RESEARCH PAPER

Chemical characterization and biological activities of ulvan extracted from *Ulva fasciata* (Chlorophyta)

Khouloud M. Barakat[1] · Mona M. Ismail[1] · Hala E. Abou El Hassayeb[1] · Nermeen A. El Sersy[1] ·
Mostafa E. Elshobary[2]

Abstract
Ulvan is a sulfated heteropolysaccharide present in the cell wall of *Ulva* species with a unique structure and biological potential used in various fields. Chemical characterization was carried out to determine the structure of ulvan from *Ulva fasciata* Delile collected from Eastern Harbor, Alexandria coast, Egypt. *Ulva* contains 31.5% carbohydrate with a total ulvan content of 43.66% of total carbohydrate (13.75 g/100 g DW) and sulfate content of 20.45% of ulvan. FTIR spectrum presented signals of the sulfate ester (C–O–S) and sulfate groups (S=O), typical for ulvan. GC–MS revealed that ulvan was mainly composed of rhamnose and fucose. ^{1}H-NMR spectra of ulvan showed identical behavior of monosaccharides nature with peaks characteristic of sulfated polysaccharides at 3.2–5.3 ppm region. Scanning electron micrographs (SEM) demonstrated amorphous architecture, and the sulfated nature of polysaccharides was emphasized by EDX analysis. The extracted ulvan showed significant antimicrobial activity against human and fish pathogens as well as antifouling bacteria with minimum inhibitory concentrations (MIC) of 8 µg/mL. The extracted ulvan exhibited potent antioxidant activity with a scavenging effect of 84.93% for 2,2-diphenyl-1-picrylhydrazy free radical (DPPH). Moreover, it showed anti-arthritic properties for the first time with a maximum inhibition of 86.04% with IC_{50} of 43.21%, indicating their potential value for the health and food industry.

Keywords Anti-arthritic · Antimicrobial activity · DPPH · *Ulva* · Polysaccharides · SEM–EDX

1 Introduction

The field of functional food is currently attracting the attention of researchers, scientists, governments, politicians, and numerous global agencies to increase public understanding and use of these food items owing to their established health-promoting effects and low-toxicity concerns. Marine algae have been considered promising resources for new bioactive compounds in addition to their nutritional significance. It has been suggested that bioactive substances derived from marine species, such as algae, are more biologically effective than those derived from terrestrial sources (Safafar et al. 2015; El-Zokm et al. 2021). This is attributed to the higher bacterial populations in seawater than in air and the intense battle among marine algae and bacteria to survive in such an environment (Bixler and Bhushan 2012; Shannon and Abu-Ghannam 2016). In addition, algal biomass is naturally grown and collected from coastal regions in the sea and consider a sustainable, renewable and costless material that can be used as a source of valuable chemicals and biopolymers (Barakat et al. 2021; Hassan et al. 2021).

Pigments, phycobiliproteins, fatty acids, proteins, polysaccharides, vitamins, phenols and sterols are among the bioactive compounds produced by marine algae. These bioactive compounds have several applications, including

✉ Mostafa E. Elshobary
   mostafa_elshobary@science.tanta.edu.eg

   Khouloud M. Barakat
   kh2m2@yahoo.com

   Mona M. Ismail
   mona_es5@yahoo.com

   Hala E. Abou El Hassayeb
   dr.lola2010@yahoo.com

   Nermeen A. El Sersy
   nermeen_ok@yahoo.co.uk

1 National Institute of Oceanography and Fisheries, NIOF, Egypt

2 Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

antioxidant, antimicrobial (Elshobary et al. 2020; Osman et al. 2012), aquaculture (El-Khodary et al. 2020; Zaki et al. 2021; El-Sayed et al. 2022), anti-inflammatory (Sarithakumari et al. 2013), anticancer activities (Chen et al. 2019), plant biostimulants (Ashour et al. 2021; Hassan et al. 2021), immunostimulatory (Tabarsa et al. 2018) and biofuel (Abomohra and Elshobary, 2019; Huo et al. 2020; El Shafay et al. 2021; Elshobary et al. 2021). Polysaccharides are intriguing materials for creating innovative systems for bioapplications, such as drug delivery and tissue engineering, as they have multiple functionalities in their structures and have interesting physicochemical properties and considerable biological activity (Shelke et al. 2014; Tziveleka et al. 2019). Marine seaweeds produce large quantities of indigestible sulfated polysaccharides, such as agar, alginate, carrageenan, ulvan, fucoidan, laminarin, etc., that are considered safer and non-immunogenic (Venkatesan et al. 2016; El-Seesy et al. 2021; El-Sayed et al. 2021; Ismail and Amer 2021), which have been employed in the food industry and are utilized as colloidal materials for improving the rheological characteristics of food products (Yang et al. 2020).

Among the marine flora encountered in Egypt, some macroalgae can become invasive or proliferative and have profound adverse ecological impacts (Shabaka 2018; Barakat et al. 2021). Over the past few years, seaweeds have been observed in the Eastern Harbor, Alexandria, Egypt. Seaweeds are driven to the shore by local wind and tides, resulting in economic losses and the damage of coastal marine habitats. Among these seaweeds, green algae species are diverse and are valuable components of the human diet in many countries worldwide. Specifically, the genus *Ulva* (sea lettuce) is rich in carbohydrates, vitamins, essential amino acids, minerals and soluble dietary fiber when eaten fresh or cooked (Pereira 2011; Ismail and Mohamed 2017; El-Zokm et al. 2021). Ulvan is one of the main cell wall sulfated polysaccharides that contributes from 9 to 36% dry weight of the biomass of common seaweeds of Ulvaceae genera. Its carbohydrate structure is complex and diverse, with the main constituents being rhamnose, glucuronic and iduronic acids, as well as xylose (Kidgell et al. 2019). The incorporation of rhamnose, a rare sugar found in bacteria and plants, and its sulfate content and uronic acids, distinguishe ulvan from other algal sulfated polysaccharides, such as fucoid and carrageenans (Alves et al. 2013). These distinct structural properties, combined with the wide range of biological activities and tunable physicochemical properties, have recently received much interest to ulvan as a promising material for therapeutic applications, such as antioxidant, anticoagulant, immunomodulating, antifouling and antihyperlipidemic capabilities (Mo'o et al. 2020).

