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Review

Cardioprotective activity of polysaccharides derived from marine algae: An overview

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Polysaccharides are underexploited marine bioresources and a source of natural ingredients for functional foods. Cardioprotective property of polysaccharides derived from marine algae possess good nutrient and medicinal benefits and could be used as an alternative source of dietary fibre. Bioactive sulphated polysaccharides are the main components of soluble fibre in marine algae, hence making it a valuable source. This review gives an overview of the cardioprotective properties of polysaccharides derived from marine algae. Recent studies have provided evidence that polysaccharides (chitin and fucoidan) from marine algae can play a vital role in cardioprotective activity. Further research work, especially clinical studies, are needed in order to gain a better knowledge of the structure–function relationship whereby these polysaccharides can exert potent cardioprotective activities at safe levels.

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Introduction

Polysaccharides are emerging ingredients in biomedical applications because they are biodegradable, water soluble and functionally active. Polysaccharides for such applications are available from diverse sources including agricultural produce, microorganisms and marine resources. As more than 70% of the world's surface is covered by oceans, the wide diversity of marine organisms offer a rich source of natural products. The marine environment can be a source of functional materials, including polyunsaturated fatty acids (PUFA), polysaccharides, essential minerals and vitamins, antioxidants, enzymes and bioactive peptides (Kim & Wijesekara, 2010; Pomponi, 1999). Among marine organisms, algae are rich sources of structurally diverse bioactive compounds with various biological activities. Recently their importance as a source of novel bioactive substances has been growing rapidly, and research has revealed that compounds of marine algal origin exhibit antioxidant, immunomodulating, anticoagulant and antiulcer activities (Barrow & Shahidi, 2008).

Marine microorganisms are now considered to be efficient producers of biologically active and/or chemically novel compounds, and no "supply issue" should arise since scaled-up production can normally be achieved through the use of bioreactors, which can currently be designed to accommodate almost any capacity (Burgess, Jordan, Bregu, Spragg, & Boyd, 1999; Jensen & Fenical, 1994). Investigations in shake flasks are conducted with the prospect of large-scale processing in reactors. Different bioprocess engineering approaches are currently used for the production of polysaccharides from microorganisms. The major modes of operation in laboratory bioreactors and pilot plants are batch, fed-batch and continuous fermentation.

Seaweeds are macroscopic algae found attached to the bottom in relatively shallow coastal waters. They grow in intertidal, shallow and deep sea areas up to a depth of 180 m, and also in estuaries and brackish waters on solid substrates such as rocks, dead corals, pebbles, shells and other plant materials. They form one of the important living resources, and are grouped under three divisions, namely, green algae, brown algae and red algae. About 624 species have been reported in India (Anantharaman, 2002). Red seaweeds contribute 27.0%, brown 0.2% and others 72.8%. About 206 species of algae have been reported, especially from the mangrove environment. They are abundant on hard substrates and commonly extend to depths of 30–40 m.

Seaweeds are the only source of certain phytochemicals, namely agar–agar, carrageenan and algin, which are extensively used in various industries such as the food, confectionary, textile, pharmaceutical, dairy and paper industries, mostly as gelling, stabilizing and thickening agents. They are also used for human consumption, animal feed and as manure in several countries (Ismail & Hong, 2002). Edible marine algae, have attracted special interest as good sources of nutrients; one particularly interesting feature is their richness in sulphated polysaccharides (SPs), the uses of which span from the food, cosmetic and pharmaceutical industries to microbiology and biotechnology (Ren, 1997). These chemically anionic SP polymers are widespread not only in marine algae but also occur in animals, including both mammals and invertebrates (Mourao, 2007; Mourao & Pereira, 1999). Marine algae 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 amount of SPs present in different marine algae also varies. The major Polysaccharides found in marine algae include fucoidan and laminarans in brown algae, carrageenan in red algae and ulvan in green algae. This review focuses on polysaccharides derived from marine algae and presents an overview of their cardioprotective activities.

Cardiovascular diseases and nutraceuticals

Worldwide, the burden of chronic diseases such as cardiovascular diseases, cancer, diabetes and obesity is increasing rapidly. In 2001, chronic diseases contributed approximately 59% of the 56.5 million total reported deaths in the world and 46% of the global burden of disease. Cardiovascular diseases (CVD) is the name for the cluster of disorders afflicting the heart and blood vessels, including hypertension (high blood pressure), coronary heart disease (heart attack), cerebrovascular disease (stroke), heart failure and peripheral vascular disease. In 1999, CVD alone contributed to a third of global deaths and by 2010 it would become the leading cause of death in developing countries. The majority of CVD are preventable and controllable. It has been reported that low intake of fruits and vegetables is associated with a high mortality from CVD (Rissanen *et al*., 2003; Temple & Gladwin, 2003). Many research studies have identified a protective role for a diet rich in fruits and vegetables against CVD (Hu & Willett, 2002).

