases and certain types of cancers. Vitamins with a strong antioxidant capacity can be used as the first line therapeutic defense against cancer before cancer treatment (Simon, 2002).
The antioxidant activity of carotenoids is associated with its binding capacity with singlet oxygen by conjugated double bonds systems. The maximum protection is given by carotenoids with more than nine double bonds (Ribeiro et al., 2011). The effectiveness of carotenoids as antioxidants is also dependent upon their interaction with other coantioxidants, especially vitamins E and C. It was demonstrated that β-carotene is 32 times less reactive toward peroxyl radical than α-tocopherol and 11 times less reactive toward carbon-centered radical. Therefore, β-carotene is less potent as an antioxidant than α-tocopherol. Carotenoids may, however, lose their effectiveness as antioxidants at high concentration or at high partial pressures of oxygen. It is unlikely that carotenoids actually act as prooxidants in biological systems; they rather have a tendency to lose their effectiveness as antioxidants (Young and Lowe, 2001).

In certain experimental studies, there is an indication for the correlation between the diet rich in carotenoids (β-carotene) and a diminishing risk of cardiovascular diseases and some type of cancer—lung cancer, and probably also cancer of the esophagus, stomach, colon, rectum, breast, and cervix (Kohlmeier and Hastings, 1995; Krinsky, 1991). Presumably, they are capable of seeking for free radicals and neutralizing them and thus they inhibit cell proliferation (Simon, 2002).

Despite the low lipid content in seaweed, the presence of vitamin E is relevant as it acts as a strong antioxidant which prevents the formation of free radicals. α-Tocopherol, the most important member of tocol group, is capable of fixing free radicals via its phenol group in the structure and thus is considered to play an important role in oxidation of biological membranes, lipoproteins, and fat deposits, controlling or reducing lipid peroxidation (Sánchez-Machado et al., 2002).

It was discovered that vitamin E is associated with lower mortality from cerebrovascular diseases as this vitamin improves endothelial dysfunction and ameliorates vascular health and reduces vascular damage (Houston, 2005). There is also evidence that vitamin E may protect against cancer—the reduction in the risk of both lung and cervical cancers (Iso and Kubota, 2007; Simon, 2002). Vitamin E may protect against the development of cancer through several mechanisms such as reacting with genotoxic radicals, reducing mutagenic activity, inhibiting carcinogenic nitrosamine formation, protecting cell membranes against peroxidation, and/or enhancing the immune system (Weber et al., 1997).

Ascorbic acid inhibits the oxidation quite efficiently for a long period and also neutralizes free nitrites which are a substrate for carcinogens. Vitamin C acts as a potent synergist in the presence of α-tocopherol and spares this antioxidant.

It is believed that ascorbic acid prevents cancer by neutralizing free radicals before they can damage DNA and initiate tumor growth, or it may act as a prooxidant helping body’s own free radicals to destroy
tumors in their early stages (Naidu, 2003). It appears that there is a very strong relationship between vitamin C and the reduction of stomach cancer, and a possible relationship with the reduction of the risk of mouth, pharyngeal, lung, and gall bladder cancer in men (Iso and Kubota, 2007; Simon, 2002). The dietary intake of vitamin C is also inversely correlated with systolic and diastolic blood pressure as it reduces blood pressure in hypertensive patients, hyperlipidemics, and diabetics. Combination of vitamin C with other antioxidants (vitamin E, β-carotene) provides synergic antihypertensive effects (Houston, 2005).

**IV. CONCLUSIONS**

There is a great prospective potential of the usage of vitamins as nutraceuticals from natural sources such as seaweed, or application of seaweed as a part of functional foods. Not only do vitamins possess considerable vitamin functions for the body, but they also possess other important qualities such as antioxidant activity and other benefits for human health. Certain groups of people (e.g., people on special diet, smokers) are prone to vitamin deficiency. Therefore, nutraceuticals or functional foods could help them to retrieve good health condition. The reason of vitamin deficiency can be caused not only by insufficient intake from foodstuffs but also by increased requirement, poor absorption, and inadequate utilization. Seaweeds are generally a good source of some B group vitamins (B₁, B₂, B₁₂) and vitamins with antioxidant activity, vitamins C and E, and the provitamins A, carotenoids (β-carotene). The highest content of vitamin B₁₂, which is deficient in vegan diet, is found in great amount in seaweed, mainly in red algae *Porphyra* sp. (nori). Vitamin C with antioxidant function shows the reduction of the risk for stomach cancer. Despite the low lipid content in seaweeds, their fat contains high level of vitamin E. It was found that vitamin E has a positive influence on mortality from cerebrovascular diseases and on the reduction of risk of both lung and cervical cancers. There is also an indication of the relation between the diet rich in β-carotene and a lower risk of cardiovascular diseases and reduction of lung cancer.

Therefore, research and following usage of seaweed vitamins as nutraceuticals or ingredients for functional food show prospective possibilities for developing food industry also for consumers who can benefit from added health properties of foods.

**REFERENCES**


# CHAPTER 29

## Seaweed Minerals as Nutraceuticals

**Ladislava Mišurcová,***1 Ludmila Machů, † and Jana Orsavová‡

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### Abstract

Seaweed is known as an abundant source of minerals. Mineral composition of seaweed is very changeable because of many exogenous and endogenous factors and differs also within the same species.

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Principally, seaweed is an excellent source of some essential elements. Mainly, iron and iodine are in high concentration. Seaweeds could be prospective as functional foods and also producers of mineral nutraceuticals.

I. INTRODUCTION

Seaweeds are well-known source of many different bioactive compounds with many health benefit activities. Thus, seaweeds are categorized into the group of functional foods. Because of abundant amounts of many minerals, seaweeds could be utilized as nutraceuticals. The most abundant elements in seaweed tissue are iron (Fe) and iodine (I). Mineral composition of seaweeds is very changeable according to many exogenous and endogenous factors, and it is obviously corresponding with the concentration of minerals in the seawater or a growth medium.

Minerals are structural components and significantly important elements that perform many necessary functions in the living body, including the cell transport and wide range of metabolic processes serving as various catalytic metalloenzymes cofactors. This chapter is focused on some trace elements that are abundantly contained in seaweed. Common feature of all trace elements is their important participation in the formation of binding site of metalloenzymes, where each element plays specific roles in living systems and many of them have a lot of beneficial functions.

II. FUNCTIONS OF IODINE, IRON, ZINC, AND MANGANESE IN THE HUMAN BODY

A. Iodine

Dietary iodine is essential for the production of thyroid hormones, thyroxine and triiodothyronine, which regulate many important physiological processes in humans (Haldimann et al., 2005). More than 1.9 billion individuals are estimated to have inadequate iodine nutrition; the lowest iodine deficiency is in America and the highest in Europe (de Benoist et al., 2003).

Iodine deficiency has effects on growth and development because of inadequate production of thyroid hormones. Health consequences of iodine deficiency are goiter, increased occurrence of hypothyroidism in moderate-to-severe iodine deficiency or decreased occurrence of hypothyroidism in mild iodine deficiency, and increased susceptibility of the thyroid gland to nuclear radiation. Abortion, stillbirth, congenital
anomalies, perinatal and infant mortality, or endemic cretinism may occur in neonates. Iodine deficiency during child and adolescent age could cause delay of physical development and impairment of mental function or iodine-induced hyperthyroidism in adults as well (Zimmermann and Crill, 2010). In severe iodine deficiency, hypothyroidism and developmental brain damage are the dominating disorders (Laurberg et al., 2010).

Excess iodine could lead to thyrotoxicosis and may be connected with hyperthyroidism, euthyroidism, hypothyroidism, or autoimmune thyroid disease (Bürgi, 2010; Laurberg et al., 2010). However, thyroid possesses the adaptation mechanisms regulating thyroid hormones synthesis and secretion and protecting from thyrotoxicosis (Wolff, 1989).

B. Iron(Fe)

Iron is an essential element for humans because of its participation in fundamental cell functions. Iron is the most abundant transition metal in the body, which takes part in the utilization of oxygen, and as a component of numerous enzymes, it affects many vitally important metabolic processes, including oxygen transport, DNA synthesis, and electron transport (Lieu et al., 2001; Puntarulo, 2005). The main part, 60–70% of Fe is bound to hemoglobin in circulating erythrocytes, 10% of Fe is present in the form of myoglobins, cytochromes, and iron-containing enzymes, and 20–30% of surplus Fe is stored as ferritins and hemosiderins (Lieu et al., 2001). Iron is stored in the liver, spleen, and bone marrow in specific proteins (Puntarulo, 2005).

Iron deficiency is considered as the most common nutritional disorder worldwide, which results mainly from excessive bleeding (Deegan et al., 2005; Puntarulo, 2005), but partly can be induced also by plant-based diets of vegans, which contains less bioavailable Fe (Martínez-Navarrete et al., 2002). Iron deficiency adversely affects the cognitive performance, behavior, physical growth, the immune status, and morbidity from infections of all age groups. Iron-deficient humans have impaired gastrointestinal functions and altered patterns of hormone production and metabolism (Walker, 1998; WHO, 2001).

Homeostatic mechanisms are very important for the prevention of accumulation of excess Fe that is believed to generate oxidative stress by catalysis of variety of chemical reactions involving free radicals, which could result in cell damage (Pietrangelo, 2002; Puntarulo, 2005). Excess Fe accumulation may promote cancer and increase the cardiovascular risk (Martínez-Navarrete et al., 2002). Iron overload can be observed in some cases including an excessive dietary iron intake, inherited diseases, for example, idiopathic hemochromatosis, congenital atransferrinemia, or the medical treatment of thalassemia (Fontecave and Pierre, 1993).
C. Zinc

Zinc (Zn) is one of the most important essential elements that occurs in hundreds of zinc metalloenzymes and in thousands of protein domains as zinc-fingers (Maret and Sandstead, 2006; McCall et al., 2000; Tapiero and Tew, 2003). Zinc is necessary for growth and development; it is a structural ion of biological membranes; it has roles in gene expression and endocrine function, DNA synthesis, RNA synthesis, and cell division (O’Dell, 2000; Salgueiro et al., 2002). Zinc is an antioxidant, regulates immune response, and has a role in vitamin A metabolism (Rink and Haase, 2007; Salgueiro et al., 2000). Zinc interacts with important hormones involved in bone growth and enhances the effects of vitamin D on bone metabolism (Salgueiro et al., 2002). The majority (85%) of Zn in the whole body is deposited in muscles and bones, 11% is in skin and liver, and the remaining amount is in other tissues. High level of Zn is present in the brain (Tapiero and Tew, 2003). Disturbances of Zn homeostasis have been associated with several diseases including diabetes mellitus, and the alteration of Zn homeostasis in the brain may be associated with the manifestation of epileptic seizures (Chausmer, 1998; Takeda, 2000).

Zinc deficiency occurs in populations whose diets contain high concentration of phytate, a powerful chelator, and low protein (Tapiero and Tew, 2003). Zinc deficiency negatively affects the epidermal, central nervous, immune, gastrointestinal, skeletal, and reproductive systems (Salgueiro et al., 2000, 2002; Tapiero and Tew, 2003; Verstraeten et al., 2004).

The exposure to elevated levels of Zn and zinc-containing compounds may cause many adverse effects in the gastrointestinal, hematological, and respiratory systems together with the alterations in the cardiovascular and neurological systems of humans (Nriagu, 2011). An excessive Zn intake leads to acute adverse effects like diarrhea, vomiting, and headache. Zinc chronic toxicity is reflecting in the effects like functional impairment in immunological response, reduced copper status, altered Fe function, or cholesterol metabolism (Scherz and Kirchhoff, 2006).

D. Manganese

Manganese (Mn) is an essential trace element required for a variety of biological processes. The highest Mn levels are concentrated in tissues with high-energy demand, such as brain, and in retina and dark skin with the high content of pigment. Further, bone, liver, pancreas, and kidney contain obviously high Mn concentration, too (Aschner and Aschner, 2005).

Mn is involved in the metabolism of protein, lipid, and carbohydrate, and performs as various enzymes cofactors. Mn is needed for normal immune function, regulation of blood sugar and cellular energy, reproduction, digestion, bone growth, aids in defense mechanisms against free
radicals, and together with vitamin K, it supports blood clotting and hemostasis and finally, it is essential for the development and function of the brain (Aschner and Aschner, 2005; Takeda, 2003).

A large portion of Mn is bound to manganese metalloproteins. Approximately 3–5% of ingested Mn is absorbed and it is cleared from the blood by the liver and excreted in bile (Mergler, 1999). Manganese absorption is influenced by the presence of other trace elements, phytate, and ascorbic acid (Aschner and Aschner, 2005).

Manganese deficiency can lead to several diseases including osteoporosis, epilepsy, impaired growth, poor bone formation and skeletal defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism (Aschner and Aschner, 2005; Nkwenkeu et al., 2002).

Manganese toxicity is associated with damaged ganglia structures and leads to neuropsychiatric symptoms and behavioral dysfunction reminiscent of Parkinson’s disease, which is the most common form of parkinsonism and is caused by neurodegenerative disease, drugs, toxicants, and infections (Cersosimo and Koller, 2006; Nkwenkeu et al., 2002; Ordoñez-Librado et al., 2010). High liver Mn content has been reported in alcoholic liver disease and it may affect hepatic fibrogenesis (Rodriguez-Moreno et al., 1997).

III. REQUIREMENTS OF MINERALS BY HUMANS

An adequate intake of minerals is essential for a high nutritional quality of the diet and contributes to the prevention of chronic nutrition-related diseases and degenerative diseases including cancer, cardiovascular disease, Alzheimer’s disease, and premature aging (Fenech and Ferguson, 2001; Kersting et al., 2001). However, too high intakes of trace elements could cause toxicity and too low intakes of trace elements may result in nutritional deficiencies (Goldhaber, 2003).

Dietary Reference Intakes (DRIs) are used quite a lot and refer to a set of four nutrient-based reference values that represent the approach to provide quantitative estimates of nutrient intakes. The DRIs replace and expand on the Recommended Dietary Allowances (RDAs) for the United States and the Recommended Nutrient Intakes (RNIs) for Canada. The DRIs consist of the RDAs, the Tolerable Upper Intake Level (UL), the Estimated Average Requirement (EAR), and the Adequate Intake (AI). Generally, each of these values represents average daily nutrient intake of individuals in the diet (Goldhaber, 2003; Murphy and Poos, 2002; Parr et al., 2006; Trumbo et al., 2001; Yates et al., 1998). In addition, dietary intake data for minerals could be assessed within the context of the bioavailability and other factors affecting the utilization of elements by the human body, such as age, sex, and health aspects (Dokkum, 1995).
Table 29.1 shows the recommended daily intakes (RDIs) for the selected trace elements. Data vary according to various countries and their values are adequate of diverse dietary pattern and different levels of these elements in the population of these countries.

In light of the essentiality of trace elements in adequate dietary intakes on one side and the toxicity of trace elements in high-level intakes on the other side, there should be a set of rules for physiological benefit and safe intakes of trace elements. Table 29.2 shows the UL for selected elements suggested for the United States and Australia.