Observably, most of the biological properties of sulfated polysaccharides such as ulvan were attributed to the presence of sulfate groups (Liang et al. 2014; Ismail and Amer 2021). Occasionally, the chemical properties, structure, molecular weight and sulfate amount of such polysaccharides vary with algal species, and consequently, their biological activity; hence, their chemical characterization is significantly required (Fernando et al. 2017).

The difficulty in regulating the structural composition of extracted polysaccharides is one downside of ulvan. Indeed, the structural composition of ulvan is influenced by a number of factors, including the algal species' origin and harvest season. Thus, the current work was suggested to extract and fully characterize ulvan from the green macroalgae *Ulva fasciata* Delile and then examined as antioxidant and antimicrobial agents against pathogenic and antifouling bacteria, which are particularly problematic and more resistant to antibiotics and biocides as well as its potential as an anti-arthritic activity that was investigated for the first time.

2 Materials and methods

2.1 Sampling and identification of the green alga

Green alga was handpicked during spring 2020 from the submerged rocks and substrates in the Eastern Harbor shallow water, Alexandria, Egypt. The collected green alga was identified morphologically according to taxonomical reference guides (Aleem 1993) and confirmed with Algae Base website (Guiry and Guiry 2020). The alga was rinsed with tap water to remove impurities and then dried to a constant weight on absorbent paper at room temperature (25 ± 2 °C). The dry sample was crushed and electrically ground into a powder form and then stored in paper bags in dry places until further use.

2.2 Chemical composition of *U. fasciata* Delile

Humidity was considered as the mass loss from a sample (1 g) after drying at 100 ± 2 °C (Elektro Helios oven J84234-2, 200 °C, Stockholm, Sweden). The residual mass in this sample was heated at 600 ± 10 °C (Fornitec 0159, 1200 °C, São Paulo, Brazil) to obtain ash (Pádua et al. 2004). The crude protein content was estimated using the Kjeldahl method using the distiller-digester (Procion 110 V, Brazil) (Yokoyama and Guimarães 1975). For lipid estimation, five grams of the sample were extracted with ethyl ether in Soxhlet for 5 h. The solvent was evaporated, and the residual lipids were measured gravimetrically (Pádua et al. 2004). Fibers were estimated by digesting five grams of the sample with 200 mL of 5% HCl for 30 min. The homogenate was filtrated and washed with hot water. The residue was digested again with 200 mL of 5% NaOH under reflux for 30 min. The homogenate was filtered and washed with water until negative alkaline reaction via methyl red indicative paper.

The material was washed with 20 mL of ethyl alcohol followed by 20 mL of ethyl ether. The residue was dried at 100 °C for 2 h, and the residual fibers were calculated using the following equations, and the results were expressed as percentage odf dried seaweed (Pádua et al. 2004).

The residual fibers $= \frac{W_R}{W_S} \times 100$

where W_R and W_S were the weight of the collected residual and dried seaweed (5 g), respectively.

Carbohydrate was calculated using the phenol–sulfuric acid method (Masuko et al. 2005) modified by Elshobary et al. (2015).

2.3 Ulvan extraction

Twenty grams of seaweed powder were rinsed with 200 mL, 80% cold ethanol under continuous stirring overnight at room temperature (25 ± 2 °C) to remove phenolic, free sugars, amino acids, lipidic, and colored compounds. The mixture was centrifuged at 10 °C and $5000 \times g$ for 10 min (Heraeus, Labofuge200, Thermo Scientific, United States) and the supernatant was removed to obtain defatted algal biomass. The residue was treated with hexane to confirm the removal of colored compounds, and the colorless residue was dried at room temperature in the fume hood. The depigmented powder was mixed with distilled water (1: 20 W/V) and extracted at 85–90 °C in a closed reflux system for 20 h with continuous stirring (Yamamoto 1980). The supernatants were collected by centrifugation for 10 min at 10°C and $8000 \times g$. This process was repeated twice, and the supernatants were combined and concentrated by evaporation under reduced pressure at 60°C. The water extract was centrifuged, filtered and precipitated using 4 vol. of ethanol (99%) and kept at 4°C overnight. The precipitated polysaccharides were collected after centrifugation at 10°C and $8000 \times g$ for 10 min. The polysaccharides were washed and dehydrated with 99% ethanol and then dried at room temperature. To obtain highly purified ulvan, the dried polysaccharide was redissolved in distilled water, dialyzed against distilled water, and freeze-dried. The ulvan yield was calculated gravimetrically relative to the depigmented residue (Tako et al. 2015; Tabarsa et al. 2018).

2.4 Chemical composition of ulvan

2.4.1 Sulfated content

Total sulfate content within the ulvan was estimated following the modified $BaCl_2$ turbidimetric method (Kolmert et al. 2000). Fifty milligrams of ulvan was hydrolyzed with 3 mL of 1 N HCl at 105 °C for 2 h, and the solution was completed to 50 mL with distilled water. The hydrolyzed polysaccharide was added to 2.5 mL of conditioning reagent (conc. HCl, 95% ethanol, glycerol, water [3:0.3:1:3] with 0.75% NaCl) shaken for 1 min at room temperature. Then, 5 mL of $BaCl_2$ solution was added to the conditioned sample, stirred for 2 min with a magnetic stirrer, and the absorbance was recorded at 420 nm immediately (Schimatzu® UV-1800, Japan). The total sulfate content was expressed as a percentage based on a sodium sulfate standard.

2.4.2 UV–visible spectroscopy analysis

UV–VIS was used to demonstrate the success of the Ulvan polysaccharide purification step. At room temperature, 1 mg/mL of extracted ulvan was scanned with a UV–vis spectrophotometer in the 200 nm to 800 nm wavelength.

