Natural products- a source of therapy

Terrestrial plants, especially higher plants, have a long history of use in the treatment of human diseases. Several well-known species, including licorice (*Glycyrrhiza glabra*), myrrh (*Commiphora* species) and poppy capsule latex (*Papaver somniferum*), were referred by the first known written record on clay tablets from Mesopotamia in 2600 BC, and these plants are still in use today for the treatment of various diseases as ingredients of official drugs or herbal preparations used in systems of traditional medicine (Newman *et al.*, 2000). Furthermore, morphine, codeine, noscapine (narcotine) and papaverine isolated from *Papaver somniferum* were developed as single chemical drugs and are still clinically used. Hemisuccinate carbenoxolone sodium, a semi-synthetic derivative of glycyrrhetic acid found in licorice, is prescribed for the treatment of gastric and duodenal ulcers in various countries (Dewick, 2002). Apomorphine is a derivative of morphine isolated from poppy (*Papaver somniferum*). Subcutaneous apomorphine is currently used for the management of sudden, unexpected and refractory levodopa-induced off states in fluctuating Parkinson’s disease (Deleu *et al.*, 2004). Nitisinone is a derivative of leptospermone, an important new class of herbicides from the bottlebrush plant (*Callistemon citrinus*) and exerts an inhibitory effect for *p*-hydroxyphenylpyruvate dioxygenase (HPPD) involved in plastoquinone synthesis (Hall *et al.*, 2001). This drug has been used successfully as a treatment of Hereditary Tyrosinaemia type 1 (HT-1), a severe inherited
disease of humans caused by a deficiency of fumaryl acetoacetate hydrolase (FAH), leading to accumulation of fumaryl and maleyl acetoacetate, and progressive liver and kidney damage (Mitchell et al., 2001). Galantamine hydrobromide is an Amaryllidaceae alkaloid obtained from *Galanthus nivalis* that has been used traditionally for neurological conditions (Howes et al., 2003; Heinrich et al., 2004).

Until the development of penicillin in the early 1940s, most natural product-derived drugs were obtained from terrestrial plants. The success of penicillin in treating infection led to an expansion in the area of drug discovery from microorganisms. Terrestrial microorganisms are a plentiful source of structurally diverse bioactive substances, and have provided important contributions to the discovery of antibacterial agents including penicillin, cephalosporin, amino glycosides, tetracycline, and polyketides (Dewick, 2002). Current therapeutic applications of metabolites from microorganisms have expanded into immunosuppressive agents (e.g., cyclosporine and rapamycin), cholesterol-lowering agents (e.g., lovastatin and mevastatin), antihelmintic agents (e.g., ivermectin), an antidiabetic agent (acarbose), and anticancer agents (e.g., pentostatin, peplomycin, and epirubicin) (Newman et al., 2003; Butler, 2005; Sneader, 2005).

By the early 1950s, an impetus to learn more about marine organisms arose. The earliest biologically active substance of marine origin was a toxin named holothurin, which was extracted from a marine organism, the *Actinopyga agassizi* (Nigrelli et al., 1967). Holothurin showed some antitumor activities in mice. Since then, the search for drugs and natural products of interest from marine organisms has continued. Among the first bioactive compounds from marine sources, spongouridine and
spongothymidine from the Caribbean sponge (*Cryptotheca crypta*), were isolated serendipitously in the early 1950s (Newman and Cragg, 2004a). They were approved as an anticancer drug (cytosine arabinoside, Ara-C) and an antiviral drug (adenine arabinoside, Ara-A), respectively, 15 years later (Newman and Cragg, 2004b). The secondary metabolites of marine organisms have been studied extensively over the past 30 years. Recently, much attention has been given to marine organisms due to their considerable biodiversity that has been found in the widespread oceans that cover over 70% of the world (Jensen *et al*., 2000). Structurally unique secondary metabolites have been isolated and identified from marine organisms and, consequently, a compound based on new chemical template has been developed and launched, while numerous other candidates are in clinical trials (Butler, 2005; Newman and Cragg, 2004a; Newman and Cragg, 2004b).

Marine bacteria and fungi are prime producers of the antagonistic substances in the oceanic environment. Antibiotic, antiviral, antifungal, and anti yeast activities of these organisms had been reported (Buck *et al*., 1962). A bromo pyrrole antibiotic has been isolated from *Pseudomonas bromoutilis* (Lovell, 1966), which showed activity against many Gram-positive bacteria. *Serratia marcescens*, a widely distributed non-pathogenic bacterium, had furnished a red coloured antibiotic named prodigiosin. It exhibited high order of antibiotic and antifungal activities. The high toxicity of prodigiosin precluded its use as a therapeutic agent. Studies on the marine phytoplanktons are few because of the difficulty in growing the organisms and the low yield of secondary metabolites. However, several toxins related to saxitoxin are isolated from *Gonyaulax* species. A penicillinnase sensitive antibiotic substance named antibiotic N which is active against Gram-
negative bacteria, had been isolated from marine isolate of the fungus *Cephalosporium acremonium*.

Several metabolites of unusual structure and exhibiting biological activities have been isolated from marine animals (Blunt *et al*., 2004; Faulkner, 1994; Bhakuni, 1994). Some of these bioactive metabolites have biomedical potential. The bioactive metabolites that are of interest have been mainly isolated from marine sponges, jelly fish, sea anemones, corals, bryozoans, molluscs, echinoderms, tunicates and crustaceans. The bioactive metabolites isolated from marine animals can be divided into steroids, terpenoids, isoprenoids, nonisoprenoids, quinones, brominated compounds, nitrogen heterocyclics, and nitrogen sulphur heterocyclics. The bioactive compounds isolated from marine animals, which have steroidal nucleus are insect moulting hormones, sterols and saponins. Karlson, (1956) found that an extract of crustacean (*Cragon vulgaris*) was active in the insect (*Calliphora*) test. A large number of sterols have been isolated from marine animals. Some of the sterols, such as fucosterol isolated from marine sources have been reported to be nontoxic and have the ability to reduce blood cholesterol levels and also exhibit antidiabetic activity (Lee *et al*., 2004). The sterols also appear to reduce the tendency to form a fatty liver and excessive fat deposition in the heart. Majority of the marine natural products have been isolated from sponges, coelenterates (sea whips, sea fans and soft corals), tunicates, opisthobranch molluscs (nudibranchs, sea hares, etc.), echinoderms (starfish, sea cucumbers, etc.) and bryozoans (moss animals) (Kijjoa and Sawangwong, 2004). Sponges, the most primitive multicellular invertebrates, considered as a gold mine during the past 50 years. Cytarabine (Cytostar-U) also known as Ara-C, a compound isolated from the Caribbean sponge *Cryptotheca crypta* currently being used with other anticancer drugs in the
treatment of acute myelocytic leukaemia (AML) and lymphomas (Schwartsmann et al., 2003). Acyclovir, synthetically known as Ara-A, was modelled based on sponge-derived spongothymidine or spongouridine. Ara-A is the first sponge-derived antiviral compound in the market. Polyketide Calyculin A (a selective inhibitor of protein phosphatise 1, isolated from sponge Discodermia calyx), Manoalide (a potent anti-inflammatory marine natural product and a direct inactivator of venom phospholipase A2), Okadaic acid, a potent inhibitor of protein phosphatases, especially protein phosphatases 1 and 2 respectively isolated from Laffariella variabilis and Halichondria okadai has reached the market undergoing from basic research to long phases of clinical study (Wakimoto, et al., 2002; Lombardo and Dennis, 1985). Squalamine, an amino sterol purified from the dogfish shark, Squalus acanthias, is an inhibitor of growth factor-mediated endothelial cell proliferation and migration and angiogenesis. (Hao et al., 2003). Ecteinascidin 743, a potent antitumor agent, was isolated from the marine tunicate Ecteinascidia turbinata (Soares et al., 2005; Rinehart, 2000).

