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Review

Bioactive polysaccharides from red seaweed as potent food supplements: a systematic review of their extraction, purification, and biological activities

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ABSTRACT

Most marine macroalgae such as red seaweeds are potential alternative sources of useful bioactive compounds. Beside serving as food source, recent studies have shown that red seaweeds are rich sources of bioactive polysaccharides. Red seaweed polysaccharides (RSPs) have various physiological and biological activities, which allow them to be used as immunomodulators, anti-obesity agents, and prebiotic ingredients. Lack of summary information and human clinical trials on the various polysaccharides from red seaweeds, however limits industrial-scale utilization of RSPs in functional foods. This review summarizes recent information on the approaches used for RSPs extraction and purification, mechanistic investigations of their biological activities, and related molecular principles behind their purported ability to prevent diseases. The information here also provides a theoretical foundation for further research into the structure and mechanism of action of RSPs and their potential applications in functional foods.

1. Introduction

The ocean is rich in marine biological resources, including seaweeds, which has generated millions of dollars in turnover. Industrial production of seaweed has popularized seaweed products globally. Moreover, the importance of seaweeds has grown dramatically with recent studies showing that seaweeds can be used for the development of new drugs. Among the wide variety of seaweeds, red seaweeds are the most primitive in the phylogenetic tree (Andersen, 1992).

Although in Asia, red seaweeds (such as carrageenans) have been consumed for centuries as food products or food supplements for health promotion, this group of polysaccharides were originally described in an Irish coastal known as Carragheen, from which the name is derived (Pangestuti & Kim, 2014). Asians have widely consumed red seaweed as food, however, global interest in the food value of red seaweed is increasing, which has therefore dramatically increased the demand for red seaweed in the global market (Leandro et al., 2020). The most attractive features of red seaweed as both food and medicinal substance because it has abundant biologically active polysaccharide ingredients.

In the functional food and nutraceutical industries, diets rich in polysaccharides provide significant health benefits, reduce disease risk, and enhance well-being (Huang et al., 2019). Red seaweeds contain large amounts of polysaccharides, especially within the cell wall matrix, which are very different from terrestrial medicinal plants. Besides, red seaweed polysaccharides (RSPs) possess unique structural characteristics and biological properties, which make them attractive sources of food supplements, functional foods, and nutraceuticals. The biological activities of RSPs can be grouped into antioxidant, immunomodulatory, anticancer, and prebiotic activities. These bioactivities of RSPs could be determined by their chemical and physical structures, hence, it is important to understand these structural properties of RSPs if we are to explore their health benefits.

To isolate and purify polysaccharides from red algae, various extraction and purification approaches are applied. The selection of each extraction and purification method depends on the properties of the polysaccharide (Garcia-Vaquero et al., 2017). Moreover, these methods play a significant role in the yield, chemical structure, and bioactivity of the extracted polysaccharides (Yu et al., 2018). Thus, it is important to use the appropriate extraction and purification method that would maintain the structural integrity and biological properties of RSPs. Most importantly, RSPs could easily be used as food supplements and nutraceuticals due to their high bioavailability, low-cost, and high yield.

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Recently, we reviewed the separation, purification, characterization, and biological activities of marine algae polysaccharides (Xu et al., 2017), and highlighted the potential impact of microbial catabolism of marine algae polysaccharides in the digestive tract (Zheng et al., 2020). In the current review, we summarize the common RSPs and their extraction, purification, and biological activities. The potential use of RSPs in functional foods and the health benefits of RSPs in terms of nutrition supplements and diseases prevention are highlighted, which could offer some important insights into further research on RSPs.

2. Extraction of RSPs

To begin the extraction and purification of RSPs, the raw red seaweeds must be pre-treated by drying. After drying, the dry weight of fresh seaweed is generally 12–15% of the original weight. The common drying methods used include rotary drying, spray drying, solar heat drying, cross-flow drying, vacuum shelf drying, flash drying, incinerator drying, and toroidal drying (Show et al., 2015). The composition and yield of RSPs depend on the species of the specific red seaweed, the growth conditions, and the separation procedure. For example, when dried pretreated *Gymnogongrus tenuis* was suspended in water and extracted at 90 °C under mechanical stirring for 4–5 h, 47.7% of carrageenan type polysaccharide was obtained (Perez Recalde et al., 2016). On the other hand, when dried *Tichocarpus crinitus* were extracted twice with water (1: 60) for 2 h at 80 °C, yield of the polysaccharides fraction was only 21% (Byankina et al., 2013).

The extraction of RSPs is often carried out by hydrothermal methods, but hot water extraction has many drawbacks, such as requires the use of large volume of water, long extraction times, and low extraction yields (Chew et al., 2018). To increase yields and productivity, innovative extraction methods have recently been applied in RSPs extraction. These innovative technologies include ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), ultrasound-microwave assisted extraction (UMAE), and enzyme assisted extraction (EAE). See Table 1 for a summary of extraction procedures and yield of RSPs.

The UAE method uses acoustic cavitation to generate cavitation bubbles that results in high shear pressure, which helps to destroy cell walls, allowing the solvent to dissolve red algal material to increase the yield of RSPs (Zijlstra et al., 2015). The mechanism of UAE of RSPs was shown in Fig. 1. When compared with hydrothermal method, UAE

Table 1
Extraction procedures and the yield of red algal polysaccharides.

Source	Extraction procedure	Yield	Monosaccharides composition	Molecular weight (Da)	Ref.
Euchema denticulatum	ultrasonic-assisted extraction: water (10 g/L), ultrasound power (150 W), extraction time (15 min), and temperature (90 °C)	50–55% (dry weight)	galactose and anhydro-galactose	10^3–10^5	(Youssouf et al., 2017)
Gracilaria caudata	hot water extraction: water (15 g/L), extraction time (2 h), and temperature (100 °C)	32.8% (dry weight)	galactose and anhydro-galactose	2.5×10^5	(Barros et al., 2013)
Hypnea musciformis	ultrasonic-assisted extraction: water (4 g/L), ultrasound power (500 W), extraction time (20 min) and temperature (90 °C)	49.01%	Galactose and anhydro-galactose	–	(Gereniu et al., 2018)
	Hot water extraction: water (10 g/L), extraction time (3.5 h), and temperature (85 °C)	37.8%	–	–	(Vázquez-Delfín et al., 2014)
Gelidium sesquipedale	A combined heating-sonication method: water (100 g/L), temperature (90 °C), extraction frequency (24 kHz), extraction time (30 min)	*ca.* 10–12%	Galactose and anhydro-galactose	9.2×10^5	(Martínez-Sanz et al., 2019)
Gracilaria birdiae	A combination of enzymatic digestion, sonication and alkaline solution: 0.1 M NaOH, temperature (22 °C), sonication (30 min/60 °C/60 W), enzymatic digestion (60 °C, 12 h, pH 8.0)	413 mg	galactose, glucose, arabinose and xylose	4.5×10^4	(Fidelis et al., 2014)
Gracilaria lemaneiformis	Amylase-assisted extraction: extraction time (40 min), temperature (95 °C), pH (5.0) and thermostable A-amylase amount of 6000 U/g	49.15%(dry base)	Rhamnose, arabinose, xylose and mannose	–	(Wu et al., 2017)
	Ultrasonic-microwave-assisted extraction: ultrasonic power (50 W), extraction time (31.7 min), extraction temperature (87 °C) and a solid-to-water ratio of 1.0:60.7	34.8%	Galactose, glucose and arabinose	8×10^5	(Shi et al., 2018)
Osmundea pinnatifida	Enzyme-assisted extraction: water (2 g/mL), enzyme (100 mg), temperature (50 °C), extraction time (24 h), pH (7.0)	58%	Galactose, mannose, arabinose, xylose, rhamnose, fucose	–	(Rodrigues et al., 2015; Rodrigues et al., 2019)
Pyropia yezoensis	Microwave-assistant extraction: water (25 g/L), extraction time (15 min), temperature (120 °C), with autoclave.	499 mg	Galactose, glucose, and mannose	3.93×10^3	(Lee et al., 2016a)
Mastocarpus stellatus	Hot water extraction: water (5 g/L), extraction time (2 h), and temperature (60 °C)	14.4%	Xylose, galactose, glucose,	$(7.07–9.83) \times 10^5$	(Gómez-Ordóñez et al., 2014)
Gymnogongrus tenuis	Hot water extraction: water (2 g/L), extraction time (3 h), and temperature (80 °C)	47.7%	Anhydrogalactose, galactose	1.04×10^5	(Perez Recalde et al., 2016)
Lithothamnion muelleri	Hot water extraction: water (5 g/L), extraction time (2 h), and temperature (60 °C)	11.6%	Galactose, glucose, xylose, mannose, rhamnose and arabinose	$(4.3–6.1) \times 10^5$	(Malagoli et al., 2014)
Tichocarpus crinitus	Hot water extraction: water (10 g/L), extraction time (2 h), and temperature (80 °C)	21%	Anhydrogalactose, galactose	3.76×10^5	(Byankina et al., 2013)
Sarcodia ceylonensis	Microwave-assisted extraction: ratio of water to raw material:1:20, extraction time (20 min), temperature (70 °C), extraction power (500 W)	9.6%	Mannose, glucose, sorbose, arabinose	4.04×10^5	(He et al., 2016)
Porphyra haitanensis	Microwave assisted extraction: ratio of water to raw material (1:30, mL/g), extraction time (14 min), temperature (70 °C), microwave power (78 W)	4.90%	Rhamnose, arabinose, xylose, mannose, glucose, and galactose	–	(Chen & Xue, 2019)
	Ultrasonic/microwave-assisted extraction: extraction time (30 min), extraction temperature (80 °C), and solid–liquid ratio of 1:41.79 g/mL	20.98%	Galactopyranose	–	(Xu et al., 2020)
Solieria chordalis	Microwave-assisted extraction: extraction time (10 min), temperature (90 °C), extraction power (500 W)	29.3%	Anhydrogalactose	–	(Boulho et al., 2017)
Kappaphycus alvarezii	Pressurized hot water extraction: water (25 g/L), extraction time (5 min), and temperature (80 °C)	–	Glucose and galactose	–	(Gereniu et al., 2017)