IV. CONTENT OF MINERALS IN SEAWEED

Seaweeds are a well-known source of minerals and their levels depend on different seaweed genera. Formerly, brown seaweed was used for the production of soda and potash and it has also been a source for iodine.

<table>
<thead>
<tr>
<th>TABLE 29.1</th>
<th>RDIs of selected macroelements and microelements in various countries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EU (mg/day)</td>
</tr>
<tr>
<td><strong>Macronutrients</strong></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>800&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chloride</td>
<td>800&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Magnesium</td>
<td>375&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>700&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium</td>
<td>2000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sodium</td>
<td>1500&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Micronutrients</strong></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Copper</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fluoride</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron</td>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Manganese</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.055&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> European Commission (2008).
<sup>b</sup> Institute of Medicine (2011).
<sup>c</sup> National Health and Medical Research Council (2006).
<sup>d</sup> International Life Sciences Institute—Southeast Asia Region (2005).
<sup>e</sup> Institute of Medicine (2005).
<sup>f</sup> Institute of Medicine (1997).
<sup>g</sup> Institute of Medicine (2001).
<sup>h</sup> Institute of Medicine (2000).
production for many years (Chapman, 1980). Nowadays, seaweeds are considered as a potential material for the production of different nutraceuticals and food supplements (Martínez-Navarrete et al., 2002; Shahidi, 2009).

Mineral content of widely used seaweeds is documented and has been very changeable in different genera across all groups of brown, red, and green seaweeds. Generally, macroelements in seaweeds have been determined in relatively low concentrations, but levels of trace elements have frequently reached the high values which exceeded RDIs. Some of the seaweeds are excellent contributors, especially of iodine and iron.

A. Contribution of seaweed minerals to daily requirements
Seaweeds could be excellent contributors of some microelements to RDIs, as it is documented in Tables 29.3 and 29.4. The contents of I and Fe, Mn, and Zn have been mentioned in mg/8 g of dry matter of particular
<table>
<thead>
<tr>
<th>Brown algae</th>
<th>% RDI EU, USA, Australia, Asia</th>
<th>Red algae</th>
<th>% RDI EU, USA, Australia, Asia</th>
<th>Green algae</th>
<th>% RDI EU, USA, Australia, Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RDI—I (mg/day)</strong></td>
<td>0.15</td>
<td><strong>RDI—I (mg/day)</strong></td>
<td>0.15</td>
<td><strong>RDI—I (mg/day)</strong></td>
<td>0.15</td>
</tr>
<tr>
<td>Sargassum vachellianum&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.51</td>
<td>Gracilaria lemaneiformis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.09</td>
<td>Enteromorpha spp.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.30</td>
</tr>
<tr>
<td>S. henslowianum&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.57</td>
<td>Gracilaria lemaneiformis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.09</td>
<td>Ulva spp.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.30</td>
</tr>
<tr>
<td>Laminaria digitata&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.00</td>
<td>Gracilaria lemaneiformis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.00</td>
<td>Codium fragile&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.23</td>
</tr>
<tr>
<td>Ecklonia radiata&lt;sup&gt;e&lt;/sup&gt;</td>
<td>31.92</td>
<td>Grassila lemaneiformis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.92</td>
<td>Ulva fasciata&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.03</td>
</tr>
<tr>
<td>Ecklonia radiata&lt;sup&gt;e&lt;/sup&gt;</td>
<td>29.76</td>
<td>Grassila lemaneiformis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.76</td>
<td>Enteromorpha intestinalis&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92</td>
</tr>
<tr>
<td>Laminaria japonica&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.32</td>
<td>Grassila lemaneiformis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.32</td>
<td>Ulva rigida&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.52</td>
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<sup>a</sup> Hou and Yan (1998).
<sup>b</sup> Peña-Rodríguez et al. (2011).
<sup>c</sup> MacArtain et al. (2007).
<sup>d</sup> Taboada et al. (2010).
<sup>e</sup> Mišurcová et al. (2009).
<sup>f</sup> Kikunaga et al. (1999).
<sup>g</sup> McDermid and Stuercke (2003).
<sup>h</sup> Karez et al. (1994).
<sup>i</sup> Kumar et al. (2011).
<sup>j</sup> Smith et al. (2010).
<sup>k</sup> Wen et al. (2006).
seaweed genera from groups of brown, red, and green together with their percentage participation on the RDIs of the minerals mentioned above. According to reported data about the seaweed consumption in Asian countries, the amount of 8 g of seaweed dry matter was considered as an average daily intake (MacArtain et al., 2007; Miyake et al., 2006). The seaweed participation on RDI was calculated for EU countries, the United States, Australia, and Asia. The conversion factor of 8 g was used as a daily intake for all countries though daily seaweed consumptions are lower in EU countries, the United States, and Australia. Unfortunately, data about them were not available. The participation values of particular seaweed genera on RDI were evaluated from data from several studies (Hou and Yan, 1998; Karez et al., 1994; Kikunaga et al., 1999; Kumar et al., 2011; MacArtain et al., 2007; Mageswaran et al., 1985; McDermid and Stuercke, 2003; Mišurcová et al., 2009; Peña-Rodríguez et al., 2011; Romarís-Hortas et al., 2011; Smith et al., 2010; Taboada et al., 2010; Wen et al., 2006).

Due to high iodine concentration in seaweeds, many of them could be utilized as natural sources for the production of iodine nutraceuticals; brown seaweed of genera Sargassum, Laminaria, Ecklonia, Macrocystis, Undaria, Ascophyllum, and Durvillaea; red seaweed of genera Gracilaria, Palmaria, Chondrus, Laurencia, and Gelidium; and even green seaweed of genera Enteromorpha, Ulva, Codium, and Monostroma. Besides the well-known seaweed genera, some other seaweed genera which are used in a lesser extent—Himalanthia and Chnoospora from brown seaweed, further Corynomorpha, Polysiphonia, Sarcodia, Coralina, Cheilosporum, Leathesia, Spiridia, and Myelophycus from red seaweed genera—could be considered as an abundant source of iodine. However, the extent of utilization of these seaweeds for iodine production should be considered because of their expanse occurrence. Table 29.3 shows the participation of selected seaweeds from all seaweed groups on the RDI whose iodine value is equal for the EU, the United States, Australia, and Asia. Seaweeds with the highest content of iodine, that is, Gracilaria lemaneiformis—red macroalga, Sargassum vachellianum—brown macroalga, and Enteromorpha spp.—green alga, exceed the RDI up to 200, 300, and 400 times, respectively (Hou and Yan, 1998; MacArtain et al., 2007; Wen et al., 2006). Finally, great differences were observed not only between various seaweed genera but also within the same genus.

Further, this review is focused on the concentrations of Fe, Zn, and Mn in 8 g of dry matter of different seaweed genera and their participation on the RDIs as it is shown in the Table 29.4. Seaweeds from all groups of green, brown, and red are the excellent contributors of Fe. The highest participation on RDI was observed in green seaweed Codium fragile, red seaweed Myelophycus simplex, and brown seaweed Colpomelia sinuosa as their iron contents exceeded RDIs in a range from 3 to 10 times (Hou and
Yan, 1998). The other seaweed genera such as green *Ulva* and *Monostroma*, red *Polysiphonia, Dictyopteris, Corallina, Leathesia, Gelidium, Rhodomela,* and *Porphyra,* and finally brown seaweed genera *Punctaria, Sargassum, Laminaria,* and *Scytosiphon* contain high amount of Fe. Considering Zn and Mn, their amounts across all seaweed groups do not reach the RDIs. Their participation on RDIs was mostly in the units and tens of percents except from *Laminaria japonica, Porphyra tenera, Ceramium boydenoo,* and *C. fragile,* whose concentrations of Mn exceeded 100% of RDI (Hou and Yan, 1998; Mišurcová et al., 2009; Wen et al., 2006).

**B. Factors influencing mineral contents in seaweed**

It was observed that seaweeds have been selective biosorbents for different metals. Concentration factors (CFs) have been determined as 10–20 times higher than those in terrestrial plants. Seaweeds have much bigger ability $>10^6$ to concentrate rare earth elements than terrestrial plants which is $10^3$. The values of CFs vary by diverse elements, for example, CFs for Al, Fe, Ce, and Th are in seaweeds much higher than CFs for Na, Mg, Cl, and Br (Hou and Yan, 1998).

Unfortunately, higher amounts of some minerals in seaweed have been the result of pollution of the seawater or natural environment. Thus, many studies were conducted with respect to the contamination of seaweed by heavy metals. Because of their high sorption capacity, they were also probed for their utilization as biosorbents to remove heavy metals from the environment and to elucidate mechanisms of metal biosorption by seaweeds (Davis et al., 2003; Murphy et al., 2008; Suzuki et al., 2005). Further, these conclusions could be utilized for the understanding of the uptake mechanisms by seaweed. Finally, endogenous and exogenous factors have participated on the variability of seaweed mineral composition.

**1. Endogenous factors**

The main endogenous factor responsible for the enormous capability to absorb inorganic substances from the environment is a different structure of seaweed cell wall polysaccharides. Each of different seaweed groups possesses various structural polysaccharides such as fibrilar, nonfibrilar, and sulphated derivates with diverse number of bound sites for metal ions resulting in dissimilar mineral sorbent capacity. Structural polysaccharides show strong ion-exchange properties.

It has been reported that brown seaweeds have higher capability to incorporate minerals into their tissue than red and green seaweeds due to larger number of compounds with anionic groups in their cell walls such as alginic acids, proteins, polygalacturonic acids, and polyphenols (Connan and Stengel, 2011; Figueira et al., 2000; Michalak and
Chojnacka, 2010). On the contrary, Baumann et al. (2009) observed higher affinity of a red seaweed *Palmaria palmata* to accumulate heavy metals Cd and Cr than those of brown seaweeds. Different affinity of metals to various seaweed compounds also results in the variability of mineral distribution in seaweeds. Alkali metals are mainly bound to alginic acid; thus their concentrations are higher in algin than those in original algae similar to Zn, Cr, and Fe that are rather combined with proteins to form metalloproteins and their concentrations in original algae are lower too (Hou, 1999).

The level of minerals depends on particular seaweed genera because of the diverse affinity of many seaweed strains for each element, which also results in various mineral amounts in seaweed tissues (Baumann et al., 2009). However, differences in mineral levels in seaweed tissues were observed also within the same seaweed species influenced by the stage of the living cycle and the age of seaweed. The highest iodine concentration was deposited in the meristematic tissue at the base of the blade in diverse seaweed genera (Teas et al., 2004). The mechanism of high iodine uptake by brown seaweed from the order Laminariales was described by Küpper et al. (1998). The iodine uptake mechanism was explained by the oxidation of iodide to hypoiodous acid and molecular iodine by cell wall haloperoxidases. The oxidized iodine may cross the plasmatic membrane, and its accumulation in seaweed tissues could be 30,000 times more than the concentration of this element in seawater.

2. Exogenous factors

The range of seaweed capability to absorb minerals is based on many exogenous factors predestining diverse levels of various minerals in seaweed tissues, such as the environmental conditions (geographic location, season, wave exposure, seawater temperature, salinity, mineral levels in seawater, and finally pH of seawater).

The influence of different geographic locations could be documented on the mineral composition of edible red seaweed *Porphyra vietnamensis* from different localities of the central west coast India. The highest amount of Fe was observed in the wide range from 33.0 to 298.0 mg/100 g dry weight. Thanks to the high level of Fe, *P. vietnamensis* from these localities could be served as a food supplement to improve dietary intake of iron (Rao et al., 2007).

Seasonal variations of seaweed chemical composition are linked with the particular life stage. It was confirmed that in periods of maximum growth photosynthetic activity increased, which resulted in higher content of carbohydrates with many binding-sites for metals (Rosenberg and Ramus, 1982). Seasonal variations of mineral composition were studied in brown seaweed *Fucus vesiculosus* (Riget et al., 1995). Maximum and minimum concentrations of Cd, Cu, and Pb were found in February
(middle growth stage) and August (initial and latter growth stage), respectively. For Zn, maximum and minimum concentrations occurred in March and September, respectively. Similar conclusions were deduced by Lares et al. (2002). The highest Cd content was in June and the lowest in October in brown seaweed *Macrocystis pyrifera*. On the contrary, no significant variations were found for the major elements Na, Ca, and Mg during a full growth seaweed period (Hou and Yan, 1998).

It is evident that the main sorption mechanism of metals based on exchange of ions by the metal binding to an anion site by either replacing an existing metal or displacing a proton depends on the pH value of the solution. Thus, pH is an important parameter on the biosorption of metal ions from aqueous solutions (Antunes et al., 2003; Crist and Martin, 1999). Further, it was also established that metals differ in the ability of displacing protons. In yellow-green alga *Vaucheria*, it was determined that the capacity of proton exchange for heavy metals—Pb and Cd—increased with pH and Pb had the highest value (Crist and Martin, 1999). The pH effect on the metal sorption was established also in nonliving biomasses from green seaweed *Ulva onoi* (Suzuki et al., 2005). The sorption capacity of *Ulva* biomasses for Cd was noticeably low in highly acidic (> pH 3) and highly alkaline conditions (< pH 10).

Lower salinity affects seaweed physiology and biochemical composition by decreasing phenolic contents and increasing protein content, and these changes have been shown to influence the availability of metal-binding sites (Connan and Stengel, 2011).

V. BIOAVAILABILITY OF SEAWEED MINERALS BY HUMANS

High levels of some essential trace elements in seaweed are not sufficient to cover their RDIs due to various extent of their bioavailability by humans.

Mineral bioavailability is defined as a part of the ingested nutrient that is absorbed and consecutively utilized by humans for maintaining normal physiological functions (Fairweather-Tait and Hurrell, 1996). The biological availability of minerals depends on the diet composition and is influenced by the levels and forms of present nutrient or nonnutrient components, and finally by nutrient synergistic or antagonistic interactions (Watanabe et al., 1997). Dietary fiber, phytate, phenolic compounds could decrease the availability of minerals due to the formation of insoluble complexes resulting in the reduction of mineral absorption (Fairweather-Tait and Hurrell, 1996).

Dietary form of nutrient determines the extent of its utilization. The best iron source is iron from animal sources or heme iron (Whittaker, 2008). It was observed that 9–53% of heme iron and 1–25% of nonheme
iron were absorbed in a study on the health volunteers. But the absorption process is very specific and could be influenced by many factors; thus it can be used only for demonstration of different extent of iron absorption (Skikne et al., 1983).

Finally, reciprocal antagonistic or synergic behavior between minerals affects the range of mineral absorption in seaweed by binding-site competition. In aqueous solutions, Fe impairs the absorption of Zn. On the other hand, Ca inhibits the absorption of Fe and Zn (Fairweather-Tait and Hurrell, 1996; Maret and Sandstead, 2006; Solomons, 1986). Finally, food preparation and cooking could determine the final mineral content of the food by the loss of water-soluble minerals (Santoso et al., 2006).

