Oceanologia 64 (2022) 117—130

Available online at www.sciencedirect.com

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ORIGINAL RESEARCH ARTICLE

# Chemical characterization and biochemical activity of polysaccharides isolated from Egyptian *Ulva fasciata* Delile

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Received 30 January 2021; accepted 30 September 2021
Available online 14 October 2021

**KEYWORDS**
Ulvan;
Chemical composition;
Biological activities;
FT-IR, $^1$H NMR

**Abstract** This study gives updated information on the isolation of ulvan from green alga *Ulva fasciata* Delile in Egypt through isolation and chemical characterization of sulfate polysaccharides by two sequential extraction steps using different solvents; distilled water, HCl and $Na_2EDTA$ forming fraction I (F-I). Fraction II (F-II) was obtained from remaining seaweeds using NaOH to give $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH. All products obtained were tested for their biological activities. The highest polysaccharides total extraction yield was 11.8% for water extract (F-I and F-II). The highest protein content was found in $F_{EDTA}$NaOH (2.44%). The highest sulfate content was recorded for F-I (HCl) (21.38%). Total carbohydrates range was 11.99—63.90% for F-I and 15.06—76.65% for F-II. Monosaccharides; galactose, rhamnose, and uronic acid were detected at all fractions, with concentrations varying from 0.11 to 1.34%, from 0.61 to 1.81% and from 11.06 to 19.30%, respectively. $^1$H NMR of F-II demonstrated the signals of ring and methyl protons of polysaccharide. The appearance of the stretching bands of the sulfate ester (C-O-S) and sulfate groups (S=O) in the FT-IR spectrum of $F_{HCl}$NaOH confirmed the presence of sulfated polysaccharides, typical of ulvan. The microbial species *Vibrio damsela* was the most susceptible to $F_{DW}$NaOH, followed by *Aeromonas hydrophila* and *Vibrio fluvialis* with inhibition zones of 30, 22, 22 mm at 150 mg/ml, respectively. $F_{DW}$NaOH was the most ef-

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Peer review under the responsibility of the Institute of Oceanology of the Polish Academy of Sciences.

Production and hosting by Elsevier

https://doi.org/10.1016/j.oceano.2021.09.008

fective fraction having antifouling property. The highest antioxidant activity was observed for F-$I_{HCl}$ followed by $F_{DW}$NaOH. At concentrations 25 and 50 mg/l, $F_{EDTA}$NaOH displayed the highest anti-inflammatory activity (94.0 and 91.40%, respectively).


## 1. Introduction

Macroalgae or seaweeds are highly diversified marine organisms, providing a great variety of metabolites and natural bioactive compounds with potential therapeutic agents (Smit, 2004). Marine algae are characterized by the presence of large amounts of polysaccharides (Murata and Nakazoe, 2001) which are natural compounds, some of them associated with several biological activities and potential health benefits, making them interesting potent for the application in pharmaceuticals, therapeutics, and regenerative medicine (De Jesus Raposo et al., 2015). They protect the human against tissue damage by reactive oxygen species (ROS) (Wijesekara et al., 2011). Furthermore, they include anticoagulant and/or antithrombotic properties, and are also good antidyslipidaemic and hypoglycaemic agents, and can be powerful antibiotics (De Jesus Raposo et al., 2015).

Ulvan molecule has been designated as being a sulfated polysaccharide composed of $\alpha$- and $\beta$-(1,4)- linked monosaccharides (rhamnose, xylose, glucuronic acid and iduronic acid) with characteristic repeating disaccharide units (Lahaye, 1998; Lahaye and Robic, 2007; Paradossi et al., 1999). Ulvan contributes from 9 to 36% dry weight of the biomass of *Ulva* spp. (Lahaye and Robic, 2007) with three other cell wall polysaccharides (cellulose, xyloglucan, and glucuronan) account for up to 45% of the dry weight biomass (Lahaye et al., 1997).