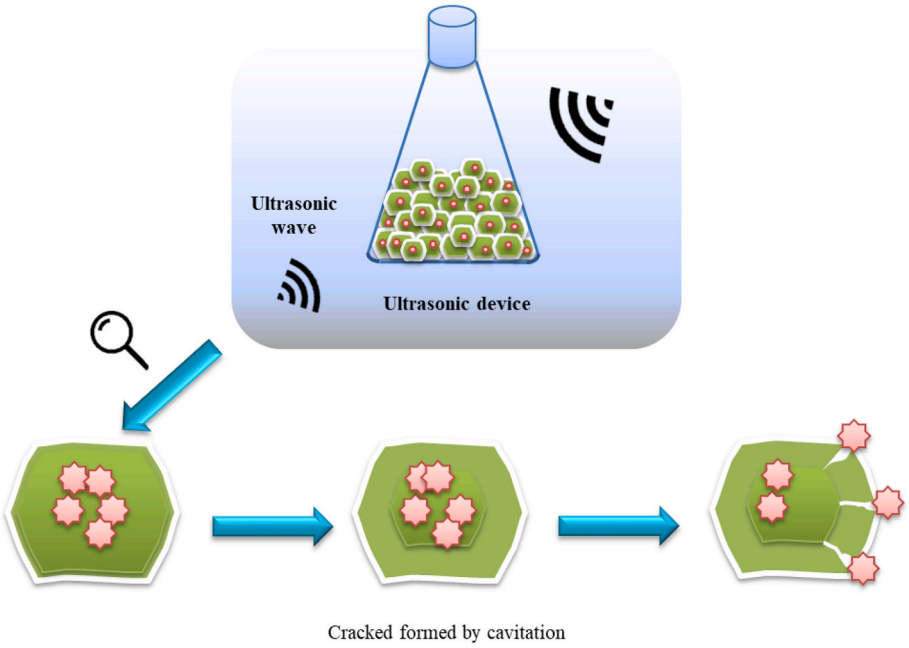

Fig. 1. The mechanism of ultrasound-assisted extraction of RSPs.

reduced the extraction time of agaran from *Gelidium sesquipedale* by 4-fold without significant effect on yield and properties, such as galactose and sulfate content (Martínez-Sanz et al., 2019). Extraction by UAE also enhances antioxidant activity compared with hydrothermal extraction (Wu et al., 2021), probably due to changes in molecular weight, sulfate content, and gel strength.

The MAE method can increase yields in less time, using less solvent, and at the same temperature. Microwave is a form of electromagnetic radiation that penetrates cell wall. The mechanism of MAE of RSPs was shown in Fig. 2. Unlike direct heating, microwave heating starts inside the material and can therefore interact with polar solvents like water in the biomass, allowing uniform heating of the whole red algal material (Vinatoru et al., 2017). When *Gracilaria lemaneiformis* polysaccharides were extracted by MAE at 70 °C and 500 W for 20 min using water to raw material ratio of 1: 20 (w/w), relatively higher extraction yields (9.618%) and less extraction time (20 min) was attained compared with hydrothermal method (9.143% extraction yields and 60 min extraction time) (He et al., 2016). Traditionally, carrageenan extraction is carried out by continuous heating up to 3 h. Thus, when carrageenan from *Hypnea musciformis* was extracted by MAE at 95 °C and 800 W for 20 min, no significant difference was found in the sulfate content compared with conventional hot water extraction (Vázquez-Delfín et al., 2014). Different extraction methods may change the structure of polysaccharides and their biological functions. For instance, the antioxidant activity of polysaccharides is higher when extracted by MAE compared with hydrothermal extraction, which is due to high content of sulfate groups, since these groups have high nucleophilic characteristics that can chelate the hydroxyl radical, thereby protecting samples against oxidative damage.

The UMAE method takes advantages of vibration cavitation in ultrasonic waves and high irradiation of microwave. Microwave provides fast and convenient heating of samples but is limited by mass transfer, while ultrasound can produce strong physical forces through cavitation but has limited heating capacity. Therefore, the combination of ultrasound and microwave radiation is a complementary method that may exhibit the advantages of both methods. Compared with conventional hydrothermal method, UMAE performs better and with advantages including higher extraction efficiency, reduced solvent requirement, and shorter extraction time (Xu, Liu, et al., 2018). Recently, we used a response surface methodology to optimize UMAE during the extraction of *Porphyra haitanensis* polysaccharide (PHP). The optimum extraction condition was at 79.94 °C for 29.64 min with an algal material to liquid ratio of 1:41.79 (g/mL), which a gave maximum yield of 20.98% (Xu et al., 2020). When *G. lemaneiformis* polysaccharides were extracted

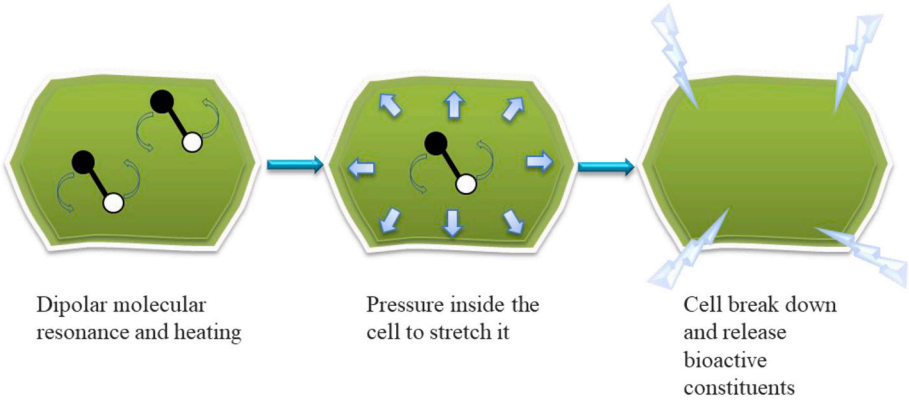

Fig. 2. The mechanism of microwave-assisted extraction of RSPs.

using UMAE, the optimum condition for extraction was 87 °C and 50 W for 31.7 min with an algal material to liquid ratio of 1.0:60.7 (w/v), yielding 34.84% polysaccharides. Contrary, the extraction conditions of conventional heating were at 90 °C for 2 h, with an algal material to liquid ratio of 1:30 (w/v), and 29.7% yield (Shi et al., 2018).

The EAE method has attracted more attention in the extraction of RSP due to its efficient, sustainable, benign, and eco-friendly technology. The fundamental principle of EAE is the destruction of cell wall under mild conditions to release intracellular polysaccharides (Nadar et al., 2018). When *Gracilaria lemaneiformis* polysaccharides were extracted by EAE with optimized conditions of 95 °C, pH 5, for 40 min and 6000 U/g α-amylase, the extraction yield reached 49.15% (Wu et al., 2017). Kulshreshtha et al. demonstrated that the EAE method improves the extraction efficiency of bioactive polysaccharide derived from *Chondrus crispus* (Kulshreshtha et al., 2015). Using EAE, higher percentage of dry matter was obtained in the supernatant extract with carbohydrases C1 and C3, which gave rise 73.4% and 71.2%, respectively. Moreover, the EAE enhanced the recovery of protein, neutral sugars, uronic acids, and sulfates (Kulshreshtha et al., 2015). However, under similar conditions of temperature and duration, hydrothermal extraction had lower yield (24.7%).

Both conventional and innovative extraction methods can be used to obtain RSPs of interest. The extracted RSPs can then be tested and applied in functional foods or other potential industrial applications. However, different extraction methods could change bioactivity, molecular weight, and composition. Thus, there is the need for novel extraction methods and improvements in conventional methods to preserve the bioactivity and yield of RSPs. Given the increasing global demand for RSPs and the integration of multidisciplinary technologies, more new innovative extraction methods will be explored that are energy saving, very efficient, and have high yield. This would industrialize and scale up RSPs extraction in the future.

3. Purification of RSPs

During RSPs extraction, non-polysaccharide components such as proteins, pigments, and other small compounds are coextracted. These non-polysaccharide components are usually removed using organic solvents such as acetone, benzene, methanol, ethanol, chloroform, and dichloromethane (Michalak & Chojnacka, 2015). On the other hand, Sevage and enzymatic methods are commonly used to remove protein from RSPs. When crude polysaccharides were extracted from *Gracilaria corticata*, the Sevage method (Seedevi et al., 2017) was used to remove denatured proteins following 6–7 times repeated treatment with chloroform and *n*-butanol reagents (Fidelis et al., 2014). Unlike the Sevage method, the enzymatic method has the advantages of not containing organic solvent, although it requires a longer digestion time and higher operational costs.

The aqueous two-phase systems (ATPS) are a recent method that improves biocompatible and a more efficient liquid-liquid extraction method for the fractionation of proteins and polysaccharides (Khan, Cheong, & Liu, 2019). Given that solutions of proteins and polysaccharides are immiscible, they form distinct phases. This method has many advantages, including low cost, easy scale-up and operation, no organic solvents, and retain bioactivities of all target components (Du et al., 2018; Yan et al., 2018). In our previous study, the ethanol/$(NH_4)_2SO_4$ aqueous two-phase system was efficient at removing proteins from *G. lemaneiformis* compared with the Sevage method. The tie line length of ATPS was 50 [284.5 mg ethanol and 212 mg $(NH_4)_2SO_4$], in the intermediate phase was about 81.1% with a relatively low amount of proteins (Shi et al., 2018).

Crude RSPs mixtures can be purified using many methods, including ethanol precipitation, biorefinery approach, membrane separation, column chromatography and so on. Ethanol precipitation is a unique and convenient method for the precipitation of polysaccharides extracted red seaweeds. For example, dried polysaccharides were obtained from *Porphyra haitanesis* and *Gracilaria lemaneiformis* by adding 4 volumes of ethanol to remove low molecular weight impurities (Liu et al., 2019). Biorefinery approach, which means an effort to valorize the side stream and utilize the whole red seaweed biomass, has recently been discussed. Removal of seaweed electrolytes can favor hybrid carrageenan extraction.

