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Sulfated Galactans From Red Seaweeds and Their Potential Applications

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Abstract – Red seaweeds (Rhodophyta) produce a variety of sulfated galactans in their cell wall matrix and intercellular space, contributing up to 50-60 % of their total dry weight. These sulfated polysaccharides are made up of galactose disaccharides substituted with sulfate, methoxyl, pyruvic acid, or non-galactose monosaccharides (e.g. xylose, glucose and mannose). They are required by the Rhodophytes for protection against pathogen, desiccation, tidal waves and extreme changes in pH, temperature and salinity. Since ancient times, sulfated galactans from red seaweeds, such as agar and carrageenan, have been consumed as human foods and later being used in traditional medicine. Nowadays, some red seaweeds are cultivated and exploited for commercial uses in various fields. In this review, different types of sulfated galactans found in red seaweeds and their current and potential uses in food, biotechnology, medical and pharmaceutical industries are discussed.

Keywords: Agar, carrageenan, red seaweed, Rhodophyta, sulfated galactan

Type of Sulfated Galactans from Red Seaweeds

Red seaweeds, especially those in the order Gelidiales and Gigartinales, are a major source of marine sulfated galactans, with at least 70-80 species of them being industrially exploited for galactan production (Delattre *et al.*, 2011). Sulfated galactans from red seaweeds are characterised into a few groups based on their stereochemistry, which include agars, carrageenans and D/L hybrids (also known as non-ideal or complex sulfated galactans) (Delattre *et al.* 2011; Jiao *et al.* 2011). They share a common alternating sequence consisting of 1,3-linked β-D-galactose and 1,4-linked α-D/L-galactose residues, with their hydroxyl groups being substituted by sulfate esters, methoxyl and pyruvic acid. The 1,4-linked galactose unit can be replaced by 3,6-anhydro sugar (Figure 1). Sulfated galactans have strong anionic properties due to the presence of negatively charged sulfate moiety, and are able to interact with other charged polysaccharides or proteins. These sulfated galactans have high molecular weight (usually more than 100 kDa), ability to form gel and many biological activities such as antiviral, anticoagulant and immune-inflammatory activities (Pomin, 2010; Jiao *et al.*, 2011).

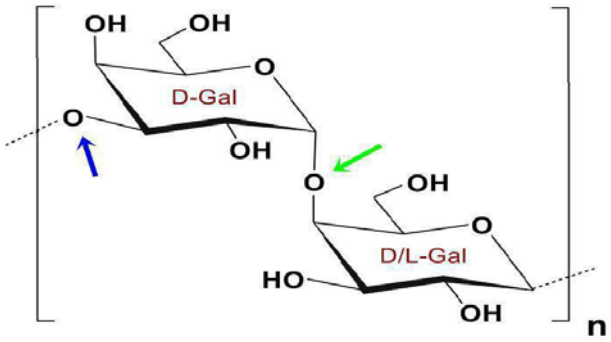

Figure 1: Chemical structure of the common repeating units of sulfated galactans in red seaweeds. Green and blue arrows show α-1,4 and β-1,3 linkages, respectively. D-Gal, D-galactose; D/L-Gal, D-galactose or L-galactose.

Agar

Agar refers to the gel-forming polysaccharide first extracted from the *Gelidium* seaweeds. The word "agar" has a Malay origin and comes from the 'agar-agar'. It is also known as "kanten" in Japanese. In Japan, agar was discovered accidentally by an inn keeper, Tarazaemon Minoya in 1658. Minoya disposed seaweed soup on snow, which turned gelatinous. Minoya's agar producing method was improved by Hambei Miyata, who produced agar strips to be sold everywhere in Japan (Okazaki, 1971). The industrial agar (food grade) in the powder form was produced from *Gelidium* in the United States and later in Spain and Portugal (Armisen & Galatas, 1987). During World War II, the shortage of *Gelidium* agars led to the discovery of *Gracilaria* species as an alternative agar source, which have comparable good agar when the seaweeds were treated with alkaline prior to agar extraction (Wu, 1990). In 1972, agar was granted the status of 'generally recognised as safe' (GRAS) by the US Food and Drug Administration (FDA). Agar is commercially extracted from *Gelidium*, *Pterocladiella*, *Gelidiella* and *Gracilaria*, but it can also be found in other genus such as *Ahnfeltia*, *Acanthopholis*, *Campylaephora*, *Ceramium*, *Phyllophora* and *Gracilariopsis* (Pereira *et al.*, 2013).