VI. CONCLUSION

Usage of mineral supplements in western countries often prevents deficiency of minerals. However, effectiveness of bioavailability of some mineral supplements has been considered as insufficient. Natural sources of many trace elements are seaweed from all algal groups. Seaweed has the enormous ability to absorb minerals from a growth medium which results in high mineral concentrations in seaweed tissues often exceeding their concentration in the seawater, especially iodine and iron occurs in very high levels.

Mineral composition of seaweed is very changeable according to different factors including the environmental conditions and specific behavior of each seaweed genus. The question of minerals availability by humans may also be considered. Among the factors influencing the bioavailability of minerals derived from seaweed matter, belong mainly to the compositions of their cell walls with different polysaccharides that could bind the elements with various powers and prevent their utilization for living processes in the human body.

The aptitude of seaweed to absorb minerals from a growth medium could be utilized to pointed production of seaweed matter enrichment with specific elements and to produce natural mineral nutraceuticals.

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Medicinal Benefits of Sulfated Polysaccharides from Sea Vegetables

Se-Kwon Kim*†,1 and Yong-Xin Li*

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Abstract
The cell walls of sea vegetables or marine algae are rich in sulfated polysaccharides (SPs) such as fucoidans in brown algae, carrageenans in red algae, and ulvans in green algae. These SPs exhibit various biological activities such as anticoagulant, antiviral, antioxidant, and anticancer activities with potential health benefits. Therefore, SPs derived from sea vegetables have great potential...
in further development as nutraceuticals and medicinal foods. This chapter presents an overview of biological activities and potential medicinal benefits of SPs derived from sea vegetables.

I. INTRODUCTION

Edible marine algae, sometimes referred to as sea vegetables, have attracted a special interest as potential sources of nutrients and one particular interesting feature is their richness in sulfated polysaccharides (SPs), the uses of which span from food, cosmetic, and pharmaceuticals industries to microbiology and biotechnology (Ren, 1997). These chemically anionic SP polymers are not only widespread in sea vegetables but also occur in animals such as mammals and invertebrates (Mourao, 2007; Mourao and Pereira, 1999). Sea vegetables are the most important source of non-animal SPs and the chemical structure of these polymers varies according to the algal species (Costa et al., 2010). The major SPs (Fig. 30.1) found in sea vegetables include fucoidan of brown algae, carrageenan of red algae, and ulvan of green algae. In recent years, various SPs isolated from sea

![Figure 30.1](image-url)

**FIGURE 30.1** Sulfated polysaccharides derived from sea vegetables, (A) fucoidan, (B) carrageenan, and (C) ulvan.
vegetables have attracted much attention in the fields of food, cosmetic, and pharmacology. Carrageenans, a family of SPs isolated from marine red algae, are widely used as food additives, such as emulsifiers, stabilizers, or thickeners ( Campo et al., 2009; Chen et al., 2007). Ulvan displays several physiochemical and biological features of potential interest for food, pharmaceutical, agricultural, and chemical applications ( Lahaye and Robic, 2007). Compared with other SPs, fucoidans are widely available commercially important compounds from various cheap sources; hence, more and more fucoidans have been investigated in recent years to develop novel drugs and functional foods ( Li et al., 2008).

Novel extraction and separation techniques, such as supercritical CO2 extraction, ultrasonic-aided extraction, and membrane separation technology have recently been applied in the development of bioactive SPs from marine algae ( Sheng et al., 2007; Ye et al., 2006). Biological activities of SPs depend on chemical structure, molecular weight, and chain conformations ( Ye et al., 2008). The cell walls of seaweeds are rich in matrix SPs and they exhibited beneficial biological activities such as anticoagulant ( Mao et al., 2009), antiviral ( Ponce et al., 2003), antioxidative ( Ruperez et al., 2002), anticancer ( Synytsya et al., 2010), and anti-inflammation ( Na et al., 2010). This chapter focuses on SPs derived from sea vegetables and presents an overview of their biological activities with potential medicinal benefits.

II. TYPES OF SPS DERIVED FROM SEA VEGETABLES

A. Fucoidan

Fucoidan consists of fucose and sulfate groups, but the chemical composition of most fucoidans derived from brown sea vegetables is more complex. Besides fucose and sulfates, they also contain some other monosaccharides (mannose, galactose, glucose, xylose, etc.) and uronic acids, even acetyl groups and protein. Further, the structures of fucoidans from different brown sea vegetables vary from species to species ( Li et al., 2008).

B. Carrageenan

Carrageenan is a generic name for a family of linear, sulfated galactans, obtained from red sea vegetables. The backbone of carrageenan is composed of -galactose units linked alternatively to -1→4 linkages. In the food industry, carrageenans are widely utilized due to their excellent physical properties, such as thickening, gelling, and stabilizing abilities ( Campo et al., 2009).
C. Ulvan

Ulvan is the main SPs in the green sea vegetables, especially the members of the Ulvales (Ulva sp. and Enteromorpha sp.). Ulvan contains rhamnose, xylose, and glucuronic acid with sulfate groups. One particular interesting feature of ulvan in the food industry is its ability to form gels.

III. MEDICINAL BENEFITS AND BIOLOGICAL ACTIVITIES OF SPS FROM SEA VEGETABLES

A. Antioxidant effect

Uncontrolled production of free radicals that attack macromolecules such as membrane lipids, proteins, and DNA is leading to many health disorders such as cancer, diabetes mellitus, and neurodegenerative and inflammatory diseases with severe tissue injuries (Butterfield et al., 2002; Frlich and Riederer, 1995; Yang et al., 2001). Antioxidants may have a positive effect on human health as they can protect human body against damage by reactive oxygen species (ROS), which attack macromolecules. Moreover, deterioration of some foods has been identified due to oxidation of lipids or rancidity and formation of undesirable secondary lipid peroxidation products. Lipid oxidation by ROS such as superoxide anion, hydroxyl radicals, and \( \text{H}_2\text{O}_2 \) also causes a decrease in nutritional value of lipid foods and affects their safety and appearance. Therefore, in food and pharmaceutical industries, many synthetic commercial antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole (BHA), tert-butylhydroquinone, and propyl gallate have been used to retard the oxidation and peroxidation processes. However, the use of these synthetic antioxidants must be under strict regulation due to potential health hazards (Hettiarachchy et al., 1996; Park et al., 2001). Hence, the search for natural antioxidants as safe alternatives is important in the food industry (Penta-Ramos and Xiong, 2001). Recently, there is a considerable interest in the food industry as well as pharmaceutical industry for the development of antioxidants from natural sources, such as marine flora and fauna. Among them, marine algae represent one of the richest sources of natural antioxidants (Mayer and Hamann, 2002; Ruperez, 2001).

SPs not only function as dietary fiber, but they also contribute to the antioxidant activity of marine algae. It has been demonstrated that SPs have potential antioxidant activity and various classes of SPs including fucoidan, laminaran, and alginic acid have been shown as potent antioxidants (Rocha de Souza et al., 2007; Ruperez et al., 2002; Wang et al., 2008). Antioxidant activity of SPs have been determined by various methods such as 1,1-diphenyl-2-picryl hydrazil (DPPH) radical scavenging, lipid peroxidation inhibition, ferric reducing antioxidant power (FRAP), nitric
oxide (NO) scavenging, ABTS radical scavenging, superoxide radical, and hydroxyl radical scavenging assays. In addition, Xue et al. (1998) reported that several marine-derived SPs have antioxidative activities in phosphatidylcholine-liposomal suspension and organic solvents. According to Kim et al. (2007), the SPs of Sargassum fulvellum (Phaeophyceae) is more potent NO scavenger than commercial antioxidants such as BHA and α-tocophorol. Antioxidant activity of SPs depends on their structural features such as degree of sulfation, molecular weight, type of the major sugar, and glycosidic branching (Qi et al., 2005; Zhang et al., 2003). For example, low molecular weight SPs have shown potent antioxidant activity than those of high molecular weight SPs (Sun et al., 2009). Moreover, a positive correlation has reported for sulfate content and superoxide radical scavenging activity in fucoidan fractions obtained from a brown alga Laminaria japonica (Wang et al., 2008). The SP fraction obtained by acid hydrolysis (0.1 M HCl, 37 °C) of Fucus vesiculosus (Phaeophyceae) has shown the highest potential to be used as antioxidants followed by the alkali- (2 M KOH, 37 °C) and water-soluble fractions (Ruperez et al., 2002). Further, fucoidan has shown the highest antioxidant activity followed by alginate and laminaran from Turbinaria conoides (Phaeophyceae) according to FRAP and DPPH assays (Chattopadhyay et al., 2010). In addition, in vivo antioxidant activity of SPs derived from marine red alga Porphyra haitanensis in aging mice has been reported (Zhang et al., 2003).

These evidences suggest that among various naturally occurring substances, SPs prove to be one of the useful candidates in search for effective, nontoxic substances with potential antioxidant activity. SPs are by-products in the preparation of alginates from edible brown seaweeds and could be used as a rich source of natural antioxidants with potential application in the food industry as well as cosmetic and pharmaceutical areas.

B. Antiviral effect

Many species of sea vegetables contain significant quantities of complex structural SPs that have been shown to inhibit the replication of enveloped viruses including members of the flavivirus, togavirus, arenavirus, rhabdovirus, orthopoxvirus, and herpesvirus families (Witvrouw and De Clercq, 1997). The potential antiviral activity of marine algal polysaccharides was first shown by Gerber et al. (1958), who observed that the polysaccharides extracted from Gelidium cartilagenum (Rhodophyceae) protected the embryonic eggs against Influenza B or mump virus. The polysaccharides with antiviral activity were shown to be highly sulfated. The chemical structure including the degree of sulfation, molecular weight, constituent sugars, conformation, and dynamic stereochemistry is caused to determine the antiviral activity of algal sulfated polysaccharides (Adhikari et al., 2006; Damonte et al., 2004; Luscher-Mattil, 2000). In addition,
both the degree of sulfation and the distribution of sulfate groups on the constituent polysaccharides play an important role in the antiviral activity of these SPs. Algal polysaccharides with low degrees of sulfation are generally inactive against viruses (Damonte et al., 2004).

SPs derived from sea vegetables are an alternative source for searching novel therapeutic candidates for HIV. Moreover, several researchers have investigated the inhibitory effects of SPs on the herpes simplex virus strains (HSV-1 and HSV-2). Fucoidans are SPs extracted from brown sea vegetables that possess some biological activities and they show the antiviral activity against infectious diseases, such as HIV, herpes simplex virus types (HSV-1 and HSV-2), and cytomegalovirus (Witvrouw and De Clercq, 1997). In addition, SPs such as carrageenans, fucoidans, and sulfated rhamnogalactans have inhibitory effects on the entry of enveloped viruses including herpes and HIV into cells. Further, the presence of sulfate group is necessary for the anti-HIV activity and potency increases with the degree of sulfation (Witvrouw and De Clercq, 1997). This leads to a hypothesis that anionic charges on the sulfate groups may be effective in inhibiting reverse transcriptase enzyme activity of the virus. In most of the studies, antiviral activity of SPs has been determined by plaque reduction and/or virus yield inhibition assays.

Moreover, it has been reported that SPs from red sea vegetables inhibit in vitro and in vivo infections of flaviviruses, such as dengue and yellow fever viruses (Ono et al., 2003; Talarico et al., 2005). Dengue virus belongs to the family Flaviviridae, the same family as Japanese encephalitis and yellow fever viruses, which are controlled by specific vaccinations. However, until now no licensed dengue vaccination or anti-dengue agents are clinically available. Fucoidan from the marine alga Cladosiphon okamuranus (Phaeophyceae) significantly inhibits dengue virus type 2 infection (Hidari et al., 2008), and they have found that virus particles bound exclusively to fucoidan, indicating that fucoidan interacts directly with envelope glycoprotein on the virus. Hence, this could be developed as a potential inhibitory agent against the dengue virus. There are numerous advantages such as relatively low production costs, broad spectrum of antiviral properties, low cytotoxicity, safety, wide acceptability, and novel modes of action over other classes of antiviral drugs, and these suggest SPs from sea vegetables as promising drug candidates in the near future, but further studies are needed with clinical trials for these antiviral SPs.

C. Immunomodulating effect

The immunostimulating effect of SPs is mainly based on macrophages modulation. Macrophages are the residence of immune cells in the innate immune system which plays an important role in the maintenance of homeostasis by changing their function according to the tissue. As the
residence of the immune system, macrophages are a predominant source of proinflammatory factors. It is hypothesized that the origin of cancer was at sites of chronic inflammation, in part based on the hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation.

Marine algae-derived water soluble compounds such as SPs are known to have promising anti-inflammatory activities (Abad et al., 2008). However, the scientific analysis of anti-inflammatory activity of sea vegetable-derived SPs has been poorly carried out until now and a few studies were reported. For example, SPs isolated from two red algae Porphyra yezoensis and Gracilaria verrucosa stimulate phagocytosis and respiratory burst in mouse macrophages in vitro and in vivo (Yoshizawa et al., 1993, 1995, 1996). Moreover, some types of carrageenans induce potent macrophage activation (Nacife et al., 2000, 2004), while some carrageenans and fucoidan appear to inhibit macrophage functions (Van Rooijen and Sanders, 1997; Yang et al., 2006). However, SPs may have potential biomedical applications in stimulating the immune system or in controlling macrophage activity to reduce associated negative effects (Leiro et al., 2007).

D. Anticancer effect

Several studies have reported that SPs derived from sea vegetables have antiproliferative activity in cancer cell lines in vitro, as well as inhibitory activity of tumor growth in mice (Rocha de Souza et al., 2007; Ye et al., 2008). In addition, they have antimetastatic activity by blocking the interactions between cancer cells and the basement membrane (Rocha et al., 2005). SPs inhibit tumor cell proliferation and tumor cell adhesion to various substrates, but their exact mechanisms of action are not yet completely understood. Yamamoto et al. (1986) reported that the oral administration of several sea vegetables can cause a significant decrease in the incidence of carcinogenesis in vivo. Porphyran, the SPs of P. yezoensis (Rhodophyceae), can induce cancer cell death via apoptosis in a dose-dependent manner in vitro without affecting the growth of normal cells (Kwon and Nam, 2006). Moreover, the SPs purified from Ecklonia cava (Phaeophyceae) stimulate the induction of apoptosis in vitro (Athukorala et al., 2009) and have potential antiproliferative effect on human leukemic monocyte lymphoma cell line (U-937). Anticancer activity of fucoidans has been reported to be closely related to their sulfate content and molecular weight. Further, SPs from sea vegetables are known to be important free-radical scavengers and antioxidants for the prevention of oxidative damage, which is an important contributor in carcinogenesis. Therefore, it might be suggested that these SPs have potent capacities for new anticancer product developments in the pharmaceutical industry as novel chemopreventing agents for cancer therapy.
E. Other medicinal benefits

Fucoidan extracted from the marine brown sea vegetable *Undaria pinnatifida* has significantly induced osteoblastic cell differentiation and has potential in use as a functional food ingredient in bone health supplements (*Cho et al.*, 2009). Moreover, fucoidan from *C. okamuranus* (Phaeophyceae) protects gastric mucosa against acid and pepsin. Therefore, fucoidan can be developed as a potential antiulcer ingredient in functional foods (*Nagaoka et al.*, 2000; *Shibata et al.*, 2000).

