Algal Research 39 (2019) 101422

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Review article

Ulvan: A systematic review of extraction, composition and function

Joel T. Kidgell[a,*], Marie Magnusson[b], Rocky de Nys[a], Christopher R.K. Glasson[b]

[a] MACRO – The Centre for Macroalgal Resources and Biotechnology, College of Science and Engineering, James Cook University, Townsville, Australia
[b] School of Science, University of Waikato, Tauranga, New Zealand

ARTICLE INFO

Keywords:
Biotechnology
Green algae
Bioactivity
Sulfated polysaccharides
Seaweed

ABSTRACT

Species of green macroalgae (Chlorophyta) of the genus *Ulva* are edible seaweeds with a range of health promoting bioactive components. *Ulva* is high in dietary fibre which promotes gastrointestinal health and is linked to a reduction in the incidence of chronic diseases. The fundamental active constituent of *Ulva* is the soluble fibre ulvan, a gelling sulfated polysaccharide with biological activities including immunomodulating, antiviral, antioxidant, antihyperlipidemic and anticancer. Ulvan also has the capacity to modulate cellular signalling processes in both plant and animal systems leading to beneficial effects on productivity and health. Consequently, ulvan is of significant interest as a constituent in human health, agricultural, and biomaterial products. This comprehensive systematic review investigates and recommends acid extraction, ultrafiltration, sugar constituent and molecular weight analysis for the extraction, purification, and characterisation of ulvan, respectively. The biological activities of ulvans are then critically reviewed.

1. Introduction

Species of green seaweed from the genus *Ulva* have high growth rates and productivities across diverse geo-climatic conditions, with highly exploitable biochemical profiles [1–4]. As a consequence, species of *Ulva* can result in the formation of problematic "green tides" [5–9]. Therefore, they are well suited for cultivation, in particular for use in the bioremediation of nutrient rich wastewater from intensive land-based aquaculture [10–12]. Importantly, cultivation, as opposed to natural harvesting, can produce a high-quality monoculture of *Ulva* biomass, which can then generate high-quality bioproducts of consistent composition. One of the major bioproducts of interest from *Ulva* is the sulfated polysaccharide known as ulvan.

Ulvan is a cell wall polysaccharide that contributes from 9 to 36% dry weight of the biomass of *Ulva* and is mainly composed of sulfated rhamnose, uronic acids (glucuronic acid and iduronic acid) and xylose [13–21]. Species of *Ulva* have three other cell wall polysaccharides (cellulose, xyloglucan, and glucuronan), which with ulvan collectively account for up to 45% of the dry weight biomass [22]. Like ulvan, xyloglucan and glucuronan are also soluble polysaccharides but are only relatively minor constituents of the cell wall polysaccharides [14,23–25]. Interestingly, of the four cell wall polysaccharides present within *Ulva*, ulvan is the only one to contain both rhamnose and iduronic acid [16]. Rhamnose is of interest for its effect on biosynthetic pathways in the dermis [26–28] and on plant immunity [29–31]. Uronic acids (glucuronic and iduronic acids) and their sulfate esters are important constituents in mammalian glycosaminoglycans (GAGs) (e.g., heparin, heparan sulphate, and dermatan sulfate). Like GAGs, ulvan has a repeating disaccharide structure predominantly comprised of an uronic acid linked to a sulfated neutral sugar and is, therefore, a candidate for the modulation of processes and functions carried out by mammalian polysaccharides [16,32,33]. In this regard, ulvan has potential applications in biomaterial science (wound dressings, tissue engineering, biofilm prevention, and excipients), nutraceuticals (antiviral, antioxidant, antihyperlipidemic, anticancer and immunostimulatory), functional foods and agriculture, as outlined in reviews by Venkatesan *et al.* [34], Cunha and Grenha [35], Alves *et al.* [36], Lahaye and Robic [13], and Wijesekara *et al.* [37]. The biological activities of polysaccharides, including ulvan, are directly related to their chemical structure. It is, therefore, important to understand how extraction, isolation and purification procedures affect the chemical structure of the extracted polysaccharide, and as a consequence its biological activity.

This review evaluates research on ulvan with a focus on extraction and purification methods, and its biological activity and potential applications. In preparing this comprehensive, systematic review, a meta-analysis was conducted on publications identified from the search string "(Ulvan* OR ((sulfate* OR sulphate*) AND polysaccharide* AND (Ulva OR Enteromorpha)))" through Scopus and Web of Science on 13th of December 2017 (see supplementary material for methods and

* Corresponding author.
E-mail address: joel.kidgell@my.jcu.edu.au (J.T. Kidgell).

https://doi.org/10.1016/j.algal.2019.101422
Received 27 September 2018; Received in revised form 15 January 2019; Accepted 16 January 2019
Available online 29 March 2019

full access to metadata). This analysis provides a summary of the chemical structures of ulvans from different sources and the methods used to assess them, as well as providing the biological activity of ulvan, and, where possible, their relative efficacies and mechanisms of action. As a consequence, the review is divided into two major sections. Section 2 describes in detail the chemistry of ulvan, providing an overview of its physicochemical properties, extraction and purification techniques and recommended characterisation for biological activity studies. Section 3 then provides an overview of the biological activities of ulvans with a focus on the mechanistic and structural features involved. Note that in line with current nomenclature convention *Enteromorpha* is referred to as *Ulva* throughout the review [38].

2. Chemistry of ulvans

2.1. Chemical structure of ulvans

The macromolecular properties (e.g., conformation) of polysaccharides are influenced by their primary structure, which is determined by their sugar constituents and the order in which they occur, their glycosidic linkages, degree of branching, molecular weights, and the presence of functional groups, such as sulfate esters, methyl ethers, amides and amines. These structural features then determine the physicochemical properties and the biological activities of the polysaccharide. In this regard, ulvans are polyanionic heteropolysaccharides with sugar compositions that are predominantly rhamnose (45.0 mol%), glucuronic acid (22.5 mol%), iduronic acid (5.0 mol%), and xylose (9.6 mol%) (Median values, see Table 1). There is, however, a broad range in the composition of ulvan sugars reported for rhamnose (5.0–92.2 mol%), glucuronic acid (2.6–52.0 mol%), iduronic acid (0.6–15.3 mol%) and xylose (0.0–38.0 mol%) (Table 1; Fig. S2). The composition of ulvan depends on the source species, eco-physiology, and processing procedures used to prepare both biomass and ulvan (see Table 1). Compositional variation due to eco-physiological factors can then be magnified by different extraction and analytical techniques. Other monosaccharides are often reported in compositional data (e.g., glucose, galactose, arabinose, and mannose), however, their presence as a component of/or contaminant of ulvan is unclear.

The ulvan backbone is most commonly made up of α- and β-(1,4)-linked monosaccharides (rhamnose, xylose, glucuronic acid and iduronic acid) with characteristic repeating disaccharide units. [13,80–82]. The two major disaccharide repeating units are aldobiuronic acids, referred to as ulvanobiuronic acid (types A and B); minor disaccharide aldobioses, referred to as ulvanobioses (type U), are also found in ulvan (Fig. 1). Type A and B are far more common than U [13]. Ulvanobiuronic acid type A_{3s}, one of the most common disaccharide units, consists of β-D-glucuronic acid (1,4)-linked to α-L-rhamnose 3-sulfate, while in type B_{3s} α-L-iduronic acid (a C-5 epimer of glucuronic acid) is (1,4)-linked to α-L-rhamnose 3-sulfate [13,80,81,83]. Ulvanobiose U_{3s} consists of β-D-xylose (1,4)-linked to α-L-rhamnose 3-sulfate, while type $U_{2's,3s}$ consists of β-D-xylose 2-sulfate (1,4)-linked to α-L-rhamnose 3-sulfate [13,81]. While (1,4)-glycosidic linkages are the predominant bonds, (1,2)- and (1,3)-glycosidic linkages also occur [24,81]. In this regard, minor branching has been associated with glucuronic acid (1,2)-linked to the rhamnose residue of the ulvanobiuronic acid type A_{3s} [13,24]. However, there are indications that greater diversity in the structure of ulvan is likely [57,84]. For structural comparisons, a useful list of fully characterised ulvan oligosaccharides can be found in [13,24].

The conformation of ulvan in solutions is in part determined by composition and has been described using both molecular modelling and empirical evidence. Predictions of the conformation of ulvan using molecular modelling are impeded by the limited knowledge of the sequencing of the polysaccharide backbone, beyond the presence of its repeating disaccharide units, and the few longer oligosaccharides described [13,24,81,84,85]. However, theoretical calculations have determined that domains with repetitive sequences of any of the major disaccharide units (e.g., chains of A_{3s} or B_{3s}) can adopt secondary helical structures [41]. In practice the conformation of ulvan in solution is pH dependent and is also influenced by the presence of counter-ions. Due to the relative hydrophobicity of rhamnose and its overall effect on the solubility of ulvan in neutral and low pH aqueous solutions, ulvan folds into a condensed bead-like conformation [86]. In the presence of salts (e.g., NaCl) these beads aggregate. The bead conformation of ulvan reduces its intermolecular interactions resulting in the low viscosity of its solutions, as well as influencing gel strength and activity. In contrast, in high pH solutions (~13) ulvan has a more open conformation increasing the intermolecular interactions that give higher viscosities and greater gel strengths. The influence of pH over the solution properties of ulvans is a convenient mechanism by which to fine-tune its rheology to suit specific applications. Further discussion of gelling properties can be found in Lahaye and Robic [13].

While the mole ratio of constituent sugars in ulvan from a particular source and batch are defined, the molecular structure can be altered through depolymerisation and removal or addition of functional groups (e.g., sulfate esters). In this regard, molecular weights (1 - > 2000 kDa, Fig. 2b, d, f) and degree of sulfation (2.3–40%, Fig. S3) of ulvan extracts vary widely and have a large influence on physicochemical properties and biological activities. These structural features are also relatively easily manipulated and provide convenient methods for the investigation of structure-function relationships. Depolymerisation can be achieved through chemical [28,71,87–89] and enzymatic hydrolysis with ulvan lyases [23,77,90,91]. The degree of sulfation can be altered by addition of sulfate esters [49,92,93] or removal of sulfate esters by solvolysis of the ulvan pyridinium salt [55,72,94,95] or through base hydrolysis [91]. Charge can be altered through manipulation of the degree of sulfation and by derivatisation of the carboxylic acids groups (e.g., esterification and amide formation) [96,97]. Both the charge and the mole ratio of constituent sugars can be varied by reduction of glucuronic acid and iduronic acid to glucose and idose, respectively [94]. Indeed, covalent cross-linking groups can also be added to enhance gel formation and gel strength [98,99].

2.2. Extraction of ulvan

The quantitative yield and the quality of ulvan can vary significantly depending on the applied extraction and purification processes, the source of the biomass (species (Table 2), source as wild or cultivated, location) [36], storage of collected biomass, and pre-extraction processing [100]. In this regard, extraction conditions vary widely in the literature, as does the yield and quality of ulvan produced (Table 2, Fig. S3). The choice of extraction conditions is generally based around the physicochemical properties of the ulvan molecule and its specific interactions with other components of the plant cell wall [77]. Less consideration is given to the capacity for the extraction conditions to degrade ulvan or co-extract impurities such as proteins, other polysaccharides (starch, cellulose, xyloglucan, and glucuronan), and to a lesser extent lipids and pigments. Depending on the intended application of the ulvan extract, co-extraction of impurities can lead to more intensive down-stream purification procedures. We use three criteria to determine optimal conditions for the extraction of ulvan: 1. High yield; 2. High selectivity; and 3. Low degradation (i.e. ulvan is extracted without extensive hydrolysis). To address these criteria, we review the physicochemical properties of the predominant macromolecules in *Ulva*, and then review the literature and provide recommendations on the best practices for the extraction of ulvan, focusing on applications.

The extraction yield of ulvan is affected by the properties of the biomass and its pre-treatment, extraction temperature, extractants, extractant to biomass ratio, biomass particle size, and duration of extraction (Fig. 2a-f). The physicochemical properties of ulvan that influence its extraction yield include its relatively low solubility in

Table 1
Ulvan monosaccharide composition (median, minimum, and maximum in mol%) for each species of *Ulva* (1954–2018). Only Median reported where $n = 1$. For additional detail, see supplementary data set, sheet 2 "Meta-Table", and search by the "In-text reference" column.

Species	Rhamnose			Glucuronic acid			Xylose			Iduronic acid			Glucose			Galactose			Uronic acids			Reference
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	
U. arasakii	33.2						11.0												46.7			[39]*
U. armoricana	51.6	41.9	60.0	21.3	15.2	27.5	7.5	3.7	10.1	7.0	3.8	15.3	10.0	0.0	26.2	3.1	1.0	5.1	13.4	12.6	16.5	[32,40]*, [41,42]
U. clathrata	10.7			4.0												4.8						[43]
U. compressa	13.0	5.0	62.0				17.0	15.0	35.0				40.0	5.7	70.0	9.0	4.0	15.0	18.3			[44,45]*, [46]
U. conglobata	67.8	57.0	72.3	6.0			1.5	1.0	20.0				17.1	12.0	22.0	2.4	1.2	5.0				[47,48]
U. fasciata	57.4	8.2	92.2	14.7			26.1	1.5	36.3				7.7	0.7	22.8	1.9	1.6	2.2				[49–55]
U. gigantea	42.1			15.4			28.8			3.6			7.9			2.3						[56]
U. intestinalis	37.2	30.2	61.0				10.9	8.5	14.2				40.0	2.3	48.4	11.4	7.7	15.8				[57,58]
U. lactuca	33.5	8.2	60.8	22.5	2.6	52.0	7.3	0.0	35.4	6.0	5.0	6.0	28.6	2.0	87.2	1.6	0.0	1.9	16.8	16.8	47.1	[59,60]*, [61,62,63]*, [64]
U. linza	61.6	60.7	62.5	22.6	17.2	27.9	10.5	9.6	11.3				10.9									[65]
U. meridionalis	39.0						17.0						6.0			6.0			28.0			[66]*
U. ohnoi	47.1	41.1	53.1	28.6	27.8	29.4	5.5	5.3	5.7	8.8	7.4	10.1	2.5	1.5	3.5	2.6	1.2	4.0				[4]
U. olivascens	53.8			16.7			15.1			3.8			7.6			3.0						[32]
U. pertusa[a]	25.3	15.2	80.7	27.0			7.7	2.7	21.0				4.7	1.0	27.4	0.0						[48,67–69]
U. prolifera	67.8	57.1	87.6				3.8	2.4	8.8				31.9	3.6	67.8	4.0						[70,71]
U. rigida	26.9	10.7	58.3	31.7	19.0	37.0	10.8	4.3	38.0	6.0	2.5	7.0	7.6	1.6	46.1	0.9	0.3	1.7	18.3	10.3	46.0	[14,32], [72]*, [73]*, [74,75], [76]*
U. rotundata	46.8	7.2	55.0	22.5	15.0	28.9	15.0	4.4	25.0	4.0	0.6	9.0	5.3	0.0	62.8	3.0	0.0	4.5				[32,42,77]
U. scandinavica	42.2			11.6			9.6			4.0			30.7			2.0						[32]
U. sp.	33.0	27.9	54.8	13.4	11.3	21.5	12.4	1.7	16.9	3.7	3.3	5.9	5.6	0.1	38.1	1.0	0.0	2.1				[32,78,79]
U. spp.	28.2						3.2						5.9			1.3			19.3			[20]*
Pooled total[b]	45.0	5.0	92.2	22.5	2.6	52.0	9.6	0.0	38.0	5.0	0.6	15.3	9.5	0.0	87.2	2.1	0.0	15.8	17.1	10.3	47.1	$n = 44$

*Content of uronic acids was determined by colourimetric assay.
[a] *pertusa* is currently regarded as a synonym of *australis*.
[b] Ulvan composition pooled across species.

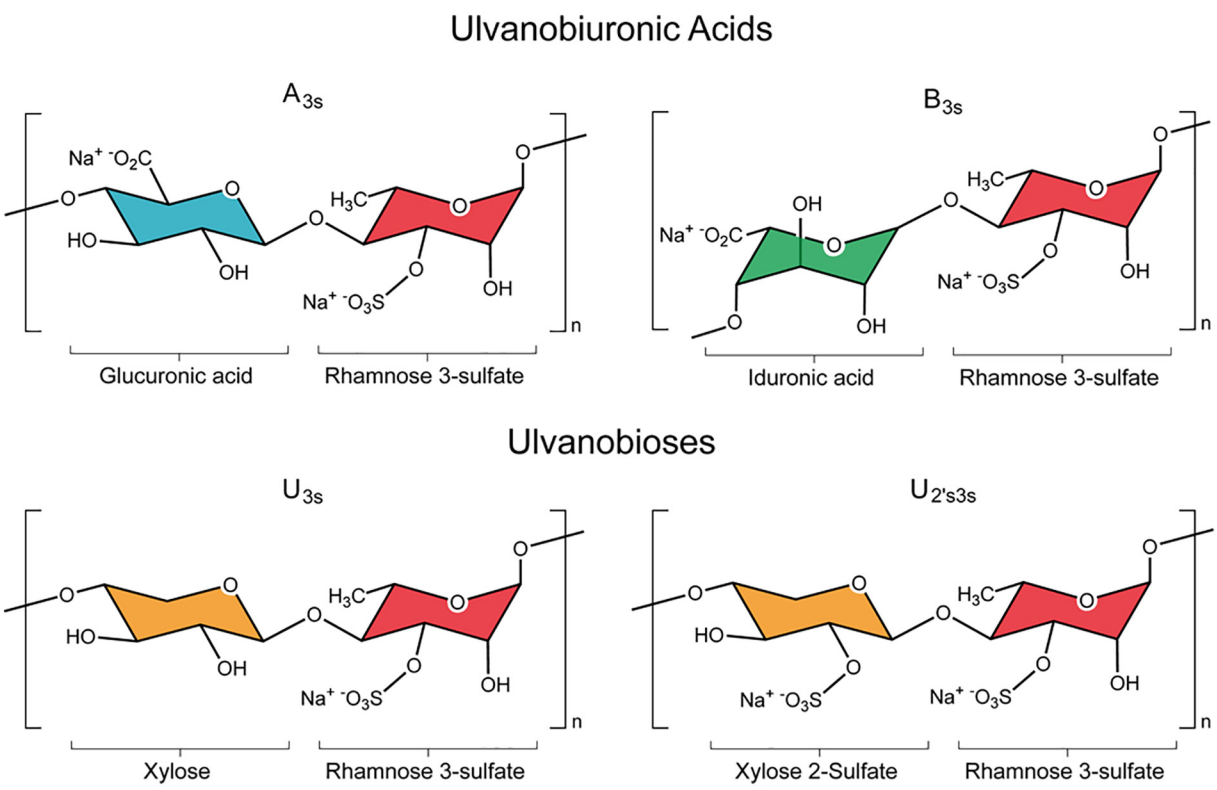

Fig. 1. Nomenclature and structure of the major repeating disaccharide units that comprise ulvan. Ulvanobiuronic acid A_{3s} contains glucuronic acid (blue) attached to rhamnose 3-sulfate (red), while the similar B_{3s} also contains rhamnose 3-sulfate but has iduronic acid (green) in the place of glucuronic acid. Ulvanobioses are comprised of rhamnose 3-sulfate attached to xylose (orange). Xylose can contain a sulfate group, as seen in $U_{2's,3s}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

aqueous conditions and its stabilisation in the plant cell wall, predominantly through its interaction with divalent cations (e.g., Ca^{2+}), borate, hydrogen bonding, and entanglement [77]. The solubility of ulvan and its intermolecular interaction are pH dependent [86]. Extraction solutions at pH > pKa of both uronic acids (~3.28) and sulfate esters (~2.0) promote a high charge density on ulvan and theoretically an increase in its solubility. Conversely, extraction solutions at pH < pKa of both uronic acids and sulfate esters theoretically reduce its solubility. In practice, the bead-like conformation of ulvan leads to formation of aggregates at neutral to acid pH, and these aggregates disperse at pH < pKa of the uronic acids allowing for greater solubility of ulvan [77]. The solubility of glucuronan, a homopolysaccharide consisting of repeating (1,4)-linked glucuronic acids, is also pH dependent with expected enhancement in its solubility above the pKa of glucuronic acid. In this regard, both glucuronan and xyloglucan are more soluble in alkaline solutions. The solubility of water soluble protein is also pH dependent. Extraction solutions with pH equal to the isoelectric point (pI) of the protein result in its reduced aqueous solubility. In this regard, the pI for aqueous and alkaline soluble proteins from macroalgae is generally between pH 3–4 [159]. However, lower pI values for soluble proteins from *Ulva* (pI = 2.25) occur [160]. Given these physicochemical properties of macromolecules in *Ulva*, an informed decision of extraction conditions can be made to enhance the efficiency and selectivity of ulvan extraction, while limiting degradation.