The structure of sulfated polysaccharide is responsible for its distinguishing properties (El-Baky et al., 2009). It can be successfully used in many applications such as antibacterial (Boisvert et al., 2015), antiviral (Jiao et al., 2012), anti-oxidant (Courtois, 2009), anti-cancer (El-Baky et al., 2009), anti-inflammatory (Faury et al., 2011) and immune-stimulating agent (Leiro et al., 2007). All these activities increased the awareness of sulfated polysaccharide importance in different domains over the world (Hernández-Garibay et al., 2010; Lahaye et al., 1999; Robic et al., 2009a; Tako et al., 2015) and applicability, in order to understand the full range of its capabilities and boost the industrial interest in green algae (Alves et al., 2013; Kidgell et al., 2019).

In Egypt, Matloub et al. (2013) carried out a study on the physico-chemical characterizations of water-soluble polysaccharides isolated from *Ulva fasciata* as a natural anti-hyperlipidemic agent, whereas Hussein et al. (2015) investigated the biological activity of ulvan extracted from *Ulva fasciata* and *Ulva lactuca*in affecting growth and metabolism of the microalga *Chlorella vulgaris*. Furthermore, Abou El Azm et al. (2019) carried out a study to obtain bioactive compounds from the aqueous high molecular weight sulfated polysaccharides isolated from *Ulva lactuca*.

The aim of the current study is to isolate and chemically characterize sulfated polysaccharide from *Ulva fasciata* Delile by two steps sequential extraction using distilled water (DW), HCl, and $Na_2$ EDTA to form fraction I (F-I), then preparing fraction II (F-II) from remaining seaweeds. Evaluation of these different extracts through testing their biological activities (antimicrobial, antifouling, antioxidant, and anti-inflammatory) is also a main goal for this study.

## 2. Material and methods

### 2.1. Sample collection

Samples of the green alga *U. fasciata* were collected during summer (2018) from the sub-littoral zone (0.5—1 m depth) in front of Scout club located in the Eastern Harbor, Alexandria, Egypt at 29°53.10′E longitude and 31°13.3′N latitude. The samples were washed with seawater *in situ* to remove the adhered sediments and impurities, separated in polyethylene bags and stored in an ice box, at temperature 4°C. At the laboratory, the alga was rinsed immediately with tap water to get rid of the remaining impurities and epiphytes. The species of *U. fasciata* Delile was identified according to Aleem (1993). It belongs to class Chlorophyceae, order Ulvales, family Ulvaceae.

### 2.2. Extraction of polysaccharides from *U. fasciata*

Before polysaccharides extraction, the algal samples were air-dried at room temperature of 25°C and homogenized with a grinder to a particle size from 0.3—0.5 mm. The powder was stored in plastic bags at 4°C for further analysis. A weight of 20 g seaweed powder was treated with 200 ml ethanol (80%) under constant stirring overnight at ambient temperature (20°C) to remove lipids, pigments and low molecular weight compounds (Tabarsa et al., 2018). The mixture was centrifuged at 10°C and 8000 rpm for 10 min and the supernatant was discarded. The residual was rinsed with acetone and dried at room temperature.

The de-pigmented powder was extracted with 200 ml (1:10w/v) of DW, or 0.2 N HCl or 0.1 M $Na_2$EDTA by stirring in a water bath for 2 h at 60°C. The mixture was centrifuged and the residues were re-extracted twice with additional 200 ml of the same media for 60 min. and centrifuged again. The different extracts were filtered and concentrated by evaporation under reduced pressure at 60°C. The extracts were precipitated by adding three volumes of ethanol then centrifuged to recover the precipitates. The precipitates were desalted two times for 30 min, each with 15 ml 70% ethanol and two times with full concentrated ethanol. The precipitate was dried at 60°C in the oven until constant weight and grounded into fine powder to ob-

tain fraction I, F-$I_{DW}$, F-$I_{HCl}$, and F-$I_{EDTA}$ from DW, HCl, and $Na_2$ EDTA, respectively, according to the simple method described by Hernández-Garibay et al. (2010). The fraction II ($F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH) was obtained sequentially from the remaining seaweeds of each of the above treatments by extraction with 200 ml 0.1 M NaOH at 60°C for 2 h (Hernández-Garibay et al., 2010). The polysaccharides in solution were recovered, as described above.