2.4.3 Infrared spectrum of ulvan from *U. fasciata* Delile

A Fourier-transform infrared spectrophotometer (FT-IR) was used to measure the polysaccharides' FT-IR spectrum (FTS-3000; Bio-Rad Laboratories Inc., CA, USA). The polysaccharide sample was mashed in a mortar with KBr powder (which had been baked in an oven at 100 °C to remove the water) and then pressed against a transparent sheet with a tablet press for FT-IR analysis in the frequency range of 4000–400 cm^{-1}.

2.4.4 Determination of monosaccharides composition by GC–MS

Identification of the monosaccharides in the extracted ulvan was done by converting them into sugar derivatives before GC–MS analysis. About 20 mg of extracted ulvan was hydrolyzed using 2 mol/L sulfuric acid at 105 °C for 10 h. After cooling, the hydrolysate was neutralized with barium carbonate to pH 7.0. The residue was discarded after centrifugation, while the supernatant was filtered through a 0.20 μm syringe filter, lyophilized, then the dried sample was dissolved in 50 μL methanol. A silyating mixture of pyridine–hexamethyldisilazane–trimethyl chlorosilane (9:3:1 v/v/v) was mixed with 50 μL per mg of dried sample. About 2 μL from the methyl–silyl sugar derivatives was injected into GC–MS (MassHunter GC–MS 1989–2014, Agilent Technologies, Inc, NRC-Egypt). The separation and detection of the formed silyl sugar derivatives were accomplished using a previously reported method: the column used was HP5MS (30 m × 0.25 mm × 0.25 μm), detector and injector temperatures were set at 320°C, column temperature was first set at 100 °C for 1 min, then increased from 100 to 260 °C at 4 °C for 1 min, then the temperature was set for 10 min at 260 °C. Helium was used as carrier gas at 1 mL/

min. The detected masses were identified by comparing with the standard masses in NIST-2014 library (Amornrut et al. 1999).

2.4.5 ^{1}H-nuclear magnetic resonance (NMR) spectroscopy

The spectra of ^{1}H-NMR were recorded on a JEOL Ltd, Japan 500 FT-NMR spectrometer at 500.00 and 125.65 MHz, respectively. The polysaccharide (2%, W/V) was dissolved in D_2O and measured at 37 or 60℃. The chemical shifts of ^{1}H-NMR were expressed in parts per million (ppm) relative to sodium 3-(trimethylsilyl) propionic-2,2,3,3-d, acid (TSP, 0.00 ppm) as an internal standard.

2.4.6 Morphological and elemental studies of the extracted ulvan by SEM–EDX spectroscopy

Morphological and elemental studies were carried out using an Auriga field emission scanning electron microscope (SEM), a completely digital 30 kV Hi Resolution SEM connected with EDX and a range of backscattered (BS) and secondary detectors peculiar to the instrument. The X-ray spectra were stored through the latest from the Oxford offering: Oxford-Advanced AZtecEnergy package with an SDD 127 eV (Khan et al. 2008).

The sample was prepared in a Class II biosafety cabinet. Ag foil (Sigma Aldrich, 0.01 mm, 99.9% trace metals basis) was used to load the sample for SEM measurement. This conducting material was cut 1 cm×1 cm and stacked with the SEM sample holder with double-sided tape. Numerous 10 μL spots of ulvan were poured onto Ag foil. The working distance and lens aperture sizes were 8 mm and 30 μm, respectively.

2.5 Antioxidant activity of ulvan

Antioxidant activity of the extracted ulvan was determined using two standard methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging (Viturro et al. 1999; Hassan et al. 2021) and Hydrogen peroxide radical scavenging assay (Gülçin et al. 2004). These assays were performed using different concentrations (5, 10, 20, 30, 40 and 50 μg/mL) of extracted ulvan, and α-tocopherol was used as a positive control for the antioxidant activity. Concentrations of the most promising extract resulting in 50% inhibition of DPPH radicals (IC_{50}) were calculated by GraphPad Prism 6 software.

2.6 Antimicrobial activity of ulvan from *U. faciata* Delile

The well-cut diffusion technique (Osman et al. 2012) was used to evaluate the antimicrobial activity of extracted ulvan

against 11 bacterial pathogens (*Bacillus subtlis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermids* ATCC, *Enterococcus faecalis* ATCC 29219, and *Listeria sp* ATCC., *Pseudomonas aeruginosa* ATCC 9027, *Klebsilla pneumonia* ATCC, *Escherichia coli* ATCC 8739 and *Bordetella sp.* ATCC), three fungal reference strains (*Aspergillus niger*, *Penicillum notatum*, and *Fusarum solani*) and one yeast species (*Candida albicans*). A sterilized nutrient agar medium was used for bacteria and Sabouraud dextrose agar for fungi and yeast. The pre-cultured tested pathogen was mixed in agar media, poured into three sterile Petri dish plates, and allowed to solidify. About 100 μL of ulvan extract was transferred into each well of 0.7 cm and subjected to 4 °C incubation for 2 h, and then later incubated at 37 °C for 24–48 h. The results were obtained by measuring the diameter of the inhibition zone three times for each well and expressed in millimeters. Furthermore, the absolute unit (AU) was calculated according to Ibrahim (2012) as follow:

$$AU = Y^2/X^2 \tag{1}$$

where Y is the diameter of the inhibition zone around the well, which its diameter is represented by X.

Moreover, antimicrobial activities of ulvan were recorded in the form of minimum inhibitory concentration (MIC) as recommended in the guide of standards of the CLSI (Clinical Laboratory Standardization Institute) (Schwalbe et al. 2007). MIC was determined, after incubation, by choosing the lowest inhibitory concentration of different ulvan concentrations according to the presence or absence of turbidity. The lower the MICs are, the higher the activity is.