Dietary fibers support to reduce cholesterol levels and recent studies have shown that dietary fibers with ion-exchange capacity contain more potent effects on cholesterol lowering (*Guillon and Champ*, 2000). Ulvan, which belongs to the SP group from *Ulva pertusa*, is a potential antihyperlipidemic agent and has significantly reduced serum triglyceride (TG), total and low-density lipoprotein cholesterol (LDL-cholesterol), and elevated high-density lipoprotein cholesterol (HDL-cholesterol) in mice (*Yu et al.*, 2003a). According to *Yu et al.* (2003b), antihyperlipidemic activity of ulvan depends on the molecular weight of ulvan fractions; high molecular weight fraction is more effective on serum total and LDL-cholesterol, whereas low molecular weight fractions are more effective on TG and HDL-cholesterol. Ulvan contains uronic acid and sulfates, with potential capability of sequestering or binding bile acids (*Lahaye*, 1991).

IV. CONCLUDING REMARKS

Recent studies have provided evidence that SPs from sea vegetables play a vital role in human health and nutrition. Further, seaweed processing by-products with bioactive SPs can be easily utilized for producing functional ingredients. The possibilities of designing new functional foods and pharmaceuticals to support reducing or regulating the diet-related chronic malfunctions are promising. Therefore, it can be suggested that, due to valuable biological functions with medicinal beneficial effects, sea vegetable-derived SPs have much potential as active ingredients for preparation of nutraceuticals and medicinal food products.

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The Perspectives of the Application of Biofilm in the Prevention of Chronic Infections

Abdul Bakrudeen Ali Ahmed and Rosna Mat Taha

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Abstract

Biofilms are a natural part of the ecology of the earth. Many biofilms are quite harmful and must be treated or controlled. Other biofilms are beneficial and can be used to help fix serious problems. Biofilms can grow on many different surfaces, including rocks in water, foods, teeth, and various biomedical implants. This bacterial colonization may present the need for additional operations, amputation, or it may even lead to death. The fundamental
principles of bacterial cell attachment and biofilm formation are discussed. Biofilms represent a new, wide-open field practice and research that is only going to get hotter with time. Functional organic plasma polymerized coatings are also discussed for their potential as bio-sensitive interfaces, connecting metallic electronic devices with their physiological environments.

I. INTRODUCTION

Biofilm communities are composed of a range of different types of microorganisms, both autotrophic and heterotrophic. Microbial mats are specialized microbial communities composed mainly of photosynthetic prokaryotes. It is also commonly associated with living organisms, both plant and animal. Yet other biofilms are not perceived as either bad or good, but rather are recognized to be an important part of the natural environment around us. The cost of society associated with biofilm is estimated to range in the billions of dollars annually. Biofilms are responsible for 65% of soft tissue and wound infections and the main cause of endocarditic, medical implants, and cystic fibrosis-associated infections. Interestingly, these biofilms still exist off the coast of Australia, seemingly uncharged over that vast time. In industry, biofilms are related to food and drinking water contamination, metal surface corrosion, reduction heat transfer, clogging water, air filters, and pollution of environment. On the positive side, there is a great beneficial potential in controlling biofilms as these participate in bioremediation, oil recovery, biofuel production, fermentation processes, and agricultural soil enrichment. Biofilms are presently garnering much attention but more as a curiosity than as the seminal element of modern infectious disease. Biofilm must be in the forefront of our thinking when considering chronic infections such as chronic wounds (Wolcott et al., 2010c).

A. Outline of chronic infections

Chronic infections in contrast tend to be focal infections, limited in size, that wax and wane for long durations and are only partially destructive to tissues. The strategies of a single-cell, mobile, free-floating bacterium versus those of a community of bacteria encased in a self-secreted protective matrix (biofilm) are radically different and may one type of infections: “chronic.” Biofilm is intrinsically resistant to host immunity, antibiotics, and biocides, different treatment strategies will be required. Chronic infections such as chronic wounds, surgical-site infections, and infected implants will yield only to repetitive evaluation and multiple simultaneous therapies that require much persistence from the physician.
A biofilm is composed of living, reproducing microorganisms, such as bacteria, that exist as a colony or a community. With the knowledge of the microorganisms present, systemic and/or topical antibiotics can be chosen (Wolcott and Dowd, 2011).

Biofilms can form on just about any imaginable surface: metals, plastics, natural materials (such as rocks), medical implants, kitchen counters, contact lenses, the walls of a hot tub or swimming pool, human and animal tissue, etc. Indeed, wherever the combination of moisture, nutrients, and a surface exists, biofilms will likely be found as well. Biofilms are characterized by structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances. Biofilms are an important link in the energy budget of many natural communities. Both types of cells produce a polymeric extracellular slime layer which encloses the cells. This complex aggregate of cells and polysaccharide is the biofilm community.

II. MECHANISM OF ACTION ON BIOFILMS IN CHRONIC INFECTION

Biofilms communities associated with the bacterial interactions have been poorly researched in relation to wound healing, but it is likely that their effect on the wound healing process, through both direct and indirect mechanisms, is significant. Bacterial biofilms can be viewed as a specific type of persistent bacterial infection. After initial invasion, microbes can attach to living and nonliving surfaces, such as prosthetics and indwelling medical devices, and form a biofilm composed of extracellular polysaccharides, proteins, and other components. Biofilms consist of many species of bacteria and archaea living within a matrix of excreted polymeric compounds. This matrix protects the cells within it and facilitates communication among them through chemical and physical signals. Some biofilms have been found to contain water channels that help distribute nutrients and signaling molecules. This matrix is strong enough that in some cases, biofilms can become fossilized.

In nature, they play an important role in the synthesis and degradation of organic matter; the degradation of environmental pollutants; and the cycling of nitrogen, sulfur, and metals. These metabolic processes are complex and typically can only be conducted through the concerted effort of multiple metabolically distinct microbes. In industrial settings, biofilms are important in processing sewage, treatment of petroleum-contaminated groundwater, and nitrification. Chronic infection is well known to clinicians, but the role of bacteria in producing these clinical differences remains poorly understood. In addition, the bacterial cells contain biofilms are up to 500 times more resistant to antibiotics than the free cells (Al-Mazrou and Al-Khattaf, 2008).
These bacteria also resist host defense mechanisms, probably becoming a source of chronic inflammation and permanent histological changes. Free-living bacteria are generally susceptible to antibiotic treatment and to host defense mechanisms (Chole and Faddis, 2003), with their metabolism and exotoxins production, leading to a chronic inflammatory response evident in respiratory mucosal changes and persistence of adenopathies. Biofilm, regardless of the species that comprises the whole, has basic features in common. First, there is usually attachment of the bacteria to a surface. Attachment to a surface is the first committed step and the most potent signal for biofilm formation. Second, the bacteria secrete substances to protect the biofilm from environmental dangers such as bacteriophage, ultraviolet light, and desiccation in the natural world. In a host environment, this extracellular polymeric substance secreted by each bacterium provides protection against white blood cells, antibodies, and even therapeutic antibiotics in the host environment (Leid et al., 2005). The molecules secreted by each biofilm bacteria are usually polysaccharides, which prompted the name “glycocalyx” for the extracellular polymeric substance. However, in a host setting, the matrix may be composed of polysaccharides, host DNA (mainly from neutrophils), bacterial DNA, bacteria proteins (e.g., biofilm accumulation protein), or host components usually associated with plasma (e.g., fibrin, albumin). Also, the biofilm has the ability to mix each of these components to suit its needs, or even change the matrix composition to confront different treatments or threats (Wolcott and Dowd, 2011).

A third property of biofilm is that each bacterium in the biofilm has a different physiology or growth state, which is under direction of the quorum-sensing signaling system. Quorum-sensing molecules produced by an individual bacterium can act on that bacterium, other bacterium of the same species, other bacteria of different species, or even the host. The main role of quorum sensing is to direct gene expression of the different members throughout the biofilm. The base region attached to the host surface has essentially no metabolic activity, whereas the environmental edge has significantly metabolic activity, and there are various growth states in between. The molecular mechanisms under quorum-sensing control that allow the cooperation of diverse microorganisms are well established (Sauer et al., 2009). Fortunately, while the biofilm reconstitutes itself, it is more vulnerable to host immunity and to treatments, thus creating a therapeutic window. There are several known molecular pathways under quorum-sensing control for biofilm to inflame the host substrate, thus providing nourishment through exudates instead of host cell death (Wolcott et al., 2008). There are several general quorum-sensing systems (e.g., lasIR, rh1) that regulate Pseudomonas biofilm behaviors (e.g., attachment, extracellular polymeric substance production, and differentiation) and regulation of virulence factors (de Kievit, 2009). Also, a myriad of two-component systems (e.g., PhoP-Q, GacA-S, RetS, LadS, and AlgR) (Gooderham and Hancock, 2009), mainly
under the control of general quorum-sensing systems, exist that integrate environmental information to provide fine control of virulence factors and antibiotic resistance. However, the maintenance of the substantial genetic material divergent infection strategies, planktonic (predatory), versus biofilm (parasitic), is costly and conflicting.

One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. The biofilm environment provides physical protection to bacteria from a potentially hostile external environment and also a habitat where bacteria can communicate with each other (quorum sensing), which may lead to increase in virulence and propensity to cause infection (Kievit and Iglewski, 2000). Theoretically, chronic wounds offer ideal conditions for biofilm production because proteins (collagen, fibronectin) and damaged tissues are present, which can allow attachment. The biofilm, in turn, becomes a primary impediment to the healing of chronic wounds (Wolcott and Rhoads, 1996). Bacteria within a biofilm live in microcolonies that are encapsulated in a matrix composed of an extracellular polymeric substance separated by open water channels that act as a pseudocirculatory system for the delivery of nutrients and the removal of metabolic waste products (Davies, 2003). Through molecular methods, they have defined an environmental microbial reality where 99% of bacteria belong to biofilm communities. Fux et al. (2005) have discovered that biofilm phenotype bacteria offer do not grow with clinical culture methods even though they are alive. Finally, biofilm infections are overwhelmingly polymicrobial and therefore can never be adequately evaluated by a clinical culture that is structured to identify only one organism.

Biofilms also have equal potential for good behavior as agents of self-purification in streams and river, waster, and pollution treatment, or generation of carbon-neutral electricity. These critical properties come from the existence of the protective slimy matrix within which members of the community live, preventing attack from both the immune system and antibiotic, but at the same time shielding them from toxic contaminants while breaking down waste or effluent. One mechanism of reduced biofilm susceptibility is failure of the antimicrobial agent to penetrate the biofilm fully. For example, direct measurements of penetration of hypochlorite and hydrogen peroxide (H$_2$O$_2$) into model biofilms have revealed significantly retarded or incomplete penetration of both antimicrobials. H$_2$O$_2$ was able to penetrate katB, katA, and katA katB mutant biofilms to respectively increasing degrees. The major housekeeping catalase katA is important in the protection of $Pseudomonas$ $aeruginosa$ biofilms against killing by H$_2$O$_2$. Biofilms formed by KatA positive strains were incomplete penetrated by 50 mM H$_2$O$_2$ and suffered scarcely and loss in viability. The major housekeeping catalase $katA$ is important in the protection of
P. aeruginosa biofilms against killing by H₂O₂. Biofilms formed by the katA mutant were penetrated by H₂O₂ and were partially killed. Interestingly, even the katA mutant, whose biofilms were fully penetrated by H₂O₂, was significantly less susceptible in the biofilm than planktonic cells of the same strain. This indicates that some protective mechanism other than incomplete penetration is operative in P. aeruginosa biofilms treated with H₂O₂ (Brown et al., 1995; Elkins et al., 1999). However, KatB could likely contribute to the protection of biofilms against H₂O₂ if they were challenged during growth with a suitable inducing agent.

Chronic wounds may be a specific example of a chronic infection (Wolcott et al., 2008). Chronic wounds have significant biofilm on their surface, whereas acute wounds have very little biofilm on their surface (James et al., 2008). In-depth studies have demonstrated that the chronic wound bed possesses host cells that are senescent and have increased proinflammatory cytokines (Charles et al., 2009), elevated matrix metalloproteases (Rayment et al., 2008), and excessive neutrophils (Diegelmann, 2003). All of these consistent and persistent molecular and cellular findings are easily explainable as downstream events produced by biofilm infection. However, all biofilm infections are not the same, as the microbial constituents of biofilm show marked variability from wound to wound, with thousands of different species already identified (Wolcott et al., 2009). This suggests that an individual wound will possess its own unique wound biofilm that must be diagnosed before therapy. Regardless of the amount of negative influence, the resident biofilm is exerting one particular wound, by specifically targeting the biofilm, even “minor” inhibitions to healing are quashed, and healing outcomes are improved (Wolcott et al., 2010a). These strategies include debridement (i.e., sharp, energy transfer, ultrasound, and biological), anti-biofilm agents (i.e., addressing attachment such as by applying topical lactoferrin, degrading the matrix such as by using topical xylitol; Ammons et al., 2009). The specific microorganisms are reconstituting the biofilm; they are more vulnerable to conventional therapies such as antibiotics and biocides and less conventional treatments such as anti-biofilm agents (Wolcott et al., 2010b). This study demonstrates that biofilms are indeed a “right target.” Further research in this area is important to understand the relationships between biofilms communities, wound pathophysiology, infection, and healing.

III. CHRONIC INFECTION IN HUMAN AND BENEFICIAL BY BIOFILMS

Chronic wound infections are responsible for considerable morbidity and significantly contribute to the escalation in the cost of health care. In many instances, it is appropriate to treat these wounds empirically with a
combination of topical antiseptics and systemic antibodies, especially in the presence of invasive infections. Biofilms can be a serious threat to health especially in patients in whom artificial substrates have been introduced. The glycocalyx in which the bacteria live protects them from the effects of antibiotics and accounts for the persistence of the infection even in the face of vigorous chemotherapy. In addition, tissue surfaces such as teeth and intestinal mucosa which are constantly bathed in a rich aqueous medium rapidly develop a complex aggregation of microorganisms enveloped in an extracellular polysaccharide they themselves produce. The study of biofilms represents a radical new way of understanding the microbiology of virtually everything around us, from problems that afflict industry to serious public health issues.

The potential to do immense good for our world is held out to those who enter this field. In 1999, the average cost per patient for 2 years of treatment of a diabetic ulcer in the USA was an estimated $27,987 (Kruse and Edelman, 2006). More recently, the cost for the treatment of a single ulcer has increased to $8000, and the cost of an infected ulcer has increased to approximately $17,000 per year (Barone et al., 1998). Global wound care expenditures amount to $13–15 billion annually (Walksley, 2002). An estimated 1–2% of the populace in developing countries will experience a chronic wound during their lifetime (Gottrup, 2004). These wounds predominantly affect patients aged older than 60 years (Mustoe, 2004). The main decision points for the physician in treating an infectious disease include not only the tissue involved, the organisms, and host factor but also the differentiation of whether the infection is chronic. Physicians have a clear grasp of the vast clinical infection; however, current therapeutic options do not reflect that difference.