## 2.3. Chemical characterization of algal polysaccharides

A weight of 100 mg fine powder of F-I and F-II was suspended in a volume of 3 ml DW to prepare different concentrations of each fraction. All measurements of biochemical contents were conducted in duplicates.

### 2.3.1. Determination of protein content

The protein content was analyzed spectrophotometrically at 650 nm according to the method described by Lowry et al. (1951), using standard salt-free bovine serum albumin.

### 2.3.2. Determination of carbohydrates content

Total carbohydrates content was assayed by phenol-sulfuric acid method (Dubois et al., 1956).

### 2.3.3. Determination of sulfate content

Sulfate content was determined turbidimetrically after acid hydrolysis with 0.5 M HCl by barium chloride-gelation method using $K_2SO_4$ as a standard (Dodgson and Price, 1962).

### 2.3.4. Monosaccharaides composition of isolated polysaccharides

Polysaccharides fractions (0.1 g) were hydrolyzed with 50 ml trifluoro-acetic acid (10 M) at 80°C for 12 h. After filtration, neutral sugars in hydrolyzed samples were analyzed by Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Phenomenex®Rezex RCM-Monosaccharide column (300 mm × 7.8 mm) operated at 80°C with Refractive Index detector operated at 40°C. The uronic acids were quantified by using HPLC (Knauer, Germany) with Rezex® column for organic acids analysis (300 mm × 7.8 mm). At 214 nm, UV detector was set. Reference sugars (glucosamine, sucrose, glucose, galactose, mannose, rhamnose, fructose, galacturonic acid, glucuronic acid) were used to identify the chromatographic peaks.

## 2.4. Structural analysis

### 2.4.1. Determination of $^1$H NMR spectra

For $^1$H NMR spectra, approximately 5 mg of each of the $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH fractions were dissolved solely in deuterated water ($D_2O$), and the spectrum was acquired at ambient temperature (198.1 K) using FT-NMR spectrometer at 300 MHz using Bruker NMR spectroscopy.

### 2.4.2. Fourier transform infrared (FT-IR)

Fourier transform infrared (FT-IR) spectra of obtained fractions were acquired in transmission mode at wave numbers ranging from 400 $cm^{-1}$ to 4000 $cm^{-1}$ using FT-IR Spectrometer (Bruker Platinum ATR Vertex 70).

## 2.5. Bioactivity of polysaccharides fractions

### 2.5.1. Antimicrobial bioassay

Three gram-negative bacterial strains were examined as pathogens, namely *Aeromonas hydrophila*, *Vibrio damsela*, and *Vibrio fluvialis* from National Institute of Oceanography and Fisheries, Alexandria, Egypt (Microbiology Lab.).

A volume of 15 ml of the sterilized media (nutrient agar CM 3 Oxoid) for bacteria was poured into sterile capped test tubes and were allowed to cool in a water bath. A 0.5 ml of inocula ($10^8$ CFU for bacteria) was added, and finally poured onto a sterile Petri dish for solidification (Mtolera and Semesi, 1996).

The well-cut diffusion technique was used to evaluate the antimicrobial activity (El-Masry et al., 2000). In nutrient agar plates inoculated with the tested microorganisms, wells have been done using a sterile 0.7 cm cork borer. Different concentrations (35, 50, 65, 75, 112 and 150 mg/ml) of the extracted polysaccharides were transferred into each well. The experiments were performed in triplicate. All plates were subjected to 4°C incubation for 2 h. The plates were incubated latterly at 37°C for 24 h. The results were obtained by measuring the inhibition zone diameter for each well and expressed in millimeters.

### 2.5.2. Antifouling activity

A volume of 100 ml sterilized seawater (filtrated and then autoclaved) was mixed separately with each fraction of $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH (50, 100, and 150 mg/l) in a conical flask containing cover glass and then was supplied with the fouling bacteria *Escherichia coli*. The mixture was incubated overnight at 30°C. Afterwards, the cover glasses were dyed with crystal violet solution (0.4%) for 10 minutes, then washed with water, and dried at room temperature and checked under the microscope. One flask was prepared without any fractions and was kept as a control (Kumaran et al. 2011).