Extensive work has been done on the secondary metabolites of marine algae (Faulkner, 2002). The green, brown and red algae had been extensively analyzed for antibacterial and antifungal activities. Chemicals responsible for antibiotic activities are widespread in macroalgae. Interesting substances in particular are the halogenated compounds such as haloforms, halogenated alkanes and alkenes, alcohols, aldehydes, hydroquinones and ketones (Lincoln et al., 1991). The depsipeptides kahalalide A and F from Bryopsis sp. were noted for their in vitro activity against Mycobacterium tuberculosis (Sayed et al., 2000). A promising antibacterial agent is a halogenated furanone, or fimbrolide that belong to a class of lactones was isolated from Delisea pulchra. It has been examined for its effectiveness as an active
ingredient in bacterial antifouling agents (Kjelleberg and Steinberg, 2001), and as a possible treatment for chronic *Pseudomonas aeruginosa* infection. Kahalalide F which is produced by *Bryopsis* sp. and subsequently assimilated by the grazer *Elysia rufescens* has anticancer and antitumor properties (Hamann and Scheuer, 1993; Hamann *et al*., 1996). It is effective in controlling tumours that cause lung, colon and prostate cancer (Horgen *et al*., 2000; Nuijen *et al*., 2000; Sparidans *et al*., 2001). The sulphated polysaccharides obtained from seaweeds are economically most important products due to their extensive use in food and medicine.

Among the bioactive compounds from marine source the polysaccharides gained more attention due to their structural diversity and wide range of therapeutic potential. Several polysaccharides were isolated from various marine source including marine invertebrates, bacteria, fungi and algae. Hyaluronic acid, chondroitin sulphate, dermatan sulphate and heparan sulphate can be found in marine invertebrates; they have been isolated from marine molluscs or echinoderms such as sea urchins or sea cucumbers (ascidians). GAGs can be extracted from marine mollusc *Amussium pleuronectus* (Linne). The structural characterization showed that they are sulphated like heparin and contain equivalent amount of uronic acid and hexosamine (Saravanan and Shanmugam, 2010). The dermatan sulphates isolated from sea urchin and chondroitin sulphates from ascidians have the same backbone structures as the mammalian GAGs but possess different sulfation patterns (Vilela-Silva *et al*., 1999; Tapon-Bretaudiere *et al*., 2002). In animal models, the fucosylated chondroitin sulphate obtained from sea cucumber was a promising molecule with possible beneficial effects in pathological conditions such as thrombosis and ischemia (Tapon-Bretaudiere *et al*., 2002). Biological properties of sulphated
fucoidans (or fucans) extracted from marine invertebrates such as sea urchins or sea cucumbers have been extensively studied. These polymers of L-fucose are homogeneous and unbranched and bear no substituent other than sulphate and they present anticoagulant and antithrombotic activities. They can act as a ligand for either L- or P-selectins like heparin or heparan sulphate. They are also active on cell growth, migration and adhesion (Berteau and Mulloy, 2003). Primary and secondary products of deep-sea microorganisms, bacteria and archaea, present a great interest to biotechnology and a potential for pharmaceutical applications (Pace, 1991; Desbruyeres et al., 1998). *Spirulina* is a microalga which offers a broad range of applications such as a nutritive or pharmaceutical additive with no risk to health. Clinical studies suggest that compounds in the microalgae have therapeutic functions and especially polysaccharides with anti-inflammatory effects (Matsui et al., 2003). Chitosan is a copolymer of (1-4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. It is obtained by de-acetylation of the natural occurring chitin. Chitin is extracted from the exoskeleton of marine organisms, mainly crabs and shrimps, as described by (Burrows et al., 2007). The major applications of chitosan are in biomaterials, pharmaceuticals, cosmetics, metal ion sequestration, agriculture, and foodstuff treatment (flocculation, clarification etc. because of its efficient interaction with other polyelectrolytes). Development of chitosan chemistry is relevant in biomedical science, particularly in the topic of drug delivery (Kumar et al., 2004; Muzzarelli and Muzzarelli, 2005).

Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. Due to their low content in lipids, high concentration in polysaccharides, natural richness in minerals, polyunsaturated fatty acids and vitamins as well as their content
in bioactive molecules, marine algae are known to be a good source of healthy food. Unlike the land plants, these algae have no roots, leaves or vascular systems; however they nourish themselves through the process of osmosis. Two major types of algae that have been identified are the microalgae which are found in both benthic and littoral habitats and also throughout the ocean waters as phytoplankton and the macroalgae or seaweeds which occupy the littoral zone. Seaweeds grow in the intertidal as well as in the sub-tidal area up to a certain depth where very little photosynthetic light is available. Seaweeds are classified into green algae (chlorophyta), brown algae (phaeophyta) and red algae (rhodophyta) on the basis of chemical composition. The colour in case of green seaweeds is due to the presence of chlorophyll a and b in the same proportions as the 'higher' plants; beta-carotene (a yellow pigment) and various characteristic xanthophylls (yellowish or brownish pigments). The dominance of the xanthophylls pigments, fucoxanthin, is responsible for the colour of brown seaweeds. This compound masks the other pigments such as Chlorophyll a and c and other xanthophylls. Phycoerythrin and phycocyanin mask the pigments such as Chlorophyll a and beta-carotene and are responsible for the colour of red seaweeds. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. They are an excellent source of vitamins such as A, B1, B12, C, D and E, riboflavin, niacin, pantothanic acid and folic acid as well as minerals such as Ca, P, Na, K (Dhargalkar and Pereira, 2005)