Due to the reduced conductivity, washing of the seaweed before extraction can remove electrolytes from it, thus favor hybrid carrageenan extraction to a large extent (Bahari et al., 2021). Membrane separation is commonly used to remove salts and low molecular weight compounds from crude extracts or purified fractions. The extracts from *Grateloupia livida* were extensively dialyzed with distilled water for 3 days by membrane molecular weight cut-off of 3.5 kDa to remove small molecular weight impurities (Tang et al., 2017). The membrane separation method is also used to obtain desired RSPs fractions based on their molecular weighs. Different membrane molecular weight cut-off in the range of 3.5–12 kDa were used to fractionate κ-carrageenan to obtain 6.8 kDa, 17 kDa, and 380 kDa molecular weight of κ-carrageenan, respectively (Liang et al., 2014). To achieve large-scale purification of RSPs, the membrane separation method is expected to gain support in industries in the future due to its operational flexibility, cost-effectiveness, low energy requirements, and easy availability of membrane materials. Column chromatography, which comes in the form of anion-exchange and gel filtration chromatography, is one of the most efficient methods for purifying RSPs (Du et al., 2018; Yan et al., 2018). For instance, anion-exchange chromatography can be used for the separation of neutral RSPs from negatively charged polysaccharides through salt gradient elution or change in pH. On the other hand, gel filtration chromatography is suitable for the fractionation of RSPs with different molecular weights. A homogenous polysaccharide with molecular weight of 43 kDa was obtained when crude polysaccharides from *Gracilaria corticata* were purified by gel filtration chromatography using a High-Resolution Sepharose 4-LB Fast Flow column (Seedevi et al., 2017). High purity RSPs can be obtained by column chromatography purification, which can enable researchers to evaluate the biological functions and understand the structure-function relationship of RSPs and their related mechanism of bioactivities. However, the major bottlenecks in the use of column chromatography include high cost and resin-regeneration, which prevent its use for industrial scale purification. Thus, these challenges in the use of column chromatography purification of RSPs could increase the prices of the final products (*i.e.*, functional foods and pharmaceutical products), hence, the fundamental goal for the use of RSPs in industries is to develop green, sustainable, and high efficiency purification techniques. To this end, there is the need to bridge the technology gap between academia and industries, which would allow co-operation to establish efficient and sustainable methods for obtaining high quality RSPs products at industrial scale. Such cooperation would help churn out functional foods and pharmaceutical products from RSPs that are acceptable and affordable to consumers.

4. Physicochemical and structural features of RSPs

The chemical structures of RSPs can be determined using combinations of chemical analysis, spectroscopy, and chromatography techniques, including methylation analysis, periodate oxidation, Smith degradation, infrared spectroscopy, gas chromatography (GC), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance spectroscopy (NMR). RSPs are the main source of sulfated polysaccharides in seaweeds. Thus, the chemical structure of most RSPs contains sulfated galactan types of polysaccharides, *i.e.*, agarans, porphyran, and carrageenans.

Porphyran is mainly isolated from *Porphyra* species, such as *Porphyra yezoensis*, *Porphyra haitanensis*, *Porphyra suborbiculata*, and *Porphyra tenera* (Bhatia et al., 2013). Porphyran is made up of repeating disaccharide units of alternating 4-linked α-L-galactopyranose-6-sulfate (L6S) and 3-linked β-D-galactopyranose (G) residues, with substitutions such

as sulfate and/or methyl groups at different positions at *O*-4,6 of the latter residues (Correc et al., 2011). The molecular weight of porphyran derived from various species ranges from 6 × 10^5 Da in *Porphyra yezoensis* (Yu et al., 2015) to 7860 Da in *Porphyra vietnamensis* (Bhatia et al., 2015). These molecular weight differences could be due to environmental factors, collection time, and extraction processes. The chain configuration of polysaccharides refers to the shape and size of polysaccharides in solution, including random coil, single helix, double helix, triple helix, worm-like shape, rod-like shape, and aggregate (Zhang et al., 2011). The use of high-performance size-exclusion chromatography coupled with viscometer, refractive index detector, and multi-angle laser light scattering revealed that polysaccharides extracted from *Gracilaria lemaneiformis* have a flexible chain in aqueous solution with molecular weight of 1.570 × 10^5 Da and intrinsic viscosity of 133.94 mL/g (Veeraperumal et al., 2020). In fact, most RSPs have relative high proportion of repeating sequences, which makes them amenable to analysis by 1D NMR and 2D NMR. To obtain more acute chemical structures, the strategy of partial reductive degradation is used to produce oligosaccharides from RSPs followed by size-exclusion chromatography purification prior to NMR analysis.

Carrageenan, which is found in *Eucheuma* sp., *Chrondrus crispus*, *Hypnea* sp., and *Gigartina* sp. (Li et al., 2014), contains alternate units of 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose, and is usually classified into six forms, *i.e.*, κ-, ι-, λ-, μ-, ν-, and θ-carrageenan (Yegappan et al., 2018). The first three forms (κ, ι, and λ) are the most important types of carrageenan. The differences among these carrageenans lie in the position and number of sulfate groups on the galactose unit. The κ-carrageenans are composed of alternate units of 3-linked 4-sulfate-β-D-galactopyranose and 4-linked anhydro-α-D-galactose (Sun et al., 2015), whereas ι-carrageenan and λ-carrageenan are similar to κ-carrageenan in structure but with two and three sulfate groups, respectively. In various species of red seaweeds, carrageenans with different molecular weights ranging from approximately 200 to 800 kDa have been found (McKim et al., 2019). For instance, the carrageenan extracted from *Eucheuma spinosum* has molecular weight of about 840–901 kDa (Diharmi et al., 2017), while that from *Gymnogongrus tenuis* is about 100 kDa (Perez Recalde et al., 2016). Biological studies of RSPs must be interpreted from their chain configuration assessed by combination of analytical ultra-centrifugation, high performance size exclusion chromatography, light scattering, and so on. For instance, according to the Mark-Houwink-Kuhn-Sakurada analysis, the change in intrinsic viscosity [η] with molecular weight has a flexible coil configuration for lambda-carrageenan in the molecular weight ranges of 340–870 kDa (Almutairi et al., 2013). Structure characterization of RSPs sometimes requires determination of the attached sulfate groups along their repeating backbones. Thus, comparison of the methylation of native RSPs to their desulfated counterparts is a good strategy to help verify the position of attached sulfate groups. Hydrolysis of the permethylated and desulfated polysaccharides to yield partially methylated monomers, followed by reduction and acetylation steps to produce partially methylated alditol acetates products, allows the analysis and quantification of the products by GC–MS.

Linear mannan and xylomannans are structural components of cell wall and intercellular matrices in some species of red seaweeds. However, sulfated galactans are the main polysaccharide types so far derived from red seaweeds. These polysaccharides have different chemical structures, molecular weights, functional groups, and chain configurations, which could be due to species differences, growth environment, and testing conditions of different red seaweeds, hence, the different biological activities of RSPs. It is therefore vital to understand the structures of RSPs, to be able to explore their structure-activity relationships and interpret differences in biological activities between various RSPs.

The general structures of three forms of carrageenan (κ, ι, and λ), which are also the most important types, and porphyrin, linear-mannan, which mentioned above, are shown in Fig. 3.

5. The remarkable range of biological activities of RSPs

Throughout history, red seaweeds have been used in Asian countries as food raw materials, food ingredients, food supplements, and feed. There is now a growing trend in the consumption of red seaweed in other countries, probably due to its delicious taste and health benefits. Beside the growing global demand of red seaweeds as functional foods, its products, RSPs, have attracted considerable research attention. *In vitro* and *in vivo* studies have demonstrated the health promoting effects of RSPs in human (Germic et al., 2019; Yegappan et al., 2018). Thus, given the potential effects of RSPs in disease prevention and as healthy food supplements, we highlight the biological activities of RSPs and some mechanistic studies on their involvement in the regulation disease prevention pathways.

5.1. Immuno-modulatory activities

The immune system is the primary defense system of the body that helps destroy abnormal cells (such as cancer cells) and protect against pathogens and foreign molecules. RSPs have attracted global attention because they do not have side effects and possess distinct biological activities, especially immuno-modulatory activities. *In vitro* and *in vivo* studies have shown that RSPs exhibit immuno-modulatory activities

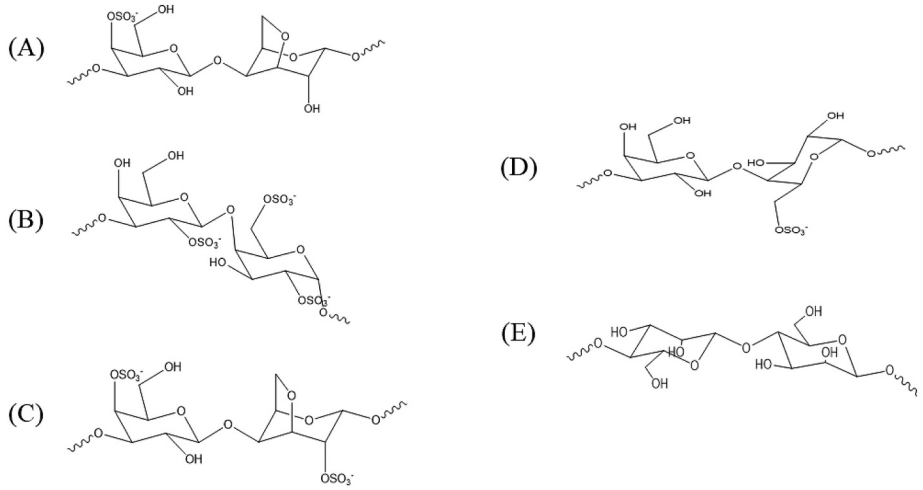

Fig. 3. The general structures κ-carrageenan (A), λ-carrageenans (B), ι-carrageenans (C), porphyran (D), linear-mannan (E) which are found in red seaweeds.

through multiple signaling pathways and targets (Huang et al., 2019). The effects of RSPs on the thymus index, spleen index, and serum interleukin level, revealed by *in vivo* studies, are illustrated in Fig. 4. *In vitro* studies have been used to explore the effects of RSPs on the immune activation of macrophages, lymphocytes, and natural killer (NK) cells and their mechanisms, such as increase in the secretion of cytokines [interleukin (IL)-1, IL-6, tumor necrosis factor, interferon (IFN), *etc.*], and their role in trauma healing, anti-tumor, and other therapeutic effects.

Macrophages are one of the major immune cells that regulate immune system by presenting active mediators and antigens (Germic et al., 2019). Phagocytosis is a major role of macrophages in the immune system. RSPs enhance phagocytosis by RAW264.7 cells *via* activation of NF-κB and p38MAPK signaling pathways. Polysaccharides purified from *G. lemaneiformis* with 3.06% sulfate content could significantly improve the proliferation and pinocytic capability of macrophage RAW264.7 cells and promote the production of ROS, NO, IL-6, and TNF-a by activating mRNA expressions of iNOS, IL-6 and TNF-α (Ren et al., 2017). Oral administration of κ/β-carrageenan from *Tichocarpus crinitus* to mice at 100 mg/kg/day could significantly increase serum levels of IFN-γ, IL-1β, IL-4, and IL-12 as well as change the motility and morphology of murine peritoneal macrophages (Cicinskas, Kalitnik, et al., 2020).