Agar can be divided into agarose and agaropectin based on the polarity. Agarose (also known as agarobiose) is composed of neutral disaccharides of D-galactose and 3,6-anhydro-L-galactose, which contributed up to 70 % of the agar polysaccharides. Agaropectin has similar structure as agarose but the hydroxyl group of L-galactose unit is heavily substituted with charged groups, such as sulfate, methoxyl, and pyruvate ketal (Lee *et al.*, 2017a). It acts as a precursor for agarose during enzymatic polymerization and desulfation processes (Armisen *et al.*, 2009). The agar solubility and ability of agar to form gel is due to the relative hydrophobicity of the galactose disaccharide. The degree of side chain substitution varies with seaweed species, e.g. *Gracilaria* was found to have a higher sulfate substitution compared to *Gelidium*. The contemporary largest agar producers are *Gracilaria* (91 %) and *Gelidium* (9 %) (Porse & Rudolph, 2017), with a lower agar gel strength in the former. *Gracilaria* agar is in high demand due to depleted *Gelidium* stocks (Armisen, 1995). The low gel strength in *Gracilaria* agar is due to high sulfate content, but can be improved by alkaline hydrolysis using sodium hydroxide (Freile-Pelegrin & Murano, 2005) or enzymatic hydrolysis using sulfohydrolase (Shukla *et al.*, 2011).

Carrageenan

The word 'carrageenan' originated from the name of an Irish Village, Carragheen, where the carrageenan-containing seaweed *Chondrus crispus* (Irish moss) was found (Bixler, 1994). Carrageenans are mainly extracted from *Chondrus*, *Eucheuma*, *Gigartina* and *Hypnea*. They are high molecular weight polymers consisting of repeating disaccharide D-galactose, which are 1,3-linked β-D-galactose and 1,4-linked α-D-galactose. The structures of carrageenan are complicated by different sulfate substitutions and the presence of anhydro-galactose sugar. Carrageenans are divided into three main families e.g. kappa, iota and lambda carrageenans (Craigie, 1990). Kappa- and iota-carrageenans are mainly extracted from *Kappaphycus alvarezii* (also known as *cottonii*) and *Eucheuma denticulatum* (also known as *spinosum*), respectively, while *Gigartina pistillata* and *C. crispus* are the principle source for lambda carrageenan (de Ruiter & Rudolph, 1997; van de Velde & de Ruiter, 2002).

These carrageenans can be further divided into sub-families and named by different Greek letters, such as delta-, theta-, nu-, mu- and pi-carrageenans, which had been largely described and reviewed (van de Velde *et al.*, 2004; Delattre *et al.*, 2011; Jiao *et al.*, 2011). However, majority of the natural carrageenans from red seaweeds exist in hybrid form, such as κ/β-hybrids (Yang *et al.*, 2011), κ/ι- hybrids (Hilliou *et al.*, 2009), κ/μ-hybrids (Jouanneau *et al.*, 2010), or ν/ι-hybrids (van de Velde, 2008), but could be varied according to the physiological states of seaweeds (Chiovitti *et al.*, 1998; Falshaw *et al.*, 2001). Some forms of carrageenan can be obtained after chemical treatment, for example θ-carrageenan with anhydride bridge was obtained after alkaline hydrolysis of sulfate ester of λ-carrageenan (Doyle *et al.*, 2010). In addition, side chain modifications with methoxyl and pyruvic acid groups and the presence of cations (e.g. sodium, calcium, potassium and magnesium) increase the structural complexity of the carrageenan. Pyruvate has been reported to be present in the *pi*-carrageenans from some *Gigartina* species (Hirase & Watanabe, 1972; McCandless & Gretz, 1984) while methoxyl groups occur in carrageenans from the *Grateloupiaceae* family (Nunn & Parolis, 1968).

Other sulfated galactans

Complex galactans are also D/L-hybrids as in agar but the galactose sugar could be ramified by other monosaccharides such as mannose, glucose, xylose and arabinose, as well as charged residues in the form of sulfate, methoxyl and pyruvic acid. For example, β-D-xylosyl group was first found to attach at the O-6 position of D-galactose unit in *Corallina officinalis*, which is known as xylogalactans or corallinan (Cases *et al.*, 1992). Xylogalactans were subsequently being identified in other species in the Corallinales order, such as *Lithothamnion heterocladum* (Navarro *et al.*, 2011), *Calliarthron cheilosporioides* (Martone *et al.*, 2010) and *Jania rubens* (Navarro *et al.*, 2008). Some other examples of highly complex sulfated galactans which contain more than two types of monosaccharides were summarized in Table 1**.**

Table 1: Examples of some highly complex D/L-hybrid sulfated galactans found in red seaweeds.