These considerations apart, nutraceuticals in the form of antioxidants, dietary fibres, omega-3 polyunsaturated fatty acids (*n*-3 PUFAs), vitamins, and minerals are recommended together with physical exercise for prevention and treatment of CVD. It has been demonstrated that molecules such as polyphenols present in grapes and wine alter cellular metabolism and signalling, which is consistent with a role in reducing arterial disease (German & Walzem, 2000).

Flavonoids are widely distributed in onions, endive, cruciferous vegetables, black grapes, red wine, grapefruit, apples, cherries and berries (Hollman, Hertog, & Katan, 1996). Flavonoids in plants, available either as flavones (the flavonoids apigenin found in chamomile), flavanones (hesperidin-citrus fruits; silybin-milk thistle) or flavonols (Quercetin and kaempferol-tea; rutin-buckwheat) or flavonglycosides (ginkgo) (Majoa, Guardiaa, Tripolia, Giammancoa, & Finotti, 2005) play a major role in curing cardiovascular diseases (Cook & Samman, 1996; Hollman, Feskens, & Katan, 1999).

Cholesterol has long been implicated as a significant risk factor in cardiovascular disease (Fig. 1). Sterols, known as phytosterols, occur in most plant species. Although green and yellow vegetables contain significant amounts, the sterols are concentrated in their seeds. Phytosterols compete with dietary cholesterol by blocking its uptake as well as facilitating its excretion from the body. Phytosterols in the diet have the potential to reduce morbidity and mortality from cardiovascular disease (Dutta, 2003). For example, *Fagopyrum esculentum Moench* (common buckwheat or sweet buckwheat) originated in Asia. Buckwheat seeds possess proteins, flavonoids, flavones, phytosterols, thiamin-binding proteins, etc. Buckwheat proteins are beneficial in constipation and obesity, and more importantly lower cholesterol and high blood pressure (Siquan & Zhang, 2001). Dietary fibre prepared from defatted rice bran has laxative and cholesterol-lowering qualities with attendant benefits towards prevention or alleviation of cardiovascular disease, diabetes, diverticulosis and colon cancer. It has been suggested that rice bran is a good fibre source (27%) that can be added to various food products (Hamid & Luan, 2000). Fatty acids of the omega-3 series (*n*-3 fatty acids) present in fish are well-established dietary components affecting plasma lipids and major cardiovascular disorders such as arrhythmias (Sidhu, 2003).

Globally, there are many marine polysaccharide-based preparations used in traditional systems of medicine for the treatment of CHD and its sequelae. The majority of marine polysaccharides are used alone or in combination with other compounds. Most of these marine polysaccharide-based medicines have not been scientifically investigated and there is little scientific or clinical data supporting their therapeutic use. However, for some specific seaweeds (dietary supplements and marine polysaccharide products) there are significant clinical data suggesting that the use of these products confers anticoagulant (Mao *et al*., 2009), anti-viral (Ponce, Pujol, Damonte, Flores, & Stortz, 2003), antioxidant (Ruperez, Ahrazem, & Leal, 2002), anti-cancer (Synytsya *et al*., 2010), anti-cholesterol and anti-free radical (Spolaore, Cassan, Duran, & Isambert, 2006) or cardioprotective effects (Thomesa, Murugan, Balu, & Ramasamy, 2010).

Marine algae that have adapted in extreme marine environments are one potential source for discovery of novel chemicals with health benefits. However, this potential remains to a large extent unexplored. Therefore, to facilitate discussion of this issue, the following review examines the