Seaweed cultivation has become a major industry in Asia thereby making the utilisation of these plants to the maximum extent (David, 2002). Seaweed is traditionally consumed in Far East countries and in Hawaiian
Islands, while in the West the principal use of seaweeds is as sources of phycocolloids, thickening and gelling agents for various industrial applications including food. Chemical composition of seaweeds varies with species, habitat, maturity, salinity, temperature, light intensity and environmental condition (Floreto and Teshima, 1998). In comparison to cultivated vegetables, edible seaweeds are potentially good sources of non-starch polysaccharides, minerals, trace elements and certain vitamins (Wong and Cheung, 2000). Seaweeds have been studied for long for production of industrially important polysaccharides like agar, carrageenan, fucoidan etc. However they have not been looked upon as a source of lipids as these are found in relatively small quantities (Ramavat et al., 1997).

The green macroalgal genera _Ulva_ and _Enteromorpha_ are well known for their wide distributions from marine to fresh water all over the world (Canter-Lund and Lund 1995; van den Hoek et al., 1995). _Ulva_ includes approximately 100 species and _Enteromorpha_ includes some 80 species (Guiry and Nic Dhonncha 2002). Only a single morphological feature has distinguished _Ulva_ and _Enteromorpha_: _Ulva_ has a distromatic flat blade, while _Enteromorpha_ has a monostromatic tubular thallus (van den Hoek et al. 1995). The cosmopolitan genus _Ulva_ Linnaeus, commonly known as the “sea lettuce”, is represented by species distributed in all oceans and estuaries of the world (Guiry and Guiry, 2008). _Ulva sps_ is rich in cell-wall polysaccharides, including cellulose and water-soluble polysaccharides that contain sulphate groups. The main type of water-soluble polysaccharide is ulvan, the main component of which is a disaccharide formed by β-D-glucuronic acid (1, 4)-L-rhamnose 3 sulphate (Lahaye , 1998; Paradossi et al., 1999).
Polysaccharides widely exist in the plants, microorganism (fungi and bacteria), algae, and animals and they represent a structurally diverse class of macromolecules of relatively widespread occurrence in nature. Polysaccharides offer the highest capacity for carrying biological information because they have the greatest potential for structural variability (Sharon and Lis, 1993). Polysaccharides or polyglycans are polymers of monosaccharide residues that are joined together by glycosidic bonds, which are formed by the elimination of elements of water, between the hemiacetal hydroxyl group of one residue and a primary or secondary hydroxyl group of an adjacent residue (Laere et al., 2000). The monomer species may be simple monosaccharides or sugar derivatives such as N-acetylaminosugars, uronic acids or ester sulphate sugars. Uronic acids are constituents of hemicelluloses, pectin, gums, mucilage and other plant polysaccharides. Uronic acids occur widely in nature and much of the carbohydrate materials in plants contain this important component (Ridley et al., 2001). Recently, polysaccharides from mushroom, plant and microbe have received considerable attention due to their biological activities, such as anti-tumour (Tong et al., 2009; Yang et al., 2005), antivirus (Yim et al., 2004), anti-oxidation (Rout and Banerjee, 2007; Tsai et al., 2007; Wang and Luo, 2007), anti-complementary (Xu et al., 2007), anticoagulant (Yoon et al., 2003), immunostimulant and immunological activities (Yang et al., 2008), which made them possible to be used in many fields including food, cosmetics, biomedicine, agriculture, environmental protection and wastewater management (Bravin et al., 2006).

Polysaccharide exhibit various biological activities affected by different chemical structures and in general, it is interesting and important to elucidate the relation among chemical structures, chain conformations of polysaccharides and their biological activities. However, polysaccharides are
usually composed of various monosaccharides linked with different glucosidic bonds. Some polysaccharides have hyper branched structures. Moreover, polysaccharides often have high molecular weights, and tend to form aggregates in solution that can mask the behaviour of individual macromolecules. In consequence, to characterize the chemical structures and chain conformations of polysaccharides is not an easy task (Liqun and Li, 2009). The chemical structures of polysaccharides, such as the sugar composition, type of glycosyl linkage and the branch structures, may be characterized by spectral analysis, chemical analysis and chromatography. FTIR spectroscopy is used to investigate the vibrations of molecules and polar bonds between the different atoms. Structures of polysaccharides, such as monosaccharide types, glucosidic bonds and functional groups, can be analyzed using FTIR spectroscopy (Mathlouthi and Koenig, 1986; Zhang, 1994). NMR spectroscopy, especially liquid-state NMR, has become recognized as an important developing tool for chemical structural analysis of polysaccharides (Bubb, 2003). The size of polysaccharides in solution can be measured by dynamic light scattering (DLS) method (Goh et al., 2006; Li et al., 2006; Ma et al., 2008). Fluorescence correlation spectroscopy (FCS) is interesting to determine the conformations and sizes of polysaccharides at lower concentration (Meunier and Wilkinson, 2002). A powerful tool for the structural elucidation of sulphated polysaccharides is NMR spectroscopy, which can provide structural details such as the monosaccharide components, linkages, anomeric configurations, and positions of branching or sulfations. This can be done by combining various 1D and 2D-NMR techniques. The use of NMR in the structural analysis of red algal galactans and green algal ulvans has been considerable (Lahaye and Robic, 2007; Usov et al., 1980). In part, this has been due to the relative high
proportion of repeating sequences (identical sulphation pattern) in these polysaccharides that make them amenable to analysis by $^{13}$CNMR. For example, Gonçalves et al., (2005) described the structural elucidation by NMR of positional isomers of sulphated oligosaccharides obtained from agarans and carrageenans. This entailed partial reductive hydrolysis to produce oligosaccharides from repetitive galactans followed by separation by anion exchange and gel-filtration chromatography prior to 1D and 2D NMR analysis. Effort has also been made to elucidate the structures of fucans by NMR. 1D-NMR of the sulphated fucans from Saccharina latissima (formerly Laminaria saccharina) was found to be 1→3 linked α-L-fucopyranose with a sulphate group at C4 and branched at C2 (Usov et al., 1998). More recently, they confirmed the complex structure of this fucoidan with a more detailed structural investigation by 2D-NMR (Bilan, et al., 2010). These studies also revealed the presence of three additional sulphated polysaccharide types, a fucogalactan, a fucoglucuronomannan and a fucoglucuronan, that appear to occur in minor amounts in their preparation.