Immuno-modulatory activity correlates with molecular weight, hence, the high viscosity, large molecular weight, complex structure, and poor solubility of RSPs makes them unconducive to human absorption and utilization. On the other hand, very low molecular weight may lead to the inability to form polymeric structures required for activity, thus reducing immune activity. Enzymatic degraded products of *Porphyra haitanensis* polysaccharides (217 kDa) had superior activities at enhancing the proliferation, phagocytosis, and NO secretion by RAW264.7 macrophage cells compared to higher molecular weight polysaccharides (Li et al., 2020). For instance, comparison of the immuno-modulatory activity of four different molecular weight fractions purified from *P. haitanensis*, namely PHPD-I (329 kDa), PHPD-II (203 kDa), PHPD-III (128 kDa), and PHPD-IV (10 kDa), revealed that the lowest molecular weight fraction (*i.e.*, PHPD-IV) had the highest immuno-modulatory activity (Gong et al., 2020). Similarly, low molecular weight degraded products of carrageenan did not only retain the biological activity of their precursors, *i.e.*, production of cytokine IL-1β, IL-6, IL-18, and TNF-α, but also had increased efficacy dose-dependently. Thus, degradation of carrageenans could possibly be a solution to overcome the limitations of their chemical and physical properties to increase their biomedical application (Cicinskas, Begun, et al., 2020). In addition, chemical modification of carrageenan may improve their bioactivities and thus facilitate their applications in various biological systems (Jiang et al., 2021).

In terms of mechanistic studies, RSPs can modulate the mitogen-activated protein kinase (MAPK) and nuclear factor kappa-B (NF-κB), which are involved in immune cells activation. The MAPK signaling pathway controls and regulates biological responses of cells such as proliferation, differentiation, and apoptosis in eukaryotic cells (Liang & Yang, 2019). MAPK includes four cascades, *i.e.*, the extracellular regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), p38 mitogen-active protein kinase, and ERK5 mitogen-activated kinase (Johnson &

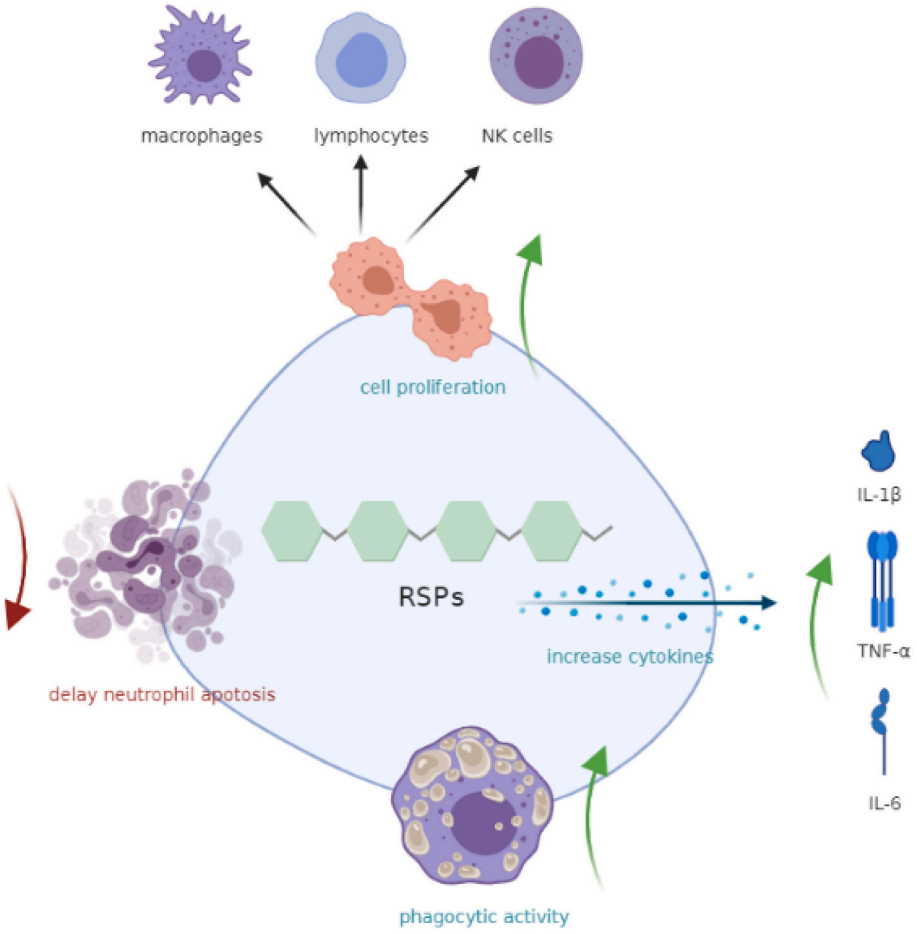

Fig. 4. *In vitro* studies of the effects of red seaweed polysaccharides (created with BioRender.com). The *in vitro* studies of the effects of RSPs include four major aspects: cell proliferation (mainly macrophages, lymphocytes and natural killer cells), cytokines increasing (such as interleukin-1β, interleukin-6 and tumor necrosis factor-α), to enhance phagocytic activity and to delay neutrophil apoptosis.

Lapadat, 2002). The PHPD-IV-4 fraction purified from *P. haitanensis* could significantly up-regulate the levels of phosphorylated ERK1/2, JNK, and p38 in a dose-dependent manner, suggesting that the immunomodulatory mechanism of macrophages was dependent upon MAPK phosphorylation (Gong et al., 2020). Similarly, polysaccharides derived from *Grateloupia livida* (Harv.) Yamada, which consist of a mixture of carrageenan and agarose, could significantly activate ERK, JNK, c-JUN, and P38 after administration for 1 h, indicating that these polysaccharides activate macrophages to initiate immune response (Liu et al., 2020).

NF-κB is another important immune signaling pathway that regulates diverse immune processes including T cell differentiation and proliferation. Polysaccharides extracted from *P. haitanensis* and *Porphyra yezoensis* activate NF-κB signaling pathways in splenic cells and are involve in Treg cell differentiation (Fu et al., 2019). Similarly, treatment of RAW 264.7 cells with κ-carrageenan polysaccharides upregulated the expression of TLR4 and translocated the main subunit of NF-κB (p65) (Shu et al., 2017).

From the foregoing, it is clear that RSPs are important marine natural products that have great potential as immunomodulation agents. Thus, RSPs could be good immuno-modulatory agents, because of their natural origin, which makes them generally safe, easily available, and less expensive.

5.2. Anti-obesity activities

Obesity is a global public health problem that affects the quality of life and overall life expectancy of approximately 650 million people (Albury et al., 2020). The obese are also at increased risk of various health problems, including atherosclerosis, hypertension, heart disease, stroke, chronic inflammation, insulin resistance, diabetes mellitus, and even cancers (Martel et al., 2017). Long-term use of anti-obesity drugs has severe side effects, such as cardiovascular problems, gastrointestinal adverse effects, liver failure, and acute kidney injury (Dietrich & Horvath, 2012). Thus, researchers are exploring more effective and safe pharmacological options for the prevention and treatment of obesity. Dietary regimen is a key in the treatment of diabetes and obesity, hence, diets rich in polysaccharides and low in fat have been recommended in recent years. The expanding unique biological activities of RSPs suggest that they could be explored as effective anti-diabetic drugs.

Given that inhibition of lipid synthesis is one potential anti-obesity strategy, the beneficial effects of RSPs on blood lipid profile could be leveraged to reduce risk of cardiovascular events. High-fat diets induce high body weight, body fat percentage, serum cholesterol and abnormal levels of adipocytokines. Mice models fed on high-fat diets supplemented with 5% ι-carrageenan extracted from *Sarconema filiforme* (du Preez et al., 2020a) or with 5% κ-carrageenan extracted from *Kappaphycus alvarezii* (Chin et al., 2019) had decreased body weight, systolic blood pressure, abdominal fat, liver fat, and plasma total cholesterol concentrations. Interestingly, *G. lemaneiformis* polysaccharides could inhibit cholesterol synthesis by attenuating SREBP-2 and HMGR expression, and increasing the conversion of cholesterol to bile acids by upregulating the expression of LxRα and CYP7A1 simultaneously (Huang et al., 2019).

Although high-fat diets are associated with obesity, weight gain, abnormal serum lipid, and so on, adipose tissues are also essential sources of energy. Under conditions of excess calories or lipids, both the number and volume of adipocytes increases. In hamsters, polysaccharides derived from *Gelidium amansii* have demonstrated anti-obesity activities due to their ability to regulate plasma adipocytokines by lowering the weight of adipose tissues. When 3% hot water extract of *Gelidium amansii* was added as feed, the polysaccharides were able to significantly decrease the weight of adipose tissue, body, liver, and lower plasma leptin, total cholesterol, and triglyceride levels in obese hamsters. The possible mechanism is that the polysaccharides enhance the phosphorylation of liver AMPK and increases lipolysis rate, thereby reducing the accumulation of triglycerides and lipoprotein lipase activity in adipose tissues (Yang et al., 2019). Obesity increases cardiovascular risk by elevating blood sugar levels and lowering insulin concentration. Given the positive effects of RSPs on lowering fasting blood glucose, improving glucose tolerance, increasing levels of C-peptide, and liver glycogen, RSPs extracted from *Gracilaria lemaneiformis* could be used to treat glucose metabolism disorders (Wen et al., 2017). Similarly, RSPs from *Porphyra* spp. could effectively decrease the postprandial blood sugar level in rats from 14.70 mmol/L to 5.35 mmol/L (Zeng et al., 2020).

The key enzymes that inhibit starch metabolism are α-amylase and α-glucosidase, which catalyze the hydrolysis of starch into glucose. These key enzymes are also therapeutic targets for overweight- and obesity-associated metabolic complications like diabetes and cardiovascular disease (CVD). RSPs from *G. lemaneiformis* could effective inhibit α-amylase activity with IC_{50} value of 3.94 μg/mL (Wen et al., 2017). Similarly, sulfated RSPs from *Bangia fusco-purpurea* inhibited α-amylase and α-glucosidase in a concentration-dependent manner with IC_{50} value of 1.26 μg/mL and 1.34 μg/mL, respectively. Moreover, kinetic analyses of this polysaccharide showed competitive inhibition against α-amylase and non-competitive inhibition against α-glucosidase (Jiang et al., 2019).

