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Review article



Is green algae polysaccharide a 'green path' to health?

Sammueal Ong Jun Kai [a,b], Matthew Myint [a], Chee Fan Tan [a], Yi Fan Hong [a], Meiyappan Lakshmanan [c,d,e], Ying Swan Ho [a,f], Thomas T. Wheeler [g], Xuezhi Bi [a,f,h], Ian Walsh [a], Sean Chia [a,*], Kuin Tian Pang [a,b,i,j,**]

[a] Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, #06-01 Centros, Singapore 138668, Republic of Singapore
[b] Department of Biomedical Engineering, National University of Singapore, 4 Engineering Drive 3 Block 4, #04-08, 117583, Republic of Singapore
[c] Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras, India
[d] Centre for Integrative Biology and Systems medicinE (IBSE), Indian Institute of Technology Madras, India
[e] Robert Bosch Centre for Data Science and AI (RBCDSAI), Indian Institute of Technology Madras, India
[f] Food, Chemical and Biotechnology Cluster, Singapore Institute of Technology, Singapore 138683, Republic of Singapore
[g] Cawthron Institute, Nelson 7010, New Zealand
[h] Duke-NUS Medical School, National University of Singapore, Singapore 169857, Republic of Singapore
[i] Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR), 30 Biopolis Street, #07-01 Matrix, Singapore 138671, Republic of Singapore
[j] School of Chemistry, Chemical Engineering, and Biotechnology, Nanyang Technology University, 62 Nanyang Drive, N1.2-B3, 637459, Republic of Singapore

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ABSTRACT

There is an increased interest in green algal polysaccharides as they have displayed many beneficial bioactivities. Relevant bioactivities found include promotion of gastrointestinal health, antioxidant, anti-inflammatory, immunomodulatory, anti-hyperlipidaemia, anti-osteoporotic, hypoglycaemic and anti-ageing. Among the many green algae species, the polysaccharides from the three main groups of green algae are more commonly studied. Namely, green algae from the Ulvales order, the Bryopsidales order and the *Chlorella* genus. As such, this review starts by providing a background on the popularity of these three groups of algae and introducing the basic structures of some of their polysaccharides. Polysaccharides extracted from green algae that have exhibited bioactivity that might make them a great nutraceutical are then highlighted. The current lack of comparisons between the effects of whole algae (which is popularly used as a health supplement, especially for *Chlorella* species) and polysaccharide extracts is also mentioned, which could hamper progress towards using green algal polysaccharide extracts instead of whole algae. Finally, the possible advantages of polysaccharide extracts as compared to whole algae are mentioned, one of which is the possibility of fine-tuning the dose that is not achievable by taking whole algae supplements due to the possible toxicity when taking whole algae at high levels.

1. Introduction

Algae produce an estimated 50 % of all oxygen on Earth. They also form a large part of the primary food chain in the ocean, supporting aquatic life [1]. Humans have consumed algae since ancient times, with historical records of consumption dating back to as early as the fourth century in Japan [2], and archaeological evidence for the consumption of algae (seaweed) was found as early as 14,000 years ago in Monte Verde [3]. Algae consumption is commonplace in the Asian diet, and many different species of algae are eaten. *Saccharina japonica* (formerly *Laminaria japonica*) (Japanese kelp), *Undaria pinnatifida* (wakame) (Phaeophyceae) and *Pyropia tenera* (formerly *Porphyra tenera*) (laver) (Rhodophyta) are some of the most well-known ones. The health benefits of algae are further confirmed in the modern era by more robust studies. For example, in a double-blinded, placebo-controlled study, *Chlorella*, a green algae popularly used as a supplement, was shown to have an immunostimulatory effect by Jung et al. [4]. There is rising interest in studying algae as it has been identified as a possible solution

* Corresponding author.
** Correspondence to: K.T. Pang, Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, #06-01 Centros, Singapore 138668, Republic of Singapore.
E-mail addresses: sean_chia@bti.a-star.edu.sg (S. Chia), zach_pang@bti.a-star.edu.sg (K.T. Pang).

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for environmental sustainability [5]. Though there are over 50,000 known species of algae [6], the number of algae species that have health benefit analysis studies is small. As such, there could be many unrecognised health benefits and as-yet undiscovered and precious bioactive compounds in algae waiting to be uncovered.

There are four commonly known groups of algae that are grouped based on the photosynthetic pigment they possess. They are red algae (Rhodophyta), green algae (Chlorophyta), brown algae (Phaeophyceae) and blue-green algae (Cyanobacteria). The first three types are eukaryotes, while the last are prokaryotes. Algae contain several types of bioactive compounds such as proteins, lipids, vitamins, antioxidants, inorganic elements, phytochemicals (carotenoids) and polysaccharides [7]. As these compounds are not mutually exclusive, categorisation of algae based on the compounds they have is hard, and categorisation is complex. Of these, polysaccharides are particularly interesting as they have been shown to exhibit various biological activities like anticoagulant, antiviral, antioxidative, and anti-inflammatory. Polysaccharides from algae are mainly categorised as structural or storage, and their composition varies widely due to several factors. They also have interspecies and intraspecies variability that is mainly due to time of collection (algae have different development and reproductive stages) and diverse habitats. Other natural factors contributing to variability include light intensity and nutrient levels [8,9]. This variation means potential for the existence and discovery of more diverse types of bioactive polysaccharides.

Of the four main types of algae, green algae show great promise, evidenced by the *Chlorella* clinical trial mentioned before. However, to our knowledge, there has not been utilisation of polysaccharides from green algae as nutraceuticals, despite the various bioactivities they have displayed, such as antioxidant [10–12], anti-inflammatory [13], and immunomodulatory [14,15]. These activities are elaborated upon in Section 3 of the paper. Thus, this review paper will focus on green algal polysaccharides and introduce the various bioactivities that could make it a prime source of nutraceuticals. Firstly, the polysaccharide structure of commonly studied green algae will be described. Next, a compilation of the various biological activities displayed by green algae polysaccharide extracts that might be beneficial to human health. The review will also focus on the potential of green algae polysaccharides as a nutraceutical, that is, when ingested by humans through the gastrointestinal tract. Thus, studies which administer the green algae polysaccharides in contexts which are unlikely to be relevant to nutraceuticals are omitted in this review, such as nasal sprays and injections.

2. Polysaccharides in green algae

Polysaccharides are polymers, and their monomers are monosaccharides bound together by glycosidic bonds. The great variety of polysaccharides is due to the many different types of monosaccharides and multiple stereochemical designations of the glycosidic bond between adjacent sugars. Monosaccharides are molecules that contain 3–7 carbon atoms, and due to their structure having multiple asymmetric centres, many isoforms and hence monosaccharide units are possible [16,17]. Additionally, being polymers, a lot of the variation comes from the chain length and, therefore, molecular weight. The diversity of polysaccharides found is also attributed to the various patterns of methylation, pyruvylation and sulfation of the polysaccharide chains [18,19]. There is relatively high carbohydrate content in green algae, with a study showing various species of green algae having 42.1 %–61.5 % of dry mass as carbohydrates [20]. Most of the carbohydrates in algae exist in polysaccharide form, which serve either one of two main functions: structural or storage. Most algal polysaccharides are part of the algal cell wall, except for storage carbohydrates, which are found as starch in the plastid for green algae [19]. In the following subsection, well-characterised polysaccharides of commonly studied green algae species will be introduced.

For green algae, most studies are focused on three main types of algae: ulvans from the genus *Ulva*, polysaccharides from the order Bryopsidales, and green microalgae from the genus *Chlorella* [21,22].

2.1. Ulvan

Ulvans are extracted from Ulvales, which are common seaweeds that are distributed worldwide. One genus in particular, *Ulva*, is particularly known as they are already grown and collected for food consumption [23]. Moreover, the high production of *Ulva spp.* in eutrophic coastal waters has attracted interest in research for new uses of these algae [24]. Ulvans are sulfated heteropolymers (Fig. 1) that are extracted from Ulvales such as *Ulva spp.* and can represent 38–54 % of dry algal matter [25]. The main constituents of ulvan are L-rhamnose, D-xylose, D-glucose, D-glucuronic acid, L-iduronic acid and sulfate in varying proportions depending on the sample origin [23,26]. The ulvans extracted from different *Ulva* species share a common main repeating disaccharide unit known as ulvanobiouronic acid, but as mentioned before, structure variations (charge density, molecular weight, *etc.*) between species are huge, hence the term heteropolymers. Particularly, ulvans have huge potential to display unique properties as they contain rare sugars like rhamnose and iduronic acid [23]. Bioactivities such as immunomodulating and antioxidant are present in a more detailed review on ulvans [27].

Other than *Ulva,* ulvan-like cell wall polysaccharides are also extracted from *Monostroma* (Ulotrichales), though they are more commonly referred to as sulfated rhamnan sulfate or just sulfated polysaccharides [28,29].

2.2. Polysaccharides from Bryopsidales

Green algae from the order Bryopsidales, which consists of the genera *Codium, Bryopsis and Derbesia*, are also highly studied for their polysaccharides. *Codium* is the main focus of research, due to its global distribution and popularity as food in Asian countries such as Japan, China and Korea [30].