### 2.5.3. Total antioxidant capacity

The determination of total antioxidant capacity was also conducted spectrophotometrically on various fractions at 695 nm using phosphomolybdenum reagent according to the method described by Prieto et al. (1999). The antioxidant activity was expressed as L-ascorbic acid equivalents (μM AAE/g).

### 2.5.4. Anti-inflammatory activity

The anti-inflammatory activity was studied using the inhibition of albumin denaturation technique (Mizushima and Kobayashi, 1968; Sakat et al., 2010) with minor modifications. Two concentrations of each fraction of the extracted polysaccharides were prepared (25 and 50 mg/l). A volume of 2.25 ml of bovine serum albumin (BSA) (5%, w/v aqueous solution) was added to 0.25 ml of each fraction, control (without extract), and standards. Samples, control and standard solutions (Diclofenac sodium Voltaren® ampoule Novartis Pharma 1000 μg/ml) were adjusted to pH 5.5 using a small amount of 0.1 M HCl or 0.1 M NaOH. The solutions were incubated at 37°C for 30 min and then transferred to 70°C water bath for 10 min. Following incubation, the solutions were left to cool down at room temperature (25°C)

then 5 ml of phosphate buffer was added to the above solutions. The turbidity of the above solutions was measured by using a UV-Visible spectrophotometer at 660 nm (UNICO-UV Visible Spectrophotometer Model UV-2000 USA). The inhibition % was determined for protein denaturation as follows:

$$\text{Inhibition}\% = \left[\left\{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample/standard}}\right\}/\text{Abs}_{\text{control}}\right] \times 100 \quad (1)$$

The activity of each fraction was compared with three concentrations (100, 250 and 500 μg/ml) of the standard commercial anti-inflammatory agent 'Diclofenac sodium'.

#### 2.5.5. Statistical analysis

Three microbial species (*A. hydrophila*, *V. damsela* and *V. fluvialis*) were randomly distributed as main plots according to the split-split plot design. Six concentrations (35, 50, 65, 75, 112, and 150 mg/ml) occupied sub-plots and six fractions (F-$I_{DW}$, F-$I_{HCl}$, and F-$I_{EDTA}$, $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH) were assigned in the sub-sub plots. Statistical analyses of the experimental data were performed according to Gomez and Gomez (1984) by using the Statistical Analysis System (SAS, 2007) version 9.1.3. Comparison between treatment means was carried out by using the least significant differences at the 0.05 level of probability ($LSD_{0.05}$).

## 3. Results and discussion

### 3.1. Biochemical composition of different algal fraction

The yield and the chemical composition of crude polysaccharides fractions obtained from *U. fasciata* varied according to the extraction media (Table 1). The total extraction yield for polysaccharides (F-I+F-II) was found to be 11.81% (w/w of algae dry weight) for water extract which was higher than that of EDTA and HCl fractions being 10.03 and 9.72%, respectively. The yield of F-$I_{HCl}$ and F-$I_{EDTA}$ (2.38 and 3.29%) was lower than $F_{HCl}$NaOH and $F_{EDTA}$NaOH (7.34 and 6.74%), respectively, whereas the DW fractions showed the inverse pattern (Table 1). Polysaccharides extraction at pH<pKa decreases its solubility (Kidgell et al., 2019). This explains the decrease in F-$I_{HCl}$ yield % (2.38%). $F_{HCl}$NaOH and $F_{EDTA}$NaOH demonstrated high yield % (Table 1). This is attributed to an increase in solution solubility at pH levels greater than pka (Kidgell et al., 2019; Robic et al., 2009b). In this study, the total extraction yield (%) ranged from 9.72 to 11.81%, which was within the ranges reported by Lahaye and Robic (2007), Hernández-Garibay et al. (2010), and Wahlström et al. (2020). This variation in extraction yield may be due to environmental variation (Kidgell et al., 2019), seaweed species, and extraction procedures (Costa et al., 2010; Kaeffer et al., 1999).