In industrialized countries, lifestyle changes in terms of increased consumption of processed foods, especially unhealthy meat products, contribute significantly to obesity. Increasing interest in healthy foods by consumers has prompted meat industries to develop new low-fat healthy products that conform more with nutritional guidelines. Due to the unique characteristics of carbohydrate-based hydrocolloids, such as gelation, texture, water binding, adhesion, and chewiness, they are potentially useful in the manufacture of low-fat meat products (Ganesan et al., 2019). Thus, as hydrocolloids, RSPs have been added to various meat products to prevent meat product spoilage and obesity (Gullón et al., 2020).

Given these unique properties of RSPs to improve intestinal microflora dysbiosis by regulating lipid metabolism, they have features similar to some weight loss medications, and could even be more effective without side effects. Therefore, RSPs could potentially be used as promising anti-obesity and anti-diabetic therapies.

5.3. Antioxidant activities

In biological systems, inappropriate production of reactive oxygen species (ROS), including hydrogen peroxide, hydroxyl radicals, and superoxides, may induce cellular oxidative damage. Moreover, oxidative damage could lead to the development of various diseases such as neurodegeneration, CVD, cancer, diabetes, and kidney diseases (Lushchak, 2014). A balance between ROS levels and radicals scavenging maintains normal cellular functions to prevent diseases associated with oxidative damage. Various studies have screened anti-oxidants from natural resources in the hope of developing their potential applications in functional foods and pharmaceuticals to be used in protecting against different types of cancers and related health problems.

In vitro radicals scavenging assays are easy, low cost, and well-developed methods for measuring the free radicals scavenging ability of RSPs. Many of these methods (Table 2), including 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide, reducing power, ferric reducing antioxidant power (FRAP), and oxygen radical absorption capacity (ORAC), have been used in determining the anti-oxidant abilities of RSPs.

Many studies have reported on the antioxidant potential of RSPs from *Gracilaria* genus. For instance, purified polysaccharides fractions from *Gracilaria rubra* have strong scavenging activity (approximately 50% at 2.5 mg/mL concentration) against ABTS and superoxide radicals, and can also inhibit lipid peroxidation (Di et al., 2017). The RSPs from *Gracilaria fisheri* showed DPPH radical scavenging activity in a concentration-dependent manner with IC_{50} of 3.0 mg/mL (Imjongjairak

Table 2
Summary of *in vitro* radical scavenging assays used in determining the antioxidant properties of red seaweed polysaccharides and their related mechanism of action.

Methods	Mechanism of action	Sources and references
2,2-Diphenyl-1-picrylhydrazyl	Measures decrease in the absorbance of DPPH radical at 517 nm caused by RSPs, which is due to the scavenging of the DPPH radical by hydrogen donating ability. Hydrogen donating ability of RSPs allows the donated hydrogen to form a stable and non-radical form of DPPH-H molecule.	*Porphyra haitanensis* (Khan et al., 2020), *Gracilaria chouae* (Khan, Qiu, et al., 2019), *Pterocladia capillacea* (Fleita et al., 2015), *Gracilaria debilis* (Sudharsan et al., 2015), *Gracilaria gracilis* (Olasehinde et al., 2019), *Gracilaria rubra* (Di et al., 2017), *Kappaphycus alvarezii* (Suganya et al., 2016), *Solieria filiformis* (Sousa et al., 2016), *Gracilaria filiforms* (Venkatesan et al., 2019), *Gracilaria fisheri* (Imjongjairak et al., 2016), *Gelidium amansii* (Xu, Kan, et al., 2018)
2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulphonic	Measures decrease in absorbance at 734 nm. RSPs donate a hydrogen atom by converting ABTS radical to the non-radical species.	*Porphyra haitanensis* (Khan et al., 2020), *Gracilaria chouae* (Khan, Qiu, et al., 2019), *Gracilaria gracilis* (Olasehinde et al., 2019), *Gracilaria corticata* (Seedevi et al., 2017), *Turbinaria ornata* (Saravana Guru et al., 2015), *Pyropia yezoensis* (Lee et al., 2016b), *Gelidium sesquipedale* (Martínez-Sanz et al., 2019), *Gelidium corneum* (Abdala Díaz et al., 2019), *Gelidium amansii* (Xu, Kan, et al., 2018)
Ferric reducing antioxidant power	Measures increase in absorbance at 593 nm. The ferrous ions from Ferric reducing antioxidant power reagent is reduced by RSPs in the presence of 2,4,6-tris(2-pyridyl)-*s*-triazine to formed an intense blue Fe^{2+}–2,4,6-tris (2-pyridyl)-*s*-triazine complex with absorption at 593 nm.	*Mastocarpus stellatus* (Gómez-Ordóñez et al., 2014), *Solieria filiformis* (Sousa et al., 2016), *Turbinaria ornata* (Saravana Guru et al., 2015)
Hydrogen peroxide (H_2O_2), hydroxyl radical	Reaction of RSPs with a specific amount of exogenously added H_2O_2 where RSPs eliminate the H_2O_2. Measures decrease in absorbance at 505 nm. RSPs chelates the metal ion and to prevent it reacting with H_2O_2 to form hydroxyl radicals.	*Pterocladia capillacea* (Fleita et al., 2015), *Gracilaria debilis* (Sudharsan et al., 2015), *Gracilaria corticata* (Seedevi et al., 2017), *Gracilaria rubra* (Di et al., 2017), *Kappaphycus alvarezii* (Suganya et al., 2016), *Gracilaria filiforms* (Venkatesan et al., 2019), *Ahnfeltiopsis pygmaea* (Fernando et al., 2017), *Gracilaria foliifera* (Fernando et al., 2017), *Gracilaria edulis* (Fernando et al., 2017),
Superoxide anion	Decreased absorbance of reaction mixture indicates increased superoxide anion-scavenging activity	*Gracilaria debilis* (Sudharsan et al., 2015), *Gracilaria rubra* (Di et al., 2017), *Turbinaria ornata* (Saravana Guru et al., 2015)
Metal chelating	Metal ion accelerates lipid oxidation by breaking down hydrogen and lipid peroxidase to reactive free radicals (Fento type reaction). Measures decrease in absorbance at 562 nm. RSPs compete with ferrozine for Fe^{2+} to result in decrease in the absorbance.	*Gracilaria gracilis* (Olasehinde et al., 2019), *Chondrus canaliculatus* (Jaballi et al., 2019), *Gracilaria corticata* (Seedevi et al., 2017), *Porphyra yezoensis* (Isaka et al., 2015)
β-Carotene-linoleic acid	Measures inhibition of the oxidative degradation of β-carotene (color despairing).	*Chondrus canaliculatus* (Jaballi et al., 2019)
Reducing power	Reduce iron (III). RSPs form a complex color with potassium ferricyanide, trichloroacetic acid, and ferric chloride. Measures increase absorbance at 700 nm by formation of Perl's Prussian blue.	*Chondrus canaliculatus* (Jaballi et al., 2019), *Kappaphycus alvarezii* (Suganya et al., 2016)
Oxygen radical absorbance capacity	The hydrogen atom of RSPs transfer reaction is assayed using 2,2′-azo-bis (2-amidinopropane) dihydrochloride as peroxyl radicals source and fluorescein and c-phycocyanin as molecular probes. Measures decrease in absorbance of fluorescence intensity.	*Ahnfeltia plicata* (Matsuhiro et al., 2014), *Gracilaria birdiae* (Torres et al., 2019)
Total antioxidant	Reduction of molybdenum VI to molybdenum V by RSPs and the subsequent formation of a green phosphave/molybdenum V complex at acidic pH.	*Gracilaria corticata* (Seedevi et al., 2017), *Kappaphycus alvarezii* (Suganya et al., 2016), *Turbinaria ornata* (Saravana Guru et al., 2015), *Gracilaria filiforms* (Venkatesan et al., 2019)
Photochemi-luminiscence	Transfer of one hydrogen to the biological superoxide radical $O_2^{-\bullet}$	*Mastocarpus stellatus* (Gómez-Ordóñez et al., 2014)
Nitric oxide		*Kappaphycus alvarezii* (Suganya et al., 2016), *Gracilaria edulis* (Arulkumar et al., 2018)

et al., 2016). Similarly, RSPs from *Solieria filiformis* had high scavenging activities against DPPH and hydroxyl radicals with good dose-dependent reductive capacity (Sousa et al., 2016). In our previous studies, sulfated polysaccharides from *Porphyra haitanensis* had relatively high ABTS radical scavenging potential (53.16% at 2 mg/mL), medium DPPH radical scavenging (34.63% at 2 mg/mL), and low hydroxyl radical scavenging (23.80% at 2 mg/mL) activity (Khan et al., 2020).

In vivo antioxidant defense mechanisms can be classified into enzymatic and non-enzymatic defense systems. Enzymatic antioxidant defense systems mainly include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferases (GST), and glutathione reductase, whereas non-enzymatic defense systems, mainly include glutathione, lipid peroxidase, and L-ascorbic acid. These antioxidant defense systems usually work together with endogenous enzymes to enhance the antioxidant function of the body and therefore effectively scavenge ROS.

In vivo studies have shown that the antioxidant activities of native carrageenan from *Kappaphycus alvarezii* can ameliorate alloxan induced oxidative stress response in Wistar albino rats (Sanjivkumar et al., 2020). Native carrageenan supplementation increased the activities of antioxidant enzymes, such as CAT, SOD, GST, and glutathione in the kidneys of alloxan induced diabetic rats (Sanjivkumar et al., 2020). Similarly, sulfated polysaccharides from *Gracilaria caudata* had therapeutic effects on oxidative stress induced by intraperitoneal injection of 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) in Wistar rats, shown by increased levels of CAT and SOD (Alencar et al., 2019).

Some synthetic antioxidant food additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), could induce cytotoxicity, apoptosis, and exert endocrine disrupting effects. For example, tert-butyl hydroquinone induces peroxide production and therefore is potentially carcinogenic (Yang et al., 2018). Research interest is growing in finding alternative sources of antioxidants in the food industry. Thus, given that RSPs demonstrate important role as radical scavengers (*in vitro*) and prevent oxidative damage in macroalgae (*in vivo*), RSPs could be leveraged as antioxidant compounds with huge health benefits.

5.4. Anti-cancer activities

Cancer incidence is increasing in greater proportion in both developed and developing countries. However, current anti-cancer drugs are not only toxic to cancer cells, but also normal cells and tissues. These anti-cancer drugs have severe side effects on normal mitotic cells with cytotoxic effects, which cause vomiting, alopecia, anemia, and nerve changes. Thus, the search for naturally-derived new effective non-toxic

antitumor drugs has received increased attention.