Extraction yield can vary significantly between populations of *Ulva* due to eco-physiological variation (light, temperature) [42], and, therefore, comparisons of extraction yields should be evaluated on a single harvest of biomass. In terms of biomass pre-treatment, the reduction of salt in the biomass by warm water extraction enhances the extraction efficiency of ulvan [4]. The mechanism here is twofold, the reduction of salt reduces the aggregation properties of ulvan, and osmotic shock increases the exposure of cell wall components to the extractant. Other pre-treatments, such as pigment and lipid removal, have no effect on either extraction efficiency or the quality of ulvan extracted and for this reason are considered unnecessary, unless they are targeted products. Finally, to increase the interaction of extractant and biomass cell wall, and, therefore, enhance extraction efficiency, biomass is dried and finely milled.

The solubility of ulvan in aqueous solutions is enhanced by extraction at high temperatures (80–90 °C). The temperature is usually capped below the boiling point of water for convenience and to prevent higher temperatures promoting degradation (e.g., depolymerisation and desulfation) [161]. However, high temperature extractions in water generally have low extraction yields due to the interactions of ulvan with other cell wall components [77]. As a result, extractants, such as chelators and acids, are used to overcome the structural integrity of the plant cell wall, thus, enhancing the extraction efficiency of ulvan. Chelators, such as oxalates and EDTA, remove divalent cations (e.g., Ca^{2+}) that promote the cross-linking of ulvan in the cell wall. Extractions using chelators are generally conducted at or near neutral pH and at high temperatures (80–90 °C). Reported yields vary widely (see Fig. 2), but the extraction efficiency (and selectivity) is improved when the pH is adjusted to 4.5 [77]. In this regard, extractions in strong acid (e.g., HCl; Fig. 2) generally produce higher extraction yields [4,77,162]. Mechanistically, extractions at pH below the pK_a of glucuronic acids disperse ulvan aggregates, facilitating its extraction. Notably, high temperatures (80–90 °C) are still required to facilitate high extraction efficiencies at low pH, however, isolation methods influence yields (see Section 2.4). Extraction pH also plays a significant role in the selectivity of ulvan over other macromolecules, as discussed above.

The physicochemical properties of the macromolecular constituents of *Ulva* support extraction pH as an important factor in the selectivity of the extraction process for ulvan. At pH below the pKa of uronic acids (~3.28) the extraction of ulvan is enhanced (see rationale above), and

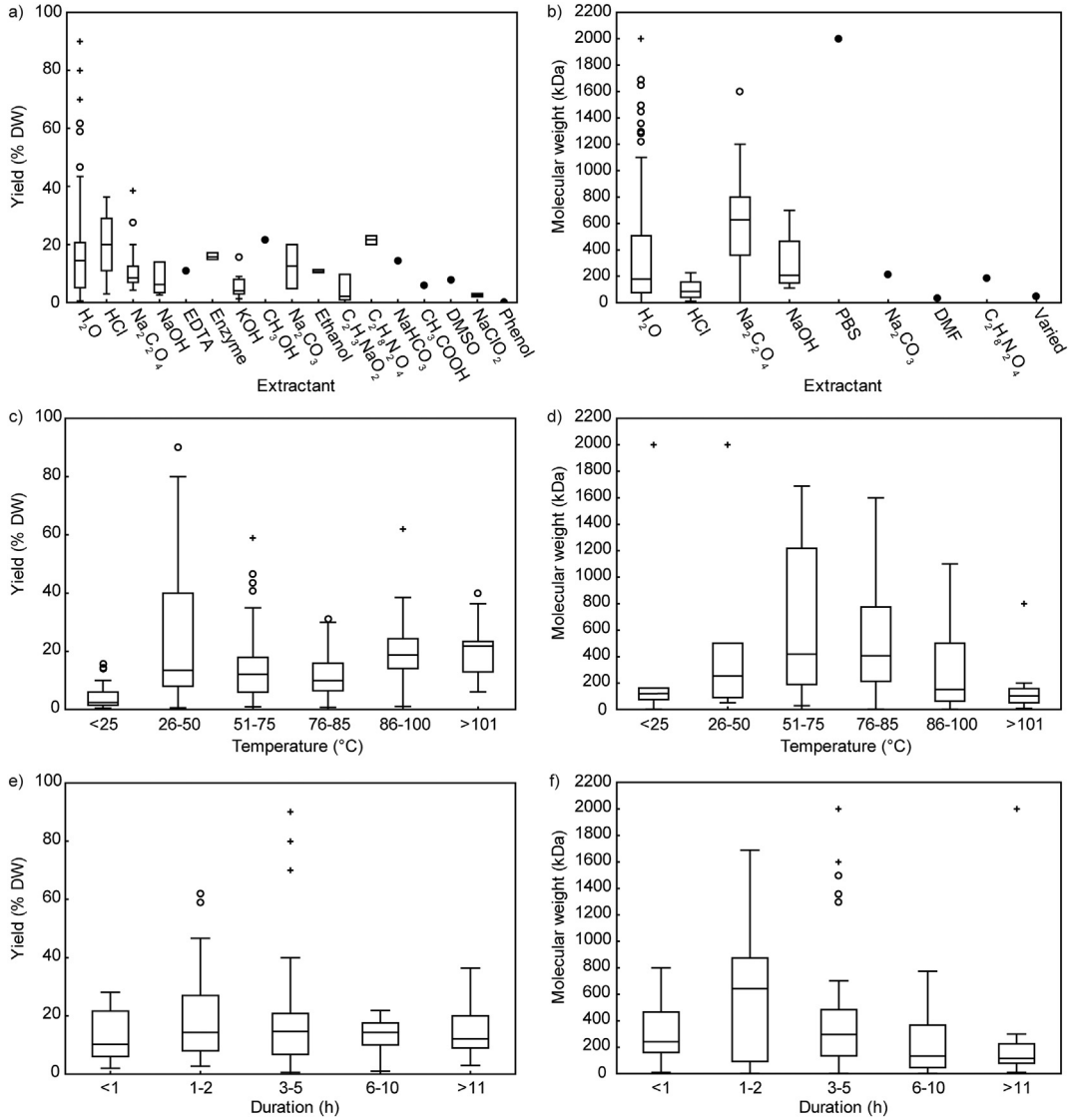

Fig. 2. Box plots of the median and variability in ulvan yield (a,c,e) and average molecular weight (b,d,f) resulting from extractant applied (a,b), temperature (c,d) and duration (e,f) of the extraction procedure. Yield is presented as % of dry weight; average molecular weight in kDa. "PBS" = Phosphate buffered saline; "DMF" = anhydrous *N*,*N*-dimethylformamide; "Varied" = multiple extractants. Boxes represent the interquartile range (IQR) with the mean as a line within the box; whiskers represent the limits of non-outlier data; open circles are outliers, calculated by ± 1.5*IQR; crosses are extreme values, calculated by ± 3*IQR; closed circles represent values for which $n = 1$.

the solubilities of other macromolecules, such as glucuronan, xyloglucan, and soluble protein, are minimised [4,77,133,162]. For example, HCl extracts of fresh *U. ohnoi* had superior selectivity for ulvan (306–333 μg rhamnose/mg extract) over protein (4–7 μg protein/mg extract), when compared with sodium oxalated extracts (114–162 μg rhamnose/mg extract and 41–59 μg protein/mg extract) [4]. The selectivity for ulvan over other polysaccharides can also be inferred by comparing the molar ratios between rhamnose (an ulvan specific monosaccharide), and uronic acids or xylose (both of which are constituents of multiple polysaccharides) of extracts obtained using different extractants [4,77]. For example, ulvan extracted from *Ulva ohnoi* had a molar ratio of 1:0.71 for rhamnose to uronic acids when HCl was used as the extractant, and 1:0.88 when oxalate was used as the extractant, demonstrating increased co-extraction of glucuronan in the latter [4]. Similarly, the mole ratio of rhamnose to uronic acids for ulvan extracted from *Ulva rotundata* with 0.05 M HCl (pH 1.3) was 1:0.83 compared to 1:0.99 when extracted with 0.02 M ammonium oxalate (adjusted to pH 4.6) [77]. These latter studies indicate a quantitative measure of selectivity can be provided using pH, resulting in a more accurate composition of the ulvan attained. It is, however, important to note that while selectivity for ulvan over other macromolecules was enhanced at low pH, significant degradation of its structure also occurred.

Degradation of the structure of ulvan during the extraction procedure may reduce or enhance its functionality depending on the intended application. For this review, we focus on the versatility of the ulvan extract, and as ulvan can be readily degraded after its extraction using chemical and enzymatic approaches (see above), an ulvan sample with minimal degradation is considered the most versatile product. There are two structural features that are most susceptible to degradation during the extraction of ulvan; its degree of polymerisation and degree of sulfation. In this regard, depolymerisation of the polysaccharide is more facile than desulfation of the polysaccharide under the range of extraction conditions used to extract ulvan. Extraction parameters that influence the degree of depolymerisation include pH, temperature, and duration (Fig. 2d, f). In this regard, high temperature (80–90 °C) extractions at low pH (~1.3–1.5) lead to significantly higher levels of depolymerisation than extractions at higher pH (with or

Table 2
Ulvan extract median, minimum, and maximum yield (% algal dry weight) and composition (% w/w) for each species of *Ulva* (1954–2018). Only Median reported where $n = 1$. For additional detail, see Supplementary data set, sheet 2 "Meta-Table", and search by the "In-text reference" column.

Species	Yield			Total carbohydrate			Protein			Ash			Sulfate			Reference
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	
U. arasakii				54.9	54.9	54.9							0.4			[39]
U. armoricana	40.0	7.0	90.0	48.7	31.2	78.0	7.3	1.5	13.9	17.0	2.9	18.6	14.4	2.7	19.1	[32,40,42,101–104]
U. clathrata	6.8	0.6	61.9	31.3	26.7	44.6	8.2	0.4	15.3	19.0			10.6	5.6	35.8	[43,105–108]
U. compressa	9.0	0.7	23.0	42.0	23.5	54.0	12.0	8.0	27.8	19.2	10.0	40.0	6.7	3.0	13.0	[44–46,104,105,109]
U. conglobata	1.1	0.9	1.3				3.9	3.8	4.5	16.3	15.4	18.5	23.2	11.9	35.2	[47,48,110]
U. fasciata	13.0	0.5	40.0	48.8	1.4	84.7	4.2	0.2	11.8	14.9	12.4	20.9	18.6	2.3	27.5	[49–55,105,111–114]
U. flexuosa	2.7															[115]
U. gigantea				79.6			5.8						11.9			[32]
U. intestinalis	12.0	2.2	59.1	39.0	4.9	92.2	5.1	0.0	15.0	21.6	12.5	29.4	29.8	2.9	40.0	[57,116–124]
U. lactuca	16.6	1.0	36.4	37.7	4.5	84.1	2.9	0.0	33.1	15.7	0.8	47.2	14.3	5.8	32.2	[15,59,60,62–64,91,105,109,111,125–139]
U. linza	9.8	6.5	15.1	51.0	47.9	60.0	1.9	0.5	3.2				17.7	12.5	21.3	[65,87,140–143]
U. meridionalis	18.7						6.8	4.0	9.5	12.9			10.0			[66,95]
U. ohnoi	6.3	4.3	8.2	54.7	42.3	67.0	2.8	0.4	5.1	25.9	23.3	28.5	14.1	11.5	15.7	[4,144]
U. olivascens				63.4			4.9	4.9	4.9				13.8			[32]
U. pertusa[a]	22.5	1.9	43.4	60.7	33.4	72.3	3.4	1.4	4.8	26.3	22.6	29.9	17.1	11.6	24.0	[48,67–69,83,84,93,97,110,143,145–147]
U. prolifera	20.3	0.6	46.7	56.1	49.2	93.3	1.9	0.0	13.9				16.8	7.7	22.0	[70,71,110,148–153]
U. reticulata	4.5	1.2	8.3	35.7	20.1	46.1	10.1	9.4	20.8	19.5	19.3	19.7	14.6	10.1	17.8	[73,105,111,154,155]
U. rigida	10.9	1.3	38.5	59.5	39.8	75.6	10.0	0.2	25.9	18.1	8.1	25.0	19.8	14.3	37.3	[14,32,73,76,82,88,111]
U. rotundata	8.0	0.1	27.5	35.9	22.3	70.8	12.6	1.0	35.5				11.4	2.4	17.3	[32,42,77,100,156]
U. scandinavica				56.3									13.1			[32]
U. sp.	9.7	5.0	14.4	72.0	25.6	91.9	7.8	4.0	11.6	20.8	6.6	23.0	13.9	7.8	18.5	[28,32,78,79,85,157,158]
U. spp.	12.2	12.2	12.2	41.7			4.3	4.3	4.3	23.7			15.8			[20]
Pooled total[b]	12.0	0.1	90.0	46.1	1.4	93.3	6.6	0.0	35.5	17.1	0.8	47.2	15.5	0.4	40.0	

[a] *pertusa* is currently regarded as a synonym of *australis*.
[b] Ulvan yield and composition pooled across species.

without an added extractant) [4,77,125,133]. However, small changes in pH, temperature, and duration all have a significant effect on the degree of depolymerisation [77,125,133,161]. For example, ulvan was extracted from *U. lactuca* using a factorial experimental design that varied temperature (80 °C or 90 °C), pH (pH 1.5 or 2), and time (1 − 3h) [133]. The harshest extraction condition (90 °C at pH 1.5 for 3 h) resulted in higher yield at the cost of significantly greater depolymerisation than the milder extraction (80 °C at pH 2 for 1 h). Furthermore, at pH 1.5 for 1 and 2 h, significantly higher depolymerisation occurred for extractions conducted at 90 °C than at 80 °C, but a higher yield was also obtained. The same temperature dependence was present for 2 and 3 h extractions at pH 2.

The combination of extraction temperature, solvent pH and extraction duration interact to influence extraction yield and quality (e.g., purity and molecular integrity). Higher extraction temperatures enable greater solubilisation of ulvan (Fig. 2c), low pH improves the selectivity for ulvan, and increased extraction duration can increase ulvan yield. However, compromises must be made to protect the integrity of the ulvan structure. For example, while high temperature results in higher yields, a combination of high temperature, low pH and long extraction duration lead to significant depolymerisation (Fig. 2d, f). However, small adjustments in pH have a large influence on depolymerisation, while small changes in temperature do not. Therefore, using the data available in the literature the following extraction conditions are recommended based on the criteria listed above: 1. High extraction yield; 2. High selectivity; and 3. Low degradation:

- Temperature: 80–90 °C
- pH range: 2–4.5
- Duration: 1–3 h

2.3. Isolation and purification

Methods for the isolation and purification of ulvan vary significantly and are linked to both the availability of resources and the focus of the study. Here we regard isolation as the separation of the extract from residual biomass and the removal of solvent, and purification as the separation of ulvan from other solutes (e.g., salts and other macromolecules). In practice, these steps are not mutually exclusive. It is also important to note that isolation and purification methods influence the physicochemical properties and biological activities of ulvan. We briefly review commonly used methods for the isolation and purification of ulvan that yield two desirable outcomes: 1. High recovery, and 2. Pure product. Methods for the isolation and purification, both chemical and enzymatic, of polysaccharides have been comprehensively reviewed [163]; our focus is to briefly review commonly used isolation and purification methods for ulvan, and comment on their effectiveness.

Following the extraction of ulvan, the extract is separated from the residual biomass by filtration and/or centrifugation prior to the isolation of ulvan by precipitation, evaporation, or concentration by ultrafiltration followed by lyophilisation. For industry, spray drying is another efficient process [38]. Precipitation of ulvan with ethanol is by far the most common isolation method used in the laboratory. Typically, addition of ethanol (e.g. 70–96%) to a concentrated extract is adequate to precipitate ulvan, while ethanol-soluble compounds, such as pigments and metabolites, remain in solution [164]. Although ethanol precipitation is a convenient method, it is limited by the low solubility of salts and higher solubility of low molecular weight ulvan in ethanol-water mixtures. Precipitation methods are also particularly difficult to replicate and in the absence of dialysis or ultrafiltration can lead to high levels of salt in the ulvan extracts [59,133]. Consequently, precipitation can lead to incorrect interpretations of yields, physicochemical properties, and biological activities. Efficient desalting of ulvan extracts can be achieved by dialysis, ultrafiltration, or even chromatography.

Dialysis and ultrafiltration allow for the reduction (removal) of excess salts and small molecules contaminating ulvan extracts. Dialysis is highly effective in the removal of excess salt from analytical samples and for other applications that require very low residual ash. Ultrafiltration is generally employed as a two-step process; concentration (volume reduction) followed by diafiltration (washing or buffer exchange). Both dialysis tubing and membrane filters for ultrafiltration are available with a variety of pore sizes (or molecular weight cut-off (MWCO)). The range of MWCO's used for ulvan generally fall between 3.6 and 12 kDa [92,111]. For dialysis, pore size selection is based around the retention of ulvan, however, for ultrafiltration pore size selection is based on both the retention of ulvan and the rate of permeate flow (which decreases as pore size decreases). The latter extends the time associated with the ultrafiltration processes. The balance between ulvan retention and time efficiency is optimised at ~10 kDa MWCO [4,100,125].

Given the high level of impurities in many ulvan extracts, it is surprising that chromatographic techniques are not applied more widely for purification prior to characterising the physicochemical properties and biological activities of ulvan. In this regard, the polyanionic and polydisperse nature of ulvan lends itself to anion-exchange chromatography (AEC) and size-exclusion chromatography (SEC), respectively. AEC is effective for the removal of proteins and neutral polysaccharide impurities from ulvan samples. Both weak anion-exchangers (e.g., diethylaminoethyl (DEAE) or tertiary amine functionalised media) [25,111] and strong anion-exchangers (e.g., quaternary (Q) amine functionalised media) [77] have been successfully employed to purify ulvan. SEC is widely used as an analytical technique for determining molecular weights and molecular weight distributions (see Section 2.4), however, it has wider applications. For example, SEC can be used to fractionate oligosaccharides and polysaccharides for the elucidation of fine structure [23], and to generate size classes for structure-function relationships [29,165]. With regards to the former, SEC has been used to fractionate ulvan oligosaccharides for fine structure analysis using NMR techniques [81]. A large range of size exclusion media is available targeting different molecular size ranges. Media commonly used for purification of ulvan samples are Sepharose CL-6B [29], Sephacryl S-400/HR [165], Sephacryl S-1000 [166], and Sephadex G-200 [46].

In summary, ideally the choice of extraction techniques will limit the co-extraction of macromolecular impurities (see Section 2.2) and reduce the necessity for intensive purification techniques. However, due to salt content (introduced by extractants and inherent in marine species) and similarities in the physical properties of ulvan and contaminating polysaccharides and proteins, purification is necessary. In general, salt is the most significant impurity and is most effectively removed by dialysis or ultrafiltration techniques. Depending on the extraction process employed, protein may also need to be actively removed, either by chemical or enzymatic approaches [36,163], or using chromatographic techniques. The latter is more reproducible and allows for highly purified fractions of ulvan. In practice, a strategic selection of extraction procedures, and isolation and purification procedures should be made based on the available resources. For example, in a resource limited situation one might select a more selective acid extraction, concentrate by evaporation, purify using dialysis, and isolate by precipitation. In a resource rich situation, a selective acid extraction is ideal, although a less selective extraction process can be tolerated, with concentration and diafiltration with ultrafiltration. Subsequent purification by anion-exchange chromatography, and lyophilisation or spray drying to isolate the purified ulvan can be applied.