A. Periodontal diseases

Periodontal diseases are perhaps the most common chronic inflammatory diseases in humans. It is an inflammatory destruction of the tooth-supporting (periodontal) tissues, as a result of oral bacteria colonizing the tooth surfaces in the form of polymicrobial biofilm communities (Marsh, 2005). Depending on the localization of the biofilm in relation to the gingival margin, this can be either “supragingival” or “subgingival.” Biofilms or their released products can cause an inflammatory response by the periodontal tissues, aiming to eliminate this bacterial challenge (Feng and Weinberg, 2006). Human dental plaque has been exposed to 5% sucrose for 5 min, after which Gram’s iodine (0.33% iodine in 0.66% KI) was applied. The sucrose solution was applied to the left central incisor (which appear on the right), while the right central incisor served as a control. Iodine selectively binds to alpha-1,4 glucans (iodophilic
polysaccharide, i.e., glycogen or amylase) which results in brown to purple staining. The ability of oral bacteria to store iodophilic polysaccharides or glycogen-like molecules inside their cells is associated with dental caries since these storage compounds may extend the time during which lactic acid formation may occur. This prolonged exposure to lactic acid results in decalcification of tooth enamel. However, biofilms also contribute to bio-corrosion are associated with tooth decay and are responsible for infections of the human body. With regard to bio-corrosion, sulfate-reducing bacteria (SRB), such as Desulfovibrio vulgaris, contribute to the corrosion of steel. The presence of Streptococcus mutans in dental plaque is a hallmark of dental caries. Also, biofilms account for more than 80% of all microbial infections of human body. Nevertheless, the use of oral biofilms rather than individual oral bacterial species provides a more accurate view of the pathogenic events that take place in periodontal or periapical diseases, such as bone resorption.

B. Wound inflammation

Chronic wounds are an important problem worldwide. These wounds are characterized by a persistent inflammatory stage associated with excessive accumulation and elevated cell activity of neutrophils, suggesting that there must be a persistent stimulus that attracts and recruits neutrophils of the wound. The cellular inflammatory response against the bacteria in the chronic wounds, the amount of neutrophils accumulated at the site of infection, was evaluated through differential neutrophil counting on the tissue sections from wounds containing either P. aeruginosa or Staphylococcus aureus. Such bacteria are morphologically and physiologically different from free-living planktonic bacteria and have been implicated in numerous chronic infections ranging from cystic fibrosis to prostatitis (Costerton et al., 1995, 1999). The existence of biofilms in an acute partial-thickness wound (Serralta et al., 2001) and in chronic human wounds (Bello et al., 2001) has been documented.

Wound healing and infection is influenced by the relationship between the ability of bacteria to create a stable, prosperous community within a wound environment and the ability of the host to control the bacterial community. Within a stable, climax biofilm community, interactions between aerobic and anerobic bacteria are likely to increase their net pathogenic effect, enhancing their potential to cause infection and delay healing. Chronic wounds are invariably polymicrobial, yet most research to date has focused on the role of specific potential pathogens in wounds (e.g., P. aeruginosa) rather than the effect of interactions between different species (Percival and Bowler, 2004).
C. Kidney relevance

Biofilm is a complex, dynamically interactive multicellular community protected within a heterogeneous exopolysaccharide matrix. Its formation results in the genesis or perpetuation of infection, enhancement of inflammation, and tissue damage or death. Industrial financial losses result from biofilm formation; however, the consequences in the medical realm are equally devastating. The relation of biofilm to patients with chronic kidney disease is often covert and extends beyond the colonization of hemodialysis circuits and vascular accesses. Urinary tract device and vascular access related biofilms may also increase the burden of cardiovascular risk borne by chronic kidney disease patients, synergizing with the chronic inflammatory state already incurred by these individuals. Current anti-infective strategies are aimed at rapid killing planktonic forms of microorganisms without specifically targeting the sessile forms that perpetuate their planktonic brethren (Tapia and Yee, 2006).

D. Diabetic foot ulcer

Biofilms have been implicated in numerous chronic infections including cystic fibrosis and prostatitis. Through interactions within a biofilm, the resident population of bacteria is likely to benefit from increased metabolic efficiency, substrate accessibility, enhanced resistance to environmental stress and inhibitors, and an increased ability to cause infection and disease. Dermal wounds often provide an ideal environment for bacteria to exist as a community, which may have a significant effect on wound healing. The conditions under which species of microorganisms can survive in nature are determined by physiological and ultimately genetic competence. Consequently, species of bacteria often rely on close relationships with other species for survival and reproductive success. A biofilm forms when bacteria attach to a surface and subsequently encase themselves in an exopolymeric material (Costerton et al., 1999). Some bacteria are morphologically and physiologically different from free-living planktonic bacteria and have implicated in numerous chronic infections ranging from cystic fibrosis to prostatitis (Costerton et al., 1995). The existence of biofilms in an acute partial-thickness wound (Serralta et al., 2001) and in chronic human wounds (Bello et al., 2001) has been documented.

A diabetic foot ulcer is an excellent example of a chronic wound that responds well to management using biofilm principles (Dowd et al., 2008). Bacteria within biofilms have been reported to be up to 500 times more resistant to antibiotics than planktonic (unattached, freely living) cells (Donlan, 2001; Donlan and Costerton, 2002). Most of the chronic wound pathogens, such a methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas*
spp., are typical biofilm producers. Bacteria that reside within mature biofilms are highly resistant to many traditional therapies. Bacteria in biofilms grow more slowly, and slower growth may lead to decreased uptake of the drug and other physiologic changes that could impair drug effectiveness (Mandell et al., 2005). Currently, one of the most successful strategies for the management of biofilm-related conditions is physical removal of the biofilm, such as frequent debridement of diabetic foot ulcers (Davis et al., 2006).

E. Eye infection

A recent small case series determination that with a trained eye, biofilm can be visualized in chronic wounds and that its appearance is quite different from that of slough. Because of the differing biochemical compositions of biofilm and slough, different management strategies are required for the removal and control of these substances. Pulsed larvicide and enzymatic (proteolytic) debriding agents were efficacious in removing slough but were ineffective against biofilm. Physical debridement (sharp or use of a sterile gauze pad) was more effective than other modalities in removing biofilm, and the daily application of a nontoxic antiseptic solution prevented biofilm redevelopment (Hurlow and Bowler, 2009).

F. Otolaryngologic diseases

Tonsillectomy is often the choice as a consequence of obstruction of the upper airway, obstructive sleep apnea syndrome, growth delay, poor school performance, feeding difficulties, and other associated clinical features (Vandenberg and Heatley, 1997). The failure of the antibiotic treatment in tonsillitis produced by susceptible organism (Brook, 2001), even though it can be thought of a consequence of antibiotic resistance (Flemming et al., 2007), might be due to the presence of biofilms that can, therefore, be considered as an etiologic factor, among others. The knowledge about biofilms existence is sustaining a new concept to explain chronic infections (Vlastarakos et al., 2007). Hence, otolaryngologists are physicians trained in the medical and surgical management and treatment of patients with diseases and disorders of ear, nose, throat (ENT), and related structures of the head and neck. Otolaryngologic diseases represent one of the most frequent problems in children. Among them, tonsillitis is one of the most common childhood pathologies and represents a real challenge because it is becoming resistant to common treatment (Nixon and Bingham, 2006; Vlastarakos et al., 2007).
Since the presence of fluid sheer force (shaking) in batch biofilms may be an important factor in bacterial resistance and chronic tonsillitis (Potera, 1999). The matrix provides mechanical stability for prolonged periods by hydrophobic interactions cross-linking by multivalent cations (Kania et al., 2007). Biofilms are also a place where genetic material is easily exchanged because of the proximity of the cells maintaining a large gene pool. Al-Mazrou and Al-Khattaf (2008) reported that many of these bacteria have the ability to form biofilms that are matrix-encased communities adapted to surface persistence.

G. Immunity

An immune system is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens and tumor cells. Biofilms are a part of most chronic infections, including killers such as cystic fibrosis and endocarditic in the heart. In cystic fibrosis, excess mucus production in the airways gives sanctuary to bacteria such as P. aeruginosa, which actually mop up the dead carcasses of white blood cells sent by the immune system, enabling them to construct their protective biofilm coat. In this case, the immune system is the architect of its own problems, helping create the shield used to repel its own agents, as well as resisting antibiotics. Indeed, resistance against antibiotics itself one of the biggest problems of all associated with biofilms (Singh et al., 2002).

IV. CONCLUSION

Chronic wounds predominantly affect patients aged older than 60 years, and with the aging of the population, their prevalence will continue to increase. Most chronic wounds are invariably colonized, and therefore, superficial swabs cultures should be avoided. Ideally, quantitative or semiquantitative tissue cultures should be obtained to guide antibiotic therapy. Topical antibiotics are not recommended in most guidelines because they can provoke delayed hypersensitivity reaction, super infection and, more importantly, select for resistance. The study of biofilms has emerged over the past three decades in various disciplines such as biotechnology, bioengineering, or infectious disease research, leading to rapid progress, but also fragmentation and duplication of effort. Presently, included among these novel weapons of microdestruction are molecular blockading techniques, electrical enhancement of anti-infective and bacterial interference. Future treatments of infections must ultimately target these reservoirs of infection aiming for their complete eradication.
REFERENCES


CHAPTER 32

Osteoporosis Treatment: Marine Algal Compounds

Jayachandran Venkatesan* and Se-Kwon Kim*†,1

Abstract

Osteoporosis is one of the most common bone diseases that occur due to imbalance during bone formation and bone resorption. About half of all women over the age of 50 will have a fracture on the hip, wrist, or vertebra. Research and treatment of osteoporosis are challenging for researchers and physicians. There are several types of treatments for osteoporosis including most famous bisphosphonates, estrogen agonists/antagonists, parathyroid hormone, estrogen therapy, hormone therapy, and recently developed RANKL inhibition. In the recent days, much attention has been paid for marine algal extracts and compounds for osteoporosis treatment. In this chapter, we extensively deal with marine algae compounds and their rich mineral constituents for osteoporosis treatment.

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I. INTRODUCTION

Bone is made up of seven hierarchical structures and consists of hydroxyapatite and collagen as major constituents (Venkatesan and Kim, 2010a,b; Venkatesan et al., 2011a,b; Weiner and Wagner, 1998). Defects in bone can occur due to many reasons such as motor accident, birth defect, osteoporosis, arthritis, bone gangrene, and low calcium level. Among this, osteoporosis is one of the most common bone diseases that occur due to imbalance of bone formation and bone resorption. Osteoporosis disease mainly occurs in woman rather than man at an elderly age (around 50–60) in the hip, wrist, and vertebral area. In bone, there are four kinds of cells which are playing most important function for bone remodeling. They are

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Function</th>
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<tbody>
<tr>
<td>Osteoblast</td>
<td>Bone formation</td>
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<tr>
<td>Osteoclasts</td>
<td>Bone resorption</td>
</tr>
<tr>
<td>Osteocytes</td>
<td>A mature osteoblast which no longer secretes matrix</td>
</tr>
<tr>
<td>Osteoprogenitor</td>
<td>Immature cells which differentiate to make osteoblasts</td>
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Osteoblast and osteoclast cells are responsible for bone formation and resorption, respectively. Calcium and phosphate are the two minerals that are essential for normal bone formation. Osteoblasts secrete a calcifiable matrix which contains minerals, collagen, and also small amount of non-collagenous proteins including osteopontin, osteonectin, bone sialoprotein, and osteocalcin (Gay et al., 2000). The function of osteoclast is to remove bone tissue by removal of its mineralized matrix and breaking up the organic bone (90% collagen). An increase in the number of osteoclast cells and their function normally induces bone osteoporosis, indicating that osteoclast plays a pivotal role in bone homeostasis (Miyamoto and Suda, 2003).

Cancellous bones are soft and present in the inner side of bone, whereas cartial bones are hard and found in the outer area of bone. Cancellous bone is normally found in hips, vertebral column, and wrist. Owing to an improper function of osteoclasts and osteoblasts, cancellous bones are more easily affected rather than cortical bone, which in turn results in osteoporosis (Watson, 1979).

II. TREATMENT FOR OSTEOPOROSIS

The goals of osteoporosis treatment are to control pain from the disease, reduce bone loss, and prevent bone fractures with medicines or hormone therapies. There are several types of treatments for osteoporosis including most famous bisphosphonates, estrogen agonists/antagonists, parathyroid...
hormone, hormone therapy, and recently developed receptor activator of nuclear factor-\(\kappa\)B ligand (RANKL) inhibition. Estrogen agonists/antagonists in combination with estrogen for prevention and treatment of osteoporosis have also been studied (Stovall and Pinkerton, 2008). Bazedoxifene for the prevention of postmenopausal osteoporosis (Gennari et al., 2008), parathyroid hormone (Black et al., 2003; Finkelstein et al., 2003; Horwitz et al., 2010; Neer et al., 2001), estrogen therapy (Eskridge et al., 2010; Genant et al., 1997; Lindsay, 1987; Lindsay and Tohme, 1990), hormone therapy (Engel et al., 2011; Pentti et al., 2009), and recently developed RANKL inhibitory (McClung, 2006, 2007) treatment options are currently available for osteoporosis treatment.

Among this bisphosphonates are the primary drugs used to both prevent and treat osteoporosis in postmenopausal women. Bisphosphonates taken orally, once a week or once a month, include alendronate (Fosamax), ibandronate (Boniva), and risedronate (Actonel). Bisphosphonates given through a vein (intravenously) are taken less often (Gass and Dawson-Hughes, 2006; Recker et al., 2009; Society, 2003). Bisphosphonates inhibit bone resorption and are therapeutically effective in diseases of increased bone turnover, such as Paget’s disease and hypercalcemia of malignancy (Hughes et al., 1995). In the recent years, a number of research articles have been published related to the treatment of osteoporosis (Barzel, 1988; Hodsman et al., 2005; Njeh et al., 1997; Pfeifer et al., 2004; Prestwood et al., 1995; Rubin and Bilezikian, 2003).

The adverse side effects of bisphosphonates are renal toxicity, acute-phase reactions, gastrointestinal toxicity, hypocalcemia, ocular complications, asthma erythema, phlebitis, altered taste, and central nervous system side effects. The osteonecrosis of the jaw is the emerging one (Diel et al., 2007; Tanvetyanon and Stiff, 2006). To overcome this kind of problem, researchers are now turning toward nature-based drugs.

## III. MARINE ALGAE

Marine algae are generally known as sea weeds; they contain abundant active compounds. There are commonly found in seashore area in all shapes and classified into three different kinds which are red, green, and brown algae as protists, chromists, and plantae, respectively (Hedgpeth, 1957).