Polysaccharides mainly exert their anti-cancer activities through activation of host immune response (Khotimchenko et al., 2020). RSPs exert the anti-cancer activities of RSPs is however exerted through multiple mechanisms, with short or long-term feeding of RSPs shown not to induce significant toxicological responses. Schematic representation of anti-cancer activities of RSPs is shown in Fig. 5. Recent studies have shown that intra-tumoral injection of λ-carrageenan inhibits tumor growth of B16-F10 and 4 T1 bearing mice. This antitumor feature of λ-carrageenan is related to its ability to improve immune response by increasing levels of tumor-infiltrating M1 macrophages, dendric cells, and activation of $CD4^+$ and $CD8^+$ T lymphocytes in spleen and enhance the secretion of IL-17A and TNF-α (Luo et al., 2015). Two different RSPs from *Porphyra haitanensis* and *Porphyra yezoensis* have also been shown to enhance immune response in immunosuppressed mice by regulating the Th1 and Th2 responses to inhibit cancer progression (Fu et al., 2019). *In vivo* studies using the Sarcoma-180 bearing mice models, polysaccharides from *Sarcodia ceylonensis* had anti-tumor activity, as the RSPs could significantly increase the thymic and splenic indices of the S180 mice to strongly promote the secretion of IL-2, TNF-α, and IFN-γ (Fan et al., 2014).

Besides the direct inhibition and killing of tumor cells by RSPs, other important properties include inducing apoptosis, blocking cell cycle, and affecting cell signal transduction of cancer cells. For instance, porphyran extracted from *Pyropia yezoensis* had no toxicity on normal human liver cells HL-7702 but could inhibit the proliferation of cancer cells such as Hep3B, HeLa, and MDA-MB-231 (He et al., 2019). Similarly, our previous study revealed that porphyran extracted from *Porphyra haitanensis* inhibits the growth of colon cancer cells HT-29, LoVo, and SW-480 with no toxic effects on normal human cells HaCaT. Th results further revealed that porphyran induced cell death (*e.g.*, in HT-29 cells) by arresting cells at the G0-G1 phase (Yao et al., 2020). Sulfated polysaccharides extracted from *Laurencia papillosa* induced G1-phase arrest and G2/M phase apoptosis in MDA-MB-231 cells, coupled with decreased levels of cyclins D1, D2, and E1 transcripts and their related cyclin dependent kinases, *i.e.*, Cdk2, Cdk4, and Cdk6 (Murad et al., 2016).

The anti-cancer activity of RSPs is also believed to be due to their ability to enhance antioxidant activity. For instance, κ-carrageenan from *Kappaphycus alvarezii* (molecular weight of 67 kDa) could prevent colon carcinogenesis by inducing apoptosis in the colon cancer cell line HCT116. The induction of apoptosis in HCT116 by κ-carrageenan is probably through ROS-mediated mitochondrial pathway coupled with downregulation of XIAP and upregulation of caspase3 (Raman & Doble, 2015).

Many reports on the anti-cancer activities of RSPs are linked with their ability to activate the immune system, inhibit cancer cells, and enhance antioxidant activity (Table 3). Cancer occurrence and development is a complicated process that involves various cells such as immune cells, inflammatory cells, cancer cells, fibroblasts, vascular smooth muscle cells, and so on. Thus, there is huge promise in the use of RSPs as potent anti-cancer agents and efficient adjuvants in anti-cancer therapeutics.

The safety of carrageenan has been raised by some researchers, who believe that it may cause or promote inflammation, specifically colitis (McKim, 2014). However, the methodology used in some of these studies have been questioned, given that some of the published experiments and *in vitro* findings could not be replicated by others (McKim Jr. et al., 2016; Weiner et al., 2007). Carrageenans also have the potential to activate coagulation due to high concentrations, which produces procoagulant effects (Jiang et al., 2021; Melo & Mourão, 2008). Thus, the size or molecular weight of the polysaccharide needs to be taken into consideration. Low molecular weight fragments could be used to obtain carrageenan derivatives with no side effects. Further breakdown of the obtained oligosaccharides can also somehow eliminate the side effects of polysaccharides. Nonetheless, further studies are required to investigate the safety of carrageenans or their acute toxicology, which must be systematically carried out in specific portions of the population,

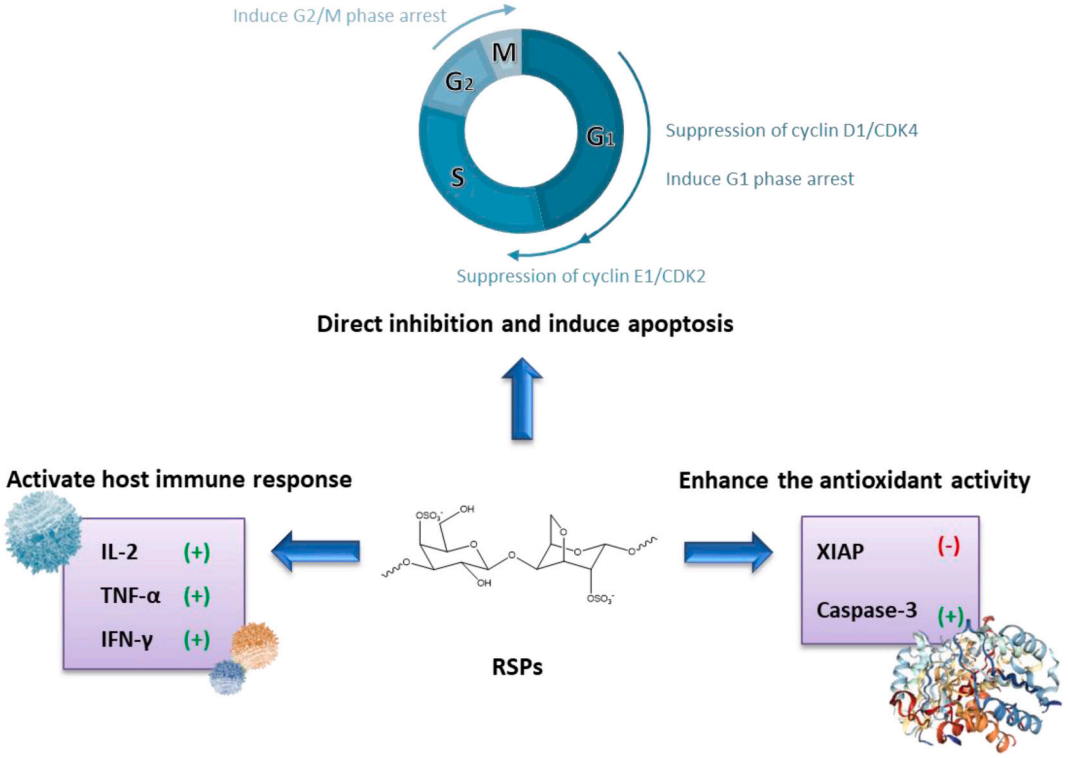

Fig. 5. Schematic representation of anti-cancer activities of RSPs IL: interleukin; TNF-α: tumor necrosis factor alpha; TR: interferon gamma; XIAP: X-linked inhibitor of apoptosis protein (−): RSPs down-regulate the expression of signaling molecules or proteins (+): RSPs up-regulate the expression of signaling molecules or proteins.

establishing the doses, experimental duration, and long-term effects.

5.5. Prebiotic activities

The human gastrointestinal microbiome contains distinct microbial communities with highly diverse microbial populations. These microbes play important protective, immunologic, and metabolic functions in the body. We have learned that diseases associated with dysbiosis of the gut microbiota can be improved by therapeutic modalities involving the microbiota, and we still need to find new tools and approaches (Pan et al., 2021). About a decade ago, studies showed that specific naturally-derived polysaccharides can provide prebiotic activity by accelerating energy metabolism of gut microbiota and regulate gut microbiota homeostasis.

In the gastrointestinal tract, the main physicochemical properties that affect the physiological effects of RSPs are viscosity, solubility, molecular weights, chemical structures, adsorption, and water retention quality. Generally, the components or structures of RSPs are not easily degraded during digestion until they enter the large intestine. The schematic representation of prebiotic activities of RSPs is shown in Fig. 6.

Our previous study revealed that, when RSPs from *Porphyra haitanensis* are incubated with saliva, simulated gastric acid, and small intestinal fluids, no degradation or change in molecular weight of RSPs occurs, indicating that this type of RSPs cannot be degraded in the digestive tract (Xie & Cheong, 2021; Xu et al., 2020). Similarly, polysaccharides from *Gracilaria rubra* had no effect on simulated small intestine digestion (Di et al., 2018). The inability to digest some RSPs is due to the absence of the corresponding enzymes in the upper gastrointestinal tract.

Although RSPs escape adsorption and digestion by host enzymes in the upper gut, they are metabolized by microbiota in the cecum and colon (Zheng et al., 2020). Short-chain fatty acids (SCFAs) are the main colon bacterial fermentative metabolites, which mainly consist of acetate, propionate, and butyrate. SCFAs could have wide-ranging effects on various aspects of host physiology, including effect on gastrointestinal epithelial cell integrity, glucose homeostasis, appetite regulation, immune function, and lipid metabolism (Koh et al., 2016). *In vitro* fermentation methods are usually used to stimulate conditions similar to the human large intestine by incubating substrates with human fecal matter in anaerobic fermenter systems. In our previous studies on the fermentation behavior of polysaccharides from *Porphyra haitanensis* and *Gracilaria lemaneiformis* using an *in vitro* fermentation model with human fecal inoculum, high levels of SCFAs were produced during the fermentation, with acetic acid, propionic acid, and butyric acid being the predominant metabolites. The molecular weight and intrinsic viscosity of the polysaccharides also decreased due to fermentation by the gut microbiota (Xie et al., 2021; Xu et al., 2019; Zhang et al., 2020). The fermentation behavior of polysaccharides may be related to the prebiotic effect and human intestinal health. Using *in vitro* fermentation, Bajury et al. showed that RSPs extracted from *Kappaphycus alvarezii* significantly increased total SCFAs production, particularly acetate and propionate (Bajury et al., 2017).

In vivo methods have also been used to try and explain the mechanism of digestion and fermentation behavior of RSPs and evaluate their proposed prebiotic functions. For instance, sulfated polysaccharides from G*elidium pacificum* had beneficial effect on mice with antibiotic-associated diarrhea and markedly increased the concentration of acetates, propionates, and total SCFAs (Cui et al., 2020). Similarly, BALB/c mice fed with high-fat diets had a significant decrease in SCFAs level, while after treatment with polysaccharides from *Gracilaria lemaneiformis*, the SCFAs level increased (Sun et al., 2018).