2.4. Characterisation

In general, the elucidation of polysaccharide structures is often complicated by the presence of multiple monosaccharide constituents, which may include neutral, acidic and amino sugars, a variety of glycosidic linkages, high molecular weights, branching of constituent

sugars, a variable degree of sulfation and substitution patterns, and complex macromolecular properties (e.g., aggregation). The presence of contaminating polysaccharides further complicates structural elucidation of a particular polysaccharide. Therefore, it is important to start with as pure a sample as possible, and even then full structural elucidation is challenging - 'Structural characterisation' is a more fitting term. Adequate structural characterisation of an ulvan sample is essential for interpreting its physicochemical properties and biological activities. To achieve this a measurement of composition (e.g., total carbohydrate content, protein content and ash) and the identifying structural features of ulvan (e.g., sugar composition, glycosidic linkages, degree of sulfation) are required. Comprehensive reviews of the techniques used to elucidate the chemical structure and macromolecular properties of bioactive polysaccharides are provided elsewhere [167–169]. In this review we focus on the methods used to characterise ulvan and propose the minimum analysis and characterisation required to progress beyond bioprospecting.

The analysis of the chemical structure of ulvan is fundamentally based on the quantitative determination of the major constituent monosaccharides (rhamnose, xylose, glucuronic acid and iduronic acid) and degree of sulfation. The latter is most commonly carried out using a turbidimetric assay [170] but can be measured using ion chromatography, high performance liquid chromatography (HPLC) coupled with conductivity [20], elemental analysis, or Fourier transform infrared spectroscopy (FTIR) [171]. Constituent monosaccharides are predominantly quantified using chromatography techniques, in particular gas chromatography (GC) [20,47,49,59,72], HPLC [70,95], and high-performance anion-exchange chromatography (HPAEC) [4,32]. Depending on the chromatographic technique employed the sample is subjected to pre-treatments. Primarily, accurate quantification of constituent monosaccharides requires complete hydrolysis of ulvan. However, due to the resistance of the aldobiuronic glycosidic linkage to hydrolysis [16,17,172], and the susceptibility of rhamnose, glucuronic acid, iduronic acid and xylose to degradation in concentrated strong acids (e.g., Saeman hydrolysis) [173,174], traditional acid hydrolysis methods, such as with trifluoroacetic acid (TFA), are both ineffective and degradative. A traditional acid hydrolysis will not cleave all of the aldobiuronic linkages and as a result the neutral and uronic acid residues involved in these linkages will not be detected. As ulvan is predominately comprised of repeating aldobiuronic acid disaccharides, a traditional acid hydrolysis will inaccurately characterise ulvan. However, hydrolysis of ulvan using traditional methods is ubiquitous in the literature. A two-step hydrolysis in methanolic HCl followed by aqueous trifluoroacetic acid is an effective alternative to reduce the hydrolysis-induced damage to constituent monosaccharides [4,175]. A chemo-enzymatic degradation involving hydrolysis with a mild acid followed by β-D-glucuronidase cleavage of aldobiuronic acid is also an effective solution [13,16]. Both HPLC [70,87,95] and HPAEC [4,32] methods are used to quantify the monosaccharides in the hydrolysis samples without further derivatisation. HPLC methods generally provide less resolution than HPAEC methods [176], but both allow the assessment of the effectiveness of the hydrolysis method employed [175]. HPAEC-PAD (pulsed amperometric detection) of methanolic-HCl/TFA hydrolysates also has the major advantage of separating and quantifying acidic and neutral sugars (and amino sugars) in a single run, without further derivatisation [175]. GC analysis requires that the constituent sugars be converted to volatile derivatives, such as alditol acetates [20,49,72,83] and trimethylsilyl (TMS) ethers [59]. For the former, reduction of monosaccharide C1-aldehyde is conducted prior to the preparation of alditol acetate. In general, C6-carboxylic acids are not reduced under the conditions employed to reduce aldehydes and, therefore, are not converted to alditol acetates. However, GC–MS analysis of alditol acetates, prepared from ulvan hydrolysates that included the pre-hydrolysis reduction of C6-carboxylic acids with sodium borodeuteride (NaBD4) to generate 6,6′-dideuterio-sugars, allows for the determination of the total uronic acid content [169]. Studies that do not include this conversion generally measure uronic acid content using colourimetric assays [177,178]. Alternatively, TMS ethers of sugars can be prepared using reagents such as *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, allowing for the quantification of both neutral and acidic sugars [59]. However, TMS derivatisation of un-reduced sugar residues can yield several peaks in the GC chromatogram corresponding to different pyranose anomers, which complicates interpretation. A solution to this problem is to follow the preparation of alditol acetate derivatives with reduction to the alditol prior to preparation of the TMS derivative. For sample preparation, HPAEC methods (e.g., HPAEC-PAD) provide the simplest and most comprehensive route for sugar constituent analysis; however, excellent results can also be obtained using either GC or HPLC methods.

While sugar constituents are important in determining the physicochemical properties and biological activities of a polysaccharide, glycosidic linkages and functional group substitution patterns, and sugar sequence are arguably more important. Glycosidic linkage (methylation) analysis is generally conducted using GC–MS methods [25,168,179] and provides information on the linkage positions and substitution patterns of constituent sugars. Briefly, methylation analysis involves the preparation of partially methylated alditol acetates followed by quantitative analysis using GC–MS. To achieve this effectively, the uronic acid components of ulvan are reduced prior to the methylation step [168,169]. Determination of glycosidic linkages and sugar sequence can then be achieved using 2D NMR techniques on hydrolysed ulvan fragments (disaccharides and oligosaccharides) [24,167,180–182]. In many cases, ^{1}H and ^{13}C resonances characteristic of ulvan structures (e.g., repeat disaccharide units) can be assigned by comparison with published data [4,49,77,84,128,183,184]. In addition, the anomeric configuration and position of substituents (e.g., sulfate groups) can be inferred by location of both ^{1}H and ^{13}C resonances [167,181].

The physicochemical properties and biological activities of polysaccharides are also fundamentally linked to molecular weight. Therefore, a measure of average molecular weight (MW) and molecular weight distribution (MWD) is integral to the characterisation of polysaccharides. In this regard, size exclusion chromatographic (SEC) methods are the most commonly employed as they allow a measure of both MW and MWDs. Firstly, as a minimum requirement for the determination of average MW and MWD's, SEC is coupled to a concentration dependent (CD) detector, most commonly refractive index and UV. For ulvan, calibration of these setups is usually achieved with narrow polydispersity polymer standards (e.g., dextrans [28,68,69,88,133,145], pullulans [59,161,185] and polyethylene oxides [61]). However, due to difference in the macromolecular properties, commonly used calibration standards are unlikely to yield accurate results, and accurate measurements of ulvan (and other complex polymers) MW and MWDs require online molecular weight detectors (e.g., multiangle laser light scattering (MALLS) and viscosity (VISC)) [4,57,61,86,128]. Both SEC-MALLS and SEC-VISC also allow analysis of macromolecular properties, such as aggregation behaviour [86,186]. Comprehensive reviews on the theory and variety of detection methods in SEC analysis are provided by [186,187].

These methods for the characterisation of ulvan can represent a significant investment in both equipment and experimental time. However, high-throughput determination of the composition of ulvan extracts is desirable in some applications (e.g., bioprospecting studies, ulvan composition comparisons, and for quality control in industry) and requires an alternative approach. The rapid determination of ulvan in samples is commonly carried out using colorimetric assays that probe sugar constituents (e.g., uronic acids, rhamnose, and xylose) and protein content [188,189], and a turbidimetric assay for determination of sulfate ester content [170]. Common and relatively accurate colorimetric methods for uronic acid determination include the carbazole/sulfuric acid [177] and sulfamate/*m*-hydroxydiphenyl techniques [178]. However, due to a lack of specificity for targeted sugars,

chemical colorimetric methods for quantitative measurements of neutral sugars (e.g., rhamnose and xylose) are hampered by interference. In this regard, highly specific enzymatic assays for monosaccharides typically present in ulvan including rhamnose [190], xylose [191] and uronic acids [192] are available. However, to gain an understanding of composition multiple characterisation assays are necessary resulting in little time savings. In this regard, a truly high-throughput method would require only a single measurement on an un-degraded sample with minimal preparation.

Chemometric methods coupled with spectral analysis, such as IR, Raman, NIR, and NMR, are effective for the rapid (single measurement) compositional analysis of polysaccharides [171,193]. For example, FT-IR spectra in the range of 1770–600 cm^{-1} coupled with the multivariate analysis method of partial least squares (PLS) analysis was used to develop calibrated FT-IR-PLS-models that allowed the sulfate content and the content of the major monosaccharides in ulvan hydrolysates to be determined [171]. The key ulvan spectral features used for the development of FT-IR-PLS-models were the uronic acid carboxylic groups (1650–1600 cm-1 (ν_{asym} C═O); 1425–1400 cm^{-1} (ν_{sym} C═O)) [171,183], sulfate ester groups (1260–1215 cm^{-1} (ν_{asy} S═O); 850–835 cm^{-1} and 795–785 cm^{-1} (C-O-S)) [25,83,183], and the glycosidic linkage between the two major sugars, rhamnose and glucuronic acid (1055–1030 cm-1 (C-O-C)) [171]. While, the broader application of the FT-IR-PLS-models for ulvans is untested, this analytical approach represents a rapid characterisation method for ulvan.

Ulvan has a broad range of biological activities; however, the characterisation of ulvan is often of low resolution leading to uncertainty in the origin of the activities tested. At the research level total characterisation methods are essential in understanding structure-activity relationships. The minimum characterisation data recommended is sugar constituent analysis (preferably suitable for the detection of neutral and acidic sugars), molecular weight analysis, and measures of contaminants, such as protein and ash. To obtain further insight into structure-activity relationships, glycosidic linkage analysis should also be conducted. In the next section we review the biological activities of ulvans and comment on the structural attributes that influence its efficacy.

3. Biological activities of ulvans

3.1. Overview

Ulvan has demonstrated significant biological activities in both animal and plant systems in in vitro and in vivo studies (Table 3). In animals, sulfated polysaccharides, such as free glycosaminoglycans (GAGs) and proteoglycans (protein linked GAGs), are intricately involved in a broad range of biological processes and have significant structural similarities with ulvan [194,195]. Therefore, the capacity of ulvan to mimic GAGs is a logical link. Sulfated polysaccharides are absent in terrestrial plants and the rationale for the biological activity of ulvans towards plants is less clear; however, ulvan has structural similarities with plant rhamnogalacturonans [196] and rhamnolipids from phytopathogenic bacteria [197], providing an insight to its activity. Importantly, the structural features of ulvan (e.g., molecular weight, degree of sulfation, sulfation pattern, constituent sugars, linkages, isomers, and degree of branching) influence its bioactivity. Therefore, ulvan obtained from different species of *Ulva*, and species from different environments, display significantly varied bioactivity profiles. We review the biological activities attributed specifically to ulvan, focusing on activities with potential biomedical applications and the developing body of technologies in plant physiology, horticulture, and agriculture.

3.2. Cytotoxicity

Cytotoxicity of supplements, nutraceuticals, therapeutic agents or adjuvants, and biomedical materials is a critical consideration for product development, including a natural product such as ulvan. In this regard, the cytotoxicity of ulvan has been investigated by dosing a range of macrophage cell lines (e.g., RAW 264.7, J774A.1, and peritoneal) [57,67,70,124], gut cells (e.g., IPEC-1) [101,102,224], fibroblast cells (e.g., mouse C3H [L929]) [225], Vero cells [207], Swiss mice and Wistar rats [129]. Ulvan is largely non-toxic, with ulvan fractions from *U. pertusa* [57,67], *U. intestinalis* [122,124], *U. armoricana* [101,102], *U. lactuca* [129,225,226], *U. clathrata* [107], *U. compressa* [199] and *U. prolifera* [70,227] having > 50% cell viability for cell cultures dosed with ≥500 μg/ml of sample.

3.3. Immunomodulating activity

Molecules that influence the immune system are known as immunomodulators and often act by influencing inflammation. The inflammatory effects of ulvan have been investigated in vitro using macrophage cell types (e.g., RAW 264.7, mouse peritoneal [122], J77A.1 [124] and fish head kidney [223]), tissues (e.g., intestinal epithelial cells [101,102]), and in vivo using animal models (e.g., Wistar rats [209], mice [147,218], and chickens [228]). Inflammation is dependent on cytokine production and is largely the result of the activation of NF-kB (a protein complex that controls transcription) by pathogen-associated molecular patterns (PAMPs) on toll-like receptors (TLRs) [102,229,230]. Lipopolysaccharides (LPS) on the cell wall of gram-negative bacteria are potent activators of TLRs and are the most commonly employed PAMP class in immunomodulation studies. Activated NF-kB induces transcription of genes coding for cytokines, thus, initiating an inflammatory response [229]. Evaluation of the influence of ulvan on inflammation has been conducted using a number of probes, including signalling molecules (e.g., cytokines; TNF-α, IL1, IL2, IL6, IL10, IL12, CXCL1, CXCL12, CXCL14 and CCL22), active metabolites (e.g., PGE2, NO, HOCl), immunoglobulins (e.g., IgM, ICAM and VCAM-1), enzymes (e.g., COX-2, iNOS-2, HO-1 and MPO) and transcription related molecules (e.g., NF-kB, mRNA) [57,67,70,72,101,122,124,144,218,227]. A pro-inflammatory response is initiated by immune cells (e.g., macrophages) through the release of cytokines (e.g., IL-1, IL-2 IL-6, IL-18, IL-12, prostaglandin E2 (PGE2), and tumour necrosis factor alpha ([TNF]-α)) [231]. Anti-inflammatory cytokines, such as IL-4, IL-10, IL-11, and IL-13, can inhibit the release of pro-inflammatory cytokines to prevent damage from excessive macrophage activation. This simplistic representation of the roles of immune cells and cytokines is useful but cytokine activity is complicated, involving multiple effects and interacting pathways [231].

Macrophage cells (e.g., RAW 264.7, mouse peritoneal, J77A.1, and fish head kidney) are often used to study the effect that ulvan (and other bio-actives) have on the inflammatory response [57,67,72,74,118,122,124,144,227]. For example, RAW264.7 cells exposed to 50 μg/ml of purified ulvan from *U. intestinalis* upregulated the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-12), the anti-inflammatory cytokine IL-10 and enzymes (e.g., iNOS and COX-2) and their products (e.g., NO and PGE_2); a response of equivalent magnitude to the LPS (1 μg/ml) positive control [57]. Importantly IL-10 inhibits pro-inflammatory cytokines (including, TNF-α, IL-1β, IL-6, and IL-12), which might act to limit any potential harmful repercussions from an over-stimulated inflammatory response. Ulvan isolated from *U. pertusa* [67] *U. rigida* [72,227] and *U. prolifera* [70] also activate RAW 264.7 cells, upregulating the production of cytokines (e.g., TNF-α, IL-1β, IL-6, IL10), enzymes (e.g., iNOS and COX-2) and their products (e.g., NO and PGE2). Consistent with RAW 264.7, macrophage J774A.1 cells treated with 200 μg/ml crude ulvan extracted from *U. intestinalis* stimulated the production of pro-inflammatory cytokines TNF-α and IL-1β to a similar degree as the LPS (1 μg/ml) positive control [124]. A similar pro-inflammatory response was also recorded when peritoneal macrophages from male ICR mice were treated with crude ulvan (100–400 μg/ml) from *U. intestinalis* [122]. Ulvan

Table 3
Ulvan bioactivities for each species of *Ulva* reported in publications that also detailed the extraction procedure and/or characterisation of the polysaccharide (1954–2018). For additional detail, see supplementary data set, sheet 2 "Meta-Table", and search by the "In-text reference" column.

Species	Bioactive						Other		
	Anti-coagulant	Immunomodulating	Anti-cancer	Antioxidant	Anti-viral	Antihyperlipidemic	Biomaterial	Plant defence	Various[b]
U. arasakii									
U. armoricana		[101,102]		[40]	[40]			[103]	[101,104]
U. clathrata	[43,65,105]	[108]			[107]				[198]
U. compressa	[105]				[199]		[200]		[104,198]
U. conglobata	[47]								
U. fasciata	[55,105,201]	[202,203]	[53,113]	[52,53,112,113,204]		[202,203,205]		[49–51,206]	[54,114]
U. flexuosa									
U. gigantea									
U. intestinalis		[57,118,121,122,124]	[58,119,121,122]	[116–118,124]	[116,207]	[116]			[208]
U. lactuca	[60,105,137]	[127,129,209]	[128,137,139,209,210]	[64,126,127,131,137,139,204,209,211,212]	[62,127,137]	[211,213]	[61,214]	[91,106]	[134,135,138,208,215]
U. linza	[142]	[87]		[87,142,143]					[140,141]
U. meridionalis									
U. nematoidea	[216]								
U. ohnoi		[144]							
U. olivascens									
U. pertusa[a]		[67,147]	[67]	[68,69,97,143]	[147]	[68,69,83,93,145,146]			[99,217]
U. prolifera		[70,218]	[70]	[71,149,151,153]		[152,219]			[148,220,221]
U. reticulata	[105]			[222]					[155]
U. rigida		[72,223]		[88]			[76]		
U. rotundata							[200]		[198]
U. scandinavica									
U. sp.	[158]	[223]							[28,79]
U. spp.								[29]	
Total publications	11	21	13	31	9	14	4	8	20

[a] *pertusa* is currently regarded as a synonym of *australis*.
[b] Various bioactive properties not otherwise listed on the table. See supplementary data set in for details.

from *U. ohnoi* also stimulated head kidney macrophages from *Solea senegalensis* (sole fish) [144]. Overall, ulvans induce a pro-inflammatory response in animal macrophages with potential for application in animal and human supplements as non-specific immunostimulants.

The effect of ulvan on the immune response has also been investigated in fish [74,144], porcine (pig) intestinal epithelial cells [101,102], rats [129,209], mice [122,129,218,232] and chickens [228]. Despite the range of organisms assessed and extraction techniques applied, ulvan is consistently reported to increase mRNA expression [74,101,102] and promote the release of immunomodulating cytokines or enzymes [144,209,218]. For example, a porcine intestinal epithelial (IPEC-1) cell line treated with purified low molecular weight (4.4 kDa) ulvan (5–500 μg/ml) from *U. armoricana* increased the mRNA and the protein expression of cytokines (e.g., CCL20, IL-8 and TNF-α) [102]. In mechanistic studies conducted by incubating human embryonic kidney (HEK) 293 cells with ulvan (500 μg/ml), ulvan primarily stimulated TLR4, with a subsequent increase in Akt phosphorylation, which activates NF-kB leading to the production of inflammatory cytokines. A similar response was recorded for LPS-treated (100 ng/ml) cells. Increased production of NF-kB was also reported in thymus and spleens of mice (Kunming) treated with ulvan from *U. prolifera* [218]. These mechanistic insights are an important consideration in the development of new supplement (nutraceutical or therapeutic) products for animal and human use.

The immunomodulating activity and potency of ulvan varies significantly between studies of the same species, and between different species. While there is evidence that this variability is due to structural features, some is related to sample purity. In this regard, a significant increase in the potency of the immunomodulatory activity of ulvan from *U. intestinalis* occurs following purification of a crude extract [57,122,124]. However, the structural features of ulvan (e.g., molecular weight and sulfation) also influence potency. For example, the molecular weight of purified ulvan extracted from *U. pertusa* is an important determinant for RAW 264.7 macrophage activation, with higher molecular weight fractions (1450 kDa and 1690 kDa) leading to > 2-fold increase in macrophage activation compared to a lower molecular weight fraction (365 kDa) [67]. An even more pronounced decrease in head kidney macrophages stimulation was measured for low molecular weight (5.92 kDa) ulvan from *U. ohnoi* over high molecular weight ulvan (698 kDa) [144]. However, a study with ulvan from *U. intestinalis* gave contradictory results with a lower molecular weight sample (28.7 kDa) having significantly higher immunomodulatory activity than a higher molecular weight sample (87.2 kDa). This highlights the importance of the interaction of the structural features of ulvan, such as the molecular weight and degree of sulfation, in determining bioactivity [57]. Although molecular weight is considered a major factor affecting immunomodulatory activity, there is also an effect of the degree of sulfation between the high (24.5%w/w) and low (6.25% w/w) molecular weight samples [57]. The level of sulfation is often positively correlated with activity in ulvan and related sulfated polysaccharides (e.g., λ-carrageenan and fucoidan). For example, de-sulfation (partial sulfate ester removal) of ulvan from *U. rigida* resulted in an ~50% reduction in its immunomodulatory effect on RAW 264.7 macrophages [72] and Turbot peritoneal leucocytes [74], when compared to native ulvan. So far, the studies that have investigated the influence of the structural features of ulvan on its effect on inflammation show that structure-activity correlations are species specific and further studies investigating the structure-activity relationships of ulvans across species are required to elucidate the key structural features driving activity. The latter informs the selection of species for cultivation extraction procedures and post-extraction modifications, with a focus of enhancing activity as a non-specific immunomodulatory agent.