Mass spectrometry (MS) is valuable in the structural analysis of polysaccharides, as it generates accurate molecular mass data for oligosaccharides and it can also provide sequence information. Compared with other analytical techniques, mass spectrometric methods have several advantages, including low sample consumption (e.g., picomole quantities) and short analysis time. While analysis of sulphated polysaccharides by MS can be problematic due to the labile nature of the sulphate groups, approaches based on electrospray ionization and matrix-assisted laser desorption/ionization (MALDI) are increasingly being developed to characterize sulphated oligosaccharides (Daniel et al., 2007; Fatema et al., 2010; Goncalves et al., 2010; Yang et al., 2009). Negative-ion ESI-CID-
MS/MS was used to characterize oligosaccharide fragments derived from mild hydrolysis of κ-carrageenan that revealed highly ordered disaccharide repeats leading to a complete series of exclusively odd-numbered oligosaccharides (Yang et al., 2009). Similarly, fucan oligosaccharides from A. nodosum, including a highly sulphated pentasaccharide, were analyzed successfully by negative ion ESI-MS (Daniel et al., 2007).

Due to the wide variations in their molecular weights, structural parameters and physiological characteristics, seaweed polysaccharides show diverse bioactivities, such as antiproliferative or antitumor, anticoagulant, antioxidant and anti-inflammatory (Riou et al., 1996).

Chemical structure and activities of polysaccharides isolated from various sources.

<table>
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<th>Polysaccharides</th>
<th>Chemical structures</th>
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<tr>
<td>Auricularia auricular</td>
<td>Heteropolysaccharides consisted of a backbone chain of β-1,3-D-glucose residues with various branch residues, such as mannose, glucose, xylose and glucuronic acid.</td>
<td>Antitumor, immunomodulatory, anticoagulant activity, anti-inflammatory and antioxidant activity.</td>
<td>Fan et al., (2006), Yoon et al., (2003).</td>
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<tr>
<td>Heparin</td>
<td>A low-sulphated polysaccharide showing a specific decrease of the sulphation in position 2 of the uronic acid units.</td>
<td>Anticoagulant activity primarily from its binding to the serine protease inhibitor (SERPIN) antithrombin III.</td>
<td>Luppi et al., (2005)</td>
</tr>
<tr>
<td>α-D-Glucans isolated from the cell wall of <em>S. cerevisiae</em></td>
<td>(1 → 3)-α-D-Glucans moderately branched with (1 → 6)-α-D-glucan chains.</td>
<td>To Adsorb zearealenone, reduce its bioavailability in the digestive tract, and protect animals against its adverse effects.</td>
<td>Yiannikouris et al., (2006)</td>
</tr>
<tr>
<td>Ulvan from green seaweeds</td>
<td>Composed of variable proportions of different repeating sequences mostly based on disaccharide domains made of rhamnose, glucuronic acid, iduronic acid, xylose, and sulphate.</td>
<td>Antitumor and immune modulation activities, strain-specific anti-influenza activities, and anticoagulant activities.</td>
<td>Lahaye and Robic, (2007).</td>
</tr>
<tr>
<td><em>Spirulina platensis</em> polysaccharide</td>
<td>Sulfated polysaccharide consisted of two types of disaccharide repeating units, O-hexuronosylrhamnose (aldoburonic acid) and O-rhamnosyl-3-O-methylrhamnos (acofriose) with sulfate.</td>
<td>Anti-atherogenic and anti-hromobogenic Activities.</td>
<td>Kaji et al., (2002, 2004).</td>
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Red seaweed galactans are of great commercial importance as they are used widely in the food industry because of their rheological properties as gelling and thickening agents. These sulphated polysaccharides are primarily...
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classified as agarans and carrageenans based on their stereochemistry, specifically galactans with 4-linked α-galactose residues of the L-series are termed agarans and those of the D-series are termed carrageenans (Knutsen et al., 1994). Thus, carrageenans are high molecular weight sulphated D-galactans composed of repeating disaccharide units with alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-galactopyranose (D-units) or 3, 6-anhydro-α-galactopyranose (AnGal-units). Carrageenans are normally classified according to their structural characteristics, including their sulfation patterns and the presence or absence of AnGal on D-units. There are at least 15 different carrageenan structures. The most industrially relevant carrageenans are κ, ι and λ forms. The major source of κ-carrageenan is the red seaweed Kappaphycus alvarezii (Anderson et al., 1973). Its structure was reported as alternating 3-linked β-D-galactose 4-sulfate and 4-linked AnGal units (Estevez et al., 2000). The ι-carrageenans have an additional sulphate group on C2 (O) of the AnGal residue, resulting in two sulphates per disaccharide repeating unit. Funami et al., (2007) examined ι-carrageenan extracted from Eucheuma spinosum using atomic force microscopy and suggested that ι-carrageenans were more homogeneous and flexible than κ-carrageenans. The λ-carrageenans have three sulphate groups per disaccharide unit with the third sulphate group of this form at the C6 position of the 4-linked residue, but there is no 3, 6-anhydride bridge on the 4-linked residues. Lambda-carrageenan is obtained from species of the Gigartina and Chondrus genera (Zhou et al., 2006). One of the best studied agarans is porphyran (Morrice et al., 1984), obtained from Porphyra species of red algae including Porphyra capensis (Zhang et al., 2005) and P. haitanensis (Zhang et al., 2009; Zhang et al., 2004). Porphyran typical exhibits a linear backbone of alternating 3-linked β-D-galactose and 4-linked
α-L-galactose-6-sulfate or 3,6-anhydro-α-L-galactose units. Sulphated agarans of a similar linear form are synthesized by *Polysiphonia* species, such as *P. strictissima*, *P. abscissoides* (Miller and Furneaux, 1997), *P. nigrescens* (Prado *et al.*, 2008) and *P. atterima* (Miller, 2003). The regular agaran backbone may be interrupted by different *O*-linked substitutions in addition to sulphate including methyl and xylosyl groups adding to the structural diversity. For example, the sulphated agaran from *P. nigrescens* is highly substituted on the C-6 of β-D-galactose with sulphate, but methyl ether and β-D-xylose residues were also present (Prado *et al.*, 2008). Agaran from *Acanthophora spicifera*, is highly sulphated at the C-2 position of β-D-galactose units, with some of the residues being 4, 6-pyruvylated (Gonçalves *et al.*, 2002). This agaran also contains small amounts of xylose and sulphated xylose residues (Gonçalves *et al.*, 2002; Duarte *et al.*, 2004). In addition to carrageenans and agarans, there are also red seaweed sulphated polysaccharides that have 4-linked D- and L-galactose sugars distributed within the same polysaccharide molecules, so-called DL-hybrids, and others with various substitutions involving sulphate groups, pyruvic acid ketals, and methoxyl groups (Stortz and Cerezo, 2000). Example of non-ideal sulphated galactans are xylogalactans, first described in the red seaweed *Corallina officinalis* and termed corallinan (Cases *et al.*, 1994), which are agarans that have β-D-xylosyl groups attached at the O-6 position of D-galactose units (Navarro and Stortz, 2008; Martone *et al.*, 2010). It should be noted that red seaweeds also produce other types of sulphated polysaccharides including those with mannose in their backbones (Lim and Ryu, 2009; Mandal *et al.*, 2008). For example, xylomannnan from *Scinaia hatei* consisting primarily of a backbone of α-(1→3)-linked D-mannose residues substituted at C-6, C-4, and C-2 with β-D-xylosyl residues (Mandal *et al.*, 2008).
Fucans are sulphated polysaccharides that are composed of a fucose backbone. One of the best studied fucans from brown algae is fucoidan, which was first isolated by Kylin, (1913). The fucoidan from *Fucus vesiculosus* has been available commercially for decades. Early work on its structure showed that it contained primarily (1→2) linked 4-O-sulfated fucopyranose residues (Conchie *et al*., 1950). However, 3-linked fucoses with 4-sulfated groups were subsequently reported to be present on some of the fucose residues (Patankar *et al*., 1993). Additionally, it was determined to contain branches every 2–3 fucose residues. Fucans can differ in structure among algal species and can vary even within the same species. Because of the heterogeneity in structures within seaweed, differing extraction conditions used by researchers can give rise to the isolation of distinct fucan forms (Li *et al*., 2008). Fucans have been classified into two groups (Ushakova *et al*., 2009). One group includes the fucans from *Laminaria saccharina, L. digitata, Analipus japonicus, Cladosiphon okamuranus,* and *Chorda filum* that have their central chains composed of (1→3)-linked α-L-fucopyranose residues. A second group included fucans isolated from *Ascophyllum nodosum* and *Fucus* species that have their central chains composed of repeating (1→3)- and (1→4)-linked α-L-fucopyranose residues. However, many studies have revealed more complex fucans some with branching structures. A fucoidan isolated from *Turbinaria conoides* was shown to be highly complex, with 33–34% terminals, 27–28% linked and 21–22% branched in the (1→3)-linked main chain (Chattopadhyay *et al*., 2010).