Similar to the ulvans, the structural heterogeneity of sulfated polysaccharides within the species differs [30]. They range from pyruvulated and sulfated galactans in *Codium yezoense* [31] to sulfated arabinans, sulfated galactans, and sulfated arabinogalactans from *Codium fragile* and *C. vermilara.* The sulfated polysaccharides that can be extracted from *Codium* are of great interest as they display various bioactivities

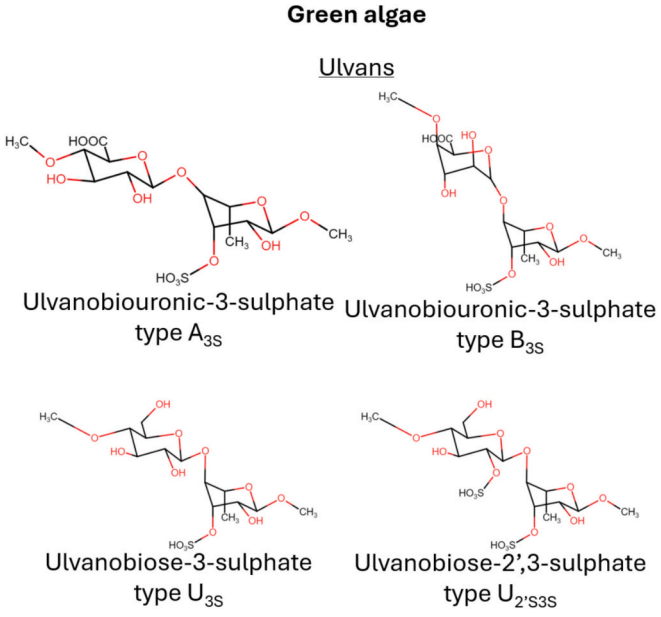

Fig. 1. Common ulvan backbone structures.

such as antitumor [32]. As the biological activities of sulfated polysaccharides are directly affected by their structure (backbone structure, molecular weight, degree of sulfation, *etc.*) [33], the wide diversity of sulfated polysaccharide structures found between species holds great potential for finding polysaccharides of various bioactivities.

2.3. Polysaccharides from Chlorella

Contrary to the macroalgae (seaweed) mentioned thus far, the *Chlorella* genus consists of unicellular organisms and is known as microalgae. *Chlorella* is grown at a large scale commercially due to its high growth rates and nutritional content. They are also attractive as the composition and type of biomolecules produced can be tuned by employing various growth conditions (such as limiting nitrogen to increase starch accumulation [34]). It is also noted that the categorisation of *Chlorella* species has changed significantly over the years, with only some species that have stayed as 'true' *Chlorella* species, such as *C. vulgaris* and *C. sorokiniana* [35]. Many species formerly referred to as *Chlorella* have been removed from the genus, exemplified by the recent recategorization of *Chlorella lobophora* to *Lobosphaeropsis lobophora*. This can create a continuity problem in studies of polysaccharides from the *Chlorella* species, which should be considered. Additionally, due to the unicellular nature of *Chlorella*, many strains of *Chlorella* may exist. For example, *Chlorella vulgaris* has over 50 different strains stored in public culture storage [36]. Hence, knowing both the species and strain of the *Chlorella* used in studies is imperative for the reproduction of results. Unfortunately, such information is not available in all studies, and perhaps more can be done to ensure such vital information is reported too.

Polysaccharides in *Chlorella* can be divided into two main types, starch-like polysaccharides (homopolysaccharides) and non-starch polysaccharides (heteropolysaccharides) [37]. Homopolysaccharides are made of only one type of polysaccharide, though the type of monosaccharide and glycosidic bonding pattern differ between different *Chlorella* species. For example, a homopolysaccharide from *Chlorella sorokiniana* mainly consisted of linear 1 → 4-linked glucan with minor branching at 1,4,6 position [38]. On the other hand, a homopolysaccharide extracted from *Auxenochlorella pyrenoidosa* (formerly *Chlorella pyrenoidosa*) was found to contain 1 → 2-linked β-D-galactofuranan monosaccharide units instead [39]. Heteropolysaccharides are polysaccharide chains that contain more than one monosaccharide type and are more complex. They can either be a neutral, acidic or amino polysaccharide depending on the substituent. As such, diverse types of polysaccharides can be obtained from the various species of the green microalgae *Chlorella,* and perhaps owing to their variety, promising immune-enhancing [40] and antioxidant [11] bioactivities have also been reported for polysaccharides extracted from *Chlorella*. Currently, *Chlorella* is more popularly consumed whole as a health supplement [41]. However, the cell wall of *Chlorella* is robust and made of cellulose, which is resistant to digestion in the human body, which is why mechanical disruption of the cells is usually done for health supplements [42]. This poses a problem as the release of polysaccharides is not guaranteed, and the bioactive polysaccharide may still be trapped in the cell walls. Hence, there is great potential in extracting polysaccharides from *Chlorella* as the active polysaccharide can be isolated and administered with greater guarantee of activity.

2.4. Polysaccharides from other green microalgae

This section covers polysaccharides from other green microalgae that have been shown to have bioactivity, which have been reported recently.

Botryococcus braunii is a microalgae which have shown great promise as a sustainable hydrocarbon producer, and also produces exopolysaccharides (EPS) [43]. *B. braunii* EPS is mainly made up of galactose, with much lower amounts of arabinose, fucose and rhamnose and uronic acids, though the composition can change depending on the strain used [44]. Recently, there have been increasing reports of EPS from *B. braunii* possessing bioactivities, such as antioxidant, anti-bacterial and anti-tumour, showing that further research into EPS from *B. braunii* can be considered given the potential shown [45–47].

Microalgae from the genus *Dunaliella spp.* have seen many industrial applications due to their low production cost and ability to synthesise high-value compounds, particularly β-carotene [48]. These microalgae also produce exopolysaccharides (EPS), and there is growing interest in their EPS as they have shown bioactivities such as immunomodulatory and antioxidant [49,50].

Another promising polysaccharide source is the *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) species, which received much attention initially as a producer of astaxanthin [51]. Promising bioactivities such as prevention of alcoholic steatohepatitis development (through gut microbiota modulation) and immunostimulatory effects have been reported for polysaccharides extracted from this species, and further research can be considered [52,53].

Finally, polysaccharides from *Brachionococcus chlorelloides* (formerly *Dictyosphaerium chlorelloides*), particularly the strain Kovacik 1978/11, have also been studied quite extensively. Numerous structural studies on the EPS of *B. chlorelloides* have been done, where it was found that the dominant heteropolysaccharide is made up of galactose, rhamnose and mannose, with a main chain consisting of an α-L-rhamno-α-D-(2-*O*-methyl)-galactan structure [54–58]. Bioactivities of the EPS include antitussive [57] and immunomodulatory [58]. Unfortunately, despite extensive structural studies, a comparison of different purified fractions to test their bioactivity was not done, and thus, the structure-activity relationship is still ambiguous. More recently, it was found that polysaccharides of the cell wall of *B. chlorelloides* also possess immunomodulatory activities [59]. Compared to EPS, cell wall polysaccharides from *B. chlorelloides* are similarly mainly made up of galactose, mannose and rhamnose. However, cell wall polysaccharides are much smaller in size as compared to EPS and do not have associated proteins, while EPS are much larger macromolecules with associated proteins (are proteoglycans).

A summary of typical green algal polysaccharide structures and the bioactivity it has shown can be found in Table 1 below.

3. Beneficial biological activities observed in green polysaccharide extracts

One of the driving reasons for the interest in algal polysaccharides is due to the many biological activities it has exhibited. In this section, the review will highlight biological activities and studies which have shown the potential for algae polysaccharides to be used as a nutraceutical.

3.1. Gastrointestinal health

The fate and effect of algae polysaccharides in the gastrointestinal system depend on their digestibility. If the polysaccharide can be digested in the upper gastrointestinal tract (mouth, stomach, small intestine), it is likely to behave as a sugar source. The relevant evaluation metrics for such polysaccharides would be the common ones for evaluating sugar sources, such as glycaemic index (GI). If the polysaccharide resists digestion in the upper gastrointestinal tract, it will likely interact with the gut microbiota in the colon.

Of the algae polysaccharide types, only the storage polysaccharides are likely to serve as a sugar source. In green algae, the storage polysaccharide is starch and is likely digestible in humans into simple sugars, as the starch from green algae has been shown to be very similar to potato starch (which is digestible in humans) [72]. Few studies regarding the bioactivity and nutraceutical value of storage polysaccharides exist, and most of the polysaccharides being studied are non-storage polysaccharides, such as ulvans in green algae. Non-storage polysaccharides normally resist digestion and remain chemically

Table 1
Summary of structural information of typical green algal polysaccharides and bioactivities shown.