3.4. Antioxidant activity

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly formed in vivo in living tissue by aerobic biogenesis or by oxidative enzymes (e.g., in response to foreign organisms) [233,234]. These highly reactive species attack all major classes of biomolecules, including lipids, DNA, proteins, and sugars. Oxidative stress has been implicated in a host of disorders including inflammatory diseases, neurodegenerative diseases, cancer, cardiovascular diseases, and aging processes. The body has several endogenous enzymatic antioxidant systems to overcome excessive ROS/RNS-mediated damage, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase and glucose-6-dehydrogenase, and non-enzymatic antioxidants including glutathione, uric acid, lipoic acid, NADPH, coenzyme Q, albumin, and bilirubin [235]. There are also exogenous antioxidants, including vitamins C and E, carotenoids, phenolic compounds, and trace elements (e.g., zinc and selenium) that play an essential role as antioxidants in living organisms. Food-based protein and polysaccharides also contribute to the antioxidant capacity of organisms, by acting as exogenous antioxidants and enhancing endogenous antioxidants [236]. In this regard, the antioxidant capacity of ulvan is mediated by radical scavenging and subsequent inhibition of lipid peroxidation, and the enhancement of antioxidant enzyme (e.g., SOD, CAT and GSH-Px) activities.

The exogenous anti-oxidant capacity of ulvan has been extensively assessed with in vitro assays using 1,1-diphenyl-2-picryl hydrazil (DPPH) radical scavenging, superoxide scavenging, ferric reducing antioxidant power (FRAP), hydroxyl radical scavenging, and lipid peroxide inhibition. Sulfate content and molecular weight have a significant effect on the anti-oxidant effect of sulfated polysaccharides from macroalgae [37]. In this regard, chemically over-sulfated (32.8% w/w sulfate) ulvan from *U. pertusa* had ~90% hydroxyl radical scavenging at 2.0 mg/ml compared to ~45% at 2.0 mg/ml of native ulvan (19.5% w/w sulfate) [92]. Similarly, radical scavenging is higher for over-sulfated ulvan from *U. linza* compared to its native form [237]. Low molecular weight ulvan also has higher antioxidant capacity relative to higher molecular weight fractions [87,88]. For example, a negative correlation was recorded between hydroxyl radical scavenging (~50–90%) and the molecular weight of ulvan (18.2–100.5 kDa) [87]. This correlation may not be a general trend [183], however, and highlights that comparative extraction conditions, purification protocols, and test protocols are necessary before concluding an overall and consistent structure-activity trend for the anti-oxidant activity of ulvan.

Although ulvan has direct ROS/RNS scavenging activity, it is its capacity to enhance the expression of the enzymatic components of the endogenous antioxidant system that affect animal health the most by ameliorating the propagation of diseases relating to oxidation. Determination of the antioxidant effects of ulvan in vivo has been made by measuring enzymatic antioxidant activities (e.g., SOD, CAT, and GSH-Px) and oxidation products (e.g., malondialdehyde (MDA) as a product of the peroxidation of polyunsaturated fatty acids; GSSH; oxidised glutathione). In this regard, ulvan from *U. lactuca* [135,139,209], *U. pertusa* [68,228,238], *U. armoricana* [102], *U. fasciata* [202,203] and *U. prolifera* [218] mitigate the production of oxidation products and enhance antioxidant enzyme activity in vitro and in vivo. For example, treatment of hyperlipidemic Kunming mice with ulvan from *U. pertusa* resulted in a reduction of MDA (29.2%) and increases in SOD (35.4%) and CAT (43.6%) relative to untreated mice [68]. Interestingly, a fraction with a molecular weight of 83 kDa exhibited higher antioxidant activity than higher molecular weight fractions (e.g., 190 kDa and 201 kDa). These findings were corroborated in a study of the effect of ulvan on egg quality and the health of laying hens [239]. Hens fed a diet supplemented with 1% ulvan showed significantly reduced serum MDA levels and increases in CAT and SOD. Ulvan treated hen groups also had increased levels of cytokines IL-6 and IFN-γ indicative of the close link between the expression of antioxidant enzymes and immune system responses. A single study demonstrated that oversulfated ulvan from *U. pertusa* had a greater capacity to influence antioxidant defence system markers (e.g., MDA, SOD, GSH-Px and CAT) in hyperlipidemic

rats relative to native ulvan [238]. Tentatively, ulvans with high sulfate content and lower molecular weights are most effective at ameliorating oxidative stress in vivo.

The mechanistic rationale for these findings is generally due to radical scavenging activity [69,135,139,238]. However, the capacity for exogenous antioxidants to significantly contribute to radical scavenging in vivo and, therefore, act as an antioxidant endogenously, has been questioned [236]. Furthermore, it was suggested that food-derived antioxidants, like ulvan, act by influencing signalling pathways that lead to the expression of the enzymes involved in the antioxidant defence system. Notably, phytochemicals, including ulvan [68,238], porphyrin [240], and fucoidan [241], have demonstrated their capacity to regulate antioxidant enzyme expression [242]. Ulvan is most likely to enhance antioxidant enzyme activity by activating the transcription enzymes (e.g., Nrf2, NF-kB, AP-1, AP-2, Sp1 and C/EBP) involved in the expression of antioxidant enzymes [236,243]. For example, ulvan from *U. armoricana* stimulates the TLR4 receptor in vitro, which results in the activation of NF-kB and the subsequent upregulation of TNF-α [102], a cytokine that modulates the expression of SOD [243]. However, further research on the mechanism(s) by which ulvan influences the expression of antioxidant enzymes is required to identify the signalling pathway(s) involved. In summary, the capacity of ulvans to boost the endogenous antioxidant system protects the body against toxic oxidation products that cause disease, such as chronic inflammation and cancer.

3.5. Anticancer

The development of cancer is a multistep process initiated by endogenous and exogenous factors, which often lead to oxidative attack on DNA, resulting in mutations that disrupt the normal regulatory pathways between cell proliferation, differentiation, and apoptosis [244]. There are a growing number of studies showing that ulvan acts as an anti-proliferation agent and promotes apoptosis in cancerous cells. Ulvan from *U. lactuca* [94,128,137,139,210], *U. intestinalis* [58,119,122,245], *U. pertusa* [67], *U. prolifera* [70], U. *tubulosa* [246] and *U. fasciata* [53,113] all demonstrate anticancer activities for a range of cancer models with murine sarcoma cancer cell line S180 [122], human cancer cell lines (e.g., HepG2 (hepatocellular carcinoma) [94,119,128,137,210], MCF7 (human breast cancer) [128,246], HeLa (cervical cancer) [128], AGS (human gastric carcinoma) [67,70], MKN45 (human gastric cancer) [113], HT-29 (human colon carcinoma) [94,210], HCT-116 (human colon carcinoma), Caco-2 (human colon carcinoma) [94,210], DLD1 (human colon carcinoma) [53,70,113]) and some cancers in animal models (e.g., rats [58,139,209], mice [122]). However, no clinical human trials have yet been conducted.

The anticancer activity of ulvan from different sources is highly variable. For example, ulvan from *U. lactuca* has significant cytotoxic activity against a number of human cancer cell lines including HepG2 (hepatocellular carcinoma), MCF7 (breast cancer), and HeLa (cervical cancer) [128]. Ulvan at a concentration of 100 μg/ml reduced the in vitro cell viability of all three cancer cell lines to 0%. Although a mechanism of action was not proposed, similar studies of anti-tumour effects of ulvan on HepG2 and MCF-7 cell-lines noted the increased expression of the pro-apoptotic tumour suppressor p53, and reduced expression of anti-apoptotic protein Bcl-2, supporting the conclusion that ulvan promotes programmed cell death (apoptosis) [119,209,210]. The antiproliferation activity of ulvan from *U. lactuca* also reduces levels of proliferating cell nuclear antigen (PCNA) in rat hepatocytes which is indicative of the reduced DNA replication associated with lower proliferation [139]. It is also important to note that many studies have registered only very low to moderate cytotoxic activity relative to traditional chemotherapy drugs [58,67,70,113,122]. For example, ulvan from *U. prolifera* only had low anticancer activity on human gastric carcinoma (AGS) and human colon cancer (DLD-1) cell lines, with dose dependent inhibition of AGS cell proliferation of 10–26% with concentrations of 200–1000 μg/ml [70]. These results do not necessarily preclude the relevance of ulvan in anticancer therapies. For example, ulvan from *U. intestinalis* also had no cytotoxic effects on sarcoma 180 tumour cells in vitro at 50–800 μg/ml, but reduced sarcoma 180 tumour weight in vivo by 61–71% in mice dosed with 100–400 mg/kg [122]. In combination with the latter, important immune organs (e.g., thymus and spleen) were enlarged in ulvan treated mice, supporting the suggestion that the antitumour activity of this polysaccharide originates from its immunomodulatory activity. In summary, the anticancer activity of ulvan appears to operate through one or more of a number of pathways, including the promotion of cancer cell apoptosis, reduction in cancer cell proliferation, and stimulation the innate immune response. Furthermore, the pathways affected are dependent on the source and/or structure of the ulvan.

There are preliminary results suggesting that both molecular weight and degree of sulfation influence the anticancer activity of ulvan [53,58,70,94]. However, at this point there are no conclusive interpretations that can be made with respect to the effect of the structure of ulvan on its anticancer activity. The generally low anti-proliferation activity of ulvan means that ulvan is unlikely to replace established chemotherapy drugs but might find application as a co-treatment, due to its broad-spectrum chemopreventative activities (e.g., immunomodulatory, antioxidant, and anticancer) [209,247]. There are also interesting potential applications for ulvan in cancer therapy. For example, as nanoparticle drug delivery systems for hydrophobic antitumour drugs [248], selenium enriched polysaccharide-protein complexes for cancer treatment [249], and pH responsive polysaccharide nanosystems that inhibit angiogenesis [250]. However, before ulvan can find applications as a co-treatment or adjunct in anticancer technologies, there is a need to ascertain the bioavailability of ulvan and whether ulvan influences the efficacy of traditional chemotherapy drugs in combination therapies.

3.6. Anticoagulant activity

The coagulation cascade is activated by two pathways, the intrinsic and extrinsic pathway, both culminating in the production of thrombin through a final, common pathway [229]. Briefly, this process involves the progressive activation and amplification of serine proteases (e.g., XIII, XII, XI, IX, X and VII) and glycoproteins, collectively termed “factors”, present within the blood. The intrinsic activation pathway is initiated when factor XII is activated by contact with an anionic surface, while activation of the extrinsic pathway occurs when factor XIII binds with tissue factor (a transmembrane receptor) released from damaged cells. Both intrinsic and extrinsic pathways lead to the activation of factor X, mobilising the common pathway and catalysing the conversion of prothrombin to thrombin, and subsequently soluble fibrinogen to insoluble fibrin that facilitates a clot. Anticoagulant compounds can inhibit all three of the above-mentioned pathways. For example, ulvan generally inhibits coagulation through the intrinsic and/or common pathways [47,60,65,93,142,180,201,216,251], while heparin can inhibit intrinsic, extrinsic, and common pathways. Mechanistic details regarding the anticoagulant activity of ulvans, and other potential anticoagulants, are commonly elucidated using three tests, the activated partial thromboplastin time (aPTT), thrombin time (TT) and prothrombin time (PT), which determine if inhibition acts on the intrinsic and/or common pathways, common pathway, and extrinsic pathway, respectively.

Ulvan extracts from *U. clathrata* [93], *U. lactuca* [60,105], *U. prolifera* [251], *U. fasciata* [105,201], *U. nematoidea* [216] *U. conglobata* [47], *U. linza* [65,142], *U. reticulata* [105] and *Capsosiphon fulvescens* [180] have demonstrated anticoagulant activity. For example, ulvan from *U. linza* led to a 3.3–6.2-fold increase in aPTT times relative to normal clotting times, and is dependent on the degree of sulfation and molecular weight [142]. With regards to the latter, a hydrolysed ulvan sample (MW = 11 kDa; SO^{3-} = 20.1%) increased aPTT by 63% compared to the native ulvan extract (MW = 108 kDa; SO^{3-} = 21.3%),

while oversulfation of the hydrolysed sample (SO^{3-} = 34.4%) led to a further 20% increase in aPTT. While similar structure-activity relationships occur for TT, there is minimal PT activity reported. However, data from a study aiming to optimise the enzymatic extraction of ulvan from *U. prolifera* with the intent to optimise anticoagulant activity demonstrated an interaction between degree of sulfation and molecular weight [251]. Namely, increasing sulfation led to high anticoagulant activity until a molecular weight threshold (< 200 kDa) was reached, after which there was a complete loss of activity [251]. In summary, the anticoagulant activity of ulvan is dependent on degree of sulfation and molecular weight [43,47,65,142,251], with a higher degree of sulfation enhancing anticoagulant activity. The relationship between molecular weight and anticoagulant activity remains unclear, and this may also be dependent on the source of the ulvan.

The potency of the anticoagulant activity of ulvan is dependent on species and ecophysiological factors, which directly affect the structure of ulvan [47,65]. The anticoagulant activities for ulvan isolated from different biomass sources are between ~2–40 times less active than heparin. For example, one of the better performers was a highly sulfated ulvan fraction (SO^{3-} = 35.2%) isolated from *U. conglobata* [47]. This sample at 2 μg/ml yielded a 2.5-fold increase in aPTT compared to a 6.2-fold increase in aPTT for 2 μg/ml of heparin. While the latter highlights the potential of ulvan as an anticoagulant, the overall efficacy of ulvan relative to commercial drugs, like heparin, is less than these previous results indicate, as it only acts on two of the three coagulation pathways. This does not diminish the relevance of the anticoagulant activity of ulvan or its capacity to be used in the preparation of anticoagulants. For example, ulvan acts on the intrinsic pathway, which activates multiple pro-inflammatory, pro-coagulant, and immunomodulating pathways [252].

3.7. Antihyperlipidemic

The human body has a sophisticated lipoprotein transport system that utilises triglyceride (TG) rich very low-density lipoprotein-cholesterol (VLDL) to shuttle fatty acids to adipocytes and muscle, resulting in cholesterol rich low-density lipoprotein-cholesterol (LDL-C) that distributes cholesterol for steroidogenesis and cell membranes [253]. Excess cholesterol is transported back to the liver by high-density lipoprotein-cholesterol (HDL-C) for recycling or conversion to bile acids (BA) followed by its removal from the body. Imbalances in the lipoprotein transport system are linked to metabolic syndrome. Patients with metabolic syndrome are often hyperlipidaemic, a condition characterised by abnormally high blood levels of lipid(s) and lipoprotein(s), which can lead to cardiovascular diseases, such as coronary artery disease and atherosclerosis. Importantly, the consumption of macroalgae and the associated sulfated polysaccharides, including ulvan, has demonstrated antihyperlipidemic activity in vivo [254–256].

To measure the antihyperlipidemic activity of foods and supplements, such as sulfated polysaccharides, the serum total cholesterol (TC), TG, HDL-C and LDL-C of hyperlipidemic rat and mice models are commonly assessed [83,93,145,146,202,211,257]. Using these parameters ulvan from *U. pertusa* [68,69,83,93,145,146,238,257,258], *U. fasciata* [116,202,203,205], *U. lactuca* [211,213], *U. prolifera* [152,219] and *Monostroma nitidum* [259] have significant antihyperlipidemic activity, reducing or maintaining low levels of TC, TG, LDL-C, and increasing or maintaining a high level of HDL-C. Both molecular weight and degree of sulfation influence this activity. For example, Wistar rats fed a high starch feed supplemented with native ulvan (151.6 kDa) from *U. pertusa* had significant reductions in serum TC (45%) and LDL-C (54%), with no significant effect on TG and HDL-C compared to control rats fed the same high starch diet [145]. However, rats fed high starch diets with low molecular weight ulvan (28.2 kDa) showed no effect on either TC or LDL-C but significantly reduced TG (78%) and raised HDL-C (61%) relative to control fed rats, the mechanisms of which are discussed below [68]. There is some early evidence that a higher degree of sulfation may enhance the antihyperlipidemic activity [257]. For example, the serum TC of female rats treated with native ulvan (SO^{3-} = 22.5%) at 250 mg/kg was reduced by 28%, while treatment with oversulfated ulvan (SO^{3-} = 40.6%) reduced serum TC by 44% relative to the hyperlipidemic control group [257]. Derivatisation of native ulvan (e.g., acetylation) also influences antihyperlipidemic activity [146]. However, interactions between the degree of substitution and molecular weight are unclear and need to be understood to optimise structures that affect higher antihyperlipidemic activity.

The effect of molecular weight on the parameters used to assess antihyperlipidemic activity supports the conclusion that several mechanisms are operating in parallel. It has been proposed that reduction in serum TC and LDL-C in ulvan supplemented hyperlipidemic rats results in LDL-C conversion to bile acid in the liver followed by excretion [145]. This is further supported by an increase in faecal bile acid concentrations in ulvan supplemented hyperlipidemic rats. In contrast, supplementation of hyperlipidemic rats with lower molecular weight ulvan (28.2 kDa) had no effect on either TC or LDL-C but had elevated faecal bile acids and reduced TG and raised HDL-C relative to control fed rats [145], suggesting that other mechanisms, such as the upregulation of the synthesis of cholesterol in the liver, are also important. Treatment of lipid-loaded hepatocytes with an ulvan-like polysaccharide from *Monostroma nitidum* resulted in lower lipid concentrations accompanied with downregulation of the cholesterol synthesis gene for 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, upregulation of the cholesterol catabolism gene for cholesterol-7-α-hydroxylase (CYP7A1), and LDL uptake gene for LDL-receptor [259]. To corroborate these results, reduced serum TC concentrations measured in ulvan (*U. prolifera*) treated rats downregulated HMG-CoA reductase and the cholesterol regulating transcription factor sterol regulatory element binding protein 2 (SREBP-2) [219]. In a later study, reduced serum TG concentrations measured in ulvan treated rats were accompanied by downregulation of acetyl-CoA carboxylase (ACC) and its regulating transcription factor, SREBP-1c (regulates fatty acid synthesis) [260]. In theory, structurally different ulvan fractions may influence the regulatory genes (SREBP-1c and SREBP-2) to varying degrees, helping to explain the contradictory results obtained by Pengzhan *et al.* [145]. Evidence for another alternative or additional mechanism suggests that antioxidant effects may contribute to the antihyperlipidemic effects of polysaccharides [261,262]. In this mechanism, molecules with high antioxidant capacity (or those that can upregulate the endogenous antioxidant system) limit lipid oxidation by ROS, which leads to the accumulation of cholesterol due to changes in its biosynthesis and metabolism. In summary, the antihyperlipidemic activity of ulvan appears to operate by multiple mechanisms in parallel, the balance of which depends on the structural features of ulvan. This provides opportunity to optimise antihyperlipidemic supplements for more targeted applications.

3.8. Anti-viral

Viral treatments target the multiple steps in the viral replication cycles, which may be broadly thought of as entry, replication, shedding, and latency. Sulfated polysaccharides derived from marine algae, including ulvan, have promising antiviral activities [263–265]. Ulvans from *U. compressa* [199,266] *U. lactuca* [62,127,137], *U. clathrata* [107], *U. intestinalis* [207], *U. armoricana* [40] and *U. pertusa* [147] all have antiviral activity. The antiviral activity of ulvan extends to the enveloped viruses of herpes simplex virus (HSV) [40,199,266], Newcastle disease virus (NDV) [107], Japanese encephalitis virus (JEV) [127], dengue virus (DENV) [127], yellow fever virus (YFV) [127], West Nile virus (WNV) [127], influenza (H1N1) [62], avian influenza virus (AIV) [147] and measles virus (MeV) [207]. Examination of the antiviral activity of ulvan has been conducted in vitro using human larynx epithelial carcinoma cells (Hep-2) [199], African green monkey cells (Vero) [40,107,137,207], canine kidney cells (MDCK) [62], and in

vivo models using mice [127,147].