In the recent years, significant development has been paid in the isolation of active compounds from marine algae for various disease treatments such as anticancer (Kim et al., 2010), anti-inflammation (Kim et al., 2009; Zhang et al., 2010), antioxidant (Li et al., 2009a), \(\alpha\)-glycosidase and \(\alpha\)-amylase inhibitory activities (Lee et al., 2009), matrix metalloproteinase (Li et al., 2009b; Ryu et al., 2009b), and inhibitory effect of ROS generation (Kang et al., 2004).
IV. MARINE COMPOUND FOR OSTEOPOROSIS

A. Bone mineral density

Bone mineral density (BMD) level of people living in arctic and subarctic regions is lower than that of Europeans and American Whites; this has been linked to high meat diets, as a protein-rich diet may cause calcium loss (Lynnerup and Von Wowern, 1997). The BMD can alter based upon age (Maugeri et al., 2001). The habitual dietary pattern of a population has major influence on the prevalence and incidence of arteriosclerotic vascular disease (Brown, 1990). The insufficient dietary calcium is associated with a number of common and chronic diseases worldwide including osteoporosis, osteoarthritis, cardiovascular disease (hypertension and stroke), diabetes, obesity, and cancer (Kim and Mendis, 2006).

B. Rich mineral extracts of marine algae

Marine alga not only consists of organic active compounds, but it is also an abundant source of rich minerals such as calcium, magnesium, and other bone-supporting elements (Adluri et al., 2010; Kim et al., 2006). Mineral-rich extracts have been isolated from red marine algae Lithothamnion calcareum and checked as a dietary supplement for prevention of bone mineral loss. Female mice on the high-fat Western-style diet had reduced bone mineralization and reduced bone strength relative to female mice on the low-fat chow diet. The bone defects developed in the female mice fed on the high-fat Western-style diet could be reversed in the presence of the mineral-rich algal extract supplement (Aslam et al., 2010).

The effect of water-soluble extract of Sargassum horneri has been shown to have an anabolic effect on bone components due to stimulating bone formation and inhibiting bone resorption in rat femoral tissues in vitro and in vivo (Matsumoto et al., 2008).

The effect of various algae such as Undaria pinnatifida, S. horneri, Eisenia bicyclis, Cryptonemia scmitziana, Gelidium amasii, and Ulva pertusa Kjellman on bone calcification in the femoral-metaphyseal tissues of rats have been studied. As a result, bone calcium content was significantly increased (Yamaguchi et al., 2001).

C. Osteoblast differentiation

Marine collagen peptides (MCP) derived from Chum Salmon (Oncorhynchus keta) skin were investigated for the development of femurs in growing rats of both sexes (Xu et al., 2010).

The modification of chromatin structure thereby regulating gene transcription through histone deacetylases (HDACs) plays important roles in
Osteogenesis and is considered to be a promising potential therapeutic target for bone diseases. Largazole (Fig. 32.1A) exhibited \textit{in vitro} and \textit{in vivo} osteogenic activity by HDAC inhibition and significantly induced the expression of alkaline phosphatases, osteopontin expression, and increased expression of Runx2 and BMPs. Largazole showed \textit{in vivo} bone-forming efficacy in the mouse calvarial bone formation assay and the rabbit calvarial bone fracture healing model (Lee et al., 2011).

Norzoanthamine (Fig. 32.1B) is a nontoxic marine alkaloid and its collagen protective activity indicates that it provides significant therapeutic benefits. Norzoanthamine accelerates the formation of a collagen–hydroxyapatite composite and enhances collagen release from an immobilized matrix vesicle model. Norzoanthamine recognizes a peptide chain nonspecifically and stabilizes its secondary structure, and collagen has polyvalent binding sites for norzoanthamine. Collagen–norzoanthamine supramolecular association is considered to be one of the most significant modes of action for enhancement of bone formation. Norzoanthamine suppressed the proteolysis not only of collagen but also of elastin and bovine serum albumin, so it apparently has a universal

![Figures 32.1](image-url)
protective effect of guarding extracellular matrix (ECM) proteins from degradation (Hikage et al., 1998; Kinugawa et al., 2009).

Norzoanthamine has also been isolated from zoanthid Zoanthus sp. which suppresses the decrease in bone weight and strength in ovariectomized mice, indicating that it could be a good candidate as an osteoporotic drug (Kuramoto et al., 1998).

Arthritis is one of the most prevalent chronic inflammatory diseases, and it is characterized by structural and biochemical changes in major tissues of the joint, including degradation of the cartilage matrix, insufficient synthesis of ECM. Ecklonia cava (EC) is a member of the family of Laminariaceae, which is an edible marine brown alga with various bioactivities. The methanol extracts of brown alga EC, the dieckol (Fig. 32.1C) and 1-(3',5'-dihydroxyphenoxy)-7-(2''''',4''''',6'''''-trihydroxyphenox)-2,4,9-trihydroxydibenzo-1,4,-dioxin (Fig. 32.1D) have been used for arthritis treatment at in vitro level (Ryu et al., 2009a).

The effect of the fractionated extracts obtained from S. horneri on bone calcium content and osteoclast-like cell formation in vitro has also been investigated. The effects of S. horneri on bone components in the femoral diaphyseal and metaphyseal tissues of young and aged rats were studied. The oral intake of the water-solubilized S. horneri extract significantly altered the bone components of young rats in vivo (Uchiyama and Yamaguchi, 2002; Uchiyama et al., 2004).

D. Osteoclast differentiation in osteoporosis

An inhibitor of osteoclast differentiation and/or function is expected to be useful for treatment of bone lytic diseases such as osteoporosis, rheumatoid arthritis, and tumor metastasis into bone. Paenol inhibits RANKL-induced osteoclastogenesis by inhibiting ERK, p38, and NF-κB pathway (Tsai et al., 2008). Symbioimine (Fig. 32.2A) from the symbiotic marine dinoflagellate Symbiodinium sp. exhibits inhibitory effect on osteoclast differentiation (Kita et al., 2004).

Biselyngbyaside (Fig. 32.2B) has been isolated from marine cyanobacterium Lyngbya sp. and subjected to osteoclast differentiation study. Biselyngbyaside inhibited RANKL-induced osteoclastogenesis in mouse monocytic RAW264 cells and primary bone marrow-derived macrophages at a low concentration (Yonezawa et al., 2011). Effects of Spirulina algae on bone metabolism in ovariectomized estrogen-deficient rats and hindlimb-unloaded mice have also been examined (Ishimi et al., 2006).

The BMD of the whole femur and tibia of ovariectomized rats in any of the Spirulina-treated groups was not significantly different from that of the ovariectomized group, although BMD of the distal femur and proximal tibia was significantly lower in the Spirulina-treated groups than in the ovariectomized group after a 6-week experimental period (Ishimi et al., 2006).
Inhibition of osteoclastogenic differentiation by ikarisoside A (Fig. 32.2C) in RAW 264.7 cells via JNK and NF-κB signaling pathways have been recently reported (Choi et al., 2010). Lucas et al. (2003) studied the modulatory effect of bolinaquinone (Fig. 32.2D), a marine sesquiterpenoid, on acute and chronic inflammatory processes. Fucoxanthin (Fig. 32.2E) that induces apoptosis also induced osteoclast differentiation in a study conducted by Das et al. (2010).

V. CONCLUSION

Very few marine compounds have been studied and reported for osteoporosis treatment. Still much research work is needed for further implications. Although synthetic bisphosphonates compounds are more promising for osteoporosis treatment, marine algal extracts and their
compounds are excellent in the biocompatibility without side effects at *in vitro* and *in vivo* condition. Further clinical trials for marine active compounds are considered necessary for their further commercialized implications.

**ACKNOWLEDGMENT**

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Marine Algae Possess Therapeutic Potential for Ca-Mineralization via Osteoblastic Differentiation

Minh Hong Thi Nguyen,*† Won-Kyo Jung,*†,1 and Se-Kwon Kim‡§

Abstract

One of the important natural product investigations from marine algae is to focus on the pharmaceutically important compounds that can be applied in bone health. Osteoporosis is one of the bone diseases caused by an imbalance between bone formation and resorption. Promotion of osteoblast differentiation is one of the best therapeutic ways to combat osteoporosis. Osteoblasts are the cells responsible for bone formation by increasing the proliferation of the osteoblastic lineage or inducing differentiation of the...
osteoblasts. In this review, we describe the central effects of osteoblast differentiation by various bone therapy biomaterials from marine algae.

I. INTRODUCTION

Osteoblasts and osteoclasts are the bone cells, which are present and active in mature bones. These are effectively involved in interacting with the bone remodeling system and their balance activities remain the homeostasis of bone. Calcium phosphate compounds such as hydroxyapatite (Ca\(_{10}(PO_4)_{6}(OH)_2\)) is present in substantial amounts in the mineralized tissue of the vertebrates, for example, 60–70% of the mineral phase of the human bones (Constantz et al., 1995). As bones grow, osteoblasts lay down an organic matrix that is then mineralized by deposition of calcium (Ca) and phosphate to form hydroxyapatite. If this process is not properly regulated, the result can be of less mineralization or more, either of which can impair bone health. Osteoclasts are cells responsible for bone resorption that play a critical role in bone modeling and remodeling as they grow within the body. The disturbances in the relationship between these cell types can be found in many disease states. Bone diseases caused by abnormal bone homeostasis such as osteoporosis can be treated via the action of osteoclast and/or osteoblast. Therefore, identification of drugs that would affect the promotion of bone formation is the key tool for desirable therapies.

Osteoblast differentiation is regulated by various factors such as bone morphogenetic proteins (BMPs), transforming growth factor \(\beta\) (TGF-\(\beta\)), Indian hedgehog (Ihh), Noggin, fibroblast growth factor (FGF2), insulin-like growth factor 1 (IGF-1), prostaglandins, parathyroid hormone (PTH)/parathyroid hormone-like peptide (PTHrP), and leptin via various signaling pathways lead by Smads, mitogen-activated protein kinases (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-\(\kappa\)B). Osteoblast differentiation can be subdivided into three subsequent stages such as proliferation stage, extracellular matrix synthesis and maturation stage, and mineralization stage (Zamurovic et al., 2004). The phenotypic markers of each stage are distinctively expressed. Active osteoblasts have high expression of alkaline phosphatase (ALP), collagen type I, early markers of osteoblast differentiation, while osteocalcin appears late, concomitantly with mineralization.

Marine natural products have attracted the attention of scientists in the world over for the past five decades. Marine organisms such as sponges, soft coral, marine algae, sea horses, tunicates, sea snakes, sea slugs, marine mollusks, and marine microorganisms are targeted nowadays for the investigation of new drug candidates. Many of the compounds
derived from marine organisms have shown very promising biological activity. The study of bioactive marine natural products has profoundly influenced the course of discovery in various fields ranging from pharmacology to cancer medicine.

According to Mayer’s review in 2002, 2004, 2005, 2007, 2009, 2010, there has been a strong increase in studying marine compounds derived from a diverse group of marine animals, algae, fungi, and bacteria apply in pharmacology by years. In 1999, there have been 21 marine compounds demonstrating anthelmintic, antibacterial, anticoagulant, antifungal, antimalarial, antiplatelet, antituberculosis, or anti-viral activities; 23 compounds had significant effects on the cardiovascular, sympathomimetic, or the nervous system, as well as possessed anti-inflammatory, immunosuppressant, or fibrinolytic effects; and 22 marine compounds were reported to act on a variety of molecular targets. In 2003–2004, there have been highlights in the preclinical pharmacology of 166 marine chemicals: 67 marine chemicals demonstrated anthelmintic, antibacterial, anticoagulant, antifungal, antimalarial, antiplatelet, antiprotozoal, antituberculosis, or antiviral activities; 45 marine compounds were shown to have significant effects on the cardiovascular, immune, and nervous system as well as possessing anti-inflammatory effects; 54 marine compounds were reported to act on a variety of molecular targets. In 2007–2008, there have been 198 marine compounds which are part of the preclinical marine pharmaceuticals pipeline: antibacterial, anticoagulant, antifungal, antimalarial, antiprotozoal, antituberculosis, and antiviral activities were reported for 74 marine natural products; 59 marine compounds were reported to affect the cardiovascular, immune, and nervous systems as well as to possess anti-inflammatory effects; 65 marine metabolites were shown to bind to a variety of receptors and miscellaneous molecular targets. Thus, marine organisms that possess great potential of novel compounds and new drugs using for the treatment of treated numerous of disease categories are attracting attention of scientist. Each year, the increasing number of novel marine metabolites and marine natural compounds is reported in the literature indicating that the marine organisms will continue to be a prolific source of new natural products for many years to come.

Algae can be classified into two main groups as microalgae and macroalgae. Microalgae include blue green algae, dinoflagellates, bacillariophyta (diatoms), etc., whereas macroalgae (seaweeds) include green, brown, and red algae (Gamal and Ali, 2010). To the best of our knowledge, macroalgae, especially brown and red algae, are focused to show their effect on bone health. Although marine compounds derived from algae have exploited for a variety of purposes, the investigations for application in bone health are few and very recent.
II. THERAPEUTIC POTENTIAL OF MARINE ALGAE

Marine algae consist of numerous bioactive substances for known and unknown applications in medical and pharmacological fields. Natural products from marine algae can be used as pharmacological ingredients/materials for bone health or as functional foods for bone-strengthening applications. Marine algae have contributed numerous therapeutic compounds for the treatment of multiple disease categories such as antitumor (Fuller et al., 1994, Guardia et al., 1999), anticancer (Gerwick et al., 1994), antibacterial (Ali et al., 2002, Bennamara et al., 1999, Smyrniotopoulos et al., 2003), anti-inflammatory (Awad, 2000, Wiemer et al., 1991), antiviral (Barbosa et al., 2004, Wang et al., 2007), antimicrobial (Barreto and Meyer, 2006), antimalarial (Lane et al., 2007, Topcu et al., 2003). In contrast, several investigations about compounds derived from marine algae effect on bone health as well as osteoblast differentiation processing were reported. Marine algae are also known as favorite food possessing enormous nutrient values. Thus, investigations to find out the medicinally important substances as drug candidates are very much needed to the development of marine materials from marine algae, which can be proposed for the bone health and/or to treat bone diseases.

A. Ca-mineralization via osteoblastic differentiation

Calcium level contributes to be crucial in the strengthening of bone and bone homeostasis. Investigations to find out natural products that promote Ca-mineralization are interesting nowadays. Marine algae have displayed a promising natural resource for this strategy, for example, unicellular coccolithophorid algae produce elaborate calcified scales called coccolith, which consist of fine pieces of CaCO₃ (calcite) crystals, known as one of the representative biominerals. A novel polysaccharide named coccolith matrix acidic polysaccharide (CMAP) was isolated from the coccolith of a coccolithophorid alga, Pleurochrysis haptonemofera, by Ozaki et al. (2007). By using chemical analysis and NMR spectroscopy including COSY, TOCSY, HMQC, and HMBC, the structure of CMAP was determined to be a polysaccharide composed of the following unit: [L-iduronic acid (α1 → 2), mesotartaric acid (3 → 1), glyoxylic acid (1→)]ₙ (Fig. 33.1). The investigation showed that CMAP has a strong inhibitory activity on CaCO₃ precipitation and suggest that it serves as a regulator in the calcification of the coccolith. However, the effects of this compound on Ca-mineralization of osteoblasts were yet to be investigated.