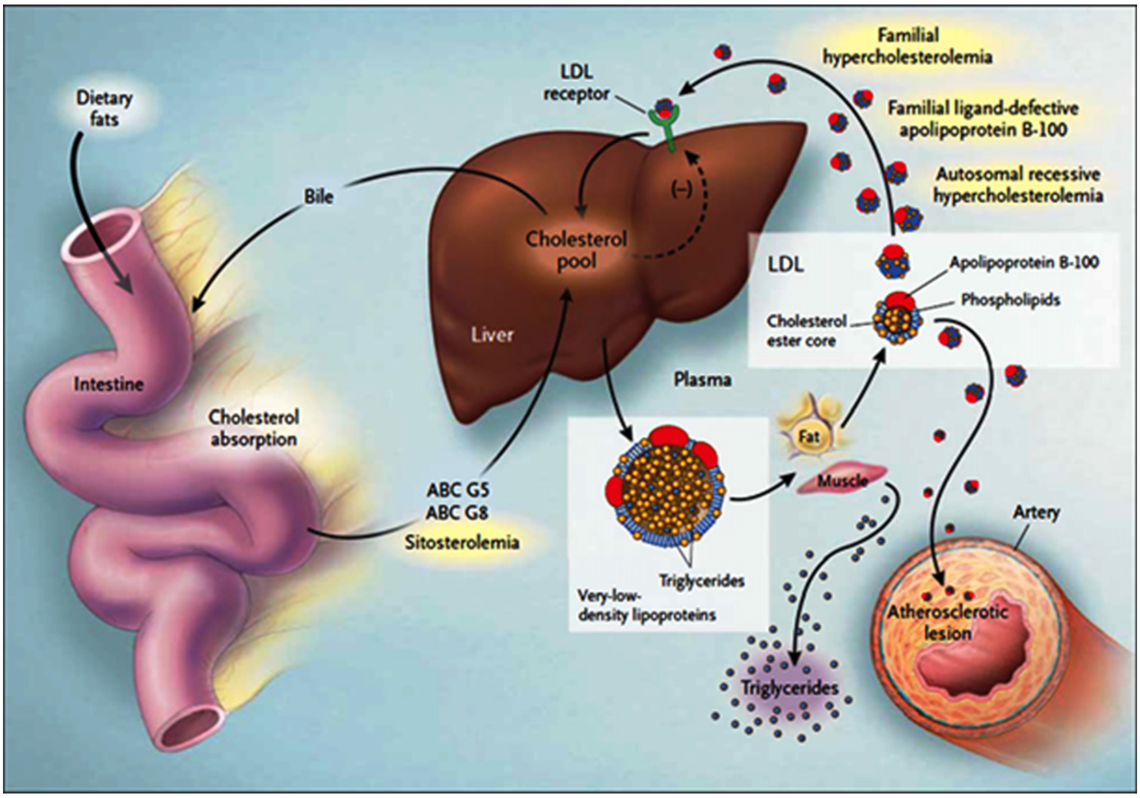

Fig. 1. The basic components of cholesterol synthesis and excretion (Elizabeth & Nabel, 2003).

existing scientific knowledge that demonstrates the suitability of marine derived polysaccharides as a functional food ingredient with cardioprotective activity.

Cardioprotective activity of marine algae derived polysaccharides

Chitin and chitosan

Chitin and chitosan are natural polyaminosaccharides, chitin being one of the world's most plentiful, renewable organic resources. A major constituent of the shells of crustaceans, the exoskeletons of insects and the cell walls of fungi, where it provides strength and stability, chitin is estimated to be synthesized and degraded in the biosphere in vast amounts, at least 10 Gt each year. Chemically, chitin is composed of (1 → 4) linked 2-acetamido-2-deoxy-β-D-glucose units (or acetyl-D-glucosamine) (Krajewska, 1991), forming a long chain linear polymer. It is insoluble in most solvents. Chitosan, the principal derivative of chitin, is obtained by varying degrees of *N*-deacetylation, depending on the source, and is consequently a copolymer of acetyl-D-glucosamine and D-glucosamine. Chitin and chitosan can be considered chemically as analogues of cellulose, in which the hydroxyl at carbon-2 has been replaced by acetamide or amino groups, respectively. Chitosan is insoluble in water, but the presence of amino groups renders it soluble in acidic solutions below pH about 6.5. It is important to note that chitin and chitosan are not single chemical entities, but vary in composition depending on the origin and manufacturing process. Chitosan can be defined as chitin sufficiently deacetylated to form soluble amine salts, the degree of deacetylation necessary to obtain a soluble product being 80–85% or higher.