The antiviral effects of native ulvans against viral targets (measured as 50% Inhibitory Concentration (IC_{50}), the concentration of ulvan required to inhibit viral yield by 50%) are mixed ranging from weak (IC_{50} > 150 μg/ml [62,199]) to significant (IC_{50} = 0.1–30 μg/ml [107,127,207]). There are some interesting results supporting the use of ulvan in antiviral therapies. Ulvan from *U. clathrata* is an effective anti-viral for NDV, a fatal virus found in chickens causing large anthropogenic food losses [107]. Treatment with ulvan provided a concentration-dependent inhibition of NDV entry into Vero cells with an IC_{50} of 0.1 μg/ml. The activity was related to inhibition of an entry protein known as NDV fusion protein F. Although ulvan from *U. pertusa* had only moderate antiviral activity (40% inhibition at 100 μg/ml) against AIV-H9N2, it had a beneficial effect in a test relevant to immunisation [147], where AIV-H9N2 vaccination combined with ulvan treatment (50 mg/kg) led to an ~100% increase in antibody titre relative to the vaccination alone. This effect was attributed to the enhancement of the humoral immune response, due to the immunomodulatory effects of ulvan.

Variations in the antiviral activity of ulvan from different sources indicate a significant effect of structure; however, there are only a limited number of studies that probe the antiviral activity of ulvan and, consequently, the understanding of its structure-activity relationships is limited. However, the required structural features of ulvan are essentially similar to other sulfated polysaccharide antivirals, where activity is optimised by a high degree of sulfation and high molecular weight [264,267]. In this regard, treatment of HSV infected Hep-2 cells with a highly sulfated (SO^{3-} = 22%) ulvan fraction from *U. compressa* resulted in 100% HSV inhibition at 100 μg/ml and had an IC_{50} of 28.2μg/ml, compared to 153 μg/ml for native ulvan (SO^{3-} = 6%) [199]. Furthermore, ulvan with higher molecular weights (34 kDa) had 2–5 times the antiviral activity of low molecular weight ulvan (< 5 kDa). However, further studies are required to verify the generality of these results. In summary, the investigation of ulvan as an antiviral is in its infancy and at this point ulvan has variable antiviral activity against specific targets, with promise as an immunostimulant co-treatment for vaccinations.

3.9. Plant defence

More recently ulvans have been found to also influence the signalling pathways involved in plant immunity. The effect that ulvans have on plant immunity has been investigated in vitro with cell cultures (e.g., wheat and rice) [50] and in vivo with various non-cropping (e.g., thale cress) and cropping (e.g., apples, beans, wheat and barley) plant species [29,49–51,91,268–272]. Ulvan from *U. fasciata* [49–51,268,271,272], *U. lactuca* [91,269] and *U. armoricana* [29,270] all enhance the inducible defences of plants, a phenomenon known as “priming”. Inducible plant defences operate by the initial recognition of microbe/pathogen-associated molecular patterns (MAMPs/PAMPs), or elicitors, by plant cell membrane bound pattern-recognition receptors (PRRs), known as MAMP/PAMP-triggered immunity (MTI/PTI). Plants also have resistance (R) genes that encode cytoplasmic receptors that recognise pathogen effector molecules designed to suppress PTI, known as effector-triggered immunity (ETI) [273,274]. Plant immune responses downstream of MTI/PTI and ETI include the production of pathogenesis-related proteins that hydrolyse pathogen cell wall constituents, plant cell wall fortification through synthesis of lignin and callose, and the production of antimicrobial secondary metabolites [275,276]. An additional response unique to ETI is the hypersensitive response (HR), which is instigated by a pulse in the production of reactive oxygen species (ROS), triggering localised cell death at the site of infection. MTI/PTI and ETI also trigger systemic acquired resistance (SAR), where the defence alert is transferred from the site of pathogen ingress to distal plant tissues leading to an alert (or primed) state that is more responsive to subsequent pathogen attacks [277]. These plant immune responses are orchestrated by a complex balance of phytohormones (e.g., salicylic acid, jasmonic acid, ethylene and abscisic acid, cytokinins, auxins, brassinosteroids, and gibberellins) dependent on the identity of the pathogen and its trophic lifestyle (i.e., biotrophic vs. necrotrophic) [274]. Therefore, a great deal of information can be found by studying how the balance of molecules involved in the plant immune response are affected by treatment with potential priming agents, such as ulvan.

The effect of ulvan treatment on plant immunity has been investigated using pathogen and plant base measures including pathogen growth and development, plant health measures, such as growth, spotting and wilting, and molecular probes/profiles, such as hormones, proteins, and DNA. Ulvan from *U. fasciata* inhibits a key stage (appressoria differentiation) in the pathogenic cycle of the anthracnose causing fungi *Colletotrichum gloeosporioides* [268]. Consistent with a priming effect in vivo, tests on ulvan pre-treatment on apple leaves reduced disease severity by as much as 50% over control plants. Similar results were obtained when ulvan (from *U. fasciata*) treated *Arabidopsis thaliana* (thale cress) was inoculated with either *Alternaria brassicicola* (black spot fungi) or *Colletotrichum higginsianum* (anthracnose fungi) [49]. Interestingly, the degree of sulfation did not influence pathogenesis. However in another study, desulfation of ulvan from *U. lactuca* significantly reduced its capacity to induce the production of the defence enzyme, phenylalanine ammonia-lyase (PAL) [91]. In this study, a > 2-fold increase in the activity of PAL occurred when tomato plants were treated with ulvan oligomers relative to native ulvan [91]. Increased PAL activity was accompanied by an increase in salicylic acid. Therefore, in this case induction of SAR appears to be salicylic acid dependent. However, a study using transcriptomics combined with hormone profiling and enzyme activity measurements found that ulvan from *U. armoricana* induced plant immunity via the jasmonic acid signalling pathway in *Medicago truncatula* (Fabaceae), *Nicotianae tabacum* (Solanaceae), and *Arabidopsis thaliana* (Brassicaceae) [29]. Salicylic acid concentrations were unaffected by ulvan treatment, while jasmonic acid concentrations increased relative to control plants. Furthermore, the mode of action of ulvan was verified by its capacity to induce the expression of jasmonic acid-dependent genes (e.g., PDF1.2 defensin and lipoxygenase NtLOX1 promoter), while failing to induce the expression of salicylic acid-dependent genes (e.g., PR1a and PR5). Regardless of the signalling pathways responsible, ulvan demonstrates significant activity as a bio-elicitor and capacity to act as a priming agent enhancing both plant health and productivity.

According to the current understanding of plant immunity the capacity of ulvan to elicit plant defences requires that it is first recognised by the plant (either directly or indirectly), probably during primary signalling events (e.g., PTI or ETI). Therefore, the assumption that ulvan has analogous structural features to existing MAMPs/PAMPs is a logical conclusion and molecules that have structural similarities with ulvan such as plant rhamnogalacturonan I and rhamnolipids from phytopathogenic bacteria [196,197] also trigger defence responses. Typically, these latter examples contain rhamnose and uronic acids (but not sulfate esters), and these moieties may be important for ulvans activity. Notably, the presence of rhamnose (in ulvan) was required to induce immune defence responses in tomato plants, while glucuronic acid (in glucuronan) had no effect [91]. In terms of molecular weight, tomato plants treated with ulvan oligomers had up to twice the PAL activity than control plants. Conclusions regarding the degree of sulfation remain unclear, with one report indicative of a positive correlation and another indicative of no effect. Clearly the optimisation of ulvan products for use as bio-elicitors is in its infancy; however, its capacity to elicit plant immune responses is promising for reducing agricultural reliance on traditional pesticide treatments.

4. Conclusion

Research into the structural and biological properties of ulvan

remain in its early stages relative to those of the other marine-derived sulfated polysaccharides carrageenan and fucoidan. However, ulvan has the potential to find widespread application, including in agriculture, human health, and biomaterials. General trends in physicochemical properties and biological activities of ulvan relative to its structural features remain ambiguous, and this is due to the limited number of systematic studies on the structure-function properties of highly refined and well characterised ulvan against highly defined target activities. Additionally, the pharmacokinetics and bioavailability of such a complicated macromolecule will require thorough investigation prior to any application in a therapeutic capacity. The structurally distinct features of ulvans across biomass sources also contributes to this ambiguity and highlights the need for a focus on highly characterised refined ulvan of a single origin, or refining process. This will then clarify the structural requirements for specific biological activities, supporting informed decisions about the selection of species for cultivation and processing for specific target applications.

Acknowledgements

The authors acknowledge James Cook University for providing a PhD scholarship to Joel Kidgell. This research is part of the Pacific Biotechnology (previously MBD Industries Ltd) Research and Development program for the Integrated Production of Macroalgae.

Declaration of Interest

Declarations of interest: none. No conflicts, informed consent, human or animal rights applicable.

Author Contributions

All authors contributed to the conception and design of the review, interpretation of the meta-data, drafting of the article and revising it critically for important intellectual content. All authors approve of the final version to be submitted.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2019.101422.

References

[1] A.R. Angell, L. Mata, R. de Nys, N.A. Paul, Variation in amino acid content and its relationship to nitrogen content and growth rate in *Ulva ohnoi* (Chlorophyta), J. Phycol. 50 (2014) 216–226.
[2] L. Mata, M. Magnusson, N.A. Paul, R. de Nys, The intensive land-based production of the green seaweeds *Derbesia tenuissima* and *Ulva ohnoi*: biomass and bioproducts, J. Appl. Phycol. 28 (2016) 365–375.
[3] M. Magnusson, C. Carl, L. Mata, R. de Nys, N.A. Paul, Seaweed salt from *Ulva*: a novel first step in a cascading biorefinery model, Algal Res. 16 (2016) 308–316.
[4] C.R.K. Glasson, I.M. Sims, S.M. Carnachan, R. de Nys, M. Magnusson, A cascading biorefinery process targeting sulfated polysaccharides (ulvan) from *Ulva ohnoi*, Algal Res. 27 (2017) 383–391.
[5] J. Blomster, S. Back, D.P. Fewer, M. Kiirikki, A. Lehvo, C.A. Maggs, M.J. Stanhope, Novel morphology in *Enteromorpha* (Ulvophyceae) forming green tides, Am. J. Bot. 89 (2002) 1756–1763.
[6] G. Coat, P. Dion, M.C. Noailles, B. de Reviers, J.M. Fontaine, Y. Berger-Perrot, S. Loiseaux-de Goer, *Ulva armoricana* (Ulvales, Chlorophyta) from the coasts of Brittany (France). II. Nuclear rDNA ITS sequence analysis, Eur. J. Phycol. 33 (1998) 81–86.
[7] M. Hiraoka, S. Shimada, M. Uenosono, M. Masuda, A new green-tide-forming alga, *Ulva ohnoi* Hiraoka et Shimada sp. nov. (Ulvales, Ulvophyceae) from Japan, Phycol. Res. 52 (2004) 17–29.
[8] F. Kong, Y. Mao, F. Cui, X. Zhang, Z. Gao, Morphology and molecular identification of *Ulva* forming green tides in Qingdao, China, J. Ocean Univ. China 10 (2011) 73–79.
[9] D.B. Largo, J. Sembrano, M. Hiraoka, M. Ohno, Taxonomic and ecological profile of 'green tide' species of *Ulva* (Ulvales, Chlorophyta) in central Philippines, Hydrobiologia 512 (2004) 247–253.
[10] R.J. Lawton, L. Mata, R. de Nys, N.A. Paul, Algal bioremediation of waste waters from land-based aquaculture using *Ulva*: selecting target species and strains, PLoS One 8 (2013) e77344.
[11] P.H. de Paula Silva, S. McBride, R. de Nys, N.A. Paul, Integrating filamentous 'green tide' algae into tropical pond-based aquaculture, Aquaculture 284 (2008) 74–80.
[12] A.E. Nardelli, V.G. Chiozzini, E.S. Braga, F. Chow, Integrated multi-trophic farming system between the green seaweed *Ulva lactuca*, mussel, and fish: a production and bioremediation solution, J. Appl. Phycol. (2018), https://doi.org/10.1007/s10811-018-1581-4.
[13] M. Lahaye, A. Robic, Structure and functional properties of ulvan, a polysaccharide from green seaweeds, Biomacromolecules 8 (2007) 1765–1774.
[14] B. Ray, M. Lahaye, Cell-wall polysaccharides from the marine green alga *Ulva* "*rigida*" (ulvales, chlorophyta). Extraction and chemical composition, Carbohydr. Res. 274 (1995) 251–261.
[15] J.W.E. Brading, M.M.T. Georg-Plant, D.M. Hardy, The polysaccharide from the alga *Ulva lactuca*. Purification, hydrolysis, and methylation of the polysaccharide, Journal of the Chemical Society (Resumed) (1954) 319–324.
[16] B. Quemener, M. Lahaye, C. Bobin-Dubigeon, Sugar determination in ulvans by a chemical-enzymatic method coupled to high performance anion exchange chromatography, J. Appl. Phycol. 9 (1997) 179–188.
[17] J.P. McKinnell, E. Percival, The acid polysaccharide from the green seaweed, *Ulva lactuca*, J. Chem. Soc. (1962) 2082–2084 Resumed.
[18] A.F. Abdel-Fattah, M. Edrees, A study on the polysaccharide content of *Ulva lactuca* L, Qualitas Plantarum et Materiae Vegetabiles 22 (1972) 15–22.
[19] C.C. Gosselin, A. Holt, P.A. Lowe, Polysaccharides of *Enteromorpha* species, Journal of the Chemical Society (Resumed) (1964) 5877–5880.
[20] M. Lahaye, M.A.V. Axelos, Gelling properties of water-soluble polysaccharides from proliferating marine green seaweeds (*Ulva* spp.), Carbohydr. Polym. 22 (1993) 261–265.
[21] E. Percival, J.K. Wold, The acid polysaccharide from the green seaweed *Ulva lactuca*. Part II. The site of the ester sulphate, J. Chem. Soc. (1963) 5459 Resumed.
[22] M. Lahaye, B. Kaeffer, Seaweed dietary fibres: structure, physico-chemical and biological properties relevant to intestinal physiology, Sci. Aliment. 17 (1997) 563–584.
[23] M. Lahaye, M. Brunel, E. Bonnin, Fine chemical structure analysis of oligosaccharides produced by an ulvan-lyase degradation of the water-soluble cell-wall polysaccharides from *Ulva* sp. (Ulvales, Chlorophyta), Carbohydr. Res. 304 (1997) 325–333.
[24] M. Lahaye, B. Ray, Cell-wall polysaccharides from the marine green alga *Ulva* "*rigida*" (Ulvales, Chlorophyta) - NMR analysis of ulvan oligosaccharides, Carbohydr. Res. 283 (1996) 161–173.
[25] B. Ray, M. Lahaye, Cell-wall polysaccharides from the marine green alga *Ulva* "*rigida*" (Ulvales, Chlorophyta). Chemical structure of ulvan, Carbohydr. Res. 274 (1995) 313–318.
[26] E. Andrès, J. Molinari, G. Péterszegi, B. Mariko, E. Ruszova, V. Velebny, G. Faury, L. Robert, Pharmacological properties of rhamnose-rich polysaccharides, potential interest in age-dependent alterations of connectives tissues, Pathol. Biol. 54 (2006) 420–425.
[27] G. Faury, E. Ruszova, J. Molinari, B. Mariko, S. Raveaud, V. Velebny, L. Robert, The α-L-Rhamnose recognizing lectin site of human dermal fibroblasts functions as a signal transducer: modulation of Ca^{2+} fluxes and gene expression, Biochim. Biophys. Acta, 1780 (2008) 1388–1394.
[28] A. Adrien, A. Bonnet, D. Dufour, S. Baudouin, T. Maugard, N. Bridiau, Pilot production of ulvans from *Ulva* sp. and their effects on hyaluronan and collagen production in cultured dermal fibroblasts, Carbohydr. Polym. 157 (2017) 1306–1314.
[29] V. Jaulneau, C. Lafitte, C. Jacquet, S. Fournier, S. Salamagne, X. Briand, M.T. Esquerre-Tugaye, B. Dumas, Ulvan, a sulfated polysaccharide from green algae, activates plant immunity through the jasmonic acid signaling pathway, J. Biomed. Biotechnol. 2010 (2010) 1–11.
[30] S. Trouvelot, M.-C. Héloir, B. Poinssot, A. Gauthier, F. Paris, C. Guillier, M. Combier, L. Trdá, X. Daire, M. Adrian, Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays, Front. Plant Sci. 5 (2014) 592.
[31] M.J. Stadnik, M.B. de Freitas, Algal polysaccharides as source of plant resistance inducers, Tropical Plant Pathology 39 (2014) 111–118.
[32] M. Lahaye, E.A.C. Cimadevilla, R. Kuhlenkamp, B. Quemener, V. Lognoné, P. Dion, Chemical composition and 13C NMR spectroscopic characterisation of ulvans from *Ulva* (Ulvales, Chlorophyta), J. Appl. Phycol. 11 (1999) 1–7.
[33] L.-A. Fransson, G.O. Aspinall (Ed.), Mammalian Glycosaminoglycans, The Polysaccharides Academic Press, New York, 1985, pp. 338–415.
[34] J. Venkatesan, B. Lowe, S. Anil, P. Manivasagan, A.A.A. Kheraif, K.-H. Kang, S.-K. Kim, Seaweed polysaccharides and their potential biomedical applications, Starch - Stärke 67 (2015) 381–390.
[35] L. Cunha, A. Grenha, Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications, Marine Drugs 14 (2016).
[36] A. Alves, R.A. Sousa, R.L. Reis, A practical perspective on ulvan extracted from green algae, J. Appl. Phycol. 25 (2013) 407–424.
[37] I. Wijesekara, R. Pangestuti, S.K. Kim, Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae, Carbohydr. Polym. 84 (2011) 14–21.
[38] J. Barbosa, S. Borges, M. Amorim, M.J. Pereira, A. Oliveira, M.E. Pintado, P. Teixeira, Comparison of spray drying, freeze drying and convective hot air drying for the production of a probiotic orange powder, J. Funct. Foods 17 (2015) 340–351.

[39] M.f. Lai, C.F. Li, C.y. Li, Characterization and thermal behavior of six sulphated polysaccharides from seaweeds, Top. Catal. 8 (1994) 215–232.

[40] K. Hardouin, G. Bedoux, A.-S. Burlot, C. Donnay-Moreno, J.-P. Bergé, P. Nyvall-Collén, N. Bourgougnon, Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed *Ulva armoricana* (Ulvales, Ulvophyceae), Algal Res. 16 (2016) 233–239.

[41] G. Paradossi, F. Cavalieri, E. Chiessi, A conformational study on the algal polysaccharide ulvan, Macromolecules 35 (2002) 6404–6411.

[42] A. Robic, J.F. Sassi, P. Dion, Y. Lerat, M. Lahaye, Seasonal variability of physicochemical and rheological properties of ulvan in two *Ulva* species (chlorophyta) from the Brittany coast, J. Phycol. 45 (2009) 962–973.

[43] X.H. Qi, W.J. Mao, Y. Gao, Y. Chen, Y.L. Chen, C.Q. Zhao, N. Li, C.Y. Wang, M.X. Yan, C. Lin, J.M. Shan, Chemical characteristic of an anticoagulant-active sulfated polysaccharide from *Enteromorpha clathrata*, Carbohydr. Polym. 90 (2012) 1804–1810.

[44] K. Chattopadhyay, P. Mandal, P. Lerouge, A. Driouich, P. Ghosal, B. Ray, Sulphated polysaccharides from Indian samples of *Enteromorpha compressa* (Ulvales, Chlorophyta): isolation and structural features, Food Chem. 104 (2007) 928–935.

[45] J.P. McKinnell, E. Percival, Structural investigations on the water-soluble polysaccharide of the green seaweed *Enteromorpha compressa*, Journal of the Chemical Society (Resumed) (1962) 3141–3148.

[46] B. Ray, Polysaccharides from *Enteromorpha compressa*: isolation, purification and structural features, Carbohydr. Polym. 66 (2006) 408–416.