Some green algae, particularly *Codium* species, are a significant source of sulphated galactans. Sulphated galactans from green algae tend to be more complex and heterogeneous in structure than their counterparts from red algae. For example, *C. fragile* and *C. cylindricum* contain sulphated
arabinogalactan and sulphated glucogalactan, respectively (Matsubara et al., 2001; Farias et al., 2008). Bilan et al., (2007) reported a highly ramified sulphated galactan from *C. yezoense* that contained a linear backbone of 3-linked β-D-galactopyranose residues containing short oligosaccharides branches through (1→6) linkages. Sulphate groups were found mainly at C-4 and in minor amounts at C-6. Polysaccharides containing sulphated galactans from other green seaweeds including *Caulerpa* and *Ulva* have been reported (Shevchenko et al., 2009; Mao et al., 2006), but the galactans are minor components. A variety of other forms of sulphated polysaccharides are synthesized by green seaweeds. This includes, for example, a water-soluble heteroglycuronan from *Enteromorpha compressa*, composed of (1→2,4)-linked rhamnose, (1→4)-linked xylose, and (1→4)-linked glucuronic acid units. Sulphate groups, when present, were situated at the C-3 of rhamnose and the C-2 of xylose. Recently, a rhamnan sulphate from *Monostroma nitidum* was shown to consist primarily of α-1, 3-linked and α-1,2-linked rhamnose residues (Percival and McDowell, 1967; Harada and Maeda, 1998; Ray, 2006).

Ulvan is the major water-soluble polysaccharide found in green seaweed of the order Ulvales (*Ulva* and *Enteromorpha* sp.) that has sulphate, rhamnose, xylose, iduronic and glucuronic acids as main constituents (Lahaye and Ray, 1996; Percival and McDowell, 1967). Ulvan structure shows great complexity and variability as evidenced by the numerous oligosaccharides repeating structural units identified in native and chemically modified ulvan preparations (Lahaye and Robic, 2007). The main repeating disaccharide units reported are ulvanobiouronic acid 3-sulfate types containing either glucuronic or iduronic acid. Additionally, minor repeat units have been reported that contain sulphated xylose replacing the uronic
acid or glucuronic acid as a branch on $O$-2 of the rhamnose-3-sulfate (Lahaye and Ray, 1996; Lahaye et al., 1997).

The most widely recognized and studied bioactivity of sulphated polysaccharides from marine algae is the heparin-like anticoagulant activity. This was first reported for fucoidan isolated from *F. vesiculosus* by Springer and colleagues who found inhibition of fibrin clot formation and antithrombin activity (Bernardi and Springer, 1962; Springer et al., 1957).

The normal physiological response that prevents significant blood loss following vascular injury is called haemostasis (Colman et al., 2006a). Familiarity with haemostasis lays the groundwork for a thorough understanding of the major disease states associated with thrombosis, such as venous thromboembolism (VTE), atherothrombosis (thrombosis triggered by plaque rupture), and cardioembolic stroke. The coagulation process that leads to haemostasis involves a complex set of protease reactions involving roughly 30 different proteins (Colman et al., 2006b). The final result of these reactions is to convert fibrinogen, a soluble protein, to insoluble strands of fibrin. Together with platelets, the fibrin strands form a stable blood clot.

Haemostasis is a finely tuned process that serves to maintain the integrity of the circulatory system (Adams et al., 2007). However, the process can go out of balance, leading to significant morbidity and mortality (Heit, 2005). Excessive coagulation leads to the formation of a thrombus, potentially obstructing blood flow. For decades, the coagulation cascade was conceptualised as having two distinct points of initiation, labelled the extrinsic and intrinsic pathways (Hoffman and Monroe, 2007). Over time, however, it has become clear that these pathways do not function in the body as parallel, independent systems.
Thrombus is a blood clot formed when there is an imbalance in the blood coagulation system. A thrombus can block the flow of blood through a vein or artery, and can detach from the vessel wall to become a life-threatening embolus when it lodges in the lungs or other vital organs (Colman et al., 2006a). Thrombosis, either arterial or venous, is a fatal and disabling consequence of cardiovascular disease, the leading cause of mortality and morbidity in developed countries (Lowe, 2008). Thrombosis occurs as a consequence of vascular injury, generally occurring at a vulnerable atherosclerotic plaque or under low-flow conditions, and imbalance between the pathways that regulate thrombus formation and/or dissolution.