2.7 In vitro anti-arthritic activity

0.05 mL of different concentrations of crude ulvan (0, 15, 30, 250, 500 and 1000 μg/mL) added to 0.45 mL of % W/V bovine serum albumin in aqueous solution and made up 0.5 mL of the reaction mixture. The pH was adjusted to 6.3 using 1 N HCl. The samples were heated for 3 min (57 °C) after incubating for 20 min at 37 °C. Allowed the sample to cool before adding 2.5 mL of phosphate buffer. At 416 nm, the turbidity generated was measured (Satoskar and Bhandarkar 2020). The percentage inhibition of protein denaturation was given by

$$\%\text{inhibition} = 1 - \left[\frac{\text{Abs Sample}}{\text{Abs control}} \right] \times 100 \tag{2}$$

Control indicates 100% protein denaturation. Diclofenac sodium was taken as standard.

Table 1 Chemical analysis of *U. fasciata* and their crude polysaccharides

	Parameter	Percentage (%)
U. fasciata contents	Moisture	16.60 ± 1.03
	Ash	23.57 ± 2.02
	Fibers	14.23 ± 0.23
	Carbohydrates	31.50 ± 1.91
	Proteins	12.66 ± 0.78
	Total lipids	5.44 ± 0.98
Purified polysaccharides	Total ulvan	43.66% of total carbohydrate
	Sulfate content	$20.45 \pm 1.98\%$

Data presented are mean $\pm$ standard error ($n=3$)

Fig. 1 UV–visible spectrum of extracted ulvan

2.8 Statistical analysis

The values of the different parameters were expressed as the mean ($n=3$). The statistical analyses were performed by SPSS statistical software program (version 23.0). These data were subjected to the analysis of the variance (ANOVA) by applying the general linear model option (Duncan's test) to determine significant differences among the samples at $p < 0.05$.
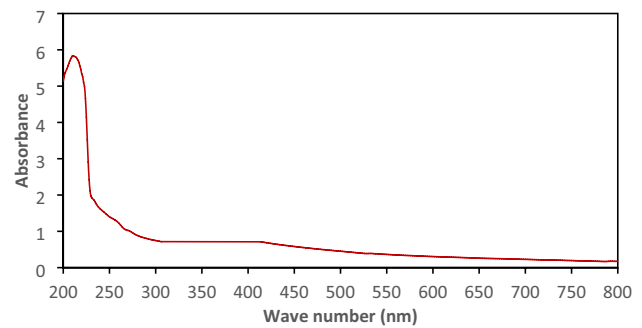
3 Results and discussion

3.1 Composition of *U. fasciata* Delile

The proximate chemical composition for the sample is summarized in Table 1. On a dry weight basis, carbohydrate 31.5% and ash 33.57% are the major contributors to the total biomass, followed by fiber 14.23% and protein 12.66%. This result agrees with that detected by El-Zokm et al. (2021) and Ismail and Mohamed (2017) for the same algal species. This result recommended this alga for use as a dietary supplement for humans and animals.

3.1.1 Characterization of the extracted ulvan

The total ulvan content after extraction relative to the *U. fasciata* dry weight was 43.66% of total carbohydrate (13.75 g/100 g DW). The sulfate content of ulvan was 20.45% of ulvan, reflecting the high content of the sulfate within the ulvan. In this context, ulvan is one of the major cell wall polysaccharides that contribute from 9 to 36%

dry weight of the biomass of *Ulva* species (Lahaye and Robic 2007). There are three polymers besides Ulvan in the *Ulva* cell wall: cellulose, glucuronan, and xyloglucan. Collectively, they present with ulvan up to 45% of the dry weight biomass (Lahaye and Robic 2007). Ulvan isolated from numerous Ulvales species have sulfate contents ranged from 14.3 to 23.2% (Thanh et al. 2016; Tabarsa et al. 2018; Tziveleka et al. 2019).

3.2 UV spectrum analysis

UV–visible spectroscopy is utilized to evaluate chromophore groups of atoms with strongly absorbing electronic transitions. Figure 1 depicts the UV–visible absorption spectrum of the ulavn tested in this study. The findings revealed that maximum absorption was measured between 200 and 220 nm. The wavelength area of 200–220 nm is frequently caused by $n-\sigma^*$ and/or $\pi-\pi^*$ transitions, which are reported in several functional groups, such as carboxyl, carbonyl, and ester of the polysaccharides. The results show ulvan had no obvious characteristic absorption peak at 260–280 nm, verifying that the extracted ulvan did not contain nucleic acid or protein, respectively (Trabelsi et al. 2009; Huo et al. 2022).

3.3 Structural characterization of sulfated polysaccharides by FTIR

The FTIR analysis of extracts showed typical polysaccharide absorption peaks containing carboxyl and sulfate groups (Fig. 2). The spectra are identical and comparable to the IR spectra of the sulfated polysaccharides obtained from ulvan of different *Ulva* species in the literature (Tako et al. 2015; Thanh et al. 2016; Tabarsa et al. 2018; Chi et al. 2020a). The FTIR spectra of the ulvan showed a wide and strong absorption band at 3375–3380 cm^{-1} corresponds to the stretch vibration of O–H in the polysaccharide resulting from intermolecular and intramolecular hydrogen bonding (Robic et al. 2009), the weak peak (shoulder band) at

Fig. 2 FTIR spectrum of the extracted ulvan
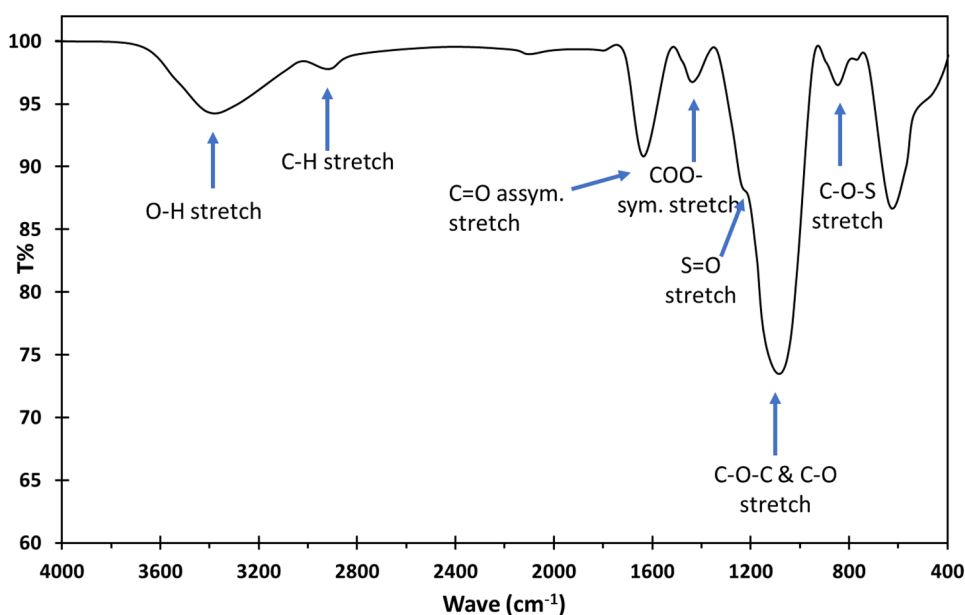
**Table 2** Monosaccharide (sugar) compositions of the tested ulvan