Species	Compound name	Molecular weight (kDa)	Monosaccharide composition	Sulfate content (%)	Type of glycosidic bonds/ linkages	Bioactivity	Ref
Caulerpa okamurae	CO-0-1	470	Man:Gal:Xyl = 4.4:4.0:1.4, trace Glc	12.78	[→4)-β-D-Manp-(1→] [→2)-β-D-Manp-(1→] [→3)-β-D-Galp-(1→], sulfate at O-6 [→2)-β-D-Xylp(1→], sulfate at O-4	Anticoagulant	[60]
Caulerpa racemosa	CR-1B	370	Gal:Glc:Man:Xyl:GlcA = 41.9:2.6:47.4:3.7:2.5 Trace amounts of Rha, Ara	46.5	Sulfated xyloarabinogalactan: 1 → 3)-galactose residues in the main chain (1 → 4)-xylose and (1 → 4)-arabinose residues in branches	Antioxidant	[61]
Chaetomorpha antennina	CA-1 A	430	Rha:Gal:Glc:Man:Xyl:IdoA:GlcA = 29.6:27.4:8.1:4.9:4:2.3:14.7	13.9	1,4-linked 3-OSO_3- α-L-rhamnose substituted with side chain 1,4-linked α- L-rhamnose β-D-glucuronic acid	Antioxidant	[61]
Codium fragile	CZS-0-1	810	Ara:Gal:Glc = 9:2:1	21	(1 → 3)-β-L-Arap, sulfate at O-4 and/or O-2 (1 → 3)β-D-Galp	Anticoagulant	[62]
Dictyosphaeria cavernosa	PSG	16.12	D-Gal	20.61	Backbone: 3-linked β-D-Galp, sulfation on C-4 and C-6, pyruvate ketals on O-4 and O-6 Branches (at C-6): 6-linked β-D-Galp(4SO4) and β-D-Galp(3,4-Pyr)-(1 → units	Anticoagulant	[63]
Halimeda opuntia	HO-2 A	80	Rha:Gal:Glc:Man:Xyl:GlcA = 1.8:73.9:8.7:9.8:1.8:4.1	12.4	Sulfated or/and pyruvylated β-D-galactans	Antioxidant	[61]
Ulva lactuca	ULP-2	N.G.	Xyl:Rha:GlcA = 49.9:30.5:19.5	20.19	[→1)-β-GlcAp-(4→] [→1)-β-Xylp-(4→] [→1)-3-O-SO_3-α-Rhap-(4→]	Anti-inflammatory	[64]
Ulva lactuca	UL-1 A	960	Rha:Man:IdoA:GlcA = 55:19:4:22	20.3	L-iduronic acid 1,4-linked 3-OSO_3- α-L-rhamnose 1,4-linked α- L-rhamnose β-D-glucuronic acid	Antioxidant	[61]
Ulva linza	ULP	474	Rha: GlcA: Xyl: Glc: Gal = 27.6:16.25:3.45:1.94:1.13	N.G	N.G	Antioxidant Gut microbiota modulation	[65]
Ulva pertusa	UPP	116.1	Rha:Fuc:Xyl:Glc:IdoA:GlcA = 35.1:2.8:4.1:5.4:13.2:39.5	20.5	[→4)-β-D-GlcA(1 → 4)-α-L-Rha3S-(1→] [→4)-β-D-Xylp3S-(1→] [→4)-α-L-IdoA-(1→]	Gut microbiota modulation	[66]
Ulva pertusa	UPP	86.3	Rha:GlcA:IdoA:Xyl:Glc:Fuc = 1:1.125:0.376:0.117:0.154:0.080		[→4)-β-D-GlcpA-(1 → 4)-α-L-Rhap3S-(1→] [→4)-α-L-IdopA-(1 → 4)α-L-Rhap3S-(1→] [→4)-β-D-Xylp3S-(1 → 4)-α-L-Rhap3S-(1→]	Immunomodulatory	[67]
Ulva prolifera (formerly *Enteromorpha prolifera*)	EP	1469.37	Man:Rha:GalA:GlcA:Glc:Gal:Xyl:Ara:Fuc = 5.0:49.4:9.8:1.0:16.1:14.5:10.4:10.4:1.3	6.94	N.G	Antioxidant	[68]
Ulva prolifera (formerly *Enteromorpha prolifera*)	F3	N.G.	Rha:Xyl:Glc = 77.11:16.33:6.56	11.14	[→4)- α-L-rhamnopyranose-3-SO_3(1→] [→α-L-rhamnopyranose-3-SO_3-(1 → 4)- β-D-xylopyranose→]	Immunomodulatory	[69]
Ulva pseudorotundata	UPRF	N.G	Rib:Rha:Xyl:Man:Gal:Glc:GlcA = 2.29:51.30:26.10:0.84:2.50:11.47:5.52	3.88	N.G	Antioxidant Antitumor	[70]
Ulva reticulata	URP-2	26.75	Xyl:Rha:GlcA = 42.94:17.03:40.04	13.89	[(→ 4)-β-GlcAp- (1 → 4)-β-Xylp(1 → 4)-α-Rhmp-3S-(1 →)]	Anti-inflammatory	[71]
Ulva rigida	UPRF	N.G	Rib:Rha:Xyl:Man:Gal:Glc:GlcA = 0.85:69.69:2.87:1.93:20.67:4.00	3.78	N.G	Antioxidant Antitumor	[70]

N.G: Not given; Man, mannose; Rib, ribose; Rha, rhamnose; GlcA, glucuronic acid; GalA, galacturonic acid; Glc, glucose; Gal, galactose; IdoA: iduronic acid; Ara, arabinose; Xyl, xylose; Fuc, fucose; GlcN, glucosamine.

unchanged after passing through the upper gastrointestinal tract. This is because humans do not produce enzymes which can effectively break down the complex non-storage polysaccharides of algae [73]. In general, these polysaccharides can serve as bulking agents that increase the sense of satiety and reduce food intake. What is arguably the most important effect, however, is the interaction of these undigested algae polysaccharides with the gut microbiota in the colon, with possible applications in microbiome-directed food intervention.

There is increasing awareness that the gut microbiome is intrinsically linked to the human immune system and general health. The gut microbiome consists of over 1500 species of microorganisms, and some species participate in processes which are beneficial to the host. For example, the *Firmicutes* and *Bacteroidetes* are the main producers of short-chain fatty acids (SCFA) from anaerobic fermentation of indigestible polysaccharides, and deficiency can lead to compromise of colonic integrity [74,75]. The main SCFAs produced by fermentation in humans are acetate, propionate, and butyrate [76]. Other than SCFAs, the gut microbiome also contributes to the synthesis of important vitamins such as biotin and thiamine in humans [75]. The composition of the gut microbiome is crucial to health, as unfavourable alterations to the gut microbiome have been linked to diseases such as diabetes and cancer [77].

Results from studies on the effect of green algal polysaccharides on the gut microbiome have been promising. *In vitro* and *in vivo* methods exist to study the effect of green algal polysaccharides on the gut microbiome. *In vitro* methods usually involve fermentation of collected faecal samples from humans to obtain the microbiome (or at least part of it), followed by the addition of the polysaccharide and observing changes in the microbiome population or products produced. Another *in vitro* approach involves observing the effect of polysaccharide supplementation on known prebiotics. *In vivo* methods are usually animal studies, which involve studying the faecal matter of animals after a given diet of polysaccharides for microbiome changes, typically paired with physiological measurements depending on the activity of interest. This section will only include studies with the investigation of microbiota population and gut environment as their main goal. Other studies where microbiota characterisation is done supplementally (mainly for exploratory purposes) will be briefly mentioned here, and their elaborations on bioactivity can be found in their respective activity sections.

Firstly, *in vitro* studies. A study by Phomkaivon et al. showed the strong prebiotic potential of water-soluble polysaccharides extracted from *Ulva rigida* [78]. It was found that polysaccharide supplementation to *Lactobacillus rhamnosus* ATCC 53103, one of the most well-known probiotics [79], could effectively increase its cell growth at a level similar to inulin, a commercial prebiotic. In a similar vein, another study found that polysaccharides from *Ulva rigida* showed prebiotic effects comparable to inulin and fructo-oligosaccharide (FOS) for four known probiotic bacterial strains [80]. The strains are *Bifidobacterium bifidum* TISTR 2129, *B. longum* TISTR 2195, *Lactobacillus casei* TISTR 1340, and L. *acidophilus* TBRC 338, where TISTR refers to the Thailand Institute of Scientific and Technological Research, and TBRC refers to the Thailand Bioresource Research Center, annotating the organisation the bacterial strain was obtained from.

Another study showed the effects of *in vitro* fermentation of ulvan polysaccharides extracted from *Ulva linza* [65]. The polysaccharides stayed relatively intact after simulated upper gastrointestinal digestion (molecular weight only decreased slightly from 474 kDa to 445 kDa) and influenced the composition of gut microbiota (collected from the faeces of healthy volunteers). Particularly, the relative abundance of several generally beneficial bacteria, such as *Bacteroides*, *Faecalibacterium* and *Parabacteroidesi*, was increased. On the other hand, the abundance of *Prevotella*, *Blautia* and *Ruminococcus* was decreased. Hence, polysaccharides from *U. linza* can be potentially used to manipulate the composition of gut microbiota based on individual needs and the current knowledge of possible effects the polysaccharides have on the gut microbiota elucidated from this study.

Liu et al. showed the metabolism of polysaccharides from *Ulva lactuca* by the human gut *Bacteroides* species *via in vitro* fermentation [81]. *Bacteroides* species was chosen as the polysaccharide significantly increased the abundance of *Bacteroides* when fermented. Seven *Bacteroides* species were evaluated, namely *Bacteroides stercoris*, *B. fragilis*, *B. vulgatus*, *B. uniformis*, *B. thetaiotaomicron*, *B. ovatus* and *B. caccae*. It was found that only *B. thetaiotaomicron* and *B. vulgatus* were able to metabolise polysaccharides from *Ulva lactuca*. Both species were found to produce acetic acid, propionic acid, cysteic acid, niacinamide and riboflavin by metabolising the polysaccharide. However, *B. thetaiotaomicron* accumulated linoleic acid too, which was not observed in *B. vulgatus*. Hence, this study shows that even within the same genus, different species of bacteria can have significantly different interactions with green algal polysaccharides, showing that further research on the metabolism mechanism of polysaccharides by gut microbiota is needed, which is still scarce for green algal polysaccharides.

Now, moving on to *in vivo* studies. Encouragingly, a clinical trial of the effect of rhamnan sulfate (RS) extracted from *Monostroma nitidum* to improve constipation was carried out by Shimada et al. [82]. In this double-blind placebo-controlled trial, RS was administered (100 mg/day) to subjects with low defaecation frequencies (3–5 times/week) for 2 weeks, and it was found that constipation was improved in subjects who took RS without observable side effects. Through gut microbiome analysis, it was found that *Clostridiales* numbers, a bacterium known for producing medium-length fatty acids which increase water absorption, dry up faeces and cause constipation, were reduced in the RS-taking group. Additionally, bacteria associated with constipation improvement, *Negativicutes* and *Acidaminococcales*, also increased in the RS-group, though the mechanism of improvement for these bacteria is unknown. This clinical trial showed the potential of RS from *Monostroma nitidum* as a safe diet supplement to improve constipation.

For *in vivo* animal studies, a study on the anti-tumour activity of *Ulva lactuca* polysaccharide (ULP) in H22 tumour-bearing mice was carried out by Qiu et al. [83]. It was found that ULP intervention reversed the trend of increased *Tenericutes* (associated with increased tumour burden) and *Parabacteroides* (abundant in early stages of hepatocellular carcinoma [84]), which contributed to decreased tumour weight and survival rates. Hence, ULP displayed anti-tumour activity which is likely mediated through microbiota modulation (elaborated in Section 4.4).

A study by Tochitani et al. investigated the effects of rhamnan sulfate extracted from *Monostroma nitidum* on the gut microbiota of BALB/c mice that were fed a normal diet [85]. The dosage used was 1 mg of polysaccharide dissolved in water that was administered through oral gavage daily. It was found that administration of *M. nitidum* polysaccharides significantly altered the composition of the gut microbiota in mice. Specifically, *Mucispirillum schaedleri*, a bacterial species found to be potentially anti-inflammatory, was significantly increased. Metagenomic functional analysis of the changed gut microbiota composition also showed a more anti-inflammatory profile. Taken together, polysaccharides from *M. nitidum* could have the potential to alter the gut microbiome to induce anti-inflammatory effects, which could have potential use in diseases such as inflammatory bowel disease (IBD).