Anticoagulants have been widely used both clinically and in vitro medical treatments. In clinical practice, they are the drugs of choice for the prevention and treatment of thromboembolic disorders, and prophylaxis of thrombotic events both pre- and post-surgery. Anticoagulants are used to improve the hemo-compatibility of medical devices and tissue engineering materials (Baumann, 2001). Recently, the concept of "vascular beautifying" has been promoted by the cosmetics industry (Wang et al., 2004); substances with anticoagulant activity are among the first choices as functional components which are being used to open up new areas of application for anticoagulants.

Heparin preparations are widely used for the treatment and prevention of arterial and venous thrombosis (Fareed et al., 2000). However, this glycosaminoglycan has several limitations due to collateral effects and limited source of material (Mourão, 2004). The anticoagulant activity of this heparin is due to its special polyanionic character (Huntington et al., 2000).
This structure, called glycosaminoglycan (GAG), is obtained by chemical processing of proteoglycan heparin present in porcine or bovine intestinal mucosa and lung. Interestingly, it is the most negatively charged compound in human body (Desai, 2004). The potential anticoagulant action of heparin is achieved mainly by potentiating of antithrombin and heparin cofactor II (Pereira et al., 1999) but it has long term side effects such as bleeding and thrombocytopenia, which may limit the use of it. The usual sources of heparin are pig or bovine tissues. Since the incidence of prion related diseases in mammals increased, better and safe alternative sources of antithrombotic compounds began to drive more attention.

Marine sulphated polysaccharides other than fucans have also been shown to possess anticoagulant activities. Reports include sulphated galactan and ulvan-like sulphated polysaccharides obtained from green algae, in particular from species of *Codium* and *Ulva* (Farias et al., 2008; Mao et al., 2006; Matsubara et al., 2001). Mao et al., (2006) described a sulphated polysaccharide from *U. conglobata* with high rhamnose content and 35% sulphate ester that prolonged clotting time through what appeared to be direct inhibition of thrombin and modulation of heparin cofactor II. Hayakawa et al. (2000) tested sulphated polysaccharides from 23 green algae species for anticoagulant activity and discovered a high rhamnose-containing sulphated polysaccharide from *Monostroma nitidum*, the purified version of which was more potent than standard heparin. Red seaweeds have also yielded a number of sulphated polysaccharides with potent anticoagulant activities (Farias et al., 2000; Pereira et al., 2005; Glauser et al., 2009). Studies on a sulphated galactan from the red seaweed *Botryocladia occidentalis* are particularly illustrative. Farias et al. (2000) reported that a 2, 3-di-\(\text{O}\)-sulfated D-galactan from *B. Occidentalis* exhibited anticoagulant
activity, comparable to heparin, which appeared to be due to inhibition of thrombin and factor X. Its activity was more potent than similar sulphated galactans, from invertebrate sources, that had only one sulphate per galactose residue. A similar polysaccharide chain from *G. crinale*, but with lower amounts of 2, 3-di-\(O\)-sulfated D-galactose, was less potent in a clotting time assay when compared with that from *B. occidentalis* (Pereira *et al.*, 2005).

The two sulphated polysaccharides did not differ in thrombin inhibition mediated by antithrombin; however, in assays where heparin cofactor II was used in place of antithrombin, the sulphated galactan from *G. crinale* was less inhibitory than that from *B. occidentalis*. Yet the sulphated galactan from *G. crinale* was a more potent anticoagulant than that from *B. occidentalis* when Factor X was the target protease. These observations suggested that the proportion and/or the distribution of 2, 3-di-sulfated galactose along the polysaccharide chain modulate the interaction of the polysaccharides with specific proteases in the coagulation system. The 2, 3-disulfated galactan from *B. occidentalis* inhibits intrinsic tenase and prothrombinase complexes that are critical for factor Xa and thrombin generation, respectively (Glauser *et al.*, 2009). The sulphated galactan interacts with the heparin-binding site on the heavy chain of factor Xa. Interestingly, the anticoagulant activities associated with the sulphated galactan and that of heparin are modulated differently by heparin cofactor II; heparin anticoagulant activity was enhanced in plasma devoid of heparin cofactor II, whereas the activity of the sulphated galactan was independent of this cofactor.

Algal sulphated polysaccharides, until recently, were largely ignored as sources of antioxidant activity. Studies over the last several years reveal that sulphated polysaccharides from a number of seaweeds have appreciable
Antioxidant capability (Costa et al., 2010; Wang et al., 2010). Oxidation is essential to many organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1984). The free radicals derived from oxygen are the super oxide anion (O$_2^-$), per hydroxyl radical (HO$_2$), hydroxyl radical (‘OH) and singlet oxygen. Reactive oxygen species (ROS) such as O$_2^-$, H$_2$O$_2$ and ‘OH have been conventionally regarded as having carcinogenic potential and have been associated with tumour promotion (Irani et al., 1997). Several mechanisms are defending against free radicals and other reactive oxygen species (ROS) in human system. Various defences are complementary to one another because they act on different oxidants or in different cellular compartments. One important line of defence is a system of enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase as well as several exogenously acquired radical-scavenging substances such as vitamins E and C and carotenoids (Diplock et al., 1998). Under normal conditions, the high concentrations of SOD maintain superoxide concentrations at a level too low to allow the formation of peroxynitrite. It is also important to mention that the antioxidant reduces glutathione (GSH). GSH is ubiquitous in aerobic tissues, and although it is not a nutrient, it is synthesized from sulfhydryl-containing amino acids and is highly important in intermediary antioxidant metabolism.

Antioxidants have been defined as “any substance that when present at low concentrations with those of an oxidizable substrate significantly delays or prevent oxidation of that substrate”. Antioxidants possess a variety of biological activities including the induction of drug metabolising enzymes,
inhibition of prostaglandin synthesis, inhibition of carcinogen induced mutagenesis and scavenging of free radicals. In order to reduce damage to the human body, synthetic antioxidants are used for industrial processing at the present time. However, the most commonly have been suspected of being responsible for liver damage and carcinogenesis (Grice, 1988; Qi et al., 2005a). Thus, it is essential to develop and utilize effective and natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella, 1993; Nandita and Rajini, 2004).