Peak no	Retinton time	Name	Area Sum %	Content %
1	11.04	L-Rhamnose	3.48	34.63
2	17.33	β-Arabinopyranose	1.66	16.52
3	21.02	L-Fucose	1.52	15.12
4	22.99	L-(+)-Rhamnopyranose	0.82	8.16
5	26.99	β-L-(-)-Fucopyranose	1.57	15.62
6	34.41	D-Mannopyranose	1.00	9.95

2925–2928 cm^{-1} is attributed to the stretch vibration of the aliphatic C–H of a methyl group and is characteristic of polysaccharides.

FTIR spectra of ulvans showed a maximum band at about 1076 cm^{-1}, which refers to the C−O stretching of the main rhamnose sugar. The sulfated nature of the polysaccharide is ascertained by the absorption band in the region 820–856 cm^{-1} which is related to stretching of axial sulfate groups related to the C–O–S group, as well as by the shoulder at 1225–1226 cm^{-1} which is assigned (ascribed) to the stretching vibration of the sulfate ester (S=O) group as confirmed by Castro et al. (2006) for ulvan from *U. rigida*, *U. pertusa* (Tako et al. 2015), *U. lactuca* (Thanh et al. 2016) and *U. clathrata* (Chi et al. 2020b). This result confirms that the ulvan from *U. fasciata* contains more than one type of sulfate group. Also, the band at the region 748–750 cm^{-1} was reported as a typical signature to sugar cycles such as ulvans (Robic et al. 2009; Hernández-Garibay et al. 2011).

3.4 Monosaccharides composition via GC–MS

Ulvan is a polysaccharide whose heterogeneous composition varies according to the algal biomass, taxonomic origin and
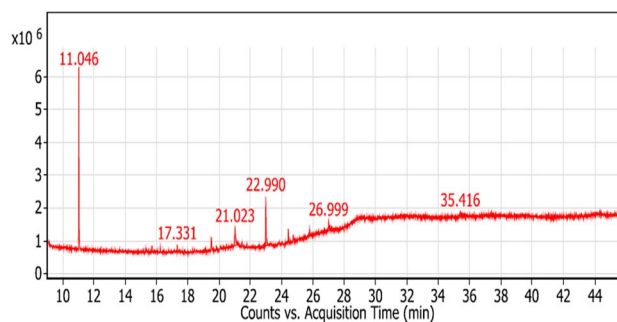
Fig. 3 GC chromatograms represent the sugar compositions for the extracted ulvan

harvesting season (Lahaye and Robic 2007). The difficulty to accurately define the composition of ulvan may be attributed to the complexity of its structure and the presence of several types of sugars (Alves et al. 2013; Sari-Chmayssem et al. 2019). Acid hydrolysis is the most effective method of depolymerizing the polysaccharides into their monomeric units, followed by their identification using the appropriate chromatographic technique (Costa et al. 2012; Elshobary et al. 2016). The monosaccharide composition of the

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Fig. 4. ^{1}HNMR spectra of the extracted ulvan
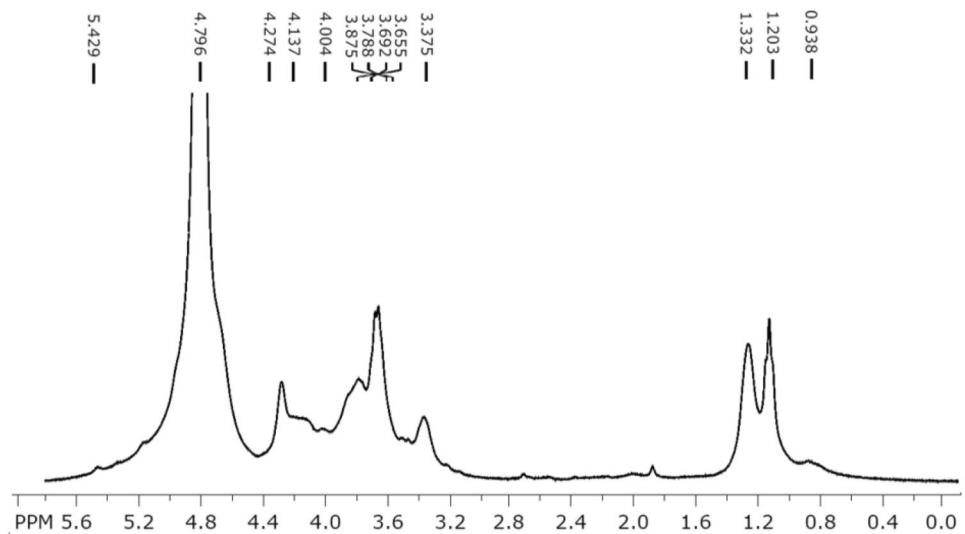
sulfated polysaccharides revealed that they might contain heterogeneous polymers due to the variation of monosaccharides. Furthermore, the polysaccharides also contain varying levels of sugars (Sari-Chmayssem et al. 2019).