Son et al. showed the ability of sulfated rhamnoglucuronan isolated from *Ulva australis* (formerly *Ulva pertusa*), termed UPP, to treat DDS (dextran sulfate sodium)-induced inflammatory bowel disease (IBD) in BALB/c mice [66]. Clinical symptoms of IBD, such as body weight loss, bloody stool and diarrhoea, were suppressed in a dose-dependent manner with prophylactic administration of UPP. Compared to the positive control treatment of mesalazine, UPP were also able to prevent a decrease in colon length due to DSS challenge in a dose-dependent manner. Analysis of the gut microbiota showed that UPP administration was able to protect the equilibrium of gut microbiota that was disrupted by DSS challenge. Specifically, prevention of a decrease in *Lactobacillaceae* and *Muribaculaceae*, and prevention of proliferation of Lachnospirales. Total SCFA, acetic acid, propionic acid, and butyric acid

levels were also significantly increased in a dose-dependent manner with UPP administration as compared to the no-treatment group. Hence, UPP has the potential to be a potent preventor of IBD, with effects likely mediated through the gut microbiota.

Another *in vivo* animal study by Chen et al. [86] investigating the hypoglycaemic and anti-ageing activities of polysaccharides from *Ulva lactuca* showed that there was alleviation of symptoms and significant gut microbiome change following administration of the green algae polysaccharide to ageing and diabetes-induced mice. The polysaccharide, termed ULP-1, resulted in a significant increase of 10 types of microbes, namely *Dubosiella, Enterococcus, Romboutsia, Bifidobacterium, Kurthia, Clostridium_sensu_stricto_1, Corynebacterium, Faecalibaculum, Aerococcus,* and *Vibrio*. In particular, *Dubosiella* has been shown to help regulate SCFA production and resist obesity and could be crucial to host glucose metabolism regulation. Levels of *Rikenella*, which is closely related to obesity, ageing, inflammation, [87] and diabetes [88], also decreased. In another similar study from the same research group, Zhu et al. showed that feeding extracted and purified polysaccharides from the green algae *Ulva prolifera* (previously *Enteromorpha prolifera*) to ageing and diabetes-induced mice improved symptoms of hyperglycaemia, which was accompanied by changes in gut microbiota. Particularly, there were significant increases in *o_Lactobacillaceae, c_Bacilli, f_Lactobacillaceae, g_Lactobacillus,* and *p_Firmicute*, showing that the *Ulva prolifera* (previously *Enteromorpha prolifera*) polysaccharide (EPP) activity could be a result of microbiome modulation of the population of these microorganisms. (Details in Section 4.6).

In a study by Liu et al., it was found that administration of polysaccharides from *Haematococcus pluvialis* to binge ethanol diet-fed mice could alleviate the symptoms of gut epithelial and vascular barrier dysfunction, particularly through the modulation of gut microbiota [53]. Particularly, polysaccharide administration increased the relative abundance of *Bacteroidetes, Ruminococcaceae*, *Roseburia* and *Lactobacillaceae*. *Roseburia*, especially, has been shown to be able to repair impaired gut barrier integrity through TLR5 (toll-like receptor 5) recognition to alleviate alcohol-induced hepatic steatosis and inflammation [89]. Confirmation that gut microbiota was the contributing factor was done through faecal microbiota transplantation, where faeces transplanted from control mice fed with PBS did not result in alleviation of ethanol-induced damage, whereas faeces transplanted from polysaccharide-fed mice resulted in alleviated symptoms. (Details in Section 3.6).

A study by Shima et al. showed that administration of rhamnan sulfate from *Monostroma nitidum* (250 mg/kg body weight dose) could decrease fasting blood glucose and plasma total cholesterol levels in BALB/c mice that were fed with a normal diet [90]. Changes to gut microbiota such as a decrease in the Firmicutes/Bacteroidetes (F/B) ratio were also observed, along with an increase in gut microbiota in the *Deferribacteraceae* and *Muribaculaceae* families. This was, however, followed by a decrease in α-diversity of the gut microbiome, which could be due to the selective increase of certain types of bacteria only. Nevertheless, rhamnan sulfate from *M. nitidum* shows potential as a health supplement, especially people with type 2 diabetes mellitus, considering its ability to reduce fasting blood glucose.

Many recent studies, especially *in vivo* ones which study bioactivity through the oral administration route (gastrointestinal pathway), are starting to include microbiome analysis as part of their methodology. This is due to growing awareness of the importance of the gut microbiome and its link to overall health. It is highly possible that for algae polysaccharides in a nutraceutical context, their bioactivities are exerted through interactions with the gut microbiota. As such, microbiome analysis might become necessary in the future for any study which administers algae polysaccharides through the oral route.

However, the study of the gut microbiome is complex. Stark differences exist in the composition and structure of the gut microbiome between different groups of people, which can be influenced by age, genetics, disease, lifestyle, diet and even method of birth [91,92]. Interestingly, it was shown that the gut microbiome can change rapidly in response to diet in a reproducible manner [93]. This means there is a system (albeit a complex one) that we can potentially manipulate through what we ingest to obtain desirable results. In this case, through the ingestion of algae polysaccharides. However, the variety of gut microbiome complicates the research on the effect of algae polysaccharides on gut microbiome since there can be great inter-individual variability, which means the possibility of different outcomes when ingesting the polysaccharide. Hence, it can be difficult to design clinical trials and validate the efficacy of algae polysaccharides in promoting good gastrointestinal health.

3.2. Antioxidant activity

Antioxidant activity of a compound is of great interest as it can help neutralise free radicals and reactive oxygen species (ROS), which in excess have been linked to diseases such as cancer and cardiovascular disease [94]. A review of antioxidants and their evaluation methods has been done by Gulcin [95], and a more targeted review of antioxidant activity for algae polysaccharides (mainly macroalgae/seaweed) has been done by Liu and Sun [96]. There are two main types of antioxidants: primary antioxidants, which directly scavenge free radicals, and secondary antioxidants, which do not directly scavenge free radicals. There are several mechanisms of secondary antioxidant action, such as binding to metal ions and scavenging reactive oxygen species. Accompanying the large variety of antioxidant action mechanisms is a myriad of techniques for their evaluation. As such, researchers usually choose a mixture of techniques to make the evaluation more comprehensive, as different methods have their specificities and shortcomings. *In vitro* assays are the most common due to their ease of performance. However, *in vivo* methods of measuring antioxidant activity have also been developed to be more indicative of actual antioxidant activity in biological systems. This section will thus be split into two main parts: *in vitro* and *in vivo* antioxidant studies.

3.2.1. In vitro determination of antioxidant activity

There are over 20 types of *in vitro* assays available for antioxidant activity, but they can be split into two main categories: hydrogen atom transfer (HAT) reaction-based assays or electron transfer (ET) reaction-based assays [96]. HAT assays measure the ability of a subject to quench a free radical by donating hydrogen. On the other hand, ET assays measure the ability of a potential antioxidant to transfer an electron to reduce any compound [97]. Common HAT assays include hydroxyl radical-scavenging, superoxide anion (O^{2-}) radical-scavenging and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging assays. Common ET assays include 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging, ferric-reducing power and Fe^{2+}-chelating power assays. As biological redox reactions mainly involve hydrogen atom transfer, HAT assays are considered to be more indicative of actual biological antioxidant activity as compared to ET assays like DPPH, though DPPH assays are popular as they are easy to perform [97]. Numerous *in vitro* experiments of antioxidant activity for green algae have been carried out and are summarised in Table 2.

Interestingly, EPS obtained from *Botryococcus braunii* showed vastly different antioxidant activity depending on its chemical race. The study by Wang et al. used a newly isolated bacterial strain which belongs to the chemical race L of *B. braunii* and showed no activity in the DPPH radical-scavenging and superoxide anion radical scavenging tests [45]. On the other hand, the study by Cheng et al. used the SAG 30.81 strain, which belongs to the chemical race A of *B. braunii*, and EPS from this strain displayed significant activity in DPPH radical-scavenging, superoxide anion radical scavenging, and hydroxyl radical scavenging too [46,109]. Hence, the chemical race of *B. braunii* used can lead to significantly different antioxidant potential of the EPS produced. With current data, it seems chemical race A may be more favourable as compared to L, though further investigation is required to confirm this.

Table 2
Summary of antioxidant activity displayed by green algal polysaccharides.