The highest sulfate content was recorded for F-$I_{HCl}$ (21.38%) followed by F-$I_{EDTA}$ (17.13%), and F-$I_{DW}$ (14.92%), with respect to F-II; $F_{DW}$NaOH showed the highest sulfate content (15.03%) followed by $F_{HCl}$NaOH (12.73%), and $F_{EDTA}$NaOH (7.76%). The present study showed higher sulfate content in polysaccharides of F-$I_{HCl}$ than that of other

**Table 1** Biochemical composition of different algal fractions of *Ulva fasciata*.

| Fractions | | Yield | Soluble carbohydrates | Total carbohydrates | Proteins | Sulfate | Monosaccharides composition | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Glucose | Galactose | Rhamnose | Glucosamine | Uronic acid |
| | | % | | | | | | | | | |
| F-I | Water | 6.02 | 0.61 | 63.90 | 1.20 | 14.92 | ND | 0.11 | 0.76 | 51.79 | 11.23 |
| | HCl | 2.38 | 1.13 | 12.62 | 1.89 | 21.38 | 0.14 | 0.17 | 0.61 | ND | 11.70 |
| | EDTA | 3.29 | 0.75 | 11.99 | 0.33 | 17.13 | ND | 0.25 | 0.68 | ND | 11.06 |
| F-II | Water | 5.79 | 0.48 | 15.06 | 1.68 | 15.03 | ND | 0.59 | 1.81 | ND | 12.67 |
| | HCl | 7.34 | 0.42 | 21.91 | 1.30 | 12.73 | 0.76 | 0.65 | 1.20 | ND | 19.30 |
| | EDTA | 6.74 | 0.33 | 76.65 | 2.44 | 7.76 | ND | 1.34 | 1.77 | 58.30 | 15.23 |