[47] W. Mao, X. Zang, Y. Li, H. Zhang, Sulfated polysaccharides from marine green algae *Ulva conglobata* and their anticoagulant activity, J. Appl. Phycol. 18 (2006) 9–14.

[48] M. Yamamoto, H. Imai, K. Mita, Y. Tadokoro, Physicochemical characterization of sulfated polysaccharides from green seaweeds: *Ulva pertusa* and *Ulva conglobata*, Agric. Biol. Chem. 44 (1980) 723–729.

[49] M.B. de Freitas, L.G. Ferreira, C. Hawerroth, M.E.R. Duarte, M.D. Noseda, M.J. Stadnik, Ulvans induce resistance against plant pathogenic fungi independently of their sulfation degree, Carbohydr. Polym. 133 (2015) 384–390.

[50] R. Paulert, D. Ebbinghaus, C. Urlass, B.M. Moerschbacher, Priming of the oxidative burst in rice and wheat cell cultures by ulvan, a polysaccharide from green macroalgae, and enhanced resistance against powdery mildew in wheat and barley plants, Plant Pathol. 59 (2010) 634–642.

[51] R. Paulert, V. Talamini, J.E.F. Cassolato, M.E.R. Duarte, M.D. Noseda, A. Smania, M.J. Stadnik, Effects of sulfated polysaccharide and alcoholic extracts from green seaweed *Ulva fasciata* on anthracnose severity and growth of common bean (*Phaseolus vulgaris* L.), Journal of Plant Diseases and Protection 116 (2009) 263–270.

[52] P. Shao, M. Chen, Y.P. Pei, P.L. Sun, In vitro antioxidant activities of different sulfated polysaccharides from chlorophytan seaweeds *Ulva fasciata*, Int. J. Biol. Macromol. 59 (2013) 295–300.

[53] P. Shao, Y. Pei, Z. Fang, P. Sun, Effects of partial desulfation on antioxidant and inhibition of DLD cancer cell of *Ulva fasciata* polysaccharide, Int. J. Biol. Macromol. 65 (2014) 307–313.

[54] P. Shao, M.P. Qin, L.F. Han, P.L. Sun, Rheology and characteristics of sulfated polysaccharides from chlorophytan seaweeds *Ulva fasciata*, Carbohydr. Polym. 113 (2014) 365–372.

[55] M.M. de Carvalho, R.A. de Freitas, D.R.B. Ducatti, L.G. Ferreira, A.G. Gonçalves, F.G. Colodi, E. Mazepa, E.M. Aranha, M.D. Noseda, M.E.R. Duarte, Modification of ulvans via periodate-chlorite oxidation: chemical characterization and anticoagulant activity, Carbohydr. Polym. 197 (2018) 631–640.

[56] P. Chandika, S.C. Ko, W.K. Jung, Marine-derived biological macromolecule-based biomaterials for wound healing and skin tissue regeneration, Int. J. Biol. Macromol. 77 (2015) 24–35.

[57] M. Tabarsa, S. You, E.H. Dabaghian, U. Surayot, Water-soluble polysaccharides from *Ulva intestinalis*: molecular properties, structural elucidation and immunomodulatory activities, J. Food Drug Anal. 26 (2) (2018) 599–608.

[58] A.A. Matloub, H.A. Aglan, S.S. Mohamed El Souda, M.E. Aboutabl, A.S. Maghraby, H.H. Ahmed, Influence of bioactive sulfated polysaccharide-protein complexes on hepatocarcinogenesis, angiogenesis and immunomodulatory activities, Asian Pac J Trop Med 9 (2016) 1200–1211.

[59] C. Costa, A. Alves, P.R. Pinto, R.A. Sousa, E.A. Borges da Silva, R.L. Reis, A.E. Rodrigues, Characterization of ulvan extracts to assess the effect of different steps in the extraction procedure, Carbohydr. Polym. 88 (2012) 537–546.

[60] A. Adrien, D. Dufour, S. Baudouin, T. Maugard, N. Bridiau, Evaluation of the anticoagulant potential of polysaccharide-rich fractions extracted from macroalgae, Nat. Prod. Res. 31 (2017) 2126–2136.

[61] A.A.A. Barros, A. Alves, C. Nunes, M.A. Coimbra, R.A. Pires, R.L. Reis, Carboxymethylation of ulvan and chitosan and their use as polymeric components of bone cements, Acta Biomater. 9 (2013) 9086–9097.

[62] G. Jiao, G. Yu, W. Wang, X. Zhao, J. Zhang, S.H. Ewart, Properties of polysaccharides in several seaweeds from Atlantic Canada and their potential anti-influenza viral activities, J. Ocean Univ. China 11 (2012) 205–212.

[63] H. Tian, X. Yin, Q. Zeng, L. Zhu, J. Chen, Isolation, structure, and surfactant properties of polysaccharides from *Ulva lactuca* L. from South China Sea, Int. J. Biol. Macromol. 79 (2015) 577–582.

[64] Y. Yuan, X. Xu, C. Jing, P. Zou, C. Zhang, Y. Li, Microwave assisted hydrothermal extraction of polysaccharides from *Ulva prolifera*: functional properties and bioactivities, Carbohydr. Polym. 181 (2018) 902–910.

[65] X.H. Qi, W.J. Mao, Y. Chen, Y.L. Chen, C.Q. Zhao, N. Li, C.Y. Wang, Chemical characteristics and anticoagulant activities of two sulfated polysaccharides from *Enteromorpha linza* (Chlorophyta), J. Ocean Univ. China 12 (2013) 175–182.

[66] A. Onda, S. Onda, M. Koike, K. Yanagisawa, S. Tsubaki, M. Hiraoka, Catalytic hydrolysis of polysaccharides derived from fast-growing green macroalgae, ChemCatChem 9 (2017) 2638–2641.

[67] M. Tabarsa, J.H. Han, C.Y. Kim, S.G. You, Molecular characteristics and immunomodulatory activities of water-soluble sulfated polysaccharides from *Ulva pertusa*, J. Med. Food 15 (2012) 135–144.

[68] W. Li, N. Jiang, B. Li, M. Wan, X. Chang, H. Liu, L. Zhang, S. Yin, H. Qi, S. Liu, Antioxidant activity of purified ulvan in hyperlipidemic mice, Int. J. Biol. Macromol. 113 (2018) 971–975.

[69] W. Li, K. Wang, N. Jiang, X. Liu, M. Wan, X. Chang, D. Liu, H. Qi, S. Liu, Antioxidant and antihyperlipidemic activities of purified polysaccharides from *Ulva pertusa*, J. Appl. Phycol. 30 (4) (2018) 2619–2627.

[70] M. Cho, C. Yang, S.M. Kim, S. You, Molecular characterization and biological activities of water-soluble sulfated polysaccharides from *Enteromorpha prolifera*, Food Sci. Biotechnol. 19 (2010) 525–533.

[71] M.J. Shi, X. Wei, J. Xu, B.J. Chen, D.Y. Zhao, S. Cui, T. Zhou, Carboxymethylated degraded polysaccharides from *Enteromorpha prolifera*: preparation and in vitro antioxidant activity, Food Chem. 215 (2017) 76–83.

[72] J.M. Leiro, R. Castro, J.A. Arranz, J. Lamas, Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh, Int. Immunopharmacol. 7 (2007) 879–888.

[73] M. Lahaye, J.-L. Gomez-Pinchetti, M.J. del Rio, G. Garcia-Reina, Natural decoloration, composition and increase in dietary fibre content of an edible marine algae, *Ulva rigida* (Chlorophyta), grown under different nitrogen conditions, J. Sci. Food Agric. 68 (1995) 99–104.

[74] R. Castro, M.C. Piazzon, I. Zarra, J. Leiro, M. Noya, J. Lamas, Stimulation of turbot phagocytes by *Ulva rigida* C. Agardh polysaccharides, Aquaculture 254 (2006) 9–20.

[75] R. Pezoa-Conte, A. Leyton, A. Baccini, M.C. Ravanal, P. Mäki-Arvela, H. Grénman, C. Xu, S. Willför, M.E. Lienqueo, J.P. Mikkola, Aqueous extraction of the sulfated polysaccharide Ulvan from the green alga *Ulva rigida* - kinetics and modeling, Bioenergy Research 10 (2017) 915–928.

[76] G. Toskas, R.D. Hund, E. Laourine, C. Cherif, V. Smyrniotopoulos, V. Roussis, Nanofibers based on polysaccharides from the green seaweed *Ulva rigida*, Carbohydr. Polym. 84 (2011) 1093–1102.

[77] A. Robic, C. Rondeau-Mouro, J.F. Sassi, Y. Lerat, M. Lahaye, Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae), Carbohydr. Polym. 77 (2009) 206–216.

[78] C. Bobin-Dubigeon, M. Lahaye, F. Guillon, J.-L. Barry, D.J. Gallant, Factors limiting the biodegradation of *Ulva* sp cell-wall polysaccharides, J. Sci. Food Agric. 75 (1997) 341–351.

[79] N. Violle, P. Rozan, H. Demais, P. Nyvall Collen, J.F. Bisson, Evaluation of the antidepressant- and anxiolytic-like effects of a hydrophilic extract from the green seaweed *Ulva* sp. in rats, Nutr. Neurosci. (2017) 1–9.

[80] E. Percival, R.H. McDowell, Chemistry and Enzymology of Marine Algal Polysaccharides, Academic Press, London, 1967.

[81] M. Lahaye, NMR spectroscopic characterisation of oligosaccharides from two *Ulva rigida* ulvan samples (Ulvales, Chlorophyta) degraded by a lyase, Carbohydr. Res. 314 (1998) 1–12.

[82] G. Paradossi, F. Cavalieri, L. Pizzoferrato, A.M. Liquori, A physico-chemical study on the polysaccharide ulvan from hot water extraction of the macroalga *Ulva*, Int. J. Biol. Macromol. 25 (1999) 309–315.

[83] Y. Pengzhan, Z. Quanbin, L. Ning, X. Zuhong, W. Yanmei, L. Zhi, en, Polysaccharides from *Ulva pertusa* (Chlorophyta) and preliminary studies on their antihyperlipidemia activity, J. Appl. Phycol. 15 (2003) 21–27.

[84] M. Tako, M. Tamanaha, Y. Tamashiro, S. Uechi, Structure of ulvan isolated from the edible green seaweed, *Ulva pertusa*, Adv. Biosci. Biotechnol. 6 (2015) 645–655.

[85] M. Lahaye, F. Inizan, J. Vigouroux, NMR analysis of the chemical structure of ulvan and of ulvan-boron complex formation, Carbohydr. Polym. 36 (1998) 239–249.

[86] A. Robic, C. Gaillard, J.F. Sassi, Y. Lerat, M. Lahaye, Ultrastructure of ulvan: a polysaccharide from green seaweeds, Biopolymers 91 (2009) 652–664.

[87] Z. Zhang, X. Wang, M. Zhao, S. Yu, H. Qi, The immunological and antioxidant activities of polysaccharides extracted from *Enteromorpha linza*, Int. J. Biol. Macromol. 57 (2013) 45–49.

[88] H. Qi, T. Zhao, Q. Zhang, Z. Li, Z. Zhao, R. Xing, Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa* Kjellm (Chlorophyta), J. Appl. Phycol. 17 (2005) 527–534.

[89] Y. Pengzhan, Z. Quanbin, Z. Hong, N. Xizhen, L. Zhien, Preparation of polysaccharides in different molecular weights from *Ulva pertusa* Kjellm (Chorophyta), Chin. J. Oceanol. Limnol. 22 (2004) 381–385.

[90] L. Reisky, C. Stanetty, M.D. Mihovilovic, T. Schweder, J.-H. Hehemann, U.T. Bornscheuer, Biochemical characterization of an ulvan lyase from the marine flavobacterium *Formosa agariphila* KMM 3901T, Appl. Microbiol. Biotechnol. 102 (16) (2018) 6987–6996.

[91] C. El Modafar, M. Elgadda, R. El Boutachfaiti, E. Abouraicha, N. Zehhar, E. Petit, Z. El Alaoui-Talibi, B. Courtois, J. Courtois, Induction of natural defence accompanied by salicylic acid-dependant systemic acquired resistance in tomato seedlings in response to bioelicitors isolated from green algae, Sci. Hortic. 138 (2012) 55–63.

[92] H. Qi, Q. Zhang, T. Zhao, R. Chen, H. Zhang, X. Niu, Z. Li, Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro, Int. J. Biol. Macromol. 37 (2005) 195–199.

[93] H. Qi, L. Huang, X. Liu, D. Liu, Q. Zhang, S. Liu, Antihyperlipidemic activity of high sulfate content derivative of polysaccharide extracted from *Ulva pertusa* (Chlorophyta), Carbohydr. Polym. 87 (2012) 1637–1640.

[94] B. Kaeffer, C. Benard, M. Lahaye, H.M. Blottiere, C. Cherbut, Biological properties of ulvan, a new source of green seaweed sulfated polysaccharides, on cultured normal and cancerous colonic epithelial cells, Planta Med. 65 (1999) 527–531.

[95] S. Tsubaki, M. Hiraoka, S. Hadano, H. Nishimura, K. Kashimura, T. Mitani, Functional group dependent dielectric properties of sulfated hydrocolloids extracted from green macroalgal biomass, Carbohydr. Polym. 107 (2014) 192–197.

[96] H.M. Qi, Q.B. Zhang, T.T. Zhao, R.G. Hu, K. Zhang, Z. Li, In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta), Bioorg. Med. Chem. Lett. 16 (2006) 2441–2445.

[97] H.M. Qi, X.L. Liu, J. Ma, Q.B. Zhang, Z.E. Li, In vitro antioxidant activity of acetylated derivatives of polysaccharide extracted from *Ulva pertusa* (Cholorophta), Journal of Medicinal Plants Research 4 (2010) 2445–2451.

[98] A. Morelli, M. Betti, D. Puppi, C. Bartoli, M. Gazzarri, F. Chiellini, Enzymatically crosslinked ulvan hydrogels as injectable systems for cell delivery, Macromol. Chem. Phys. 217 (2016) 581–590.

[99] K. Kanno, T. Tanigawa, Y. Fujita, N. Ohnaka, Y. Kubo, K. Yoshida, K. Kojima, K. Nakata, M. Shimohara, Adsorbents for apheresis prepared from polysaccharides of algae that threaten ecosystem services, Chem. Biodivers. 11 (2014) 1140–1150.

[100] A. Robic, J.F. Sassi, M. Lahaye, Impact of stabilization treatments of the green seaweed *Ulva rotundata* (Chlorophyta) on the extraction yield, the physico-chemical and rheological properties of ulvan, Carbohydr. Polym. 74 (2008) 344–352.

[101] M. Berri, C. Slugocki, M. Olivier, E. Helloin, I. Jacques, H. Salmon, H. Demais, M. Le Goff, P.N. Collen, Marine-sulfated polysaccharides extract of *Ulva armoricana* green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells, J. Appl. Phycol. 28 (2016) 2999–3008.

[102] M. Berri, M. Olivier, S. Holbert, J. Dupont, H. Demais, M. Le Goff, P.N. Collen, Ulvan from *Ulva armoricana* (Chlorophyta) activates the PI3K/Akt signalling pathway via TLR4 to induce intestinal cytokine production, Algal Res. 28 (2017) 39–47.

[103] V. Jaulneau, C. Lafitte, M.F. Corio-Costet, M.J. Stadnik, S. Salamagne, X. Briand, M.T. Esquerré-Tugayé, B. Dumas, An *Ulva armoricana* extract protects plants against three powdery mildew pathogens, Eur. J. Plant Pathol. 131 (2011) 393–401.

[104] V. Gadenne, L. Lebrun, T. Jouenne, P. Thebault, Role of molecular properties of ulvans on their ability to elaborate antiadhesive surfaces, J. Biomed. Mater. Res. A 103 (2015) 1021–1028.

[105] M. Shanmugam, B.K. Ramavat, K.H. Mody, R.M. Oza, A. Tewari, Distribution of heparinoid-active sulphated polysaccharides in some Indian marine green algae, Indian Journal of Marine Sciences 30 (2001) 222–227.

[106] R.M. Hernandez-Herrera, G. Virgen-Calleros, M. Ruiz-Lopez, J. Zanudo-Hernandez, J.P. Delano-Frier, C. Sanchez-Hernandez, Extracts from green and brown seaweeds protect tomato (*Solanum lycopersicum*) against the necrotrophic fungus *Alternaria solani*, J. Appl. Phycol. 26 (2014) 1607–1614.

[107] J.A. Aguilar-Briseño, L.E. Cruz-Suarez, J.F. Sassi, D. Ricque-Marie, P. Zapata-Benavides, E. Mendoza-Gamboa, C. Rodríguez-Padilla, L.M. Trejo-Avila, Sulphated polysaccharides from *Ulva clathrata* and *Cladosiphon okamuranus* seaweeds both inhibit viral attachment/entry and cell-cell fusion, in NDV infection, Marine Drugs 13 (2015) 697–712.

[108] P. del Rocío Quezada-Rodríguez, E.J. Fajer-Ávila, The dietary effect of ulvan from *Ulva clathrata* on hematological-immunological parameters and growth of tilapia (*Oreochromis niloticus*), J. Appl. Phycol. 29 (2017) 423–431.

[109] M. Lahaye, D. Jegou, Chemical and physical-chemical characteristics of dietary fibres from *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grev, J. Appl. Phycol. 5 (1993) 195.

[110] M. Yamamoto, Physicochemical studies on sulfated polysaccharides extracted from seaweeds at various temperatures, Agric. Biol. Chem. 44 (1980) 589–593.

[111] A.K. Siddhanta, A.M. Goswami, B.K. Ramavat, K.H. Mody, O.P. Mairh, Water soluble polysaccharides of marine algal species of *Ulva* (Ulvales, Chlorophyta) of Indian waters, Indian Journal of Marine Sciences 30 (2001) 166–172.

[112] G.M. Jose, M. Kurup, In vitro antioxidant properties of edible marine algae *Sargassum swartzii*, *Ulva fasciata* and *Chaetomorpha antennina* of Kerala coast, Pharmaceutical Bioprocessing 4 (2016) 100–108.

[113] P. Shao, X. Chen, P. Sun, In vitro antioxidant and antitumor activities of different sulfated polysaccharides isolated from three algae, Int. J. Biol. Macromol. 62 (2013) 155–161.

[114] P. Shao, J. Shao, L. Han, R. Lv, P. Sun, Separation, preliminary characterization, and moisture-preserving activity of polysaccharides from *Ulva fasciata*, Int. J. Biol. Macromol. 72 (2015) 924–930.

[115] R. Pankiewicz, B. Łęska, B. Messyasz, J. Fabrowska, M. Sołoducha, M. Pikosz, First isolation of polysaccharidic ulvans from the cell walls of freshwater algae, Algal Res. 19 (2016) 348–354.

[116] A.A. Matloub, S.S.M. Elsouda, W.M. El-Senousy, M. Hamed, H. Aly, S.A. Ali, R.S. Mohammed, K. Mahmoud, S. El-Hallouty, N.A. Ibrahim, N.A. Awad, H.M. El-Rafaie, In vitro antiviral, cytotoxic, antioxidant and hypolipidemic activites of polysaccharide isolated from marine algae, International Journal of Pharmacognosy and Phytochemical Research 7 (2015) 1099–1111.

[117] N. Peasura, N. Laohakunjit, O. Kerdchoechuen, S. Wanlapa, Characteristics and antioxidant of *Ulva intestinalis* sulphated polysaccharides extracted with different solvents, Int. J. Biol. Macromol. 81 (2015) 912–919.

[118] F. Rahimi, M. Tabarsa, M. Rezaei, Ulvan from green algae *Ulva intestinalis*: optimization of ultrasound-assisted extraction and antioxidant activity, J. Appl. Phycol. 28 (2016) 2979–2990.

[119] X.X. Wang, Y. Chen, J.J. Wang, Z.X. Liu, S.G. Zhao, Antitumor activity of a sulfated polysaccharide from *Enteromorpha intestinalis* targeted against hepatoma through mitochondrial pathway, Tumor Biol. 35 (2014) 1641–1647.

[120] H.J. Alipour, M. Rezaei, B. Shabanpour, M. Tabarsa, Effects of sulfated polysaccharides from green alga *Ulva intestinalis* on physicochemical properties and microstructure of silver carp surimi, Food Hydrocoll. 74 (2018) 87–96.