Chitosan has been reported to possess antilipidemic (Santhosh, Sini, Anandan, & Mathew, 2006), antioxidant (Xie, Xu, & Liu, 2001) and membrane stabilizing properties (Filipovic-Grcic, Skalko-Basnet, & Jalsenjak, 2001). Lamiaa and Barakat (2011) indicated that both chitosan and wheat bran used individually or in combination had strong hypolipidemic, hypotriglyceridemic and hypocholesterolemic effects in serum and liver of normocholesterolemic and hypercholesterolemic rats, inducing a reduction in plasma LDL-C levels and an increase in HDL-C levels. Furthermore, the atherogenic index markedly decreased, causing a significant reduction in the LDL/HDL ratio in all groups fed a diet supplemented with either chitosan or wheat bran. This finding is in agreement with Makni *et al.* (2008), who stated that an increase in the HDL-C or HTR ratio is one of the most important criteria for an anti-hypercholesterolemic agent. Zhang, Liu, Li, & Xia (2008) suggested that the hypolipidemic activity of chitosan functions both in healthy and hypercholesterolemic rats. Chitosan significantly lowered total cholesterol and triacylglycerol levels in the plasma and liver and increased the faecal excretion of fat and cholesterol, consistent with previous reports (Kanauchi, Deuchi, Imasato, & Kobayashi, 1994).

Furthermore, the reductions in lipid levels in plasma and liver may be due to the increased faecal lipid excretion. Also, the increased excretion of faecal fat due to chitosan was consistent with our previous *in vitro* study (Zhou, Xia, Zhang, & Yu, 2006). Thus, the reduced absorption of cholesterol and fat is an important hypolipidemic action of chitosan. The plasma and liver total cholesterol and triacylglycerol levels in the hypercholesterolemic group fed chitosan were slightly higher than those in the healthy group fed chitosan. This result might suggest that chitosan can effectively prevent hypercholesterolaemia with a high fat diet, and longer treatment was better in terms of its hypocholesterolemic effect. It is possible the beneficial effects of chitosan may lead to the development of nutraceuticals.

Fucoidans

Fucoidans are a complex series of sulphated polysaccharides found widely in the cell walls of brown macroalgae. Fucoidans are reported to display numerous physiological and biological properties. In addition, the therapeutic potential of fucoidans increases with their degree of sulfation and they can be easily extracted using either hot water or an acid solution.

Thomesa *et al.* (2010) reported results investigating whether fucoidan of *Cladosiphon okamuranus* might affect isoproterenol induced myocardial infarction in rats. Isoproterenol induced marked elevations in the serum levels of creatine phosphokinase, lactate dehydrogenase and transaminases in animals. On the contrary, fucoidan treatment protected the structural and functional integrity of the myocardial membrane as evident from the significant reduction in the elevated levels of these serum marker enzymes in rats treated with fucoidan when compared to the isoproterenol treated rats. Increases in the activities of these enzymes are diagnostic indicators of myocardial infarction, indicative of cellular damage and loss of functional integrity of cell membrane (Bhakta *et al.*, 1999). The reversal of these enzyme activities by pretreatment with fucoidan indicates its therapeutic potential against myocardial infarction.

Fucoidan treatment also reduced lipid peroxidation activity and increased enzymatic and non-enzymatic antioxidant levels in rats, indicating a protective role for fucoidan against isoproterenol induced myocardial infarction. The formation of free radicals and accumulation of lipid peroxides is one possible biochemical mechanism for the myocardial damage caused by this catecholamine. Free radical scavenging enzymes such as catalase, superoxide dismutase and glutathione peroxidase are the first line cellular defense against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical (OH•). The equilibrium between these enzymes is essential for the effective removal of oxygen stress in intracellular organelles. The second line of defense consists of non-enzymatic scavenger's, *viz*., α-tocopherol and ascorbic acid, which scavenge residual free radicals escaping decomposition by antioxidant enzymes. Improved antioxidant levels (enzymatic: SOD, CAT, GPx, GSH, GST; non-enzymatic: reduced glutathione, α-tocopherol, ascorbic acid) in the hearts of animals treated with fucoidan indicate the potential of fucoidan in the treatment of myocardial infarction. Isoproterenol administration raised LDL-cholesterol and decreased HDL cholesterol levels in the serum. Interestingly, treatment with fucoidan reversed the effects of isoproterenol. Increased total cholesterol and LDL-cholesterol and decreased HDL cholesterol are associated with higher risk for myocardial infarction. High levels of circulating cholesterol and triglycerides and their accumulation in heart tissue are associated with cardiovascular damage. Hypertriglyceridemic patients at risk for cardiovascular disease often develop a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol, which causes myocardial membrane damage (Kelly, 1999). Hypertriglyceridemia has been seen in isoproterenol treated rats in cases of ischaemic heart disease. The anti-hypertriglyceridemia activity of fucoidan signifies that the myocardial membrane was protected against isoproterenol induced damage. Furthermore, histopathological findings have confirmed the induction of myocardial infarction by isoproterenol and the protection rendered by fucoidan treatment to the cardiac muscle.