In vitro and *in vivo* studies that show the effects of RSPs on gut microbiota and SCFAs production are summarized in Table 4.

Gut microbiota imbalance or dysbiosis is related to many health problems such as inflammatory bowel disease, cancer, immune disorders, diabetes, and obesity. Dietary RSPs can regulate the composition and abundance of gut microbiota because these microbes can adaptively deploy several glycoside hydrolases and polysaccharide lyases to digest the bond types in RSPs for utilization by colonic microbiota. The effects of RSPs on gut microbial population have been explored by several *in vitro* and *in vivo* studies (Zheng et al., 2020).

Different RSPs and their quantities can have a major influence on the populations of different bacteria within the large-intestinal community (Table 4). For example, sulfated polysaccharides derived from *Gracilaria lemaneiformis* increased the abundance of *Bacteroidetes* and decreased the abundance of *Firmicutes* in *in vitro* human fecal inoculum studies (Zhang et al., 2020). The genuses *Bacteroides* have various genes that encode carbohydrate active enzymes. Thus, *Bacteroides* are broadly adapted to the degradation of polysaccharides into oligosaccharides that combines with the Sus-like proteins, before being imported into bacteria, and ultimately catabolized to produce functional metabolites SCFAs. Notably, *Bacteroidetes plebeius* could grow with porphyran, whereas *Bacteroidetes uniformis* NP1, grew on agarose, but showed reduced growth with porphyran. On the other hand, *Bacteroidetes thetaiotaomicron* VPI-3731 exhibited specific growth with carrageenan (Hehemann et al., 2012). The relative abundance of *Bacteroides plebeius* increased in the presence of sulfated polysaccharides from *Gracilaria rubra*, which could be attributed to specific enzymes that break down the chemical structure of *Gracilaria rubra* polysaccharides produced by *Bacteroides plebeius* (Di et al., 2018). Carrageenan does not only increase the growth of *Bacteroidetes*, but also significantly increased *Bifidobacterium* population, obtained from *Kappaphycus alvarezii* (Bajury et al., 2017). Among other gut microbial communities, *Bacteroides* and *Bifidobacterium* coevolved to use diverse types of polysaccharides through harmonizing distinct carbohydrate utilization systems. *Bacteroides* and *Bifidobacterium* usually share digested oligosaccharides, carbohydrate-active enzymes, and metabolites for sustaining gut microbial symbiosis and improving fitness of both communities (Singh, 2019).

In vivo studies have demonstrated similar results of gut microbiota with *in vitro* fermentation studies. For instance, in high-fat diet mice, the relative abundance of *Bacteroides*, *Ruminococcus_1*, and *Lactobacillus* in the gut microbiota, were significantly decreased compared with normal control. However, after intervention with sulfated polysaccharides from *Gracilaria lemaneiformis*, this decrease was significantly reversed (Huang et al., 2019). Similarly, in mice with antibiotic-associated diarrhea, polysaccharides from *Gelidium pacificum* had beneficial effects on gut microbiota by increasing the relative abundance of *Bacteroides*, *Bifidobacterium*, and *Oscillospira*, but decreased the relative abundance of harmful bacteria such as *Sutterella* (Cui et al., 2020).

In summary, current scientific evidence shows that RSPs have good prebiotics activities due to the absence of the necessary enzymes in the upper gastrointestinal tract. After fermentation by intestinal microbiota, RSPs could selectively be used as substrate for the growth of beneficial gut microbiota, which exploit differences in substrate preferences and competitive abilities between different gut microbial community members to affect body health. Future studies are needed to characterize more enzymes in gut microbiota, probiotics, and the *in vivo* health effects of RSPs. Furthermore, whether RSPs are degraded into harmful oligosaccharides by gut microbiota needs to be tested with *in vivo* studies. Given that dietary fiber intake is less than half the recommended levels, increased application of RSPs in food supplements is an ideal way for the promotion of public health through good health and disease prevention.

6. Quality control of RSPs

Good quality control is needed to ensure that RSPs and their related products meet safety standards and possess the requisite biological activities. The biological activities of RSPs are strongly related to their structural and molecular conformation. Thus, in RSPs related pharmaceutical products, the most common challenge with quality control is the confirmation of their structure. Generally, a combination of chemical

Table 3
Anti-tumor activities and effect of red algal polysaccharides.

Source	Cancer cell line/animal model	Inhibition rate	Mechanism of action	Structural properties	Reference
Kappaphycus alvarezii	Liver cancer cell line Hep G2, osteosarcoma cancer cell line MG63, breast cancer cell line MCF-7	IC_{50} 56.71 μg/mL IC_{50} 47.85 μg/mL IC_{50} 103.2 μg/mL	–	Carrageenan	(Suganya et al., 2016)
Laurencia papillosa	MDA-MB-231 human breast cancer cells	IC_{50} at 50 μg/mL	Apoptosis induction regulated through activation of Caspase-3 and inhibition of Bcl-2 protein	ι-Carrageenan	(Murad et al., 2016)
Laurencia papillosa	Breast cancer tumor cells MCF-7	200 μmol/L decrease in cell viability at 64.9%	Apoptosis induction regulated through the activation of caspases, PARP, P53, and Bax/Bcl-2 gene expression	κ, ι, and λ-carrageenan with molecular weight of 3.2, 5.6, and 2.58 × 10^5 Da	(Ghannam et al., 2018)
Gracilariopsis lemaneiformis	A549 lung cancer cells	IC_{50} at 50 μg/mL	Downregulation of apoptosis-related genes (PRKACB and BIRC)	A linear structure of repeated units of disaccharide agarobiose	(Kang et al., 2016)
Tribonema minus	Liver cancer cell line Hep G2	66.8% at 250 μg/mL	Induce cell apoptosis rather than affect cell cycle and mitosis of HepG2 cells	Heteropolysaccharide composed mainly of galactose with 197 kDa molecular weight	(Chen et al., 2019)
Gracilaria fisheri	Cholangiocarcinoma	IC_{50} at 8 μg/mL	Reduce cell migration, inhibit EGFR and ERK phosphorylation in EGFR/MAPK/ERK signaling pathway	Consists of 3-linked-β-D-galactopyranose and 4-linked 3,6-anhydro-α-L-galactose with partial methylation and sulfation	(Sae-lao, Luplertlop, et al., 2017)
Kappaphycus alvarezii	Human colon cancer cells HCT116	63.5% at 1000 μg/mL	Upregulate the expression levels of Bcl-2, Bcl-xL and caspase3, and downregulate XIAP and PARP-1 expression	D-galactose and 3,6-anhydro-Dgalactose, linearly linked by α(1 → 3) and β(1 → 4) glycosidic linkages	(Raman & Doble, 2015)
Gracilaria fisheri	Cholangiocarcinoma		Downregulate cell cycle proteins (cyclin-D, cyclin-E, cdk-4, cdk-2), inhibit phosphorylation of epidermal growth factor receptor and extracellular signal-regulated kinases (ERK)	Consists of 3-linked-β-D-galactopyranose and 4-linked 3,6-anhydro-α-L-galactose with partial methylation and sulfation	(Sae-Lao, Tohtong, et al., 2017)
Porphyra haitanensis	ICR mice	Gavage administration 100 mg/kg	Increase levels of TNF-α and NO	Porphyran with molecular weight of 5.82 × 10^3 Da	(Wang & Zhang, 2014)
Chondrus ocellatus	BALB/c mice	1% (w/v, 100 μL) injected every 2 days	Enhance secretion of IL-17A in spleen and increase levels of TNF-α	λ-carrageenan	(Luo et al., 2015)
Gracilaria lemaneiformis	Human cervical carcinoma HeLa	50.8% at 1000 μg/mL	–	Mainly composed of galactose with molecular weights of 85 kDa	(Shi et al., 2018)
Sarcodia ceylonensis	Kunming mice	50 mg/kg injected intraperitoneally	Promote secretion of IL-2, TNF-α and IFN-γ	Backbone of →6)-α-D-Glcp-(1 → and →6)-α-Galp-(1→ with molecular weight of 50 kDa	(Fan et al., 2014)
Gracilariopsis lemaneiformis	Human lung cancer cell line A549	IC_{50} at 8 μg/mL	Upregulates Fas/FasL	Contains D-galactose and 3,6-anhydro-L-galactose with molecular weight of 14.29–64.78 kDa	(Kang et al., 2017)
Porphyra haitanensis	Human gastric carcinoma SGC-7901 Kunming mice	70.40% at 500 μg/mL 80–160 mg/kg injected	Induce cell apoptosis	Rhamnose, arabinose, xylose, mannose, glucose, and galactose in a molar ratio of 10.25:9.38:1:12.45:9.9:11.55.	(Chen & Xue, 2019)
Porphyridium sordidum	Breast cancer tumor cells MCF-7 and MDA-MB231	IC_{50} at 32–69 μg/mL	–	Glc: Gal: Man: Rha in a molar ratio of 1: 0. 52: 0.44: 0.31	(Nikolova et al., 2019)
Pyropia yezoensis	Human cervical carcinoma HeLa	50%	Arrest the G2/M phase and reduce the expression of Cyclin B1 and CDK1	Mainly composed of 3,6-anhydro-L-galactose with molecular weight of 9.7–34.6 kDa	(He et al., 2019)
Porphyra haitanensis	Human colon cancer cell HT-29	IC_{50} at 327.3 μg/mL	Induce cell apoptosis by arresting the G0-G1 phase	Mainly comprised galactose (94.85%), molecular weight of 2.01 × 10^3 Da, intrinsic viscosity of 463.76 mL/g	(Yao et al., 2020)
Jania rubens	Human breast cancer cell MCF7	IC_{50} at 0.3125 mg/mL	Upregulates caspase 3 gene and downregulate Bcl2 gene expression	–	(Gheda et al., 2018)