Species	Monosaccharide composition	References
Kappaphycus alvarezii	Gal, Xyl, Glu	Estevez *et al.* (2004)
Cryptonemia crenulata	Gal, Xyl, Glu	Zibetti *et al.* (2005)
Ahnfeltiopsis flabelliformis	Gal, Xyl, Glu	Kravchenko *et al.* (2016)
Iridaea cordata	Gal, Glu, Fuc	Kim *et al.* (2016)
Mastocarpus stellatus	Gal, Xyl, Glu, Man	Gómez-Ordóñez *et al.* (2014)
Gymnogongrus torulosus	Gal, Xyl, Glu, Man	Estevez *et al.* (2008)
Grateloupia indica	Gal, Xyl, Glu, Fuc	Chattopadhyay *et al.* (2007)
Halymenia durvillei	Gal, Xyl, Fuc, Ara	Fenoradosoa *et al.* (2009)
Hypnea musciformis	Gal, Xyl, Glu, Ara, Man, Fuc	Cosenza *et al.* (2017)

Xyl, xylose; Glu, glucose; Fuc, fucose; Man, mannose; Ara, Arabinose.

Comprehensive screening of the cell wall diversity across a wider red algal lineage is necessary, as the current knowledge on sulfated galactans is limited to a few genus with commercial values. The red seaweeds survive in different habitats with different abiotic and biotic factors (Lee *et al.*, 2017b) thus they may have evolved and showed high plasticity in terms of cell wall chemical and metabolic composition. Identification of new and novel galactan structure will not only improve our current knowledge on these carbohydrate polymers, but could also potentially lead to the discovery of new biological applications. To achieve these objectives, a high throughput screening method and structural analysis of sulfated polysaccharides are necessary to replace the traditional nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).

Current and Potential Uses of Sulfated Galactans from Red Seaweeds

Food

Agar has been eaten as food since its discovery in 1658, while carrageenan was introduced as food additive to replace other gums when their supply reduced during the World War II (McLachlan, 1985; Armisen *et al.*, 2009). Agar and carrageenan are extensively used in the food industry because they can change food viscosity and texture. Agar and carrageenan contributed to approximately US $ 173 and 525 million per year, respectively, in food economic growth (Kraan, 2012). More than 80 % of both commercial agar and carrageenan were used as food additives (McHugh, 1987). Agar and carrageenan are soluble in hot water, and polymerize into gel when cooled. These properties are clear, neutral and tasteless, making them ideal food additives that modify food taste and appearance in color (Ruperez & Saura-Calixto, 2001). By altering the concentration, pH, temperature, salt and coupling with other hydrocolloids, desired food texture and viscosity can be achieved (Walstra, 2003). In addition, agar and carrageenan are widely used as food ingredient by the Asian countries. However, they are indigestible by human, thus providing no nutritional value (Ikeda *et al.*, 1983). Nonetheless, when eaten in large quantities, agar and carrageenan can act as dietary fiber to soften stools, promote the growth of healthy gut bacteria, reduce cholesterol and glycemic response (Gómez-Ordóñez *et al.*, 2010; O'Sullivan *et al.*, 2010; Elleuch *et al.*, 2011).

Agar and carrageenan can be used as food thickener due to their ability to form gel by trapping water molecules when their polymer units are cross-linked. The process of thickening involved the transition of freely disperse polymer chains to intertwine network of agar and carrageenan polymer chains (Saha & Bhattacharya, 2010). The transition can be achieved by increasing the concentration of polymer chains. In low concentration, the polymer chains of agar and carrageenan disperse randomly in the solution but when in high concentration, these polymer chains move closer to each other and entangle into a thicker suspension (Saha & Bhattacharya, 2010). Agar and carrageenan have been added into pie fillings, toppings for cupcakes and ice creams, as thickener. They are also added into sauces, syrups and ketchup to maintain the consistency of the texture. In addition, agar and carrageenan are used as momentary thickener in canned food (Glicksman, 1987). The gelling property of agar and carrageenan can be used to make confectionery such as jelly, candy, gummy bear, and pudding. Agar especially, can withhold sugar in large quantities without forming crystal or losing its gelling properties (Nussinovitsch *et al.*, 1991).