Species	Test in which activity was found†	Reference
Acrosiphonia orientalis	Total antioxidant capacity, DPPH radical-scavenging, ABTS radical-scavenging, Ferric-reducing power	[98]
Botryococcus braunii	DPPH radical-scavenging, Hydroxyl radical-scavenging, Superoxide anion radical-scavenging	[46]
Botryococcus braunii	ABTS radical-scavenging, DPPH radical-scavenging, Hydroxyl radical-scavenging, Superoxide anion radical scavenging	[45]
Caulerpa cupressoides var. flabellata	Total antioxidant capacity, Fe^{2+}-chelating, sSuperoxide anion radical-scavenging, Hydroxyl radical-scavenging	[12]
Caulerpa lentillifera	DPPH radical-scavenging, ABTS radical-scavenging, Superoxide anion radical scavenging, Ferric-reducing power	[99]
Caulerpa racemosa	DPPH radical-scavenging, ABTS radical-scavenging, Superoxide dismutase scavenging, Ferric-reducing power, Hydroxyl radical-scavenging	[61]
Caulerpa scalpelliformis	Total antioxidant capacity, DPPH radical-scavenging, ABTS radical-scavenging, Ferric-reducing power	[98]
Chaetomorpha antennina	*DPPH radical-scavenging*, ABTS radical-scavenging, *Superoxide dismutase scavenging*, Ferric-reducing power, Hydroxyl radical-scavenging	[61]
Chlorella vulgaris	Ferric-reducing power	[11]
Codium bernabei	ABTS radical-scavenging, DPPH radical-scavenging	[100]
Codium cylindricum	DPPH radical-scavenging, Hydroxyl radical-scavenging, Superoxide anion radical scavenging, Ferric-reducing power	[101]
Codium fragile	DPPH radical-scavenging, Ferric-reducing power	[102]
Dunaliella tertiolecta	DPPH radical-scavenging, ABTS radical-scavenging, Ferric-reducing power	[50]
Halimeda opuntia	DPPH radical-scavenging, ABTS radical-scavenging, Superoxide dismutase scavenging, Ferric-reducing power, Hydroxyl radical-scavenging	[61]
Halimeda tuna	DPPH radical-scavenging, Ferric-reducing power	[103]
Ulva australis (formerly *Ulva pertusa*)	Superoxide anion radical-scavenging, Ferric-reducing power, DPPH radical-scavenging	[104]
Ulva australis (formerly *Ulva pertusa*)*Ulva pertusa*	DPPH radical-scavenging	[105]
Ulva lactuca	Total antioxidant capacity, DPPH radical-scavenging, Ferric-reducing power, Fe^{2+}-chelating	[106]
Ulva lactuca	DPPH radical-scavenging, ABTS radical-scavenging, Superoxide dismutase scavenging, Ferric-reducing power, Hydroxyl radical-scavenging	[61]
Ulva lactuca	Total antioxidant capacity, DPPH radical-scavenging, ABTS radical-scavenging, Ferric-reducing power	[98]
Ulva linza	DPPH radical-scavenging, ABTS radical-scavenging, Hydroxyl radical-scavenging	[65]
Ulva pertusa	Superoxide anion radical-scavenging, Ferric-reducing power, DPPH radical-scavenging	[104]
Ulva pseudorotundata	ABTS radical-scavenging	[70]
Ulva rigida	DPPH radical-scavenging, ABTS radical-scavenging, Ferric-reducing power	[78]
Ulva rigida	ABTS radical-scavenging	[70]
Ulva rigida	DPPH radical-scavenging, Ferric-reducing power	[107]
Ulva stenophylloides	DPPH radical-scavenging, Ferric-reducing power	[108]

†: Tests in italics are those in which no activity was found.

In a study which investigated the production of β-1,3-xylo-oligosaccharides using xylanase from *Caulerpa lentillifera*, it was found that β-1,3-xylo-oligosaccharides obtained from *C. lentillifera* consistently showed better antioxidant activity than β-1,4-xylo-oligosaccharides (obtained from land source) *in vitro* for all antioxidant assays used (DPPH radical scavenging, ABTS radical scavenging, superoxide anion radical scavenging and ferric-reducing power) [99]. This shows the higher antioxidant potential of polysaccharides sourced from algae as compared to land sources, encouraging further research into polysaccharides from algae.

In the study which investigated the antioxidant power of EPS extracted from *Dunaliella tertiolecta*, it was found that the use of mixotrophic cultivation conditions, as compared to photoautotrophic conditions, produced EPS with much better antioxidant activities [50]. Hence, investigation of such conditions for other microalgae, such as *Botryococcus braunii*, can be considered when aiming to increase antioxidant activities of the EPS extracted.

A study by Contreras-Porcia et al. showed the potential effects of extraction temperature on the antioxidant activity of polysaccharides from *Ulva stenophylloides* [108]. It was observed that polysaccharides obtained using higher extraction temperatures displayed higher antioxidant activity in all the antioxidant assays used (DPPH radical and ferric-reducing power). This trend continued up to 220 °C, which is the highest temperature used in the study. This shows that the extraction method can potentially have a huge influence on the bioactivity of polysaccharides extracted from green algae.

Another study demonstrated that purified polysaccharide fractions isolated through the use of anion exchange chromatography displayed better bioactivity as compared to the unpurified crude polysaccharide [98]. This pattern was observed for all green algae evaluated (*Caulerpa scalpelliformis*, *Ulva lactuca*, *Acrosiphonia orientalis*), where at least one fraction, if not all, exhibited better performance in the antioxidant assays as compared to the crude fraction. Some improvements are particularly dramatic. For example, crude polysaccharides from *Caulerpa scalpelliformis* displayed a half maximal effective concentration (EC_{50}) of 145 μg/mL, while the fraction obtained from 0.3 M NaCl, termed CAU.3 N, had an EC_{50} of 21 μg/mL, which is more than 10 times more potent compared to the original crude fraction. This shows the potential of purifying polysaccharides to improve their bioactivity.

Though lower molecular weight and higher sulfation content generally improve antioxidant activity [96], this was not true in a study by Li et al. [104], which did an *in vitro* evaluation of the antioxidant activity of polysaccharides extracted from *Ulva australis* (formerly *Ulva pertusa*). In the study, two fractions of polysaccharide, F1 and F2, were purified and although F2 had both lower molecular weight (83 kDa in F2 as compared to 190 kDa in F1) and higher sulfation extent (23.99 % in F2 as compared to 15.56 % in F1), the reducing power activity and DPPH radical scavenging activity in F2 was lower than F1. This shows that molecular weight and sulfation degree are not the only factors that determine antioxidant activity, and as the author noted, the degree of branching and conformation of main chains are also important, which highlights the importance of advanced NMR techniques to elucidate accurate structures of polysaccharides for better study of the structure-activity relationship of algae polysaccharides. Yet, the complexity did not end there, as F2 did not perform better than F1 in the superoxide radical-scavenging assay. This makes the determination of the antioxidant power of different polysaccharides inconclusive even within the

same study.

In general, direct comparisons between studies can be difficult as not only are there a variety of ways to assess antioxidant activity, but there are also many confounding factors such as uronic acid content, protein content (either impurities or glycoprotein), sulfation pattern, and so on. As such, there could be a need for a more standardised series of tests for *in vitro* evaluation of antioxidant activity for algae polysaccharides, particularly one which can best mimic the *in vivo* conditions the polysaccharide will encounter as a nutraceutical, namely the gastrointestinal tract, and the subsequent common ROS the algae polysaccharide will encounter. An explanation for the choice and rationale of tests performed for assessing antioxidant capacity should also be expounded within any research work, which is not always done currently.

3.2.2. *In vivo determination of antioxidant activity*

In vivo evaluation of antioxidant capacity, sometimes known as endogenous antioxidant ability, is usually done by measuring the levels of enzymes in the antioxidant system, such as superoxide dismutase (SOD) and catalase (CAT), and/or oxidation products like malondialdehyde (MDA) [27,96].

In a study by Jiang et al. [10], polysaccharide from *Ulva australis* (formerly *Ulva pertusa*) was extracted, and interestingly, phosphorylated using the sodium tripolyphosphate method. This modified polysaccharide was termed phosphorylated ulvan (PU). Through feeding either normal ulvan or PU to mice that were fed with a high-fat diet, it was found that PU resulted in significantly higher levels of antioxidant enzyme CAT as compared to normal ulvan of the same dosage (250 mg/kg). However, levels of glutathione peroxidase (GSH-Px) and SOD in mice fed with PU decreased as compared to normal ulvan, and an increased dosage of PU to 500 mg/kg increased both GSH-Px and SOD, but resulted in a slight drop of CAT activity. Hence, this study showed that phosphorylation modification of algae polysaccharides can be used to modify the antioxidant activity of algae polysaccharides, though the modification may not result in better performance in all antioxidant activity assessments. More study into the mechanism of action is needed to investigate why phosphorylation selectively increases activity related to CAT while lowering SOD and GSH-Px.

In an *in vivo* mouse study that used an alcohol-induced liver injury model, it was found that *Ulva prolifera* (formerly *Enteromorpha prolifera*) polysaccharides were able to alleviate the oxidative stress imposed on the liver due to excessive alcohol consumption [68]. Particularly, liver total antioxidant capacity, SOD activity and GSH-Px activity were found to be significantly increased as compared to the model group. MDA level, which is a sign of oxidative stress at higher levels, was also decreased as compared to the model group. Altogether, it shows the powerful antioxidant potential of polysaccharides from *U. prolifera*. Additionally, though two types of dose groups were used (low dose = 5 mg/mL, high dose = 10 mg/mL), there was no significant difference in antioxidant effectiveness between the two groups, showing that the current low dose amount is sufficient to achieve the maximum antioxidant activity of the polysaccharide in this mouse model.

Other than mouse models, zebrafish models have also been used to investigate the antioxidant activity of algae polysaccharides. In a study by Wang et al. [110], the ability of sulfated polysaccharide extracted from *Codium fragile* (CFCE-PS) to inhibit H_2O_2-induced oxidative stress in zebrafish was investigated. Specifically, embryos that were at the 7–9 h post-fertilisation (hpf) stage were used. These embryos were incubated with different concentrations of CFCE-PS (12.5, 25, and 50 μg/mL) on a 12-well plate for 1 h. Afterwards, H_2O_2 (10 mM) was added to the embryos, and the embryos were incubated until 24 hpf. It was found that there was a dose-dependent increase in survival rates of the zebrafish, and it was postulated that the CFCE-PS protected the zebrafish from oxidative stress *via* scavenging of intracellular reactive oxygen species (ROS).

In summary, various *in vitro* and *in vivo* studies have shown that algae polysaccharides from green algae show the potential to exhibit potent antioxidant activity. One potential benefit of purified polysaccharides from raw algae is that it has been reported that trace elements such as arsenic in raw algae can negatively affect antioxidant capability [111]. Extraction and refinement can hence allow both a better understanding of the structure-activity relationship and an increase in potency of antioxidant capability by removing such undesirable trace elements. However, due to the complex nature of the antioxidant system in the body, it can be hard to compare results between studies, and perhaps a more standardised experimental procedure that can comprehensively assess the antioxidant ability of algae polysaccharides, especially in the context of the passage through the gastrointestinal tract, is needed. Modification studies, such as phosphorylation of algae, can also be considered to potentially improve antioxidant activity.