The results are depicted in Table 2 and Fig. 3, revealing the presence of various monosaccharides with different contents in the order: L-rhamnose (34.63%) > β-arabinopyranose (16.25%) > L-fucose (15.12%) > rhamnopyranose (8.16%) > fucopyranose (15.62%) > mannopyranose (9.95%), while the contents of α-D-glactopyranose is neglected. The sulfated polysaccharide from *U. fasciata* was mainly composed of rhamnose (42.79%) of L-rhamnose, and L-(+)-rhamnopyranose and fucose (30.47%) of L-fucose and β-L-(-)-fucopyranose as major compositions, while arabinose and mannose were in minor contents. The composition of ulvan from *U. faciata* was similar to those of ulvan from other studies that demonstrated rhamnose, the main sugar of ulvan, and its content ranged from 12.73 to 45% (Tabarsa et al. 2018; Van Tran et al. 2018). Ulvan of *U. fasciata* lacks uronic acid sugars, such as glucuronic or galacturonic acids, which may be due to the substitution of uronic acids sugars with sulfated sugar residues (Yaich et al. 2014; de Freitas et al. 2015; Thanh et al. 2016).

3.5 Structural analysis of the extracted ulvan by ^{1}H NMR

Ulvan is a group of heteropolysaccharides composed mainly of rhamnose, xylose, glucose, and sulfate, with low contents of galactose, mannose, and arabinose (Yaich et al. 2017). It has been documented that ulvan possess complex and heterogeneous structures lacking regularity, and the existence of sulfate groups often interrupts the NMR determination of connectivity and branching. However, the assignment of most of the proton signals in

the present investigation was based on the chemical shifts reported for ulvans in literature (Tako et al. 2015; Yaich et al. 2017; Van Tran et al. 2018). ^{1}H-NMR spectrum for ulvan extract (Fig. 4) is comparable with previous data for ulvan. Thirteen chemical signals were observed as shown in Fig. 4. Signals at 5.43 and 4.79 ppm were assigned to be α-L-idulonic acid and sulfated α-L-rhamnose, respectively (Hernández-Garibay et al. 2011; Tako et al. 2015). The two intense peaks at 1.20 and 1.33 are assigned to the protons of the methyl groups of non-sulfated and sulfated α-L-rhamnosyl residues, respectively (Nakamura et al. 2011; Tako et al. 2015). The region 3.37–4.13 ppm is probably representative of ring protons (Thanh et al. 2016), and the hybrid nature of polysaccharides, rich in rhamnose and the presence of xylose, glucose, uronic acids and galactose as observed by the overlapped and broadened signals. The sulfated nature of the α-L-rhamnose was emphasized by the signals at 1.31 and 5.43 ppm as reported for *U. clathrata* (Chi et al. 2020b).

It was a hurdle to assign all the protons bound to the –OH groups in the region of 4.5–5.4 ppm because the signals are mostly superimposed by the peak of the deuterium oxide (D$_2$O; 4.78 ppm); however, the protons linked to other groups are allocated in the region of 3.4–3.6 ppm (Barcellos et al. 2018).

3.6 Morphological and elemental studies by SEM and EDX spectroscopy

The SEM micrographs demonstrated amorphous architecture (Fig. 5) with a high percentage of C, O, and S elements. Interestingly, the suggestion above of the sulfated nature of the polysaccharides was emphasized from the EDX analysis (Fig. 6; Table 3), which indicated that the
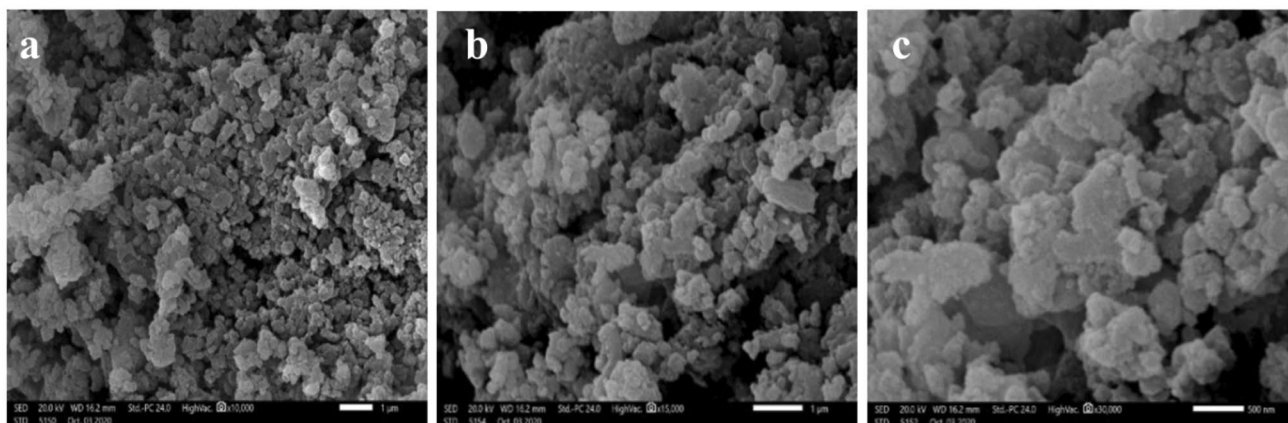
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Fig. 5 Scanning electron microscopy micrograph of the extracted ulvan at **a** 10^3, **b** 15^3 and **c** $30^3 \times$ magnification
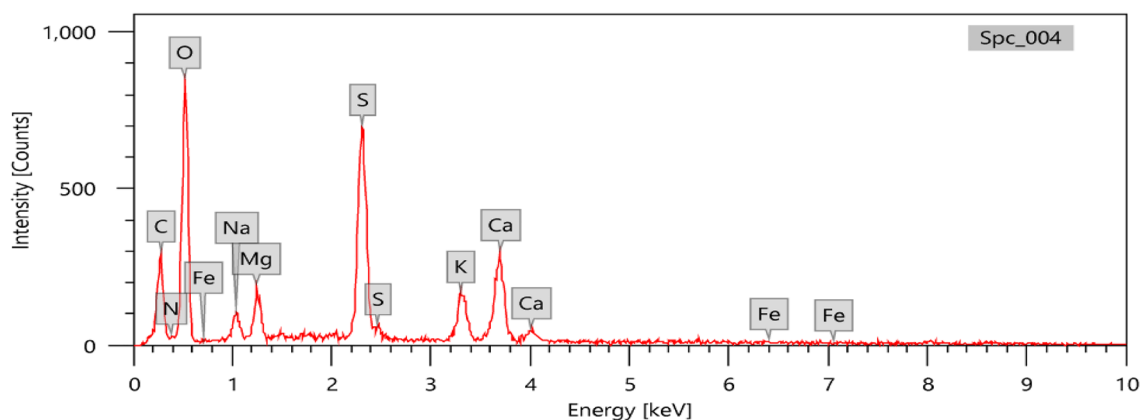