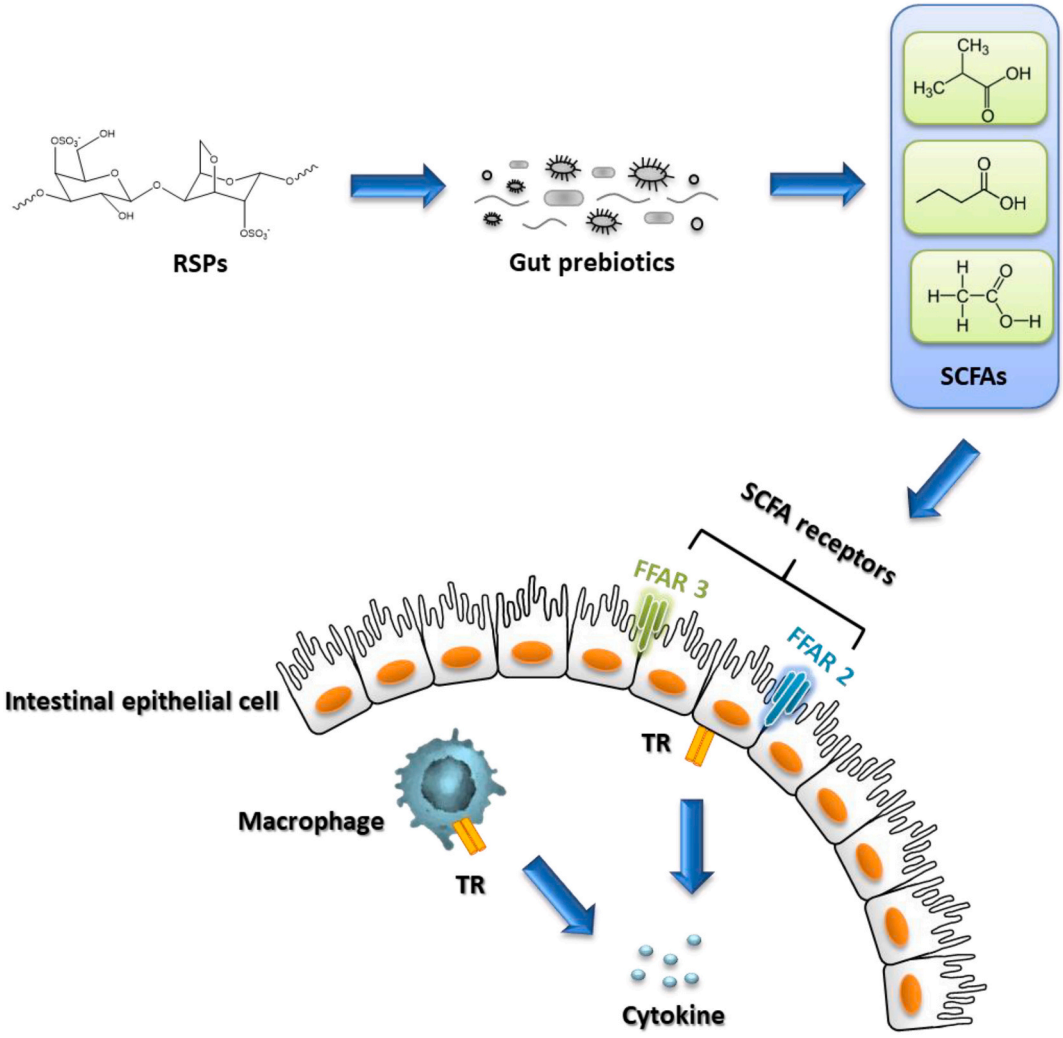

Fig. 6. Schematic representation of prebiotic activities of RSPs.
SCFA: Short-chain fatty acids; FFAR: Free fatty acid receptor; TR: Toll-like receptor.

analysis approaches such as colorimetric method, chromatographic analysis (HPLC and GC), and spectroscopy (FT-IR, UV–Vis, MS, and NMR) techniques are used to determine the structure of RSPs. However, these methods are laborious, complicated, time consuming, and require expensive equipment to obtain structural information of RSPs. A specific enzymatic method, saccharide mapping (Cheong et al., 2016), that is simple, cheap, fast, and reliable for quality control of polysaccharides derived from natural resources (Wu et al., 2015), has shown promising results in quality control of RSPs and can also discriminate polysaccharides from different resources. According to the unique structure of RSPs, α-agarases (EC 3.2.1.158), β-agarases (EC 3.2.1.81), κ-carrageenases (EC 3.2.1.83), ι-carrageenases (EC 3.2.1.157), and λ-carrageenases (EC 3.2.1.162) are potentially used for saccharide mapping and quality control. Agarases are glycoside hydrolases that can cleave the internal linkages of agarose derived from red seaweeds. The mechanism of α-agarases is to hydrolyze α-glycosidic linkages of agarose and produce agaro-oligosaccharides (Jahromi & Barzkar, 2018), while β-agarases hydrolyze β-glycosidic linkages of agarose to produce neoagaro-oligosaccharides (Park et al., 2020). Both carrageenases can catalyze the hydrolysis of carrageenans to yield a series of carrageenan-oligosaccharides (Chauhan & Saxena, 2016).

Quality control of RSPs using saccharide mapping with agarase and carrageenase follows these steps: First, the RSPs solution is extracted and prepared from samples, before being treated with a set of endo-glycosidases (agarase and carrageenase) to obtain enzymatic oligosaccharide fragments. Next, chromatographic (such as TLC, PACE, HPLC) profiles are performed and the hydrolysates oligomers are used as biological markers. Based on different functional groups, RSPs, especially those with linear backbone of 3,6-anhydro-galactose and galactose, could use this saccharide mapping strategy to ensure their quality control. In our previous study, polysaccharides derived from *Gelidium amansii*, were treated with β-agarase and enzymatic fragments

Table 4
Summary of studies on the prebiotic effects of red algal polysaccharides.

Sources	Experiment condition	Key findings	Structural information	Ref.
Porphyra haitanensis	*In vitro* fermentation by human feces	Modulate microbial community structure with notable increase in the relative abundance of Bacteroidetes and reduction in the relative abundance of Proteobacteria	–	(Xu et al., 2020)
Porphyra umbilicalis	Individual microbial fermentability test and *in vitro* fecal fermentation	Selective use by species of *Bifidobacteria*, *Lactobacilli*, and *Bacteroides*, with no growth of harmful bacteria	Porphyran	(Seong et al., 2019)
Porphyra haitanensis	*In vitro* fermentation by human feces	Increased concentration of total SCFAs. Positive effect on *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, *Defluviitalea saccharophila*, and *Faecalibacterium prausnitzii*	Mainly composed of 3,6-anhydro-L-galactose with molecular weight of 2.623×10^5 Da	(Xu et al., 2019)
Kappaphycus alvarezii	*In vitro* fermentation by human feces	Increase in total SCFA production. Increase of *Bifidobacterium* species and decrease of *Clostridium coccoides/Eubacterium rectale*	–	(Bajury et al., 2017)
Acanthophora spicifera	Individual microbial fermentability test	Promote the growth of *Lactobacillus plantarum* and suppress *Salmonella typhimurium*	–	(Ajanth Praveen et al., 2019)
Gracilaria rubra	*In vitro* fermentation by human feces	Increased the concentration of total SCFAs. Relative lower abundances of Proteobacteria and Fusobacteria, and lowering the ratio of *Firmicutes/Bacteroidetes*	–	(Di et al., 2018)
Gracilaria lemaneiformis	*In vitro* fermentation by human feces	Increased the concentration of total SCFAs. Increased the abundance of Bacteroidetes and decreased the abundance of Firmicutes	Mainly composed of 3,6-anhydro-L-galactose with molecular weight of 2.15×10^5 Da	(Zhang et al., 2020)
Gracilaria lemaneiformis	*In vitro* fermentation by human feces	Increased the concentration of total SCFAs. Increased the relative abundances of Firmicutes and Bacteroidetes, but decreased the relative abundances of Proteobacteria, Fusobacteria and Synergistetes		(Han et al., 2020)
Gracilaria lemaneiformis	High fat diet mice supplementation with polysaccharides	Increased the concentration of total SCFAs. Increased the abundance of Bacteroidetes, but decreased abundance of Firmicutes. Decreased the abundance of *Enterococus* spp., *Pseudomonas* spp., and *Ruminiclostridium* spp.	–	(Sun et al., 2018)
Gelidium pacificum	Antibiotic-associated diarrhea mice supplementation with polysaccharide	Increased the concentration of total SCFAs. Increased relative abundance of Bacteroides, Oscillospira, and Bifidobacterium, but decreased abundance of Sutterella	Comprised of 1,4-linked-α-D-Galp3S, and 1,3-linked-β-D-GalpA residues with molecular weight of 28,807 Da	(Cui et al., 2020)
Chondrus ocellatus	High-sucrose with low dietary fiber mice supplementation with algae	Altered the microbiome. Increased abundance of *Bacteroides vulgatus*	Carrageenan	(Takei et al., 2019)
Porphyra haitanensis	C57BL/6 mice, gavaged with 250 mg/kg per day	Effective for improving the intestinal environment and increase the level of *Lactobacillus bacterium*	–	(Zhang et al., 2018)
Gracilaria lemaneiformis	High-fat and high-cholesterol diet mice supplementation with polysaccharide	Increased the relative abundance of *Bacteroides*, *Ruminococcus_1*, *Lactobacillus*, while deceased the relative abundance of *Alistipes*, *Prevotellaceae_UCG-001*, and *Corprococcus_1*	–	(Huang et al., 2019)
Gracilaria lemaneiformis	High-fat diet mice supplementation with 2% polysaccharide	Increased the concentration of total SCFAs, and increased ratio of Bacteroidetes to Firmicutes	–	(Xu et al., 2020)
Sarconema filiforme	High-fat diet mice supplementation with 5% polysaccharide	Modulated gut microbiota without changing the Firmicutes to Bacteroidetes ratio.	κ-carrageenan	(du Preez et al., 2020b)

quantified by PACE. The saccharide mapping adapted by PACE is promising for large samples analysis, because of its simplicity and low cost (Xu, Kan, et al., 2018).

Quality control has always been the key issue in the commercialization of RSPs products. Therefore, saccharide mapping could be very useful in guiding the manufacture and quality control of RSPs. It could also be used in controlling the quality of RSPs related pharmaceutical and nutraceutical products.

7. Conclusion

This review provides a synthesis of the extraction, purification, physiochemical, and structural features of RSPs. Recent studies on the immuno-modulatory, anti-obesity, antioxidant, anti-cancer, and prebiotic properties of RSPs coupled with their quality control have been reviewed and summarized. To maximize the development of functional ingredients or therapeutic agents from RSPs, a sustained effort in the assessment of their biological activities is needed. In addition, specific research should explore the usage of RSPs and their possible mechanisms of action. Thus, this review sheds insight on how to explore RSPs for potential therapeutic application or in functional foods. The unique characteristics of RSPs indicate their future as resource for therapeutic and functional foods.

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