3.3. *Anti-inflammatory effects*

Inflammation is a common and normal response to potentially harmful stimuli such as pathogens, injury, and metabolic stress [112]. The inflammation response is also important as it is the precursor to many other downstream immune activities. However, when there is dysregulation of the immune system and the inflammation does not resolve, it can contribute significantly to the pathogenesis of several diseases, such as atherosclerosis. Such an inflammation is known as nonresolving inflammation [113]. Hence, discovering anti-inflammatory agents to regulate such harmful inflammatory processes is of high interest, especially ones without complications associated with traditional anti-inflammatories such as possible organ damage when using non-steroidal anti-inflammatory drugs (NSAIDs) [114,115]. Lipopolysaccharide (LPS)-induced models are commonly used to study potential inflammatory activity. LPS usually refers to bacterial LPS and is a well-studied molecule that has been attributed to being the cause of pathogenic inflammation following bacterial infection [116]. As such, LPS is usually used to induce pathogenic inflammation, and the effect of various polysaccharides on such inflamed systems is evaluated.

A study by Pai et al. showed the potential anti-inflammatory effects of polysaccharides from *Ulva lactuca*, termed ULP-2, on LPS-induced CALU-1 cells [64]. It was found that there was a dose-dependent ability to modulate cytokine secretion in CALU-1 cells. Specifically, there was a significant reduction of inflammatory TNF-α, IL-1β, and IFN-γ cytokines upon ULP-2 exposure. ULP-2 administration also restored the decreased expression of IFN-α caused by LPS induction. Hence, ULP-2 have the potential to be an anti-inflammatory nutraceutical. In another study by the same group, anti-inflammatory effects of polysaccharides from *Ulva reticulata*, termed URP-2, were also found [71]. Again, the *in vitro* CALU-1 cell model was used, and a significant reduction in inflammatory cytokines TNF-α, IL-1β, and IFN-γ were observed upon URP-2 exposure. Restoration of IFN-α was also observed. Significantly, it was found that URP-2 could reduce the expression of inflammatory IL-33 cytokines, which was not observed when ULP-2 was administered. Hence, a comparison of these two polysaccharides to find out the reason for IL-33 inhibition can be considered.

In a study by Wang et al. [13], the anti-inflammatory property of sulfated polysaccharide extracted from the green algae *Codium fragile*, labelled CFCE-PS, was investigated *in vivo* in zebrafish. It was found that there was a dose-dependent increase in survival rates for LPS-challenged zebrafish embryos after treatment with CFCE-PS. The levels of ROS and nitric oxide (NO), both involved in the inflammation process, were increased following the LPS challenge and were effectively reduced by CFCE-PS treatment.

An *in vivo* study of the anti-inflammatory effects of polysaccharides extracted from the *Ulva sp.* was also conducted, using a murine model of delayed-type hypersensitivity (DTH), also known as a type IV hypersensitivity immune response [117]. Oral treatment of mice with polysaccharides at a dosage of 250 mg/kg body weight improved DTH reaction, evident from reduced footpad swelling and cell infiltration at the site of allergen (ovalbumin) challenge. It was found that

polysaccharide administration could significantly reduce IFN-γ levels, which are associated with Th1 patterns, while significantly increasing IL-4 levels, which are associated with Th2 patterns. This ultimately shifts the polarity of the Th1/Th2 response towards Th2, which helps alleviate DTH, as increased Th1 activation is one of the hallmarks of DTH. Hence, *Ulva sp.* polysaccharides could alleviate DTH response through restoring Th1/Th2 balance, particularly through modulation of IFN-γ and IL-4 levels, which were decreased and increased, respectively.

In an *in vivo* animal study by Kraiem et al., sulfate polysaccharide extracted from *Halimeda tuna* was found to be able to effectively alleviate inflammation [103]. The model used was carrageenan-induced oedema in rat paw (Wistar rats), where a 20 mg/kg dose of *H. tuna* through oral gavage was able to display anti-inflammatory effects similar to diclofenac–sodium, a standard drug used to treat inflammation.

3.4. Immunomodulatory and potential anti-cancer properties

Green algal polysaccharides have also displayed immunomodulatory and potential anti-cancer properties. *In vitro* studies usually observe how green algal polysaccharides interact with selected cancer cell lines, and two main observations of interest are anti-proliferative and cytotoxicity. *In vivo* studies for anti-cancer properties usually involve transplanting tumours onto mice and observing the tumour growth following polysaccharide administration.

The *in vitro* immunomodulatory and antitumor studies for green algal polysaccharides are summarised in Table 3.

Hence, a substantial number of *in vitro* studies regarding the immunomodulatory activity of different species of algae from green algae have been done, with the most common cell types used being colorectal cancer cells and immune cells. However, it can be hard to compare results between studies as the cell lines used and assays performed might differ from study to study. This means it is hard to use the results of these studies to determine which algae species yield the best immunomodulatory polysaccharide. Nevertheless, green algae polysaccharides have shown promising immunomodulatory activity, and this could incentivise a comparative analysis on promising algal species.

Additionally, the methodology used by some studies has been able to elucidate some of the structure-activity relationships of the polysaccharide. For example, in the study by Tabarsa et al. [119], it was shown through deproteination and deglycosylation of the extracted polysaccharide that the protein-polysaccharide structure was necessary for the immunomodulatory function of the algae polysaccharide. Polysaccharides were extracted from the *Codium fragile* species, and the purified fraction, F2, was subject to systematic desulfation, deproteination and deglycosylation procedures to determine the structure-function relationship. F2 is likely a glycoprotein with protein moieties covalently bonded to the polysaccharide backbone, as Sevag treatment did not result in the removal of protein components. Immunomodulatory function was measured by the ability of macrophage cells (RAW264.7 cells) to release NO upon exposure to the various modified F2 fractions. It was shown that either deproteination or deglycosylation of F2 leads to loss of ability for F2 to stimulate RAW264.7 cells to release NO (a sign of immune activation), and both the protein moiety and the polysaccharide are necessary for immunomodulatory activity. A similar approach was taken by Surayot et al. [120], who performed desulfation of the polysaccharides extracted from *Codium fragile*. It was shown that the sulfate-polysaccharide structure was essential for improving NK cells' cytotoxicity against HeLa cancer cells, as neither the sulfate-only nor polysaccharide-only fraction could elicit improvement in NK cells' cytotoxicity. These studies show that the removal of certain parts of the polysaccharide is a useful tool which can elucidate the structure-activity relationship of polysaccharides and should be considered by future studies.

In vivo studies of immunomodulatory and anti-cancer activity have also been done for green algae polysaccharides. However, many

Table 3
Summary of the immunomodulatory activity of green algal polysaccharides.

Species	Model used	Suggested Mechanism	Reference
Ulva intestinalis	RAW 264.7 (mouse macrophage)	Induce macrophage IL-1β, TNF-α, IL-6, IL-10 and IL-12 production	[118]
Ulva prolifera (formerly *Enteromorpha prolifera*)	RAW 264.7 (mouse macrophage)	Induce macrophage NO production and suppress over-activation by producing anti-inflammatory cytokines (IL-10)	[15]
Ulva rigida	Colorectal carcinoma (HTC-116)	Exhibited anti-tumour activity with an IC_{50} value of 0.31 mg/mL Selectivity index (SI) was 8.03	[70]
Ulva pseudorotundata	Colorectal carcinoma (HTC-116)	Exhibited anti-tumour activity with an IC_{50} value of 0.33 mg/mL Selectivity index (SI) was 4.05	[70]
Codium fragile	RAW 264.7 (mouse macrophage)	Macrophages had higher proliferation and NO release, and likely triggered the NF-κB pathway through the degradation and phosphorylation of IκB-α and the subsequent translocation of p65/p50 complex into the cell nucleus. MAPK pathway was also likely triggered through phosphorylation of ERK1/2, p38, and JNK1/2, as well as the nuclear translocation of c-JUN and c-FOS.	[119]
Codium fragile	NK cell against HeLa cells	Polysaccharide exposure increased the proliferation of NK cells and enhanced NK cells' cytotoxicity against HeLa cells.	[120]
Codium bernabei	Colorectal carcinoma (HTC-116)	Acid media-precipitated polysaccharides showed high antiproliferative activities against HTC-116 cancer cells.	[100]
Caulerpa lentillifera	Colorectal cancer cells (HCT-8)	Showed antiproliferative activity, without toxicity to normal cells	[14]
Caulerpa chemnitzia	RAW 264.7 (mouse macrophage)	Increased macrophage proliferation and stimulated the release of cytokines IL-6, IL-1β and TNF-α	[121]
Caulerpa chemnitzia	THP-1 (human acute monocytic leukemia)	Increased macrophage proliferation and stimulated the release	[121]

(*continued on next page*)

Table 3 (*continued*)

Species	Model used	Suggested Mechanism	Reference
		of cytokines IL-6, IL-1β, and TNF-α	
Botryococcus braunii	Human promyelocytic leukemia cells (HL-60)	Induced apoptosis in cancerous HL-60 cells without significant increase in toxicity to normal cells (LO2 cells).	[47]
Dunaliella salina	Monocytes and lymphocytes (Peripheral blood mononuclear cells from human volunteers)	Proliferation inhibited at low dose, proliferation increases with increasing dose. Cell proliferation is most likely mediated through the production of TNF-α cytokine.	[49]
Dunaliella salina	RAW 264.7 (mouse macrophage)	Dose-dependent inhibitory effect on the proliferation and nitric oxide production of macrophage, with higher doses leading to less macrophage proliferation and nitric oxide production.	[49]
Haematococcus pluvialis	Mice splenocytes	Promoted LPS-induced B lymphocytes proliferation	[52]
Brachionococcus chlorelloides (formerly Dictyosphaerium chlorelloides)	RAW 264.7 (mouse macrophage)	Decreased macrophage proliferation, but induced significant release of certain interleukins and growth factors, such as pro-inflammatory cytokines (TNF-α, IL-6, IL-12) and anti-inflammatory cytokines (IL-10, T_H1)	[58]
Brachionococcus chlorelloides (formerly Dictyosphaerium chlorelloides)	B16F10 melanoma cells collected after subcutaneous inoculation into lower back of syngenic C57BL/6 mice	Induced activation of Th1 response of splenocytes (determined through significant increase in production of IFN- γ)	[59]
Halimeda tuna	RAW 264.7 (mouse macrophage)	Improved cell viability and growth of macrophage, and decreased the production of pro-inflammatory cytokines (GM-CSF and TNF-α) in LPS-stimulated macrophage	[103]
Ulva prolifera (formerly *Enteromorpha prolifera*)	THP-1 (human acute monocytic leukemia)	Inhibited the cell viability and induced apoptosis of THP-1 cells. Most likely pathway deduced was through the mitochrondrial pathway, particularly through inducing loss of mitochondrial membrane potential and mitochrondrial dysfunction.	[122]

administer the polysaccharides through injection methods [123,124] and may not be that relevant in a nutraceutical context. An *in vivo* anti-cancer study using diet-fed polysaccharides was an *in vivo* animal study on the anti-tumour activity of *Ulva lactuca* polysaccharide (ULP) carried out by Qiu et al. (as mentioned in Section 4.1) [83]. H22 tumour-bearing mice were created by injecting H22 hepatoma cells and subsequently fed with 100 mg/kg, 200 mg/kg, or 300 mg/kg of ULP based on the assignment. In general, tumour weight was significantly decreased in ULP-treated groups, as compared to the control. In addition, mice that were fed more ULP had better survival rates (90 % at the end of the experiment for 200 mg and 300 mg ULP, 70 % for 100 mg ULP and untreated control). As mentioned in Section 4.1, it was found that ULP intervention reversed the trend of increased *Tenericutes* (associated with increased tumour burden) and *Parabacteroides* (abundant in early stages of hepatocellular carcinoma [84]). Hence, ULP displayed anti-tumour activity through microbiota modulation in an *in vivo* mouse study.