Fig. 6 SEM–EDX data of the produced ulvan

Table 3 Mass and atom percentage of the detected elements using SEM–EDX

Elements	Mass%	Atom%
C	25.53 ± 0.35	35.33 ± 048
N	1.21 ± 026	14.4 ± 0.31
O	48.97 ± 0.64	50.87 ± 0.67
Na	1.85 ± 0.11	1.33 ± 0.08
Mg	2.28 ± 0.10	1.56 ± 0.07
S	10.56 ± 0.17	5.47 ± 0.09
K	3.31 ± 0.11	1.41 ± 0.05
Ca	6.22 ± 0.16	2.58 ± 0.07
Fe	0.07 ± 0.04	0.02 ± 0.01
Total	100.00	100.00
		Fitting ratio 0.0494

presence of different elements, particularly the presence of some elements like C, N, O, S, Cl, Na, Ca, K, and Fe, ascertaining the existence of the sulfate group within the third order of mass % (10.56%) after oxygen (48.97%) and carbon (25.53%) (Table 3) in extracted ulvan as confirmed by the IR and ^{1}HNMR analyses. The amorphous architecture of the ulvan may be related to the presence of calcium-bound that was difficult to remove with ethanol precipitation method. This influences the particulate shape and aggregation in the SEM micrograph.

3.7 Antioxidant activity of the extracted ulvan

Several polysaccharides produced from seaweeds have shown superior antioxidant activities, and they could be used as prospective candidates for safe, stabilized, and efficacious natural antioxidants owing to their low toxicity and lesser side effects, which is a major issue with synthetic antioxidants (Kumar et al. 2008; Casas-Arrojo et al. 2021). The antioxidant activity of polysaccharides is dependent on different factors, including the type, purity, molecular weight, structure, sidechain, and attached groups (Sun et al. 2014; Ismail and Amer 2021). In the concentration range of
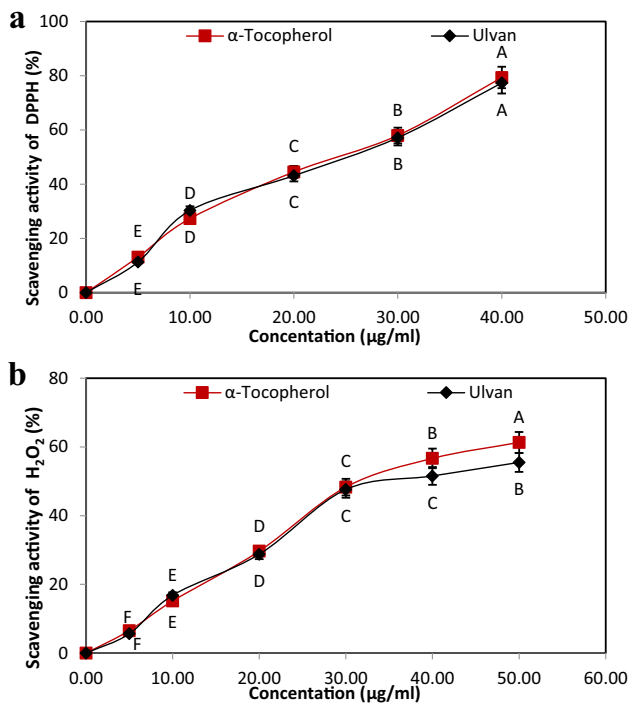
Fig. 7 Antioxidant activity of different concentrations of extracted ulvan against DPPH (**a**) and superoxide radicals (**b**). Data presented are mean ± standard error ($n = 3$). Different letters indicate significant differences at $p < 0.05$ using Duncan's test

0–50 μg/mL, the scavenging rate of H_2O_2 and DPPH free radicals increased significantly (ANOVA, $p = 0.012$ and 0.006, respectively) by increasing the concentration of ulvan. Ulvan as sulfate polysaccharide has a promising in vitro antioxidant property, with an inhibitory effect of 84.93% on DPPH and 55.5% on superoxide radicals compared to the standard α-tocopherol of 83.91 and 61.32%, respectively (Fig. 7). Yaich et al. (2017) reported that scavenging activity against DPPH of ulvan from *U. lactuca* was 75.0.2% at 400 μg/mL. In another study, ulvan from *U. intestinalis* showed 56.18% DPPH scavenger activity (Peasura et al. 2015). Compared to these results, the current ulvan exhibited greater scavenging activity toward DPPH radical than that recorded in *U. lactuca* (Yaich et al. 2017) or *U. intestinalis* (Peasura et al. 2015). Ulvan extracted from *U. fasciata* exhibited the greatest scavenging activity (IC50 = 25.26 μg/mL) toward DPPH radical less than α-tocopherol standard (IC50 = 25.91 μg/mL), which reflect its efficiency as antioxidant activity.