Phlorotannins are the group of tannins and are found only in brown algae. Phlorotannins are oligomeric compounds using phloroglucinol (1,3,5-trihydroxybenzene) as a basic unit. Some phlorotannins have been
identified as the bioactive components in *Ecklonia* species such as *Ecklonia cava*, *Ecklonia kurome*, and *Ecklonia stolonifera* (Ham et al., 2007). The compounds eckol, 2 dieckol, 6,6'-bieckol, and 1-(3',5'-dihydroxyphenoxy)-7-(2',4',6'-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin (Fig. 33.2) were isolated from *E. cava* by Ryu et al. (2009), who investigated the potent effect of these compounds on a host of commonly

![FIG. 33.1 Probable repeating structure of CMAP: ([L-iduronic acid (α1 → 2), mesotartaric acid (3 → 1), glyoxylic acid (1 → n).](image1)]

![FIG. 33.2 Structures of phlorotannins from *Ecklonia cava* (1: dieckol; 2: 1-(3',5'-dihydroxyphenoxy)-7-(2',4',6'-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin).](image2)
occurring diseases which possess an inflammatory component, including osteoarthritis, atherosclerosis, cancer, etc. These results suggested that the compounds help to stimulate the osteoblast differentiation at various stages and further confirmed that these compounds might have a therapeutic potential for the patients with osteoarthritis by stimulating production of proteoglycan. The phlorotannin derivatives showed the regulation of osteosarcoma differentiation by increasing ALP activity, mineralization, total protein, and collagen synthesis in human osteosarcoma cell (MG-63 cells). In addition, the phlorotannin derivatives could attenuate inflammatory response via MAPK pathway in chronic articular diseases.

Fucodiphlorethol G (Fig. 33.3), a new compound isolated from the methanol extract of *E. cava*, a brown alga, collected offshore in Jeju Island by Ham *et al.* (2007). By the examination of $^1$H and $^{13}$C NMR data, it was found that the structure of the compound is similar to that of trimeric phlorotannin triphlorethol-A (Fig. 33.3). Although these studies evidenced that these compounds can stimulate osteoblast differentiation at various stages, there is no clear demonstration of whether the phlorotannin compound, Fucodiphlorethol G, has direct effect on osteoblast differentiation.

The fucans of brown algae, often called fucoidans, have shown biological activities such as antioxidative, anticoagulant, antithrombotic, anti-inflammatory, antitumoral, and antiviral activities (Cho *et al.*, 2009). Berteau *et al.* (2003) studied the fucoidan derived from brown algae and other common fucoidans (Fig. 33.4). Although fucoidan was extracted from the numerous species of brown algae (Table 33.1), to the best of our knowledge, there are a few reports on beneficial effects of fucoidan on bone health or formation. Cho *et al.* (2009) extracted fucoidan from brown algae *Undaria pinnatifida* and showed that fucoidan significantly effects

---

**FIG. 33.3** Structure of phlorotannin from *Ecklonia cava* (1: fucodiphlorethol G; 3: triphlorethol-A).
FIG. 33.4 Common structures in fucoidans from brown algae. (A) The disaccharide repeating unit \((4)-\alpha-L-\text{Fucp}(2,3\text{di-OSO}_3)\rightarrow 3)-\alpha-L-\text{Fucp}(2\text{OSO}_3)\rightarrow 1\) of a fraction of \(A. nodosum\) fucoidan representing the most abundant structural feature of fucoidans from both \(A. nodosum\) and \(F. vesiculosus\). The same structure has been identified in the fucoidan of \(F. evanescens\). (B) The 3-linked, preponderantly 4-sulfated fucoidan from \(E. kurome\). (C) The quasirepeat unit identified in fucoidan from \(C. filum\). Other substituents, such as O-acetyl, and branches are present in all these fucoidans and add considerably to their heterogeneity.
osteoblastic cell differentiation. The level of ALP, osteocalcin, and BMP-2 were increased in the presence of fucoidan. It is suggested that fucoidan could be an agent to promote osteoblast differentiation and has possibility for its application in bone health supplement. Synytsya et al. (2010) determined the structure of fucoidan extracted from brown algae *U. pinnatifida* and concluded that this fucoidan is sulfated galactofucan containing β-D-galactopyranose and α-L-fucopyranose at near equal amounts (44.6 and 50.9 mol%).

**TABLE 33.1 Brown algae containing fucoidan**

<table>
<thead>
<tr>
<th>Species</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cladosiphon okamuranus</em></td>
<td>Chordariales</td>
</tr>
<tr>
<td><em>Chordaria flagelliformis, Ch. gracilis</em></td>
<td>Chordariales</td>
</tr>
<tr>
<td><em>Saundersella simplex</em></td>
<td>Chordariales</td>
</tr>
<tr>
<td><em>Desmarestia intermedia</em></td>
<td>Desmarestiales</td>
</tr>
<tr>
<td><em>Dictyosiphon foeniculaceus</em></td>
<td>Dictyotales</td>
</tr>
<tr>
<td><em>Dictyota dichotoma</em></td>
<td>Dictyotales</td>
</tr>
<tr>
<td><em>Padina pavonica</em></td>
<td>Dictyotales</td>
</tr>
<tr>
<td><em>Spatoglossum Schroederi</em></td>
<td>Dictyotales</td>
</tr>
<tr>
<td><em>Adenocystis utricularis</em></td>
<td>Ectocarpales</td>
</tr>
<tr>
<td><em>Pylayella littoralis</em></td>
<td>Ectocarpales</td>
</tr>
<tr>
<td><em>Ascophyllum nodosum</em></td>
<td>Fucales</td>
</tr>
<tr>
<td><em>Bifurcaria bifurcata</em></td>
<td>Fucales</td>
</tr>
<tr>
<td><em>Fucus vesiculosus, F. spiralis, F. serratus, F. evanescens</em></td>
<td>Fucales</td>
</tr>
<tr>
<td><em>Himanthalia lorea</em></td>
<td>Fucales</td>
</tr>
<tr>
<td><em>Hizikia fusiforme</em></td>
<td>Fucales</td>
</tr>
<tr>
<td><em>Pelvetia canaliculata, P. wrightii</em></td>
<td>Fucales</td>
</tr>
<tr>
<td><em>Sargassum stenophyllum, S. horneri, S. Kjellmanium, S. muticum</em></td>
<td>Fucales</td>
</tr>
<tr>
<td><em>Alaria fistulosa, A. marginata</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Arthrothamnus bifidus</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Chorda filum</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Ecklonia kurome, E. cava</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Eisenia bicyclis</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Laminaria angustata, L. brasiliensis, L. cloustoni, L. digitata, L. japonica, L. religiosa, L. saccharina</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Macrocystis integrifolia, M. pyrifera</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Nereocystis luetkeana</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Undaria pinnatifida</em></td>
<td>Laminariales</td>
</tr>
<tr>
<td><em>Petalonia fascia</em></td>
<td>Scytosiphonales</td>
</tr>
<tr>
<td><em>Scytosiphon lomentaria</em></td>
<td>Scytosiphonales</td>
</tr>
</tbody>
</table>
B. Marine algae-derived biomaterials for bone tissue engineering applications

The marine environment is rich with mineralizing organisms of porous structures, some of which are currently being used as bone graft materials and others are used in their early stages of development for bone repair. Some species of red algae (phylum Rhodophyta), specifically a group of coralline algae deposits calcium carbonate, have been used in bone tissue engineering (Clarke et al., 2011).

Felício-Fernandes et al. (2000) reported that calcium phosphate compounds such as hydroxyapatite were prepared by hydrothermal synthesis with phycogenic CaCO₃ as starting material. They showed that it may be suitable for use as a biomaterial. The biogenic material was obtained from algae of the Rodophycophyta. Calcium carbonate for the synthesis of calcium phosphates similar to bone can be obtained from several natural sources. Only the calcium carbonate originating from marine algae and corals shows characteristic porosity and interconnectivity that makes it like human bone.

Manufacture cell-seeded three-dimensional bone constructs based on hydroxyapatite ceramic granule calcified from red algae and mesenchymal cambial-layer precursor cells have been investigated by Turhani et al. (2005). The results showed that these 3D composites might possess suitable properties for bone reconstruction of the maxillofacial region in vivo and provide new insights into the development of novel strategies of bone tissue engineering. They conclude a positive effect of hydroxyapatite ceramic granules on mesenchymal cambial-layer precursor cell behavior in cell-seeded three-dimensional bone constructs.

Walsh et al. (2008) investigated a bioceramic from algal origin, which is suitable for bone tissue application. These reports confirmed the successful conversion of mineralized red alga to hydroxyapatite, via low-pressure hydrothermal process. Further, the synthesized hydroxyapatite maintained the unique microporous structure of the original algae, which is considered beneficial in bone repair applications. Investigations on hydrothermal transformation of mineralized red algae were performed by Walsh et al. (2010), which are shown to be a suitable candidate for conversion to calcium phosphate ceramics in terms of its physiochemical properties to hydroxyapatite that preserves the algae’s unique structure. The optimum processing parameters for the thermal heat treatment were found to be in the range of 600–650 °C, with a ramp rate of 2 °C min⁻¹. These parameters are considered as a well-strategized approach for hydrothermal conversion of Corallina officinalis to hydroxyapatite.

Calcium is an essential mineral to support bone health and serves as a major therapeutic intervention to prevent and delay the incidence of
osteoporosis. Adluri et al. (2010) examined the effect of a novel plant-based calcium supplement from marine algae, which additionally contains high levels of Magnesium and other bone-supporting minerals [commercially known as AlgaeCal (AC)]. These supplements have potential effect on proliferation, mineralization, and oxidative stress in cultured human osteoblast cells and compared with inorganic calcium carbonate and calcium citrate salts. The results demonstrated that AC can serve as a superior and bioavailable calcium supplement than the inorganic calcium sources, and the effect of AC may be due to its content of other bone-supporting minerals and their influence on ALP, DNA synthesis, helping in the proliferation and mineralization of the osteoblast cells.

C. Marine algal diet with calcium supplement

The well-known correlation between diet and health demonstrates the great possibilities of food to maintain or even improve our health (Plaza et al., 2008). Dietary factors are very important for osteoporosis, and Ca$^{2+}$ is the most important cationic mineral in the bone (Aslam et al., 2010). Different algae and their comparative composition to use as new sources of functional ingredients have been shown previously (Plaza et al., 2008). Algae have mainly been used in the Western countries as raw material to extract brown algae alginates and red algae agar and carrageenans. Thus, it is possible to conclude that these organisms have shown a high potential as natural sources of ingredients with many different biological activities.

Aslam et al. (2010) investigated whether a mineral-rich extract from the red marine algae Lithothamnion calcareum could be used as a dietary supplement for prevention of bone mineral loss. Their experiments showed that the mice receiving the mineral-rich supplement in the high-fat Western-style diet had better bone structure/function than the mice on the low-fat chow diet. The algae extract is already available as a food supplement under the name Aquamin (GRAS 000028), which is currently used in various products of human consumption in Europe, Asia, Australia, and North America.

III. CONCLUSION

Worldwide, there are about 1500–2000 species of brown algae, 5000–5500 species of red algae, 6000 species of green algae, approximately 4000 known species of microalgae. Among them, just small species groups have clarified the pharmacological potentiality. There are still more potential compounds derived from marine algae unidentified to be identified. Thus, marine algae are promising for investigations in future
toward their biomedical applications especially. Investigation of marine natural products from marine algae effect on osteoblast differentiation that can lead to regain bone homeostasis and possibility for its application in bone health supplement is beginning.

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Extracts of Marine Algae Show Inhibitory Activity Against Osteoclast Differentiation

Tomoyuki Koyama

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Abstract

Osteoclasts are multinucleated cells that play a crucial role in bone resorption. The imbalance between bone resorption and bone formation results in osteoporosis. Therefore, substances that can suppress osteoclast formation are potential candidate materials for drug development or functional foods. There have been reports that extracts or purified compounds from marine micro- and macroalgae can suppress osteoclast differentiation. Symbioimine, isolated from the cultured dinoflagellate Symbiodinium sp., had suppressive effects against osteoclast differentiation in osteoclast-like cells. Norzoanthamine, isolated from the colonial zoanthid Zoanthas sp., has been shown to have antiosteoporosis activity in ovariectomized mice. With regard to marine extracts, the fucoxanthin-rich component from brown algae has been shown to have suppressive effects against osteoclast differentiation. An extract of...
Sargassum fusiforme has recently been shown to have antiosteo-
porosis activity. This extract suppressed both osteoclast differenti-
ation and accelerated osteoblast formation in separate in vitro
experiments. It also showed antiosteoporosis activity in ovariecto-
mized mice by regulating the balance between bone resorption and
bone formation. These marine algae and their extracts may be
sources of marine medicinal foods for the prevention of osteoporosis.

I. INTRODUCTION

Osteoporosis is a disabling disorder that is characterized by decreased
bone strength, which predisposes patients to an increased risk of bone
fracture. Peak bone mass (maximum bone strength and density) is nor-
mally attained by age 20–25. Age, race, sex, environment, and lifestyle
factors such as physical activity and diet are important determinants of
bone density. With the increase in age, the rate at which bone tissue is
replaced is reduced in comparison to the rate at which it is lost, which
increases the risk for fractures.

As bone loss is a gradual and painless process, osteoporosis is often
diagnosed only after the occurrence of a fracture, which makes it chal-
lenging to reliably estimate the patient population that is at risk for
developing this disease. Worldwide, 200 million people are estimated to
suffer from osteoporosis (Cooper, 1999). While the overall number of
fractures is increasing worldwide according to the report from World
Health Organization, several reports from population-based studies
have shown that recent progress in the diagnosis and treatment of osteo-
porosis has been effective at reducing the number of hip fractures (Jaglal
et al., 2005).

Pharmacological and nutritional factors may help to prevent bone loss
with increasing age. Nutritional factors may be especially important in
the prevention of osteoporosis (Bonjour et al., 1996; Yamaguchi, 2002,
2006). While chemical factors in food and plants may also help to prevent
bone loss with increasing age, these factors are poorly understood.

There has been a major increase in lifestyle-related diseases due to the
fact that we are living longer and have adopted a Western diet.

In Japan, osteoporosis is currently treated with active form of vitamin
D₃ (Orimo et al., 1994; Schacht, 1999), estrogen (Lufkin et al., 1992;
Weinstein et al., 2003), calcitonin (Body, 2002; Kopaliani, 2005), ipriflavone
(Nakamura et al., 1992), vitamin K₂ (Sakamoto et al., 2005; Steven et al.,
2005), bisphosphonate (Hamdy et al., 2005; Matsumoto, 2004; Perez-
Lopez, 2004), and related compounds. However, although these drugs
are used to treat osteoporosis, they cannot prevent it. In addition, some of
these drugs, such as calcitonin, are not easily administered, and the emergence of drug resistance has been observed. The active form of vitamin D3 is not effective for treating hypercalcemia, and bisphosphonate inhibits bone formation. The use of estrogen should be careful, while estrogen administration for even just 6 months has been observed to result in abdominal bloating, breast pain, digestive symptoms, and irregular vaginal bleeding (Barkhem et al., 1998). These limitations of medications suggest that the prevention of osteoporosis through diet is very important.