The potential anti-tumour activity of ulvan from *Ulva lactuca* was displayed by Qiu et al. in a H22 liver cancer tumour-bearing mice model [125]. The dosage administered was 300 mg/kg, and it was found that polysaccharide administration could repress tumour growth. Interestingly, it was discovered that polysaccharide and 5-fluorouracil (5FU), a known cancer treatment that comes with side effects, could work together synergistically to repress the H22 tumour growth. This is evident from the tumour weight of the polysaccharide+5FU treatment group being significantly lower than the polysaccharide-only or 5FU-only treatment groups. Additionally, the decrease in weight gain as compared to the no-treatment group induced by 5FU treatment was also partially alleviated in the polysaccharide+5FU treatment group, where significant weight gain was observed (albeit not as much as the model or normal group). It was also found that the polysaccharide exerts its anti-tumour effect through miRNA regulation. Altogether, it shows the potential of polysaccharides from *Ulva lactuca* as an anti-tumour nutraceutical that can be used synergistically with 5FU.

Wen et al. used BALB/c mice with a CT26 (murine colorectal carcinoma) tumour allograft model to show the potential anti-cancer effect of polysaccharides from *Caulerpa lentillifera* [126]. It was found that tumour weight was reduced with the administration of polysaccharides as compared to the no-treatment group. Interestingly, a low dose of polysaccharide (200 mg/kg) yielded significantly better results than a high dose (400 mg/kg), showing that there is an optimal dose for the anti-cancer effects, which warrants further investigation.

Another *in vivo* study involves the use of cyclophosphamide (CTX)-induced immunosuppressed BALB/c mice model to investigate the immunostimulatory effects of ulvan-type polysaccharides extracted from *Ulva australis* (formerly *Ulva pertusa*) [67]. It was found that administration of *U. australis* polysaccharides could effectively alleviate the damage caused by CTX to the immune system. This was assessed through various methods, notably through restoration of the thymus weight and size, which was significantly reduced in CTX mice. In general, there was a dose-dependent increase in the effectiveness of the polysaccharides, with the highest dose of 200 mg/kg showing the best results most of the time. Additionally, this activity could have been mediated by the gut microbiota, as polysaccharide treatment was able to restore the levels of short-chain fatty acids (SCFAs) that were decreased by CTX treatment. As mentioned in Section 3.1, SCFAs are important metabolites produced by the gut microbiota, and reduced SCFA production is usually linked to impaired health. Unfortunately, no gut microbiota analysis was done in this study. Thus, further investigation is needed to confirm if the mode of action of polysaccharides is through the gut microbiota.

In a study by Son et al., the ability of sulfated-rhamnoglucuronan from *Ulva australis* (formerly *Ulva pertusa*) to activate the immune system was shown using a C3H/HeN mice model [127]. It was found that there was dose-dependent activation (the highest dosage was 200 mg/kg) of Peyer's patches (PP), which serve an important function as a germinal centre for B cell maturation and contribute to mucosal

immunity in the intestine [128]. Specifically, it was found that secretion of IgA (immunoglobulin A) and IgA-related proteins such as IL-6, IL-10 and TGF-β in the PP was significantly increased, showing the ability of polysaccharides to activate non-inflammatory related immunity. Additionally, SCFA levels were also analysed, and it was found that there was a dose-dependent increase in levels of acetic acid, propionic acid and butyric acid. This shows potential involvement of gut microbiota in the immune activation, though further analysis of the gut microbiome was not done in this study.

Pai et al. also used a CTX-induced immunosuppressed model, but on Wistar rats, to investigate the immunostimulating effects of various green algae (*Ulva reticulata*, *Ulva lactuca* and *Caulerpa racemosa*) [129]. Polysaccharide treatment could effectively reverse the body and organ weight loss caused by CTX challenge. Additionally, immunoglobulin M (IgM) and immunoglobulin G (IgG) levels, which were decreased by CTX challenge, were also significantly increased by polysaccharide treatment, showing the immunostimulant effect polysaccharides have, at least on the humoral system. It was found that there was a dose-dependent increase in the effects of polysaccharide treatment, with a limit. The optimal dosage for all polysaccharides was found to be 150 mg/kg. Of all the polysaccharide sources, it was found that polysaccharides from *Ulva lactuca* were the most potent, suggesting further research on polysaccharides from *Ulva lactuca* can be considered.

Thus, it can be seen that *in vivo* immunomodulatory activities and anti-cancer properties are likely to be mediated through the gut microbiota, and future *in vivo* studies may be hybrid-based that consider both the gastrointestinal health and the health of the overall body. Furthermore, there is a lack of *in vivo* studies for the immunomodulatory effects of green algal polysaccharides, and perhaps more effort can be directed there.

3.5. Anti-hyperlipidaemia

Hyperlipidaemia is the condition of having elevated lipids in the bloodstream. Fats, fatty acids, cholesterol, and triglycerides are some commonly known lipids. Hyperlipidaemia is one of the major causes of atherosclerosis, which is widely known as one of the major risk factors for diseases like coronary heart disease [130]. Hence, there is interest in methods to reduce lipid levels in the blood. However, not all lipids were found to be harmful. High-density lipoprotein cholesterol (HDL-cholesterol) is believed to be able to help clear excess cholesterol in the body, and an increase in levels of HDL-cholesterol is believed to be beneficial [131]. However, its counterpart, low-density lipoprotein cholesterol (LDL-cholesterol), likely contributes to the pathology of hyperlipidaemia. As such, novel methods to reduce all lipid levels except for HDL-cholesterol would be of interest. This section will highlight several *in vivo* animal studies which have shown that an algae polysaccharide-supplemented diet can improve the condition of hyperlipidaemia.

For green algae, in a study by Pengzhan et al. [132], ulvans extracted from *Ulva australis* (previously *Ulva pertusa*) were found to have anti-hyperlipaemia activity in rats that were fed a high-fat diet. Comparisons of activity between native ulvan (U), high molecular weight ulvan (U1) and low molecular weight ulvan (U2) were carried out. Ulvan and U1 significantly reduced serum total cholesterol and LDL-cholesterol compared to the untreated group, with U1 showing a greater effect. U2 significantly reduced triglyceride levels while significantly increasing HDL-cholesterol levels compared to the untreated group. Hence, U1 and U2 showed promising anti-hyperlipidaemia activity. Another study by Li et al. [104] also studied polysaccharides from *Ulva australis* (previously *Ulva pertusa*). A cholesterol-rich diet was used to induce hyperlipidaemia in mice, and extracted polysaccharides were orally fed to the mice for 28 days. Treatment with polysaccharides significantly decreased serum cholesterol, triglycerides, and LDL-cholesterol while increasing HDL-cholesterol as compared to the untreated group. In yet another study of polysaccharides from *Ulva australis* (previously *Ulva pertusa*), Jiang et al. [10] showed that phosphorylated ulvan prepared using the mixed phosphate method had better anti-hyperlipaemia activity than the normal ulvan extracted.

Chu et al. [133] conducted a study on polysaccharides from *Chaetomorpha linum* and found that the polysaccharide, termed CLP, mainly contained arabinogalactan sulfate. Mice were fed a high-fat diet to induce hyperlipaemia, and CLP was fed through feeding the mice water dissolved with CLP. It was found that there was a dose-dependent decrease in serum levels of triglyceride, total cholesterol, and LDL-cholesterol.

3.6. Other bioactivities

Kim et al. reported the anti-osteoporotic function of polysaccharides extracted from the green algae *Capsosiphon fulvescens* [134]. An *in vivo* model of ovariectomized (OVX) female mice was used as a post-menopausal osteoporosis model. The polysaccharide extracted was a sulfated glucuronorhamnoxylan polysaccharide (termed as SPS-*CF*), and oral administration of SPS-*CF* to OVX mice significantly improved the bone mineral density and serum osteoprotegerin/RANKL ratio, which shows alleviation of osteoporosis. It was found that this was likely done through dampening of osteoclastogenesis and bone resorption *via* downregulation of the TRAF6-c-Src-Pyk2-c-Cbl-gelsolin signalling pathway.