According to some studies, the key factors affecting the radical scavenging capacity of polysaccharides from marine algae are sulfate content, monosaccharide composition, and molecular weight (Li et al. 2013; Yaich et al. 2017). It was proposed that the sulfate group could activate the hydrogen atom of the anomeric carbon, thereby contributing to the polysaccharide's hydrogen-donating ability (Wang et al. 2010; Peasura et al. 2015). The high sulfate content

Table 4 Antimicrobial activity of the isolated ulvan

Tested bacteria		AU	MIC (μg/mL)
Gram-positive	*Bacillus subtilis*	ND	1025
	Staphylococcus epidermidis	1.7 ± 0.24	16
	Staphylococcus aureus	ND	1025
	Listeria sp.	2.1 ± 0.26	500
	Enterococcus faecalis	1.9 ± 0.14	16
Gram-negative	*Escherichia coli*	1.6 ± 0.09	32
	Pseudomonas aeruginosa	1.9 ± 0.04	16
	Klebsiella pneumonia	2.3 ± 0.28	8
	Bordetella sp.	ND	1025
Fungi	*Candida albicans*	2.1 ± 0.25	8
	Aspergillus niger	ND	1025
	Penicillium notatum	ND	500
	Fusarium solani	ND	1025

ND Not detected

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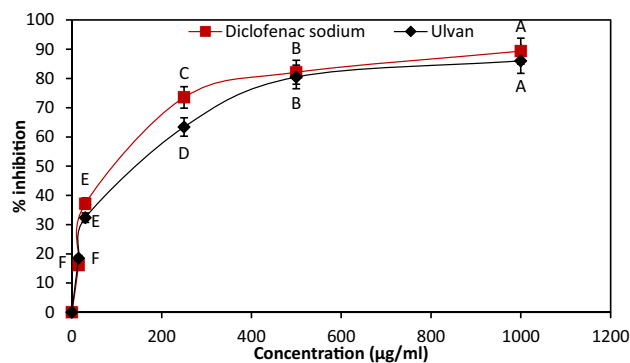

Fig. 8 Anti-arthritic activity of the extracted ulvan. Data presented are mean ± standard error ($n=3$). Different letters indicate significant differences at $p < 0.05$ using Duncan's test

of extracted ulvan (20%) could explain the higher recorded radical scavenging activity.

3.8 Antimicrobial activity of extracted ulvan

Clearly, the extracted ulvan exhibited antimicrobial activity against some Gram-positive and Gram-negative bacteria besides yeast *C. albicans*. Most of them are known as pathogenic and multi-resistant antifouling bacteria, such as the Gram-positive *E. faecalis*, *Staph. aureus* and *Staph. epidermidis*, and the Gram-negative *E. coli*, *K. pneumoniae* and *P. aeruginosa* (Donlan 2001; Shunmugaperumal 2010). The data presented in Table 4 showed that many bacterial pathogens were influenced by ulvan in low concentrations with AU ranging from 1.7 to 2.3; however, *B. subtilis Staph. aureus*, *Listeria* sp., and *Bordetella* sp. were inhibited in high concentrations of more than 1000 µg/mL. The MIC values ranged between 32 µg/mL against *E. coli* to 16 µg/mL against *E. faecalis*, and 8 µg/mL *C. albicans*. Moreover, weak suppression was detected against the tested fungal pathogens (*A. niger*, *P. notatum* and *F. solani*), even though many studies on the bioactivities of ulvan, such as antioxidant, anticoagulant, immunomodulatory, and anticancer activity, just a few studies on antimicrobial activities have been published (Van Tran et al. 2018). The sulfated polysaccharide extract of *U. armoricana* showed antibacterial activity against 42 Gram-positive and Gram-negative bacterial strains (Berri et al. 2016). However, the MIC of the most sensitive species (160–6250 µg/mL) was relatively higher than recorded in the current study. Ulvan isolated from the genus *U. reticulata* was efficacious against *E. coli*, *P. aeruginosa* and *E. cloace* (Van Tran et al. 2018). Differential antibacterial activity of ulvan could be due to their branching

architectures. However, aspects impacting ulvan's antibacterial action, such as molecular weight, the density of charged groups, and shape, must be investigated further. Noteworthy, the antibacterial activity of ulvan was primarily demonstrated by its ability to prevent the formation of biofilms on coated surfaces (Gadenne et al. 2013; Junter et al. 2016).

3.9 In vitro anti-arthritic activity

Denaturation of proteins and production of autoantigens is the main cause of rheumatoid arthritis (Shravan et al. 2011). Accordingly, inhibition of protein denaturation was examined using the extracted ulvan that showed potent inhibition of protein denaturation very close to the diclofenac sodium standard. In the concentration range of 0–1000 µg/mL, the anti-arthritic activity increased significantly (ANOVA, $p = 0.013$) by increasing the concentration of ulvan (Fig. 8). The maximum inhibition (86.04%) was recorded at 1 mg/mL with IC$_{50}$ of 43.21 µg/mL, which was insignificantly different from the diclofenac sodium standard. In this context, many studies have shown sulfated polysaccharides extracted from different seaweeds, such as *Gelidium pacificum* (Cui et al. 2019); *Corallina officinalis* and *Pterocladia capillacea* (Ismail and Amer 2021); *Ulva ohnoi* (Kidgell et al. 2020); *Ulva armoricana* (Berri et al. 2017), have anti-inflammatory properties that may be due to their inhibitory effects on the production of pro-inflammatory mediators (Berri et al. 2017; Kidgell et al. 2020). Based on these findings, Ulvan of *U. fasciata* is able to control the production of autoantigens and, as a result, inhibit protein denaturation.

4 Conclusion

It could be concluded that the use of the marine ulvan, sulfated heteropolysaccharide, from *Ulva fasciata* Delile has many potential biological activities which could be applied in both the biomedical field and food industry. First, chemical characterization was studied to assure the extracted ulvan structure using FTIR, GC–MS and ^{1}HNMR analysis and confirmed by SEM and EDX analysis to show the sulfated nature of polysaccharides due to the FTIR bands of the sulfate ester and sulfate groups, typical for ulvan. The extracted ulvan exhibited potent antioxidant activity reaching 84.93 and 55.5% against DPPH and superoxide radical scavenging, respectively. In addition, ulvan showed antimicrobial activity against microfoulers or/and pathogenic bacteria and fungi with MIC of 8 µg/mL against *K. pneumonia* and *C.*

albican, as well as anti-arthritic properties up to 86.04%. These results indicate that the extracted ulvan has a potential value for health and food applications.

Declarations

Conflict of interest The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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