[121] L.L. Jiao, P. Jiang, L.P. Zhang, M.J. Wu, Antitumor and immunomodulating activity of polysaccharides from *Enteromorpha intestinalis*, Biotechnol. Bioprocess Eng. 15 (2010) 421–428.

[122] L.L. Jiao, X. Li, T.B. Li, P. Jiang, L.X. Zhang, M.J. Wu, L.P. Zhang, Characterization and anti-tumor activity of alkali-extracted polysaccharide from *Enteromorpha intestinalis*, Int. Immunopharmacol. 9 (2009) 324–329.

[123] B. de Reviers, A. Leproux, Characterization of polysaccharides from *Enteromorpha intestinalis* (L.) link, chlorophyta, Carbohydr. Polym. 22 (1993) 253–259.

[124] N. Peasura, N. Laohakunjit, O. Kerdchoechuen, P. Vongsawasdi, L.K. Chao, Assessment of biochemical and immunomodulatory activity of sulphated polysaccharides from *Ulva intestinalis*, Int. J. Biol. Macromol. 91 (2016) 269–277.

[125] H. Yaich, H. Garna, S. Besbes, J.-P. Barthélemy, M. Paquot, C. Blecker, H. Attia, Impact of extraction procedures on the chemical, rheological and textural properties of ulvan from *Ulva lactuca* of Tunisia coast, Food Hydrocoll. 40 (2014) 53–63.

[126] O.A. Al-Amoudi, H.H. Mutawie, A.V. Patel, G. Blunden, Chemical composition and antioxidant activities of Jeddah corniche algae, Saudi Arabia, Saudi Journal of Biological Sciences 16 (2009) 23–29.

[127] Y.H. Chiu, Y.L. Chan, T.L. Li, C.J. Wu, Inhibition of Japanese encephalitis virus infection by the sulfated polysaccharide extracts from *Ulva lactuca*, Mar. Biotechnol. 14 (2012) 468–478.

[128] T.T.T. Thanh, T.M.T. Quach, T.N. Nguyen, D.V. Luong, M.L. Bui, T.T.V. Tran, Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*, Int. J. Biol. Macromol. 93 (2016) 695–702.

[129] I.W.F. de Araújo, J.A.G. Rodrigues, A.L.G. Quinderé, J.D.F.T. Silva, G.D.F. Maciel, N.A. Ribeiro, E. de Sousa Oliveira Vanderlei, K.A. Ribeiro, H.V. Chaves, K.M.A. Pereira, M.M. Bezerra, N.M.B. Benevides, Analgesic and anti-inflammatory actions on bradykinin route of a polysulfated fraction from alga *Ulva lactuca*, Int. J. Biol. Macromol. 92 (2016) 820–830.

[130] T.K. Gajaria, P. Suthar, R.S. Baghel, N.B. Balar, P. Sharnagat, V.A. Mantri, C.R.K. Reddy, Integration of protein extraction with a stream of byproducts from marine macroalgae: a model forms the basis for marine bioeconomy, Bioresour. Technol. 243 (2017) 867–873.

[131] J. He, Y. Xu, H. Chen, P. Sun, Extraction, structural characterization, and potential antioxidant activity of the polysaccharides from four seaweeds, Int. J. Mol. Sci. 17 (2016).

[132] D.G. Medcalf, T. Lionel, J.H. Brannon, J.R. Scott, Seasonal variation in the mucilaginous polysaccharides from *Ulva lactuca*, Bot. Mar. 18 (1975) 67–70.

[133] H. Yaich, H. Garna, S. Besbes, M. Paquot, C. Blecker, H. Attia, Effect of extraction conditions on the yield and purity of ulvan extracted from *Ulva lactuca*, Food Hydrocoll. 31 (2013) 375–382.

[134] A. Haug, The influence of borate and calcium on the gel formation of a sulfated polysaccharide from *Ulva lactuca*, Acta Chemica Scandinavica. Series B: Organic Chemistry and Biochemistry 30 (1976) 562–566.

[135] A. Sathivel, Balavinayagamani, B.R. Hanumantha Rao, T. Devaki, Sulfated polysaccharide isolated from *Ulva lactuca* attenuates D-galactosamine induced DNA fragmentation and necrosis during liver damage in rats, Pharm. Biol., 52 (2014) 498–505.

[136] A. Alves, R.A. Sousa, R.L. Reis, Processing of degradable ulvan 3D porous structures for biomedical applications, J. Biomed. Mater. Res. A 101 (2013) 998–1006.

[137] H.H. Abd El-Baky, F.K. El Baz, G.S. El Baroty, Potential biological properties of sulphated polysaccharides extracted from the macroalgae *Ulva lactuca* L, Academic Journal of Cancer Research 2 (2009) 1–11.

[138] T. Devaki, A. Sathivel, H.R. BalajiRaghavendran, Stabilization of mitochondrial and microsomal function by polysaccharide of *Ulva lactuca* on D-Galactosamine induced hepatitis in rats, Chem. Biol. Interact. 177 (2009) 83–88.

[139] U.K. Hussein, H.M. Mahmoud, A.G. Farrag, A. Bishayee, Chemoprevention of diethylnitrosamine-initiated and phenobarbital-promoted hepatocarcinogenesis in rats by sulfated polysaccharides and aqueous extract of *Ulva lactuca*, Integrative Cancer Therapies 14 (2015) 525–545.

[140] J. Wang, W.H. Jin, Y. Hou, X.Z. Niu, H. Zhang, Q.B. Zhang, Chemical composition and moisture-absorption/retention ability of polysaccharides extracted from five algae, Int. J. Biol. Macromol. 57 (2013) 26–29.

[141] H.A. Badr, K. Takahashi, R. Kawakami, Y. Oyama, K. Yokoigawa, K. Kanemaru, Screening and analysis of edible seaweeds in the ability to adsorb Shiga toxin, Eur. Food Res. Technol. 243 (2017) 2147–2153.

[142] X.M. Wang, Z.S. Zhang, Z.Y. Yao, M.X. Zhao, H.M. Qi, Sulfation, anticoagulant and antioxidant activities of polysaccharide from green algae *Enteromorpha linza*, Int. J. Biol. Macromol. 58 (2013) 225–230.

[143] Z.S. Zhang, F. Wang, X.M. Wang, X.L. Liu, Y. Hou, Q.B. Zhang, Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro, Carbohydr. Polym. 82 (2010) 118–121.

[144] C. Fernández-Díaz, O. Coste, E.J. Malta, Polymer chitosan nanoparticles functionalized with *Ulva ohnoi* extracts boost in vitro ulvan immunostimulant effect in Solea senegalensis macrophages, Algal Res. 26 (2017) 135–142.

[145] Y. Pengzhan, L. Ning, L. Xiguang, Z. Gefei, Z. Quanbin, L. Pengcheng, Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta), Pharmacol. Res. 48 (2003) 543–549.

[146] H.M. Qi, X.L. Liu, J.B. Zhang, Y. Duan, X.L. Wang, Q.B. Zhang, Synthesis and antihyperlipidemic activity of acetylated derivative of ulvan from *Ulva pertusa*, Int. J. Biol. Macromol. 50 (2012) 270–272.

[147] L. Song, X. Chen, X. Liu, F. Zhang, L. Hu, Y. Yue, K. Li, P. Li, Characterization and comparison of the structural features, immune-modulatory and anti-avian influenza virus activities conferred by three algal sulfated polysaccharides, Marine

Drugs (2016) 14.
[148] C. Cai, Z. Guo, Y. Yang, Z. Geng, L. Tang, M. Zhao, Y. Qiu, Y. Chen, P. He, Inhibition of hydrogen peroxide induced injuring on human skin fibroblast by *Ulva prolifera* polysaccharide, Int. J. Biol. Macromol. 91 (2016) 241–247.
[149] B. Li, S. Liu, R. Xing, K.C. Li, R.F. Li, Y.K. Qin, X.Q. Wang, Z.H. Wei, P.C. Li, Degradation of sulfated polysaccharides from *Enteromorpha prolifera* and their antioxidant activities, Carbohydr. Polym. 92 (2013) 1991–1996.
[150] Y. Yu, Y.P. Li, C.Y. Du, H.J. Mou, P. Wang, Compositional and structural characteristics of sulfated polysaccharide from *Enteromorpha prolifera*, Carbohydr. Polym. 165 (2017) 221–228.
[151] J.X. Li, Z. Chi, L.J. Yu, F. Jiang, C.G. Liu, Sulfated modification, characterization, and antioxidant and moisture absorption/retention activities of a soluble neutral polysaccharide from *Enteromorpha prolifera*, Int. J. Biol. Macromol. 105 (2017) 1544–1553.
[152] Z.L. Teng, L. Qian, Y. Zhou, Hypolipidemic activity of the polysaccharides from *Enteromorpha prolifera*, Int. J. Biol. Macromol. 62 (2013) 254–256.
[153] J. Xu, L.L. Xu, Q.W. Zhou, S.X. Hao, T. Zhou, H.J. Xie, Isolation, purification, and antioxidant activities of degraded polysaccharides from *Enteromorpha prolifera*, Int. J. Biol. Macromol. 81 (2015) 1026–1030.
[154] Q.T.M. Thu, T.H. Bang, N.T. Nu, D.V. Luong, B.M. Ly, T.T.T. Van, T.T.T. Thuy, Structural determination of ulvan from green seaweed *Ulva reticulata* collected at central coast of Vietnam, Chem. Lett. 44 (2015) 788–790.
[155] T.T.V. Tran, H.B. Truong, N.H.V. Tran, T.M.T. Quach, T.N. Nguyen, M.L. Bui, Y. Yuguchi, T.T.T. Thanh, Structure, conformation in aqueous solution and antimicrobial activity of ulvan extracted from green seaweed *Ulva reticulata*, Nat. Prod. Res. (2017) 1–6.
[156] P.N. Collén, J.F. Sassi, H. Rogniaux, H. Marfaing, W. Helbert, Ulvan lyases isolated from the flavobacteria *Persicivirga ulvanivorans* are the first members of a new polysaccharide lyase family, J. Biol. Chem. 286 (2011) 42063–42071.
[157] A. Alves, S.G. Caridade, J.F. Mano, R.A. Sousa, R.L. Reis, Extraction and physicochemical characterization of a versatile biodegradable polysaccharide obtained from green algae, Carbohydr. Res. 345 (2010) 2194–2200.
[158] V.C. Padmanaban, K.P. Prashant, M.V. Ranjith, T. Aswani, M.T. Aswani, Comparative analysis of anti-coagulant potential of marine macro algae, Int J Pharm Pharm Sci 5 (2013) 334–337.
[159] P.A. Harnedy, R.J. FitzGerald, Extraction and enrichment of protein from red and green macroalgae, Methods Mol. Biol. 1308 (2015) 103–108.
[160] A.R. Angell, N.A. Paul, R. de Nys, A comparison of protocols for isolating and concentrating protein from the green seaweed *Ulva ohnoi*, J. Appl. Phycol. 29 (2017) 1011–1026.
[161] S. Tsubaki, K. Oono, M. Hiraoka, A. Onda, T. Mitani, Microwave-assisted hydrothermal extraction of sulfated polysaccharides from *Ulva* spp. and *Monostroma latissimum*, Food Chem. 210 (2016) 311–316.
[162] E. Hernández-Garibay, J.A. Zertuche-González, I. Pacheco-Ruíz, Isolation and chemical characterization of algal polysaccharides from the green seaweed *Ulva clathrata* (Roth) C. Agardh, J. Appl. Phycol. 23 (2011) 537–542.
[163] L. Shi, Bioactivities, isolation and purification methods of polysaccharides from natural products: a review, Int. J. Biol. Macromol. 92 (2016) 37–48.
[164] A. Béress, O. Wassermann, T. Bruhn, L. Béress, E.N. Kraiselburd, L.V. Gonzalez, G.E. de Motta, P.I. Chavez, A new procedure for the isolation of anti-HIV compounds (polysaccharides and polyphenols) from the marine alga *Fucus vesiculosus*, J. Nat. Prod. 56 (1993) 478–488.
[165] H.-J. Zhang, W.-J. Mao, F. Fang, H.-Y. Li, H.-H. Sun, Y. Chen, X.-H. Qi, Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostroma latissimum*, Carbohydr. Polym. 71 (2008) 428–434.
[166] P. Ghosh, U. Adhikari, P.K. Ghosal, C.A. Pujol, M.J. Carlucci, E.B. Damonte, B. Ray, In vitro anti-herpetic activity of sulfated polysaccharide fractions from *Caulerpa racemosa*, Phytochemistry 65 (2004) 3151–3157.
[167] L. Yang, L.-M. Zhang, Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources, Carbohydr. Polym. 76 (2009) 349–361.
[168] I.M. Sims, S.M. Carnachan, T.J. Bell, S.F.R. Hinkley, Methylation analysis of polysaccharides: technical advice, Carbohydr. Polym. 188 (2018) 1–7.
[169] F.A. Pettolino, C. Walsh, G.B. Fincher, A. Bacic, Determining the polysaccharide composition of plant cell walls, Nat. Protoc. 7 (2012) 1590–1607.
[170] J.S. Craigie, Z.C. Wen, J.P. van der Meer, Interspecific, intraspecific and nutritionally-determined variations in the composition of agars from *Gracilaria* spp, Bot. Mar. (1984) 55.
[171] A. Robic, D. Bertrand, J.F. Sassi, Y. Lerat, M. Lahaye, Determination of the chemical composition of ulvan, a cell wall polysaccharide from *Ulva* spp. (Ulvales, Chlorophyta) by FT-IR and chemometrics, J. Appl. Phycol. 21 (2009) 451–456.
[172] J.N. BeMiller, Acid-catalyzed hydrolysis of glycosides, Adv. Carbohydr. Chem. Biochem. 22 (1967) 25–108.
[173] L.A. Fransson, L. Roden, M.L. Spach, Automated ion-exchange chromatography of uronic acids and uronic acid containing oligosaccharides, Anal. Biochem. 23 (1968) 317–330.
[174] H.E. Conrad, The acid lability of the glycosidic bonds of L-iduronic acid residues in glycosaminoglycans, The Biochemical journal 191 (1980) 355–363.
[175] G.A. De Ruiter, H.A. Schols, A.G.J. Voragen, F.M. Rombouts, Carbohydrate analysis of water-soluble uronic acid-containing polysaccharides with high-performance anion-exchange chromatography using methanolysis combined with TFA hydrolysis is superior to four other methods, Anal. Biochem. 207 (1992) 176–185.
[176] D.W. Templeton, M. Quinn, S. Van Wychen, D. Hyman, L.M.L. Laurens, Separation and quantification of microalgal carbohydrates, J. Chromatogr. A 1270 (2012) 225–234.
[177] B. Radhakrishnamurthy, G.S. Berenson, Effect of temperature and time of heating on the carbazole reaction of uronic acids and acid mucopolysaccharides, Anal. Chem. 35 (1963) 1316–1318.
[178] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, Anal. Biochem. 54 (1973) 484–489.
[179] J.E. Cassolato, M.D. Noseda, C.A. Pujol, F.M. Pellizzari, E.B. Damonte, M.E. Duarte, Chemical structure and antiviral activity of the sulfated heterorhamnan isolated from the green seaweed *Gayralia oxysperma*, Carbohydr. Res. 343 (2008) 3085–3095.
[180] A. Synytsya, D.J. Choi, R. Pohl, Y.S. Na, P. Capek, E. Lattová, T. Taubner, J.W. Choi, C.W. Lee, J.K. Park, W.J. Kim, S.M. Kim, J. Lee, Y.I. Park, Structural features and anti-coagulant activity of the sulphated polysaccharide SPS-CF from a green alga *Capsosiphon fulvescens*, Mar. Biotechnol. 17 (2015) 718–735.
[181] J.Ø. Duus, C.H. Gotfredsen, K. Bock, Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations, Chem. Rev. 100 (2000) 4589–4614.
[182] H.N. Cheng, T.G. Neiss, Solution NMR spectroscopy of food polysaccharides, Polym. Rev. 52 (2012) 81–114.
[183] H. Yaich, A.B. Amira, F. Abbes, M. Bouaziz, S. Besbes, A. Richel, C. Blecker, H. Attia, H. Garna, Effect of extraction procedures on structural, thermal and antioxidant properties of ulvan from *Ulva lactuca* collected in Monastir coast, Int. J. Biol. Macromol. 105 (Pt 2) (2017) 1430–1439.
[184] M. Kopel, W. Helbert, Y. Belnik, V. Buravenkov, A. Herman, E. Banin, New family of ulvan lyases identified in three isolates from the alteromonadales order, J. Biol. Chem. 291 (2016) 5871–5878.
[185] A. Morelli, M. Betti, D. Puppi, F. Chiellini, Design, preparation and characterization of ulvan based thermosensitive hydrogels, Carbohydr. Polym. 136 (2016) 1108–1117.
[186] A.M. Striegel, Multiple detection in size-exclusion chromatography of macromolecules, Anal. Chem. 77 (2005) 104 A–113 A.
[187] L.K. Kostanski, D.M. Keller, A.E. Hamielec, Size-exclusion chromatography—a review of calibration methodologies, J. Biochem. Biophys. Methods 58 (2004) 159–186.
[188] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[189] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[190] F. Pittner, P.L. Turecek, The application of immobilized α-L-rhamnosidase and L-rhamnosedehydrogenase in the analysis of L-rhamnose and α-L-rhamnosides, Appl. Biochem. Biotechnol. 16 (1987) 15–24.
[191] Z. Gong, W. Zhou, H. Shen, Z. Yang, G. Wang, Z. Zuo, Y. Hou, Z.K. Zhao, Co-fermentation of acetate and sugars facilitating microbial lipid production on acetate-rich biomass hydrolysates, Bioresour. Technol. 207 (2016) 102–108.
[192] T.S. Moon, S.-H. Yoon, M.-J. Tsang Mui Ching, A.M. Lanza, K.L.J. Prather, Enzymatic assay of D-glucuronate using uronate dehydrogenase, Anal. Biochem. 392 (2009) 183–185.
[193] T. Salomonsen, H.M. Jensen, D. Stenbæk, S.B. Engelsen, Chemometric prediction of alginate monomer composition: a comparative spectroscopic study using IR, Raman, NIR and NMR, Carbohydr. Polym. 72 (2008) 730–739.
[194] T.M. Handel, Z. Johnson, S.E. Crown, E.K. Lau, A.E. Proudfoot, Regulation of protein function by glycosaminoglycans–as exemplified by chemokines, Annu. Rev. Biochem. 74 (2005) 385–410.
[195] D. Scharnweber, L. Hubner, S. Rother, U. Hempel, U. Anderegg, S.A. Samsonov, M.T. Pisabarro, L. Hofbauer, M. Schnabelrauch, S. Franz, J. Simon, V. Hintze, Glycosaminoglycan derivatives: promising candidates for the design of functional biomaterials, J. Mater. Sci. Mater. Med. 26 (2015) 232.
[196] A.L. Varnier, L. Sanchez, P. Vatsa, L. Boudesocque, A. Garcia-Brugger, F. Rabenoelina, A. Sorokin, J.H. Renault, S. Kauffmann, A. Pugin, C. Clement, F. Baillieul, S. Dorey, Bacterial rhamnolipids are novel MAMPs conferring resistance to *Botrytis cinerea* in grapevine, Plant Cell Environ. 32 (2009) 178–193.
[197] P. Vatsa, L. Sanchez, C. Clement, F. Baillieul, S. Dorey, Rhamnolipid biosurfactants as new players in animal and plant defense against microbes, Int. J. Mol. Sci. 11 (2010) 5095–5108.
[198] S. Bigot, G. Louarn, N. Kébir, F. Burel, Click grafting of seaweed polysaccharides onto PVC surfaces using an ionic liquid as solvent and catalyst, Carbohydr. Polym. 98 (2013) 1644–1649.
[199] N. Lopes, S. Ray, S.F. Espada, W.A. Bomfim, B. Ray, L.C. Faccin-Galhardi, R.E.C. Linhares, C. Nozawa, Green seaweed *Enteromorpha compressa* (Chlorophyta, Ulvaceae) derived sulphated polysaccharides inhibit herpes simplex virus, Int. J. Biol. Macromol. 102 (2017) 605–612.
[200] V. Gadenne, L. Lebrun, T. Jouenne, P. Thebault, Antiadhesive activity of ulvan polysaccharides covalently immobilized onto titanium surface, Colloids Surf. B: Biointerfaces 112 (2013) 229–236.
[201] C. Faggio, M. Pagano, A. Dottore, G. Genovese, M. Morabito, Evaluation of anticoagulant activity of two algal polysaccharides, Nat. Prod. Res. 30 (2016) 1934–1937.
[202] M.Z. Rizk, H.F. Aly, A.A. Matloub, G.I. Fouad, The anti-hypercholesterolemic effect of ulvan polysaccharide extracted from the green alga *Ulva fasciata* on aged hypercholesterolemic rats, Asian Journal of Pharmaceutical and Clinical Research (2016) 9.
[203] M.Z. Rizk, M. El-Sherbiny, I.H. Borai, M.K. Ezz, H.F. Aly, A.A. Matloub, A.E.R. Farrag, G.I. Fouad, Sulphated polysaccharides (SPS) from the green alga *ulva fasciata* extract modulates liver and kidney function in high fat diet-induced hypercholesterolemic rats, Int J Pharm Pharm Sci 8 (2016) 43–55.
[204] A. Tariq, M. Athar, J. Ara, V. Sultana, S. Ehteshamul-Haque, M. Ahmad, Biochemical evaluation of antioxidant activity in extracts and polysaccharide