In recent years, sulphated polysaccharides from the marine algae *Porphyra haitanesis* (Zhang et al., 2003), *Ulva pertusa* (Qi et al., 2005b) and *Fucus vesiculosus* (Ruperez et al., 2002) have been demonstrated to have antioxidant activities. Their activity depends on several structural parameters such as the degree of sulfation, the molecular weight, the sulfation position, type of sugar and glycosidic branching (Melo et al., 2002). Fucans from *F. vesiculosus* exhibited considerable ferric reducing/antioxidant power (Ruperez et al., 2002) and superoxide radical scavenging ability (Rocha de Souza et al., 2007). Fucan fractions from *L. japonica* also showed significant antioxidant capabilities in superoxide radical and hydroxyl radical scavenging assays (Zhao et al., 2005; Wang et al., 2010; Wang et al., 2009). Superoxide radical scavenging activity correlated positively with the sulphate content of the polysaccharide fractions (Rocha de Souza et al., 2007; Wang et al., 2010). Antioxidant properties of carrageenans (Rocha de Souza et al., 2007) and ulvans (Qi et al., 2005b) also appeared related to sulphate content. In the latter study, high sulphate content derivatives of ulvan showed improved antioxidant activities (Qi et al., 2005a). Interestingly, metal chelating, free radical and hydroxyl radical scavenging
activities of fucan fractions appear to relate to their ratio of sulphate content/fucose (Wang et al., 2010).

Sulphated polysaccharides, including those from algae, have been shown to possess anti-inflammatory activity (Chen et al., 2008). Inflammation is considered as a primary physiologic defence mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for much chronic illness (Kumar et al., 2004b). Inflammation can accelerate the development of cancer. Inflammatory process provides the prerequisite environment for the development of malignancy includes up regulation of mediators of the inflammatory response such as cyclo-oxigenase (COX-2) leading to the production of inflammatory cytokines and prostaglandins which themselves may suppress cell mediated immune responses and promote angiogenesis. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and the mediators that increase blood flow (Ialenti et al., 1995). Several experimental models of paw oedema have been described. Carrageenan induced paw edema is widely used for determining the acute phase of inflammation where as formalin induced paw edema is for determining the chronic phase of inflammation. Drugs which are presently in use for the management of pain and inflammatory conditions are either narcotics e.g., opioids or non-narcotics e.g., salicylates and corticosteroids e.g., Hydrocortisone. All of these drugs possess well known side and toxic
effects. Therefore, the development of potent natural anti inflammatory drugs with fewer side effects is necessary.

Many algal species contain polysaccharides at relatively high concentrations and many of them have been shown to have anti inflammatory activity. *Enteromorpha intestinalis* polysaccharide has effects on clearing away heat and detoxify and eliminating inflammation. Polysaccharides isolated from various mushrooms are found to possess immunomodulatory and anti inflammatory activities (Borchers *et al*., 1999; Wasser, 2002). Sulphated polysaccharides may affect multiple targets in the immune and inflammatory systems that can have impact on disease progression and outcome including tumour progression and metastasis (Groth *et al*., 2009). One of the interests in algal sulphated polysaccharides as anti-inflammatory agents is the evidence illustrating their ability to interfere with the migration of leukocytes to sites of inflammation. For example, in a rabbit model of bacterial meningitis, leukocyte rolling was markedly reduced by intravenous infusion of fucoidan (Granert, *et al*., 1994). Similarly, intravenous addition of fucoidan reduced, in a dose-dependent manner, leukocyte recruitment to peritoneum in a rat model of peritoneal inflammation (Preobrazhenskaya *et al*., 1997). These effects were ascribed to the binding of fucoidan to L- and P selectins, cell adhesion molecules essential in the recruitment process. Both of these studies used the fucoidan from Sigma-Aldrich Chemical Co (St. Louis, MO, U.S.) that is sourced from *F. vesiculosus*. Fucans from other seaweeds including *Laminaria spp*, *Fucus spp.*, *A. nodosum*, and *C. okamuranus* also inhibit leukocyte recruitment to the abdominal cavity during acute peritonitis in rats (Cumashi *et al*., 2007). In addition to impairing the action of selectins, algal sulphated polysaccharides inhibit tissue degradative enzymes such as heparanase and
elastases that are involved in the breakdown of basement membrane integrity during inflammation (Senni et al., 2006; Parish et al., 2001).

One of the major and potentially promising activities is the potent inhibitory effect of sulphated fucans on human complement activation. The original observations showed that fucoidan fractions from A. nodosum potently inhibit both the classical and alternative pathways in human serum (Blondin et al., 1994). The interaction of algal sulphated polysaccharides with the complement system suggests that they may have utility in influencing innate immunity to reduce the pro-inflammatory state or other detrimental conditions such as allergic reactions arising during the innate immune response. In addition, there is a growing body of evidence that algal polysaccharides can regulate the innate immune response directly by binding to pattern recognition receptors (PRRs) such as the mannose receptor and toll-like receptors on phagocytic cells including macrophages (Tsuji et al., 2003). For example, λ-carrageenan stimulated mouse T cell cultures in a toll-like receptor-4 (TLR4) dependent manner (Maruyama et al., 2005) generating a T helper 1 (Th1) patterned cytokine response. However, splenocytes prepared from TLR4-deficient mice still retained some ability to produce interferon-γ in response to λ-carrageenan suggesting that PRRs other than TLR4 were also elicited. In mice immunized with ovalbumin to produce an allergic reaction, oral dosing with λ-carrageenan lead to a reduction in ovalbumin-specific IgE and serum histamine release, suggesting that λ-carrageenan might be used to ameliorate allergic reactions. Similar results were reported for mekabu fucoidan from U. pinnatifida (Lewis et al., 1988).

Algal polysaccharide has been widely used in industry (Skjak and Martinsen, 1991). In recent years, researchers have screened large amounts of
algal extracts for their anti-tumour effects. The results demonstrate that polysaccharides especially sulphated polysaccharides extracted from seaweeds have better anti-tumour activities because of their immunostimulating activity. (Choi et al., 2005; Sheng et al., 2007).

Cancer is one of the leading causes of death in the world. Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Normal cells divide and grow in an orderly fashion, but cancer cells do not. They continue to grow and crowd out normal cells. The process of carcinogenesis is divided into three stages; initiation, promotion and progression. Three main classes of genes that are likely to be the molecular targets for the development of neoplasia include proto-oncogenes cellular oncogene and tumour suppressor genes (Marshall, 1991; Boyed and Barrett, 1990).