Although fucoidans are high-molecular-weight polysaccharides, they exert biological effects in experimental animals after oral intake. Fucoidans activate lymphocytes and macrophages to render immune protection. They may exert their effects through gut-associated immunity before absorption, which is then transferred to the systemic immune response *via* lymph nodes and peripheral blood (Wu, Han, Bronson, Smith, & Meydani, 1998). Recent findings suggest that polysaccharides administered orally might also be partially absorbed intact *via* the hepatic portal vein and central lacteals into the general circulation. They accumulate in the mesenteric lymph nodes, Peyer's patches, spleen, liver and kidneys. Therefore, fucoidan has the potential to modulate the activity of the body's defence systems both at the mucous membrane and systemically.

Collectively, Thomesa *et al.* (2010) showed fucoidan was non-toxic and could protect cardiac cells from isoproterenol induced myocardial infarction. We hypothesis the protection of myocardium by fucoidan is likely due to the detoxification of isoproterenol through the antioxidant defence system and alterations in the lipid profile. Further studies are needed to determine the molecular mechanism by which fucoidan acts on myocardium to beneficially affect the cardiovascular system. Studies along this line can open new avenues for novel seaweed compounds in the treatment of cardiovascular dysfunction.

Alginate

Alginate is a natural occurring polysaccharide of guluronic (G) and mannuronic (M) acid, quite abundant in nature as structural component in marine brown algae

(*Phaeophyceae*) and as capsular polysaccharides in soil bacteria. Brown algal biomass generally consists of mineral or inorganic components and organic components, the latter mainly composed of alginates, fucans, and other carbohydrates. Alginates are also produced extracellularly by *Pseudomonas aeruginosa* and *Azotobacter vinelandii* (Johnson, Craig, & Mercer, 1997).

Over the last few years, the medical and pharmaceutical industries have shown an increased interest in biopolymers in general and alginates in particular. The reason for this increased interest is their usefulness in specific applications, as it enhances the efficient treatment of esophageal reflux and creates multiuse calcium fibres for use in dermatology and wound healing. They are also used for high and low gel strength dental impression materials. Besides this, alginate is an effective natural disintegrant and tablet binder, offering an attractive alternative for sustained-release systems. It offers advantages over synthetic polymers as it forms hydrogels under relatively mild conditions of pH and temperature, and is generally regarded as non-toxic, biocompatible, biodegradable, less expensive and abundantly available in nature; in addition, alginate meets the important requirement of being amenable to sterilization and storage. All these advantages make alginates very useful materials for biomedical applications, especially for controlled delivery of drugs and other biologically active compounds and for encapsulating cells. Calcium alginate is a natural haemostat, so alginate based dressings are indicated for bleeding wounds (Paul & Sharma, 2004).

Ulvan

Ulvan is a heteropolysaccharide composed mainly of rhamnose, xylose, glucose, glucuronic acid, iduronic acid and sulphate with smaller amounts of mannose, arabinose, and galactose. The primary repeating disaccharide units are [β-D-GlcAp A-(1 → 4)-α-L-Rhap 3S] and [α-L-Idop A-(1 → 4)-α-L-Rhap 3S]. *Ulva pertusa* is consumed by local inhabitants as a marine vegetable in Asia. It has been authorized for human consumption by French authorities due to its nutritional value, i.e. it is rich in vitamins, oligo elements, minerals and dietary fibres (Lahaye & Jegou, 1993). *U. pertusa* has been utilized for medical purposes for centuries in China. Algal extracts were traditionally used to treat hyperlipidaemia, sunstroke, urinary diseases, etc. The algal polysaccharide ulvan resists biodegradation by human endogenous enzymes and is considered to be a natural source of dietary fibres (Lahaye, 1991).

Yu *et al*. (2003) reported that ulvan and its subfractions had significantly different and even opposing effects on lipid parameters. Significant reductions in serum total and LDL-cholesterol concentrations and elevations of daily bile acid excretion in ulvan-fed rats suggested a mechanism of cholesterol breakdown into bile acid, by which ulvan effectively lowered serum cholesterol levels. When a diet supplemented with ulvan was consumed, other nutritional components were more easily digested and absorbed from the small intestine and ulvan became a major component in the gut lumen. Ulvan gelled with calcium ions in the body and increased the viscosity of the lumen's contents interfering with absorption of bile acid from the ileum. LDL-cholesterol was removed from blood and converted into bile acids by the liver to replace the bile acids lost in the stool, consequently serum LDL-cholesterol was also decreased simultaneously (Marlett, McBurney, & Slavin, 2002).