Heat treatment is a common practice to sterilize food products but the treatment can cause protein denaturation which may alter the structure and nutritional value of food (Ibanoglu, 2005). One solution to prevent protein denaturation during heat treatment is to add polysaccharides as stabilizer. Carrageenan can act as protein stabilizer due to its ability to transfer heat efficiently. The interaction of protein and carrageenan and the mechanism on how carrageenan protects protein from denaturation were inconclusive (DeFreitas *et al.*, 1997). Even so, most milk products contain carrageenan which function as suspension, emulsion stabilizer, bodying, syneresis control, whey prevention, and meltdown control (Glicksman, 1987). Compared to other hydrocolloids, carrageenan can form gel at lower concentration in milk (Verbeken *et al.*, 2004).

The association of carrageenan to bowel inflammation and carcinogenicity is highly debated. Many detrimental effects of carrageenan on animal models have been thoroughly reviewed (Tobacman, 2001), but there was no report of carcinogenicity of carrageenan in human. However, it is noteworthy that degraded carrageenan (also known as poligeenan) was used in those animal studies instead of its non-degraded counterparts, the carrageenan which was widely used for food preparation (Weiner, 1991). Concern was also raised on possibility of acid hydrolysis of native carrageenan to poligeenan in stomach, and caused colon cancer (Tobacman, 2001). In 1987, the native and degraded carrageenan were listed by the International Agency for Research on Cancer (IARC) as Group 3 carcinogens (not classifiable as to their carcinogenicity to humans) and 2B carcinogents (possibly carcinogenic to humans), respectively. In 2001, the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) confirmed the safety of carrageenan during the 57th meeting, and carrageenan was granted the acceptable daily intake (ADI) of “not specified”. This ADI is generally applicable to food substance with very low toxicity, which is proven from biochemical, toxicological and carcinogenical data. Hence, the GRAS (Generally Regarded as Safe) status of carrageenan, which was granted in 1959, was maintained.

Biotechnology

Agars produced by red seaweeds are widely applied in biotechnology research. They serve well as a solid support medium for microbial culture of bacteria and fungi (Lee *et al.*, 2017b), as most microorganisms are unable to digest agar (Rivera-Posada *et al.*, 2012). Many ingredients are added to the agar (in the form of powder) for the growth of microorganisms, such as the commercially available Potato-dextrose agar (PDA) for fungal culture, Luria-Bertani (LB) agar and Blood agar plate (BAP) for bacterial culture. The bacteriological-grade agar has stable gelling and melting temperature, good transparency of solution and gel, low amount of electronegative groups and oligomers, and free from contamination by thermophilic spores and hemolytic substances (Armisen, 1991). Agar provides the nutrient medium a moist gel, making it a widely used supportive agent for plant tissue culture under sterile condition (Huang & Murashige, 1977). The agar was shown to be stable and responsive to magnetic field, thus was used as a coating for magnetic beads to absorb protein (Tong & Sun, 2001). Agarose has a low degree of chemical complexity, thus it is suitably used as matrix in DNA, RNA and protein gel electrophoresis (Renn, 1990). Meanwhile, agarose is also being used in gel filtration chromatography, by forming beads or resins with different fineness (Freifelder, 1982; Renn, 1984). Some commercially available examples of these agarose-based beads are Sepharose, WorkBeads 40 SEC, Superose and Superdex. In addition, agarose can be used as a supportive material in immunodiffusion (Renn, 1984).

Immobilization of enzymes using agar had been reported for maltase, manganese peroxidase, pullulanase, pectinase, amylase, chitosanase, pectinase and urease (Ichikawa *et al.*, 2002; Kuroiwa *et al.*, 2005; Ming *et al.*, 2006; Mulagalapalli *et al.*, 2007; Li *et al.*, 2008; Prakash & Jaiswal, 2011; Rehman *et al.*, 2014; Bilal *et al.*, 2016; Nawaz *et al.*, 2016). The use of agar matrix for immobilization of enzymes for industrial utilization offers several advantages: cheap and easy preparation, good stability and no reactivity to protein (Mulagalapalli *et al.*, 2007). Agar was also used to immobilize bacteria such as *Bacillus subtilis* PE-11, *B. circulans* ATCC 21783, *Pseudomonas sp.* strain NGK 1, *Anacystis nidulans*, *Clostridium butyricum* and fungus such as *Rhizopus nigricans* (Suzuki *et al.*, 1980; Maddox *et al.*, 1981; Monahar & Karegoudar, 1998; Khattar *et al.*, 1999; Vassileva *et al.*, 2003; Adinarayana *et al.*, 2005). Besides, agar was also immobilized with poly-allylamine hydrochloride and alizarin red S to produce non-enzymatic sensors for detection of hydrogen peroxide (Soares *et al.*, 2016).