fractions of seaweeds, Global Journal of Environmental Science and Management 1 (2015) 47–62.
[205] I.H. Borai, M.K. Ezz, M.Z. Rizk, M. El-Sherbiny, A.A. Matloub, H.F. Aly, G.I. Fouad, Hypolipidemic and anti-atherogenic effect of sulphated polysaccharides from the green alga *Ulva fasciata*, International Journal of Pharmaceutical Sciences Review and Research 31 (2015) 1–12.
[206] L. Araújo, M.J. Stadnik, L.C. Borsato, R.M. Valdebenito-Sanhueza, Potassium phosphite and ulvan in the control of 'Gala' leaf spot on apple, Tropical Plant Pathology 33 (2008) 148–152.
[207] K. Morán-Santibañez, L.E. Cruz-Suárez, D. Ricque-Marie, D. Robledo, Y. Freile-Pelegrín, M.A. Peña-Hernández, C. Rodríguez-Padilla, L.M. Trejo-Avila, Synergistic effects of sulfated polysaccharides from Mexican seaweeds against measles virus, Biomed. Res. Int. 2016 (2016).
[208] J. Spavieri, M. Kaiser, R. Casey, S. Hingley-Wilson, A. Lalvani, G. Blunden, D. Tasdemir, Antiprotozoal, antimycobacterial and cytotoxic potential of some british green algae, Phytother. Res. 24 (2010) 1095–1098.
[209] G.E.F. Abd-Ellatef, O.M. Ahmed, E.S. Abdel-Reheim, A.H.Z. Abdel-Hamid, *Ulva lactuca* polysaccharides prevent Wistar rat breast carcinogenesis through the augmentation of apoptosis, enhancement of antioxidant defense system, and suppression of inflammation, Breast Cancer: Targets and Therapy 9 (2017) 67–83.
[210] O.M. Ahmed, R.R. Ahmed, Anti-proliferative and apoptotic efficacies of ulvan polysaccharides against different types of carcinoma cells in vitro and in vivo, Journal of Cancer Science and Therapy 6 (2014) 202–208.
[211] S. Hassan, S.A. El-Twab, M. Hetta, B. Mahmoud, Improvement of lipid profile and antioxidant of hypercholesterolemic albino rats by polysaccharides extracted from the green alga *Ulva lactuca* Linnaeus, Saudi Journal of Biological Sciences 18 (2011) 333–340.
[212] H.M. Mahmoud, U.L. Hussein, U.L. Hussein, Suppression of N-nitrosodiethylamine induced oxidative renal toxicity by sulphated polysaccharide and aqueous extract of *Ulva lactuca* in rats, Int J Pharm Pharm Sci 6 (2014) 248–253.
[213] A. Sathivel, H.R.B. Raghavendran, P. Srinivasan, T. Devaki, Anti-peroxidative and anti-hyperlipidemic nature of *Ulva lactuca* crude polysaccharide on D-Galactosamine induced hepatitis in rats, Food Chem. Toxicol. 46 (2008) 3262–3267.
[214] A. Alves, E.D. Pinho, N.M. Neves, R.A. Sousa, R.L. Reis, Processing ulvan into 2D structures: cross-linked ulvan membranes as new biomaterials for drug delivery applications, Int. J. Pharm. 426 (2012) 76–81.
[215] S.S. Alam, H.A.M. Abd El-Kader, A.H. Abd El-Rahim, S.M. Hamed, A.A. Saber, The protective role of *Ulva lactuca* against genotoxic and biochemical effects induced by γ-irradiation in rats, International Journal of Pharmaceutical Sciences Review and Research 37 (2016) 40–48.
[216] G. Guerra-Rivas, C.M. Gomez-Gutierrez, G. Alarcon-Arteaga, I.E. Soria-Mercado, N.E. Ayala-Sanchez, Screening for anticoagulant activity in marine algae from the northwest Mexican Pacific coast, J. Appl. Phycol. 23 (2011) 495–503.
[217] J.M. Shi, C.L. Cheng, H.T. Zhao, J. Jing, N. Gong, W.H. Lu, In vivo anti-radiation activities of the *Ulva pertusa* polysaccharides and polysaccharide-iron(III) complex, Int. J. Biol. Macromol. 60 (2013) 341–346.
[218] J. Wei, S. Wang, G. Liu, D. Pei, Y. Liu, Y. Liu, D. Di, Polysaccharides from *Enteromorpha prolifera* enhance the immunity of normal mice, Int. J. Biol. Macromol. 64 (2014) 1–5.
[219] R. Ren, J. Gong, Y. Zhao, X. Zhuang, Y. Ye, W. Lin, Sulfated polysaccharides from *Enteromorpha prolifera* suppress SREBP-2 and HMG-CoA reductase expression and attenuate non-alcoholic fatty liver disease induced by a high-fat diet, Food and Function 8 (2017) 1899–1904.
[220] W.T. Lin, W.X. Wang, D.D. Liao, D.M. Chen, P.P. Zhu, G.X. Cai, A. Kiyoshi, Polysaccharides from *Enteromorpha prolifera* improve glucose metabolism in diabetic rats, Journal of Diabetes Research 2015 (2015) 1–12 (Article ID: 675201).
[221] Q. Kong, S.Y. Dong, J. Gao, C.Y. Jiang, In vitro fermentation of sulfated polysaccharides from *E. prolifera* and *L. japonica* by human fecal microbiota, Int. J. Biol. Macromol. 91 (2016) 867–871.
[222] H. Balaji Raghavendra Rao, A. Sathivel, T. Devaki, Antihepatotoxic nature of *Ulva reticulata* (Chlorophyceae) on acetaminophen-induced hepatoxicity in experimental rats, J. Med. Food 7 (2004) 495–497.
[223] R. Castro, I. Zarra, J. Lamas, Water-soluble seaweed extracts modulate the respiratory burst activity of turbot phagocytes, Aquaculture 229 (2004) 67–78.
[224] M. Roselli, M.S. Britti, I. Le Huërou-Luron, H. Marfaing, W.Y. Zhu, E. Mengheri, Effect of different plant extracts and natural substances (PENS) against membrane damage induced by enterotoxigenic Escherichia coli K88 in pig intestinal cells, Toxicol. in Vitro 21 (2007) 224–229.
[225] A. Alves, R.A. Sousa, R.L. Reis, In vitro cytotoxicity assessment of ulvan, a polysaccharide extracted from green algae, Phytother. Res. 27 (2013) 1143–1148.
[226] M. Alves, C. Dore, A.J.G. Castro, M.S. do Nascimento, A.K.M. Cruz, E.M. Soriano, N.M.B. Benevides, E.L. Leite, Antioxidant, cytotoxic and hemolytic effects of sulfated galactans from edible red alga Hypnea musciformis, J. Appl. Phycol. 24 (2012) 1217–1227.
[227] J.-K. Kim, M.L. Cho, S. Karnjanapratum, I.-S. Shin, S.G. You, In vitro and in vivo immunomodulatory activity of sulfated polysaccharides from *Enteromorpha prolifera*, Int. J. Biol. Macromol. 49 (2011) 1051–1058.
[228] J.K. Hong, M.H. Bong, J.C. Park, H.K. Moon, D.W. Kim, S.C. Lee, J.H. Lee, Antioxidant and immunomodulatory effects of *Ulva pertusa* Kjellman on broiler chickens, Journal of Animal Science and Technology 53 (2011) 419–428.
[229] H.P. Rang, J.M. Ritter, R.J. Flower, G. Henderson, Rang & Dale's Pharmacology, Churchill Livingstone, 2016.
[230] P.N. Moynagh, Toll-like receptor signalling pathways as key targets for mediating the anti-inflammatory and immunosuppressive effects of glucocorticoids, J. Endocrinol. 179 (2003) 139–144.
[231] J.M. Cavaillon, Pro-versus anti-inflammatory cytokines: myth or reality, Cell. Mol. Biol. 47 (2001) 695–702.
[232] C. Zhu, S. Zhang, C. Song, Y. Zhang, Q. Ling, P.R. Hoffmann, J. Li, T. Chen, W. Zheng, Z. Huang, Selenium nanoparticles decorated with *Ulva lactuca* polysaccharide potentially attenuate colitis by inhibiting NF-κB mediated hyper inflammation, Journal of Nanobiotechnology 15 (2017) 20.
[233] E. Birben, U.M. Sahiner, C. Sackesen, S. Erzurum, O. Kalayci, Oxidative stress and antioxidant defense, World Allergy Organization Journal 5 (2012) 9–19.
[234] S.B. Nimse, D. Pal, Free radicals, natural antioxidants, and their reaction mechanisms, RSC Adv. 5 (2015) 27986–28006.
[235] J. Bouayed, T. Bohn, Exogenous antioxidants–double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses, Oxidative Med. Cell. Longev. 3 (2010) 228–237.
[236] H.J. Forman, K.J. Davies, F. Ursini, How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging in vivo, Free Radic. Biol. Med. 66 (2014) 24–35.
[237] Z.S. Zhang, X.M. Wang, S.C. Yu, L. Yin, M.X. Zhao, Z.P. Han, Synthesized oversulfated and acetylated derivatives of polysaccharide extracted from *Enteromorpha linza* and their potential antioxidant activity, Int. J. Biol. Macromol. 49 (2011) 1012–1015.
[238] H.M. Qi, Y.L. Sun, Antioxidant activity of high sulfate content derivative of ulvan in hyperlipidemic rats, Int. J. Biol. Macromol. 76 (2015) 326–329.
[239] Q. Li, J. Luo, C. Wang, W. Tai, H. Wang, X. Zhang, K. Liu, Y. Jia, X. Lyv, L. Wang, H. He, Ulvan extracted from green seaweeds as new natural additives in diets for laying hens, J. Appl. Phycol. 30 (2018) 2017–2027.
[240] Q. Zhang, N. Li, G. Zhou, X. Lu, Z. Xu, Z. Li, In vivo antioxidant activity of polysaccharide fraction from *Porphyra haitanesis* (Rhodephyta) in aging mice, Pharmacol. Res. 48 (2003) 151–155.
[241] A.R. Phull, S.J. Kim, Fucoidan as bio-functional molecule: insights into the anti-inflammatory potential and associated molecular mechanisms, J. Funct. Foods 38 (2017) 415–426.
[242] X.G. Lei, J.H. Zhu, W.H. Cheng, Y. Bao, Y.S. Ho, A.R. Reddi, A. Holmgren, E.S. Arner, Paradoxical roles of antioxidant enzymes: basic mechanisms and health implications, Physiol. Rev. 96 (2016) 307–364.
[243] L. Miao, D.K. St Clair, Regulation of superoxide dismutase genes: implications in disease, Free Radic. Biol. Med. 47 (2009) 344–356.
[244] M. Valko, M. Izakovic, M. Mazur, C.J. Rhodes, J. Telser, Role of oxygen radicals in DNA damage and cancer incidence, Mol. Cell. Biochem. 266 (2004) 37–56.
[245] A.A. Matloub, A.H. Salama, H.A. Aglan, M.M. AbouSamra, S.S.M. ElSouda, H.H. Ahmed, Exploiting bilosomes for delivering bioactive polysaccharide isolated from *Enteromorpha intestinalis* for hacking hepatocellular carcinoma, Drug Dev. Ind. Pharm. 44 (2018) 523–534.
[246] Z. Hu, P. Hong, Y. Cheng, M. Liao, S. Li, Polysaccharides from *Enteromorpha tubulosa*: optimization of extraction and cytotoxicity, Journal of Food Processing and Preservation 42 (2018) e13373.
[247] C. Gerhauser, A. Alt, E. Heiss, A. Gamal-Eldeen, K. Klimo, J. Knauft, I. Neumann, H.R. Scherf, N. Frank, H. Bartsch, H. Becker, Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop, Mol. Cancer Ther. 1 (2002) 959–969.
[248] J. Li, F. Jiang, Z. Chi, D. Han, L. Yu, C. Liu, Development of *Enteromorpha prolifera* polysaccharide-based nanoparticles for delivery of curcumin to cancer cells, Int. J. Biol. Macromol. 112 (2018) 413–421.
[249] X. Sun, Y. Zhong, H. Luo, Y. Yang, Selenium-containing polysaccharide-protein complex in Se-enriched *Ulva fasciata* induces mitochondria-mediated apoptosis in A549 human lung cancer cells, Marine Drugs 15 (2017).
[250] F. Yang, X. Fang, W. Jiang, T. Chen, Bioresponsive cancer-targeted polysaccharide nanosystem to inhibit angiogenesis, Int. J. Nanomedicine 12 (2017) 7419–7431.
[251] J. Cui, Y. Li, S. Wang, Y. Chi, H. Hwang, P. Wang, Directional preparation of anticoagulant-active sulfated polysaccharides from *Enteromorpha prolifera* using artificial neural networks, Sci. Rep. 8 (2018) 3062.
[252] A.T. Long, E. Kenne, R. Jung, T.A. Fuchs, T. Renné, Contact system revisited: an interface between inflammation, coagulation, and innate immunity, J. Thromb. Haemost. 14 (2016) 427–437.
[253] C.H. Lee, P. Olson, R.M. Evans, Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors, Endocrinology 144 (2003) 2201–2207.
[254] S. Mohamed, S.N. Hashim, H.A. Rahman, Seaweeds: a sustainable functional food for complementary and alternative therapy, Trends Food Sci. Technol. 23 (2012) 83–96.
[255] L. Wang, X. Wang, H. Wu, R. Liu, Overview on biological activities and molecular characteristics of sulfated polysaccharides from marine green algae in recent years, Marine Drugs 12 (2014) 4984–5020.
[256] G. Jiao, G. Yu, J. Zhang, H.S. Ewart, Chemical structures and bioactivities of sulfated polysaccharides from marine algae, Marine Drugs 9 (2011) 196–233.
[257] H. Qi, J. Sheng, The antihyperlipidemic mechanism of high sulfate content ulvan in rats, Marine Drugs 13 (2015) 3407–3421.
[258] H. Qi, X. Liu, K. Wang, D. Liu, L. Huang, S. Liu, Q. Zhang, Subchronic toxicity study of ulvan from *Ulva pertusa* (Chlorophyta) in Wistar rats, Food Chem. Toxicol. 62 (2013) 573–578.
[259] M.H. Hoang, J.-Y. Kim, J.H. Lee, S. You, S.-J. Lee, Antioxidative, hypolipidemic, and anti-inflammatory activities of sulfated polysaccharides from *Monostroma nitidum*, Food Sci. Biotechnol. 24 (2015) 199–205.
[260] R. Ren, J. Gong, Y. Zhao, X. Zhuang, Y. Ye, F. Huang, W. Lin, Sulfated polysaccharide from *Enteromorpha prolifera* suppresses SREBP-1c and ACC expression to lower serum triglycerides in high-fat diet-induced hyperlipidaemic rats, J. Funct. Foods 40 (2018) 722–728.

[261] L. Gesquière, N. Loreau, A. Minnich, J. Davignon, D. Blache, Oxidative stress leads to cholesterol accumulation in vascular smooth muscle cells, Free Radic. Biol. Med. 27 (1999) 134–145.
[262] M.M. Ali, F.G. Agha, Amelioration of streptozotocin-induced diabetes mellitus, oxidative stress and dyslipidemia in rats by tomato extract lycopene, Scand. J. Clin. Lab. Invest. 69 (2009) 371–379.
[263] Q. Shi, A. Wang, Z. Lu, C. Qin, J. Hu, J. Yin, Overview on the antiviral activities and mechanisms of marine polysaccharides from seaweeds, Carbohydr. Res. 453-454 (2017) 1–9.
[264] M. Witvrouw, E. De Clercq, Sulfated polysaccharides extracted from sea algae as potential antiviral drugs, General Pharmacology: The Vascular System 29 (1997) 497–511.
[265] E.B. Damonte, M.C. Matulewicz, A.S. Cerezo, Sulfated seaweed polysaccharides as antiviral agents, Curr. Med. Chem. 11 (2004) 2399–2419.
[266] J.B. Lee, K. Hayashi, M. Maeda, T. Hayashi, Antiherpetic activities of sulfated polysaccharides from green algae, Planta Med. 70 (2004) 813–817.
[267] T. Ghosh, K. Chattopadhyay, M. Marschall, P. Karmakar, P. Mandal, B. Ray, Focus on antivirally active sulfated polysaccharides: from structure-activity analysis to clinical evaluation, Glycobiology 19 (2009) 2–15.
[268] L. Araujo, A.E. Goncalves, M.J. Stadnik, Ulvan effect on conidial germination and appressoria formation of *Colletotrichum gloeosporioides*, Phytoparasitica 42 (2014) 631–640.
[269] L.G. Castellanos-Barriga, F. Santacruz-Ruvalcaba, G. Hernández-Carmona, E. Ramírez-Briones, R.M. Hernández-Herrera, Effect of seaweed liquid extracts from *Ulva lactuca* on seedling growth of mung bean (*Vigna radiata*), J. Appl. Phycol. 29 (2017) 2479–2488.
[270] S. Cluzet, C. Torregrosa, C. Jacquet, C. Lafitte, J. Fournier, L. Mercier, S. Salamagne, X. Briand, M.-T. Esquerré-Tugayé, B. Dumas, Gene expression profiling and protection of *Medicago truncatula* against a fungal infection in response to an elicitor from green algae *Ulva* spp, Plant Cell Environ. 27 (2004) 917–928.
[271] D.Z. Delgado, M.B. de Freitas, M.J. Stadnik, Effectiveness of saccharin and ulvan as resistance inducers against rust and angular leaf spot in bean plants (*Phaseolus vulgaris*), Crop Prot. 47 (2013) 67–73.
[272] M.B. de Freitas, M.J. Stadnik, Race-specific and ulvan-induced defense responses in bean (*Phaseolus vulgaris*) against *Colletotrichum lindemuthianum*, Physiol. Mol. Plant Pathol. 78 (2012) 8–13.
[273] J.D.G. Jones, J.L. Dangl, The plant immune system, Nature 444 (2006) 323.
[274] A. Robert-Seilaniantz, M. Grant, J.D.G. Jones, Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism, Annu. Rev. Phytopathol. 49 (2011) 317–343.
[275] C.M. Pieterse, A. Leon-Reyes, S. Van der Ent, S.C. Van Wees, Networking by small-molecule hormones in plant immunity, Nat. Chem. Biol. 5 (2009) 308–316.
[276] L. Burketova, L. Trda, P.G. Ott, O. Valentova, Bio-based resistance inducers for sustainable plant protection against pathogens, Biotechnol. Adv. 33 (2015) 994–1004.
[277] U. Conrath, G.J. Beckers, V. Flors, P. Garcia-Agustin, G. Jakab, F. Mauch, M.A. Newman, C.M. Pieterse, B. Poinssot, M.J. Pozo, A. Pugin, U. Schaffrath, J. Ton, D. Wendehenne, L. Zimmerli, B. Mauch-Mani, Priming: getting ready for battle, Mol. Plant-Microbe Interact. 19 (2006) 1062–1071.