When ulvan was degraded into smaller fractions, the viscosity decreased and its ability to interfere with bile acids might be expected to shrink and even disappear. As a result, its cholesterol-lowering action degenerated; this postulation was in agreement with the results for total and LDL-cholesterol concentrations in the fractionated ulvan group. However, one confusing problem was that bile acid excretion in various ulvan-fed rats showed no significant differences. To elucidate the mechanism of ulvan's action in lowering cholesterol, further studies on various enzymes, such as HMG-CoA reductase or 7-alpha hydroxylase, are required.

Carrageenan

The major species of Rhodophyceae used for the commercial production of carrageenan is *Eucheuma spinosum*. Carrageenan is a high-molecular-weight linear polysaccharide comprising repeating galactose units and 3,6-anhydrogalactose (3,6 AG), both sulphated and non-sul phated, joined by alternating α-(1,3) and β-(1,4) glycosidic links. An almost continuous spectrum of carrageenans exists, but the work of Rees and co-workers (Anderson, Dolan, & Rees, 1965) was able to distinguish and attribute definite chemical structures to a small number of idealized polysaccharides. The findings of Panlasigui, Baello, Dimatangal, and Dumelod (2003) showed that use of carrageenan as the main source of dietary fibre may have brought about hypocholesterolemic effects due to its ability to bind bile acids and cholesterol in the lower small intestines. By binding up bile acids, less cholesterol can be absorbed. Since bile is made from cholesterol, circulating cholesterol is further decreased since it is used to compensate for the lost bile. Reductions in serum cholesterol and lipids may also be due to changes in the physical properties of the intestinal contents related to the ability of fibres to provide bulk, volume and viscosity to the contents – all of which are important in slowing the rate of digestion and absorption of nutrients. Dietary fibres provide bulk since they are not digested, so they remain during transit of food through the small intestines. The water-retaining capacity of carrageenan results in an increased volume of the intestinal contents. On the other hand, the viscosity of the intestinal contents increases due to the presence of viscous polysaccharides such as carrageenan.

Laminaran

Laminaran is a polysaccharide found in brown seaweed and occurring principally in the *Laminaria* spp and two form of laminaran are recognized; they are referred to as soluble and insoluble laminaran. Microsomal polysaccharide of laminaran has been noted to produce hypercholesterolemic and hypolipidemic responses due to reduced cholesterol absorption in the gut (Panlasigui *et al*., 2003). This is often coupled with an increase in the faecal cholesterol content and a hypoglycemic response (Dumeload, Ramirez, Tiangson, Barrios & Panlasigui, 1999). Others have reported lowering of systolic blood pressure (antihypertensive responses) and lower levels of total cholesterol, free cholesterol, triglyceride and phospholipids in the liver (Nishide & Uchida, 2003).

This review has provided evidence that marine algal derived polysaccharides can play a vital cardioprotective role through various mechanisms. They can be easily utilized to produce functional ingredients. The possibilities for designing new functional foods and pharmaceuticals to support reducing or regulating chronic diet related pathologies are promising. Therefore, it is reasonable to suggest that due to its valuable biological functions with beneficial health effects, marine algal derived polysaccharides have great potential as active ingredients for the preparation of nutraceuticals, cosmeceuticals and phar maceutical products.

Future perspective

A brief review of recent advances in applications of polysaccharides of marine origin for cardioprotective activity has been reported. Many algal polysaccharides are a source of cardioprotective activities that may find therapeutic benefit. They are structurally diverse and heterogeneous, which makes studies of their structures challenging and may also have hindered their development as therapeutic agents to date. The production of a standardized commercial product based on algal polysaccharide constituents will be a challenge since their structural and pharmacological features may vary depending on species, location and time of harvest. Some of the hypolipidemic effects of seaweed polysaccharides arise through effects on bile acid sequestration in the intestinal lumen. Some algal polysaccharides are already used topically in cosmetics and there is significant interest in further development for cosmetics and cosmeceuticals products. Until now, the cardioprotective activities of marine derived polysaccharides have been observed *in vitro* or in mouse model systems. Therefore, further research studies are needed in order to investigate their activity in clinical subjects for nutraceuticals.

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