Medicine and pharmacy

Many sulfated polysaccharides produced by red seaweeds have anticoagulant activities. The 2,3-di-O-sulfated D-galactan extracted from *Botryocladia occidentalis* (Farias *et al.*, 2000) and *Gelidium crinale* (Pereira *et al.*, 2005), and carrageenan-type sulfated galactan from *Solieria filiformis* (Andrade *et al.*, 2017) were able to inhibit thrombin and factor X in blood coagulation. Its anticoagulant effects are comparable to commercially used heparin. Heparin used in the treatment of venous thrombosis and other thromboembolic disorders has obvious limitations, including contamination by pathogenic agents (as it is isolated from pig and bovine intestine), and causes side effects such as bleeding and deficiency of platelets (Warkentin *et al.*, 1995). Red algal sulfated galactans may be used as *in vivo* therapeutic agent from non-mammalian to replace heparin, but their effects on platelet aggregation must be tested clinically as sulfated fucans from brown seaweeds were known to cause platelet aggregation (reviewed in Mourao & Pereira, 1999).

Pathogenic viral infection is one of the leading causes of human death worldwide, thus diverse types of antiviral agents especially those derived from natural products are desired for treatment of viral diseases (Kitazato *et al.*, 2007). Marine algae have been widely described in the literature as a source of antiviral agents. Sulfated galactans isolated from red seaweeds were able to inhibit the propagation, reverse transcription or replication of dengue virus type-2 (Pujol *et al.*, 2002; Rodriguez *et al.*, 2005; Talarico *et al.*, 2007; Talarico & Damonte, 2007), herpes simplex virus (HSV) (Damonte *et al.*, 1996; Carlucci *et al.*, 1997; Carlucci *et al.*, 1999; Cscaceres *et al.*, 2000; Duarte *et al.*, 2001; Pujol *et al.*, 2002; Carlucci *et al.*, 2004; Matsuhiro *et al.*, 2005; Rodriguez *et al.*, 2005; Chattopadhyay *et al.*, 2007; Ghosh *et al.*, 2009), human immunodeficiency virus (reviewed in Schaeffer & Krylov, 2000) and human metaneumovirus (Mendes *et al.*, 2014). These highly sulfated galactans can bind to the viral envelope protein and inhibit envelop protein-target cell receptor interaction in the initial step of viral infection (Chen *et al.*, 1997). Hence, the potency of antiviral activity of sulfated galactans is positively dependent on their sulfation level as well as their molecular weight (Witvrouw & De Clercq, 1997).

The introduction of antioxidants has received tremendous attention due to increasing demand to develop anti-aging and anticarcinogenic compounds in pharmaceutical and food industries. Antioxidant activity was reported from many algal sulfated polysaccharides, including those from *Gracilaria caudata* (*G. caudata*; Costa *et al.*, 2010), *Porphyra haitanensis* (Zhang *et al.*, 2004), *G. birdiae* (Souza *et al.*, 2012), *Gigartina acicularis*, *Eucheuma cottonii* (currently known as *Kappaphycus alvarezii*) and *E. spinosa* (currently known as *Eucheuma denticulatum*) (de Souza *et al.*, 2007). The sulfate content in the red algal polysaccharides correlated with their antioxidant abilities (de Souza *et al.*, 2007). These galactans are natural antioxidants which were able to protect the human cells from oxidative damage by reactive oxygen species, and replace the commercial antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene which may cause liver damage (Grice, 1988).

Many sulfated galactans were reported to have anticancer or antitumor properties. For example, sulfated galactans isolated from *Champia feldmannii* (Lins *et al.*, 2009) and *C. ocellatus* (Zhou *et al.*, 2004; Zhou *et al.*, 2006) have inhibitory effects on tumor growth in mice transplants with Sarcoma 80 tumors and H-22 tumor, respectively. Sulfated galactans isolated from *Gracilaria fisheri* have been demonstrated to exhibit antiproliferation effects on the cholangiocarcinoma cell line thus could be potentially developed for clinical therapy for cancer treatment (Sae-Lao *et al.*, 2017). Carrageenans also help in activating immune mechanisms by eliciting the production of specific antibodies. Interestingly, the efficacy in tumor inhibition is also positively related to the degree of sulfation (Hu *et al.*, 2006) and molecular weight (Yuan & Song, 2005) of the sulfated galactans.