The potential hypoglycaemic and anti-ageing activities of polysaccharides from *Ulva lactuca* were also shown by Chen et al. [86]. Ageing and diabetes were induced in mice through a high-sugar, high-fat diet intervention, 40-day intraperitoneal injection of D-galactose (D-gal, 45 mg/kg) (to accelerate the ageing process in tissues), and streptozotocin (STZ, 70 mg/kg) (to simulate lack of insulin) was started on the 30th day with 3-day intervals. The extracted and purified polysaccharide (using anion-exchange chromatography) was termed ULP-1. Low-dose (100 mg ULP-1/kg, ULPL) and high-dose groups (200 mgULP-1/kg, ULPH) were assigned. It was shown that low-dose ULP significantly improved the hyperglycaemia and glucose tolerance impairment caused by administration of D-gal, STZ and a high-fat diet. In gut microbiome analysis, 10 types of gut microbiota showed a significant increase, namely *Dubosiella, Enterococcus, Romboutsia, Bifidobacterium, Kurthia, Clostridium_sensu_stricto_1, Corynebacterium, Faecalibaculum, Aerococcus,* and *Vibrio*. In particular, *Dubosiella* has been shown to help regulate SCFA production and resist obesity and could be key to regulating host glucose metabolism. Levels of *Rikenella*, which is closely related to obesity, ageing, inflammation, [87] and diabetes [88], also decreased. Interestingly, virtual protein docking of the ULP with potential target proteins in the GLP-1/GLP-1R pathway (involved in insulin and energy homeostasis [135]) was carried out. It was found that ULP-1 was predicted to bind particularly strongly to GLP-1 and GLP-1R, suggesting strong interactions. Hence, through increasing beneficial microorganisms while decreasing harmful ones, ULP is shown to have the potential to alleviate hypoglycaemic and ageing conditions.

In a similar vein to the previous study, the hypoglycaemic and anti-ageing activity of polysaccharides from green algae *Ulva prolifera* (previously *Enteromorpha prolifera*) was investigated by Zhu et al. [136]. The method of age and diabetes induction is similar to the study above by Chen et al. (same research group). The extracted and purified polysaccharide was labelled as EPP, and fed to mice in low doses (100 mg ERP/kg, EPL) and high doses (200 mg ERP/kg, EPH). EPP administration was found to significantly improve hyperglycemia, oral glucose tolerance test results (OGTT), malondialdehyde (MDA) levels, and serum insulin levels in Type 2 diabetes mellitus mice compared to the untreated group. OGTT is used as an indicator of the glucose processing ability of mice, and EPP-treated mice had much lower blood glucose levels as compared to the untreated group. MDA is an indicator of free radicals, with EPP having much lower MDA, indicating less harmful free radicals in mice. As mentioned in Section 4.1, it was found that there were significant increases in *o_Lactobacillaceae, c_Bacilli,*

f_Lactobacillaceae, g_Lactobacillus, and *p_Firmicutes*, showing the EPP activity could be a result of microbiome modulation of the population of these microorganisms.

The potential for polysaccharides from *Haematococcus pluvialis* (HPP) to prevent alcoholic steatohepatitis development was investigated by Liu et al., where a binge ethanol diet was used to induce alcoholic damage [53]. The HPP dosage was 400 mg/kg, and it was found that the reduced expression of tight junction protein zonula occludens-1 (Zo-1) caused by ethanol-diet was restored with HPP administration. Additionally, PV-1 (plasma lemma vesicle associated protein), an indicator of gut vascular barrier function, was found to be elevated by ethanol-diet. This increase was counteracted when HPP was administered. Hence, HPP administration was able to improve vascular barrier dysfunction through up-regulation of Zo-1 protein and suppressing PV-1 expression. As mentioned in Section 3.1, HPP administration increased the relative abundance of *Bacteroidetes, Ruminococcaceae*, *Roseburia* and *Lactobacillaceae*. *Roseburia*, and confirmation that gut microbiota was the contributing factor was done through faecal microbiota transplantation, showing that the ability to prevent steatohepatitis development shown by HPP is likely mediated through gut microbiota modulation.

The potential for polysaccharides from *Auxenochlorella pyrenoidosa* (formerly *Chlorella pyrenoidosa*) to alleviate high temperature stress-induced injury in a *Drosophila melanogaster* (fruit fly) model was shown by Chang et al. [137]. *D. melanogaster* is popularly used as a model for age-related disease due to its short lifespan, ease of care, and a genome that shares 70 % of known human disease genes [138]. It was observed that polysaccharides from *A. pyrenoidosa* could alleviate the oxidative stress induced by increased temperatures, reflected by significantly increased survival rates of the flies. This amelioration was mainly mediated through the Keap1-Nrf2 signalling pathway to improve flies' tolerance to high-temperature environments. In the backdrop of global warming, such studies may become useful to develop nutraceuticals to protect humans from the negative effects of heightened temperatures.

Hamzaoui et al. showed the ability of polysaccharides from *Chaetomorpha linum* to alleviate zinc and copper-induced testicular toxicity in male SWISS mice [139]. It was found that 200 mg/kg body weight oral gavage of the polysaccharides could restore the reduced sperm viability caused by zinc and copper poisoning, induced through $ZnSO_4$ and $CuSO_4$, respectively. It was hypothesised that this is likely due to the antioxidative effects the polysaccharides had, evident from the increased levels of GPx and decreased levels of MDA, showing improved oxidative profile in the testicles. Hence, polysaccharides from *Chaetomorpha linum* have the potential to protect against reproductive toxicity induced by zinc and copper poisoning.

In summary, algae polysaccharides display various bioactivities which could be highly beneficial to humans if consumed. However, most studies are in the *in vitro* and animal stages, with few clinical studies to verify their activity in the human body and assess possible side effects. Additionally, although studies on the effect of algal polysaccharides on gut microbiota are complex, they also show the greatest potential in areas such as anti-ageing. Hence, it could be beneficial for humans if more resources were to be channelled into this promising field.

4. Challenges and future directions

One major challenge of research in algae polysaccharides is that the composition, structure and yield of the polysaccharides obtained differ based on many factors, such as the environment of growth and time of harvest [8]. This can cause complications in attempts to reproduce studies, as it is hard to keep track of and account for these variables, especially for wild algae. The extent of difference possible is exemplified by a study by Wang et al. [140], which compared the anti-allergic effect of porphyran extracts from different harvest timings in an *in vivo* rat system. It was found that the composition of extracts and anti-allergic effect indeed differed between harvest timings, showing the importance of such factors. Hence, to account for such variations, either developing a method to keep track of algae growth conditions in the wild or growing algae in a controlled environment would likely be required. If not, this variation problem will likely persist.

Another challenge would be the optimisation of the extraction process for commercial use. Though this review has highlighted the many bioactivities green algal polysaccharides possess, algae also contain many other valuable compounds like proteins, phenols and vitamins. Ideally, extraction methods for polysaccharides should not destroy other valuable compounds in the algae to maximise commercial value. Hence, even after identifying polysaccharides of value, significant effort must be invested to investigate the optimisation of the extraction process. The extraction process should maximise both the yield of polysaccharides and the co-extraction of other valuable compounds that can be recovered. This sentiment is echoed in another review on algae polysaccharides for nutraceutical applications [141].

On the aspect of safety, the current lack of adverse reports could be just due to a lack of safety studies in green algal polysaccharides and should not be taken for granted. Green algae polysaccharides are rarely consumed outside of the few clinical trials involving green algal polysaccharides. As such, much care must be taken to properly assess the safety of novel algae polysaccharides even if they display favourable bioactivity in preliminary tests.

Currently, many green algae nutraceuticals involve giving whole algae extracts. Due to batch-to-batch variation in composition for whole algae cultivated, the type and level of compounds contained can differ. This is exemplified by a study which showed that there was significant variability within batches of commercial *Chlorella* from the same company [142]. This means that the nutritional value and beneficial effects can vary. A possible consequence of this variation is that the dosage of certain compounds can be too high, and that can lead to undesirable side effects. This issue can be largely circumvented if extracted green algal polysaccharides are used instead, as they can be more reliably characterised. Extracts also unlock the potential of fine-tuning dosage in nutraceuticals to maximise the desired bioactivity. This might not be possible for whole algae nutraceuticals, especially since other constituents in whole algae may be toxic at high dosages. For example, *Chlorella* supplements have been found to contain carcinogenic microcystin that limits the amount of *Chlorella* supplement one can take daily to stay below the safe intake levels of microcystin [143].

Another possible challenge in the adoption of green algal polysaccharide extracts over whole algae is that not all studies administer whole algae and compare them to isolated algal polysaccharides, which makes it hard to determine if the bioactivity displayed by the polysaccharide extract has been unlocked, enhanced, or made worse as compared to whole algae. Thus, it would be ideal if future studies had such comparisons to be able to better understand the bioactivity of green algal extracts and make a stronger case for polysaccharide extracts.

5. Conclusion

This review has introduced the structures of some of the most studied groups of green algae polysaccharides, and the significant bioactivities displayed by various polysaccharides extracted from various species were also described. The review has also revealed a sore lack of clinical trials using pure algae polysaccharides in the diet. This is in stark contrast with the many clinical trials done or ongoing for whole algae [144]. However, as extraction is shown to improve bioactivity as compared to just using the whole algae, the future where algae polysaccharides are widely used as nutraceuticals is certainly promising. Having isolated polysaccharide extracts can also allow more flexibility with dosage, allowing fine-tuning of dosage that is not feasible with whole algae extracts (especially high dosages, considering other compounds that are present in whole algae, which may be toxic at high levels). Dealing with extracts can also potentially address batch-to-batch issues in whole algae, as it is easier to determine chemical characteristics of extracted and purified products. However, current studies rarely

compare whole green algae with the polysaccharide extracts, which makes it hard to determine if extraction of polysaccharides enhanced, worsened, or unlocked new bioactivity as compared to whole algae. Hence, though the potential for green algal polysaccharides as a green and sustainable nutraceutical is certainly there, more comprehensive studies to show the advantage of polysaccharide extracts as compared to whole algae are needed.

CRediT authorship contribution statement

Sammueal Ong Jun Kai: Writing – review & editing, Writing – original draft, Validation. **Matthew Myint:** Writing – review & editing, Visualization. **Chee Fan Tan:** Writing – review & editing. **Yi Fan Hong:** Writing – review & editing. **Meiyappan Lakshmanan:** Writing – review & editing. **Ying Swan Ho:** Writing – review & editing. **Thomas T. Wheeler:** Writing – review & editing, Resources. **Xuezhi Bi:** Writing – review & editing, Resources. **Ian Walsh:** Writing – review & editing, Resources. **Sean Chia:** Writing – review & editing, Visualization. **Kuin Tian Pang:** Writing – review & editing, Writing – original draft, Resources, Conceptualization.

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Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

No data was used for the research described in the article.

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