Bone health and the prevention of osteoporosis-related fractures are key elements in the strategy for managing patients undergoing menopause. A detailed knowledge of bone health and related diagnostic and therapeutic options falls within the domain of the gynecologist as part of a multidisciplinary approach.

Osteoporosis-related fractures are common and will affect at least one-third of women over 50 years of age (Johnell and Kanis, 2005). It is estimated that osteoporosis affects 75 million people in Europe, the United States, and Japan, and this is estimated to increase by 240% by 2050.

Bone metabolism is characterized by two opposing activities: bone formation and bone resorption (Martin, 2002). Once formed, the bones in adults are continuously remodeled, and the remodeling rate is between 2% and 10% of the skeletal mass per year. Bone mass depends on the balance between resorption and formation within the remodeling unit. As osteoporosis is characterized by a decrease in bone mass with deterioration in the architecture of bones, it is the result of an imbalance between bone formation and resorption.

Bone remodeling is disturbed under a variety of pathologic conditions that affect the skeleton, including postmenopausal osteoporosis and rheumatoid arthritis, in which there is a local and/or systemic alteration in the levels of hormones or proinflammatory cytokines that are known to stimulate or inhibit bone resorption in vitro and in vivo. Parathyroid hormone has been recognized as a stimulator of bone resorption since the early 1980s (Rodan and Martin, 1981). Studies with genetically altered mice and other animal models of bone diseases over the past 10 years have greatly increased our knowledge of factors that regulate the formation and activity of osteoclasts. In particular, identification of the receptor activator of nuclear factor-κB ligand (RANKL)/RANK/osteoprotegerin signaling system in the mid- to late-1990s represented a major breakthrough that clarified the role played by osteoblasts in this process. Moreover, it has become increasingly clear that osteoclasts are not simply trench-digging cells; instead, they have important regulatory functions as immunomodulators in pathologic states and also help to control osteoblast function (Martin and Slims, 2005).
II. METHODS FOR ASSAYING OSTEOBLASTS AND OSTEOCLASTS IN VITRO

In general, these activities have been evaluated using an experimental system, which leads to the differentiation of mature osteoclasts, in which bone marrow cells are cultured in the presence of osteoblasts.

Bone remodeling is a continuous process that helps to repair the microdamage to the bone matrix and adjusts the bone architecture to maintain bone strength. In this tightly regulated process, osteoclasts, which are multinucleated cells derived from the myeloid hemopoietic lineage, comprise the principal cell population that is involved in bone resorption, whereas osteoblasts, which originate from multipotent mesenchymal stem cells, carry out bone formation. The overall integrity of bone is controlled by biochemical factors, which include hormones, cytokines and other proteins, and mechanical factors. Perturbations of this complex but well-coordinated process result in skeletal abnormalities characterized by increased bone loss or excessive bone formation.

Osteoclast-like multinucleated cells can be differentiated in vitro from cocultures of mouse bone marrow cells and calvarial osteoblastic cells by treatment with the osteotropic factor 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) (Miura et al., 2002). It has been shown that RANKL induces osteoclast formation in a culture of bone marrow cells in the presence of macrophage colony-stimulating factor without the need for osteoblasts. A mouse macrophage RAW264 cells are also known to differentiate into osteoclasts in the presence of RANKL (Hsu et al., 1999). When osteoblast/stromal cells are stimulated by osteotropic factors such as 1,25(OH)₂D₃, RANKL is expressed and induces the differentiation of osteoclast progenitors by binding to RANK (Jimi et al., 1999).

Osteoblasts were isolated from 3-week-old mice that had been killed by cervical dislocation. The calvariae were digested in 2 ml of an enzyme solution containing 0.2% collagenase (Wako, Osaka, Japan) for 5 min at 37°C in a shaking water bath. The supernatant was discarded and 2 ml of the enzyme solution was added. After the mixture was shaken at 37°C for 20 min, the supernatant was carefully collected and transferred to a new tube. This digestion of calvariae by collagenase was repeated three times. The collected supernatant (6 ml) was placed in a centrifuge at 1500 × g for 5 min to collect osteoblastic cells. Cells were resuspended in α-minimal essential medium (α-MEM) (MP Biomedicals, Germany) containing 10% fetal bovine serum (FBS) and cultured to confluence in culture dishes for about 1 week. The cells were then detached from the culture dishes using trypsin–EDTA, suspended in α-MEM containing 10% FBS, and used for the coculture as osteoblastic cells.

Femoral and tibial bone marrow cells were collected from 7-week-old mice that had been killed by cervical dislocation. The tibiae and femora
were removed and dissected free of adhering tissues. The bone ends were removed, and the marrow cavities were flushed by slowly injecting media at one end using a 26-gauge needle. The calvariae and bone marrow cells were washed and used in the coculture.

Mouse calvarial cells (1.3 × 10^5 cells/ml) were cocultured with bone marrow cells (5.0 × 10^6 cells/ml) in α-MEM containing 10% FBS in 48-well plates (Corning Inc., NY, USA). The culture volume was made up to 250 μl per well with α-MEM supplemented with 10% FBS, in the presence of 10 ng/ml 1,25(OH)2D3 (Biomol, PA, USA), with or without samples. All cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO2 in air. Half of the medium was changed after coculture for 3 days.

To count multinucleated cells, cultured cells were stained as described below. After cells were cultured, adherent cells were fixed with 10% formaldehyde in phosphate-buffered saline (–) for 20 min. After cells were treated with 95% ethanol for 1 min, the well surface was dried and treated with tartrate-resistant acid phosphatase (TRAP)-staining solution [0.1 M sodium acetate buffer (pH 5.0) containing 50 mM sodium tartrate, 0.1 mg/ml naphthol AS-MX phosphate (Sigma Chemical Co., St. Louis, USA), and 1 mg/ml fast red violet LB salt (Sigma Chemical Co.)] for 30 min. TRAP-(+) multinucleated cells were then counted under a microscope.

Cell viability was evaluated using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma Chemical Co.) assay. After culture, cells were treated with 1 mg/ml MTT for 2 h, precipitated dye was solubilized in dimethylsulfoxide, and the absorbance at 570 nm was measured.

### III. MICROALGAE

The symbiotic microalga *Symbiodinium* sp., which is a type of symbiotic zooxanthellae, is found in a wide range of marine invertebrates. Dinoflagellates are widely known to be a rich source of biologically active and structurally unique secondary metabolites (Kita and Uemura, 2005, 2006; Uemura, 2006). Some dinoflagellates were cultured under artificial conditions with seawater medium, and the alga body was then centrifuged and extracted with 80% aqueous EtOH to collect metabolites. Symbioimines (Kita et al., 2004, 2005), symbidinolide (Kita et al., 2007), and symbiospirols (Tsunematsu et al., 2009) have been isolated from the same strain of *Symbiodinium* sp. derived from the marine acoel flatworm. One of these compounds, symbioimine (Fig. 34.1), an amphoteric iminium metabolite, has been shown to be an antiresorptive and anti-inflammatory drug (Kita et al., 2005). Its ability to suppress osteoclast differentiation (EC_{50} = 44 μM) was demonstrated in RAW264 cells. In addition, symbioimine (10 μM) also inhibited cyclooxygenase-1 and -2 activities by 5% and 32%, respectively.
The zoanthamine alkaloids are a structurally unique family of natural products that exhibit antiosteoporotic, antibiotic, anti-inflammatory, and cytotoxic biological activities. Although they are isolated from soft coral of the order zoantharia, symbiotic algae may play an important role in their biosynthesis. Norzoanthamine (Fig. 34.2) was isolated along with some analogs from a Zoanthus species collected off the Ayamaru coast of the Amami Islands in Japan (Fukuzawa et al., 1995). Norzoanthamine and its hydrochloride salt have been shown to prevent bone loss in ovariectomized mice, a pharmaceutical model for postmenopausal osteoporosis (Kuramoto et al., 1996). As ovariectomized mice do not produce sufficient estrogen, they quickly lose bone mass and strength within a few weeks. However, oral treatment of mice with norzoanthamine HCl at doses of

![FIGURE 34.1 Structure of symbioimine. Symbioimine, an amphoteric iminium compound, was isolated from symbiotic algae of the marine acoel flatworm Amphiscolops sp. which was collected at Sesoko Island, Okinawa, Japan.](image1)

![FIGURE 34.2 Structure of norzoanthamine. Norzoanthamine, an antiosteoporotic marine alkaloid, was isolated along with some analogs from a Zoanthus species collected off the Ayamaru coast of the Amami Islands in Japan.](image2)
0.08–2.0 mg/kg/day for 4 weeks led to a significantly higher retention of femur weight than in the control group (Kuramoto et al., 1996, 1998; Uemura, 2006). Further, these preventive effects were not accompanied by an increase in uterine weight, which is a serious side effect of treatment with 17b-estradiol (Yamaguchi et al., 1999). In vitro studies with norzoanthamine showed that it had no effect on the formation of osteoclasts, and the suppression of IL-6 secretion, which has been suggested by in vitro experiments (Kuramoto et al., 2000), has not yet been demonstrated in vivo (Behenna et al., 2008). Further investigations will be needed to elucidate the mechanism of the antiosteoporosis action of norzoanthamine.

IV. MACROALGAE

The suppressive effects of macroalgae extract against osteoclast differentiation have been reported as described below.

Uchiyama et al. (2004) investigated the effect of the water-soluble extract from marine algae Sargassum horneri on osteoclastic bone resorption and osteoblastic bone formation in vitro. They found two components in the crude extract: heat-labile component with a molecular weight of 1000 and heat-stable component with a molecular weight of 50,000. The former component increased the calcium content in rat femoral-diaphyseal tissues at 25 µg/ml (Uchiyama and Yamaguchi, 2003; Yamaguchi et al., 2001), and the latter suppressed 1,25(OH)2VD3-induced osteoclast-like cell formation. Their research group also reported that the S. horneri extract helped to prevent bone loss in streptozotocin-induced diabetic rats in vivo (Uchiyama and Yamaguchi, 2003). Interestingly, these two active components in S. horneri extract were thought to regulate bone metabolism to prevent osteoporosis.

Brown sea algae contain the characteristic carotenoid fucoxanthin. Dietary fucoxanthin has been shown to exhibit various beneficial effects. Das et al. (2010) reported that fucoxanthin from edible brown algae significantly suppressed the differentiation of RAW 264.7 cells at 2.5 µM, without any cytotoxic effects. They concluded that fucoxanthin is helpful for the prevention of diseases associated with abnormal bone metabolism, as fucoxanthin induced apoptotic cell death in osteoclast-like cells at a concentration that was nontoxic to osteoblast-like cells. Using the same bioassay system, our research group found that the methanol extract of Sargassum fusiforme (SME) suppressed osteoclast differentiation and accelerated osteoblast differentiation. Osteoclast differentiation was estimated by TRAP- (+) multinucleated cell formation in osteoblastic cell/bone marrow cell coculture. Osteoclast formation was induced by the presence of 1,25(OH)2D3 in the coculture. As shown in Fig. 34.3, SME
reduced the number of multinucleated osteoclast cells in a dose-dependent manner without any cytotoxic effects. The addition of SME reduced the number of TRAP-(+) multinucleated cells, without cytotoxicity, to 60% at 25 μg/ml and 6% at 50 μg/ml (Fig. 34.3).

The effects of SME on osteoblast cells were also investigated in vitro as shown in Fig. 34.4. Osteoblasts are differentiated from stem cells by their own mediators, alkaline phosphatase (ALP), osteopontin, and osteocalcin. Eventually, mature osteoblasts become calcified to form bone tissue. The addition of bone morphogenetic protein 2 accelerated the differentiation of osteoblasts in a dose-dependent manner at 25–100 ng/ml. On the other hand, SME increased ALP activity, which accelerated the differentiation of osteoblasts in a dose-dependent manner.

The effect of SME on bone resorption in vivo was examined in ovariectomized mice, an experimental model of postmenopausal osteoporosis (Masuda et al., 2008). Femurs were isolated from mice at 4 weeks after ovariectomy. The amount of cancellous bone was measured on photographs of the ground bone. The average length of the cancellous bone area on ground sections of femurs in the sham-operated group (5.4 mm) was much greater than that in the ovariectomized group (2.7 mm). The weight loss and decrease in cancellous bone area in the ovariectomized group were likely due to bone resorption enhanced by estrogen deficiency. However, oral administration of SEM at 500 mg/kg/day for 4 weeks prevented this decrease, as shown in Fig. 34.5.

FIGURE 34.3  Effect of methanolic extract of S. fusiforme (SME) on osteoclast formation. Closed and open columns indicate cell viability and osteoclast formation, respectively. TRAP-positive multinucleated cells that had more than three nuclei were counted. Cell viability was determined by MTT assay. Data are expressed as the mean ± SE of four cultures. ***p < 0.005 versus control using Student’s t-test.
FIGURE 34.4 Effects of methanolic extract of *S. fusiforme* (SME) on osteoblast differentiation *in vitro*. Osteoblasts are differentiated from stem cells by their own mediators *in vitro*. Differentiation rates were evaluated by measuring alkaline phosphatase (ALP) in comparison with control cells without samples. Bone morphogenetic protein 2 (BMP2) was used as a positive control to accelerate the differentiation of osteoblasts. Data are expressed as the mean ± SE of four cultures. *p < 0.05 and ***p < 0.005 versus control.

FIGURE 34.5 Effects of methanolic extract of *S. fusiforme* (SME) on bone loss in ovariectomized mice. Four-week-old female ddY mice were subjected to ovariectomy or sham operation under anesthesia. Oral administration of the vehicle or SME dissolved in distilled water (DW) was started from the day after surgery and continued for 5 days per week for 4 weeks. After 4 weeks, mouse body weight was measured and the animals were sacrificed to retrieve their femurs and uterine weight. Dry weight was measured using the right femur after drying at 60 °C for 24 h. Cancellous bone of the distal femur was measured on a photograph of the ground femur. Data are expressed as the mean ± SE of four cultures. *p < 0.05 and ***p < 0.005 versus control (SME = 0 mg/kg).
SME both promoted osteoblast differentiation and inhibited osteoclast differentiation in vitro. Its ability to suppress bone loss has also been demonstrated in vivo. SME has been suggested to regulate bone turnover by influencing both osteoblasts and osteoclasts. These two effects are thought to involve other compounds. As these effects were associated with the methanol extract, the active components are thought to be nonpolar, low-molecular-weight molecules. It is expected that the active components will be identified in the near future.

V. CONCLUSION

Extracts and purified compounds from various marine algae have been reported to suppress osteoclast differentiation. Further studies to elucidate the detailed mechanisms and the responsible components may hopefully show that these marine algae are a potential source of marine medicinal foods to prevent osteoporosis and related diseases.

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