In cancerous cells they lost the capacity to stop division *i.e.*, apoptosis. Apoptosis is a complex process that involves many different signalling pathways and results in a multitude of changes in the dying cells. The apoptotic machinery is triggered as a result of a shift in the balance of anti- and proapoptotic proteins. Up regulation of antiapoptotic proteins, down regulation of proapoptotic proteins, and decreased expression of caspases may lead to decreased apoptosis. Evasion of apoptosis is recognized to facilitate cancer development by blocking differentiation, promoting angiogenesis, and increasing cell motility, invasion, and metastasis (Ghobrial et al., 2005). Dysregulation of apoptotic signalling can play a vital role in diseases with insufficient apoptosis leading to cancer. The proapoptotic member of the Bcl-2 family such as Bim, a BH3 induces apoptosis by binding to and inhibiting the function of antiapoptotic proteins such as Bcl-
XL and Bcl-w. In addition, Bim is reportedly inducing cytochrome C release from the mitochondria (O’Connor et al., 1998). The release of cytochrome C from the mitochondria is also induced by caspase 8, an initiator caspase that links the death receptor and mitochondrial pathways of apoptosis. Caspase 3 is an effector caspase that executes cell death by cleavage of proteins, vital for cell survival (Philchenkov, 2004).

Induction of apoptosis is one of the active strategies to arrest proliferation of cancer cells. Radiation and chemical agents like tamoxifen, capable of inducing apoptosis, have been used to treat cancer (Woynarowska et al., 2000; Zhang et al., 2000). Many chemo preventive agents exert their anticarcinogenic effects by inducing apoptosis. Anticancer drugs are known to cause severe adverse effects such as immune system damage, which constrains their use in tumour treatment. Therefore, it is very important to investigate novel anti-tumour drugs with improving immunity potential without harming the host (Yang et al., 2008). The apoptosis inducing effect of plant extracts may be attributed to up regulated immune surveillance, increased macrophage, and activations of death inducing signal complex. Natural dietary constituents such as curcumin and resveratrol have been reported to induce apoptosis in malignant cells in vitro. The marine phytochemicals also can activate the macrophages and induce apoptosis. Fucoidan from Laminaria japonica can restore the immune functions of immunosuppressed mice, and it is immunomodulator acting directly on macrophage and T lymphocyte.

In the last few decades, the biological activities of polysaccharides have attracted more and more attention in biochemistry and medicine. Chief among the most promising bio pharmacological activities of polysaccharides
are their immunomodulatory and antitumor effects. The earliest polysaccharide reported to have antitumor activity was isolated in 1943 from the bacterium Serratia marcescens and became known as Shear’s polysaccharide (Whistler et al., 1976). The search for potential polysaccharides as antitumor agents probably stems from dissatisfaction with cancer chemotherapy and radiotherapy. A large numbers of chemical compounds which have been identified as specific agents for killing cancer cells are also toxic to normal cells. Many of the potential anticancer drugs have considerable side effects and therefore have little clinical use. Hence, the discovery and identification of new safe drugs which are active against tumours becomes an important goal of research in biomedical sciences. Numerous antitumor polysaccharides have been discovered from mushrooms, fungi, yeasts, algae, lichens and plants.

The reinforcement of host immune response has been recognized as a possible means of inhibiting tumour growth without harming the host. For this reason, extensive study has been undertaken on polysaccharides isolated from seaweed (Ooi and Lin, 2000). Polysaccharides from the Sargassum genus have been reported to have antitumor activity (Leandro et al., 2011). Algal sulphated polysaccharide from Chondrus ocellatus was found to possess antitumor activities (Mou et al., 2003).

Alginate constitutes the dominant structural compound of the cell wall and intercellular matrix in brown seaweeds (Kloareg and Quatrano, 1988). It is reported that alginites can stimulate production of the cytokines, tumour necrosis factor-a, interleukin-1 and interleukin-6 from human monocytes (Otterlei et al., 1991; Espevik et al., 1993), and display inherent antitumor activity against murine tumour models in vivo (Fujihara et al., 1984; Fujihara
and Nagumo, 1992). The anti-tumour effects of *G. lucidum* polysaccharide in vivo using a mouse model was studied and the underlying mechanisms for the treatment of cancer involve a direct cytotoxic effect of this polysaccharide on cancer cells and a cell-mediated immune response induced by this agent (Zhu, 2007).

The active components contained in algal polysaccharides are mainly sulphated ones (Okai, 1998). Most studies support that sulphated polysaccharides can enhance the innate immune response by promoting the tumoricidal activities of macrophages and natural killer cells (Yim, *et al.*, 2005). Antigen presenting cells migrate into and out of tumour tissue to present tumour antigen to T-helper cells, as well as to produce cytokines, such as interleukin-1β and TNFα that stimulate T-helper cells. As a result, T-helper cells promote the activity of cytotoxic T-cell, which has the strong cytotoxic effect on tumour cells. Sulphated polysaccharides can enhance the adaptive immune response by promoting such process (Yim *et al.*, 2005; Sullivan *et al.*, 2000). Recent studies have implicated that sulphated polysaccharides recognize a range of cell adhesion systems. Sulphated polysaccharide can bind to CD2, CD3, and CD4 in T lymphocytes and enhance the proliferative response of T lymphocytes (Miao, *et al.*, 2005; Miao *et al.*, 2004).

Polysaccharides having antitumor action differ greatly in their chemical composition and configuration and physical properties. Antitumor activity is exhibited in a wide range of glycans extending from homopolymers to highly complex heteropolymers. Although it is difficult to correlate the structure and antitumor activity of complex polysaccharides, some possible relationships can be inferred. It has been reported that most of
the antitumor polysaccharides such as lentinan and schizophyllan show the same basic β-glucan structure with different types of glycosidic linkages. Therefore it is obvious that some structural features such as β-1, 3-linkages in the main chain of the glucan and further β-1, 6-branch points are needed for antitumor action. The β-glucans containing mainly 1, 6-linkages have fewer activities. Glucans with high molecular weight appear to be more effective than those with low molecular weight. There are antitumor polysaccharides with other chemical structures, such as hetero-β-glucan, heteroglycan, β-glucanprotein, α-manno-β-glucan, α-glucan, α-glucan-protein, and heteroglycan-protein complexes. Therefore, it is difficult to identify which polysaccharide structure is essential for antitumor action. It has been shown that the molecular mass, the degree of branching, conformation and chemical modification of antitumor polysaccharides significantly affect their antitumor and immunomodulatory activities. These investigations raise the possibility that antitumor polysaccharides may not always be multiple enhancers of the host defence system, and that high molecular mass is required for extensive enhancement of immunological and antitumor activities (Yim et al., 2005).