In recent years, there is a high demand for new antibacterial compounds as many pathogens have developed specific resistance and adapted to the existing drugs after multiple treatments (Pierre *et al.*, 2011). Yamashita *et al.* (2001) demonstrated the antibacterial effects of commercial ι-carrageenan on *Salmonella* spp., *Vibrio mimicus*, *Escherichia coli*, *Aeromonas hydrophila*, and *Staphylococcus aureus*, but the ability to inhibit those bacteria was lost when the carrageenan was desulfated (90 % of the sulfate moieties were removed). Similarly, ι-carrageenan extracted from *E. denticulatum* showed antibacterial activity against *Streptococcus pyogenes* and *S. aureus* (Al-Haj *et al.*, 2009). Due to these antibacterial effects, carrageenan was also applied as surface coating of fruits for the prevention of bacterial growth to preserve the quality of the fruits during long storage periods (Plotto *et al.*, 2006; Bico *et al.*, 2009; Plotto *et al.*, 2010).

Conclusions

Red seaweeds are rich sources of marine sulfated polysaccharides which have many biological potentials and applications (Table 2). In the past, sulfated galactans have been widely used as thickening agent in food industry and as media/separation matrix in biotechnology research. Elucidation of antiviral, anticoagulant and antioxidant potentials of red algal sulfated galactans was initiated, but further developments and trials are required before they can be commercialised for therapeutical and regenerative medicine applications. Meanwhile, some aspects of these sulfated galactans such as high variability of pharmacological features (based on species, location and time of harvest) and low bioavailability have to be addressed (Jiao *et al.*, 2011). The standardization and improvement of the extraction and purification methods using innovative extraction technology (e.g. ultrasound, microwave and enzymatic extractions), specifically for each type of sulfated galactans are also needed. Although the presence of sulfate groups in the sulfated polysaccharide contributes to a weak agar gel which is not suitable for industrial exploitation, but sulfated polysaccharides contribute to many biological activities important for medical and pharmaceutical applications. Understanding on the biochemical and metabolic pathways for the galactan biosynthesis (including the establishment of algal genomes and transcriptomes) is also important to develop tools for industrial-scale modification of the cell wall component of desired algal species.

Table 2: A summary on the usage/applications of sulfated galactans extracted from red seaweeds.

Industry	Examples of red seaweeds (common name)	Usage and potential applications	References
Food	*Fucus vesiculosus*, *Laminaria digitata* (Kombu), *Undaria pinnatifida* (Wakame), *Chondrus crispus* (Irish moss), *Porphyra tenera* (Nori)	• ingredient in food preparation • food additives to modify the food taste and appearance in color • food thickener • food stabiliser to avoid protein denaturation during sterilisation process	Ruperez & Saura-Calixto (2001), Saha & Bhattacharya (2010), Verbeken *et al.* (2004)
Biotechnology	*Gelidium* spp, *Pterocladiella* spp, *Gelidiella* spp, *Gracilaria* spp.	• solid medium for microbial culture (bacteria, fungi etc.) and plant tissue culture • coating for magnetic beads used in protein absorption • separation matrix in nucleic acid and protein gel electrophoresis • gel matrix in gel filtration chromatography • immobilizes various substances (e.g. enzymes and bacteria)	Mulagalapalli *et al.* (2007), Tong & Sun (2001), Renn (1984)
Medicine and pharmacy	*Botryocladia occidentalis*, *Gelidium crinale*, *Gigartina skottsbergii*, *Sebdenia polydactyla*, *Gracilaria caudata*, *Porphyra haitanensis*, *Gigartina acicularis*, *Eucheuma cottonii* (currently known as *Kappaphycus alvarezii*), *Eucheuma spinose* (currently known as *Eucheuma denticulatum*), *Champia feldmannii*, *Gracilaria fisheri*	• anticoagulants • antioxidants • anticancer/tumor drugs • antiviral agent • antibacterial drug	Pereira *et al.* (2005), Carlucci *et al.* (1997), Ghosh *et al.* (2009), de Souza *et al.* (2007), Souza *et al.* (2012), Costa *et al.* (2010), Zhang *et al.* (2004), Lins *et al.* (2009), Al-Haj *et al.* (2009); Sae-Lao *et al.* (2017)

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