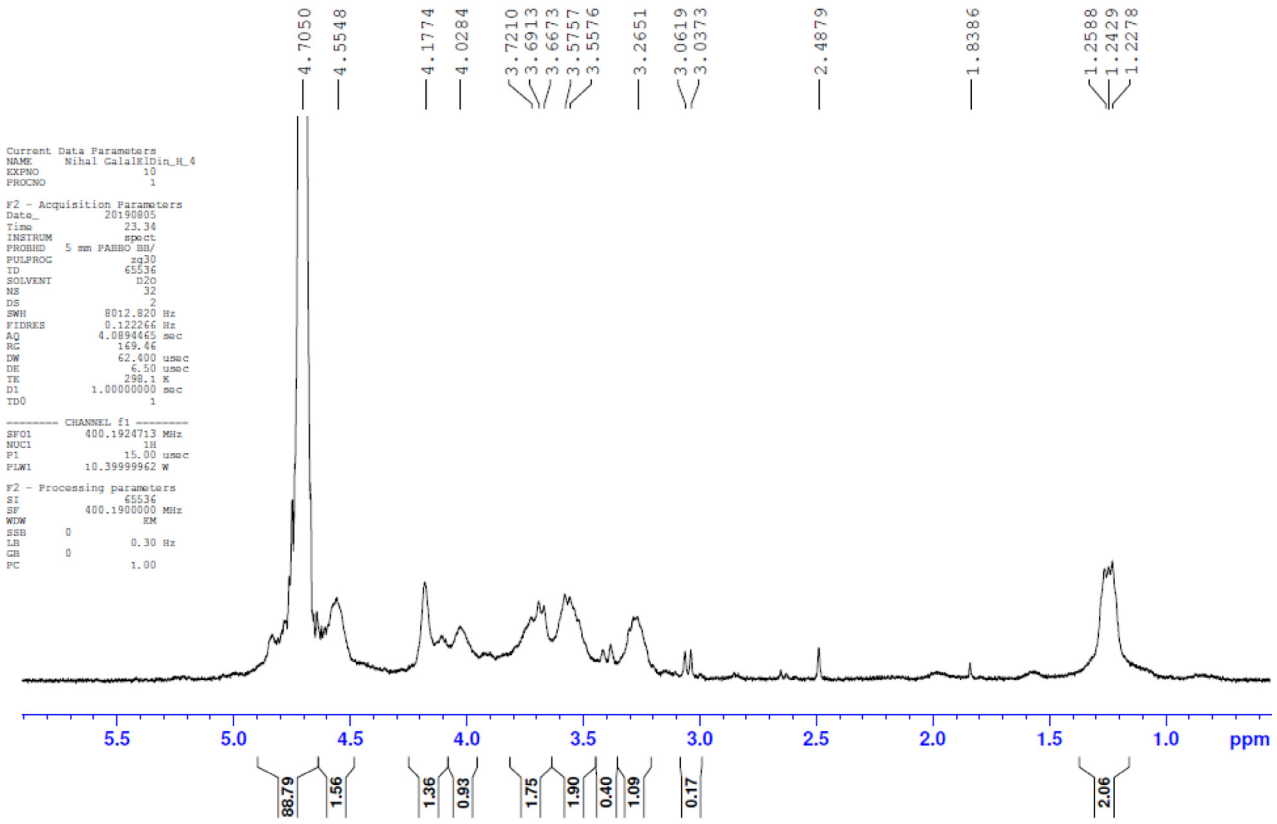


**Figure 1** $^{1}$H NMR spectrum of $F_{DW}$NaOH extract.

fractions while Wahlström et al. (2020) found that the extraction of polysaccharides using HCl resulted in low sulfate content. This variation may be caused by extraction conditions such as the acid concentration, temperature, and solid/liquid ratio.

The protein content did not differ significantly between F-I and F-II for water (1.20 and 1.68%) and HCl (1.89 and 1.30%) extracts, respectively while in case of $F_{EDTA}$NaOH contained a higher amount of protein content (2.44%) than F-I (0.33%). Since proteins were released by the breaking of hydrogen bridges in the alkaline solution, $F_{EDTA}$NaOH had higher protein content than the corresponding F-$I_{EDTA}$ (Robic et al., 2008). Proteins, in general, are an impurity that may occur in the extracts (Glasson et al., 2017).

The total carbohydrates range was 11.99—63.90% for F-I and 15.06—76.65% for F-II. F-II exhibited higher values of total carbohydrates than F-I, except for F-$I_{DW}$. The total carbohydrates content in $F_{EDTA}$NaOH was 76.65% followed by F-$I_{DW}$ (63.90%). In case of F-$I_{DW}$, extraction media resulted in a low salt content of algal biomass, which decreased polysaccharide aggregation properties, in addition to the exposure of cell wall components through osmotic shock, resulting in a high yield percent and total carbohydrates content (Kidgell et al., 2019).

In addition, monosaccharides; galactose, rhamnose, and uronic acids were detected at all fractions, with concentrations varying from 0.11 to 1.34%, from 0.61 to 1.81% and from 11.06 to 19.30%, respectively. Cell wall rigidity can be increased by the presence of large quantities of rhamnose-containing polysaccharides (Rashidi and Trindade, 2018). The presence of glucose and glucosamine below the detection limit was noticed in most fractions. The recorded concentrations for glucose were 0.14 and 0.76% in F-$I_{HCl}$ and $F_{HCl}$NaOH, respectively. According to Wahlström et al. (2020), polysaccharides extracted in acid media had higher glucose content. Glucosamine concentrations of 51.79 and 58.30% were indicated for F-$I_{DW}$ and $F_{EDTA}$NaOH, respectively. Glucosamine (an amino sugar) was reported by Rashidi and Trindade (2018) as a component of cell wall polysaccharides from Chlorophyta. In general, sequential extracts (F-II) using alkaline media showed higher monosaccharides content than F-I.

## 3.2. $^{1}$H NMR spectra of $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH extracts

$^{1}$H NMR spectra of $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH are shown in Figures 1, 2, 3. The methyl proton of the $\alpha$-L-rhamnosyl residues was observed in the range of 1.23—1.26 ppm. The signals of the ring protons appeared at the range of 3.28—4.18 ppm. O-acetyl group was detected in fractions $F_{DW}$NaOH and $F_{EDTA}$NaOH at 2.49 and 2.49 ppm, respectively.

The overlapping signals in the spectra of $^{1}$H NMR gave an indication for the complex form of polysaccharide. The signals of ring and methyl protons in the current study have been mentioned for polysaccharide from *Ulva* sp. by Hernández-Garibay et al. (2010) and Lahaye and Robic (2007). The observed O-acetyl group found in $F_{DW}$NaOH and $F_{EDTA}$NaOH may be considered as a part of the structure of polysaccharide or as an impurity (Monsur et al., 2017).

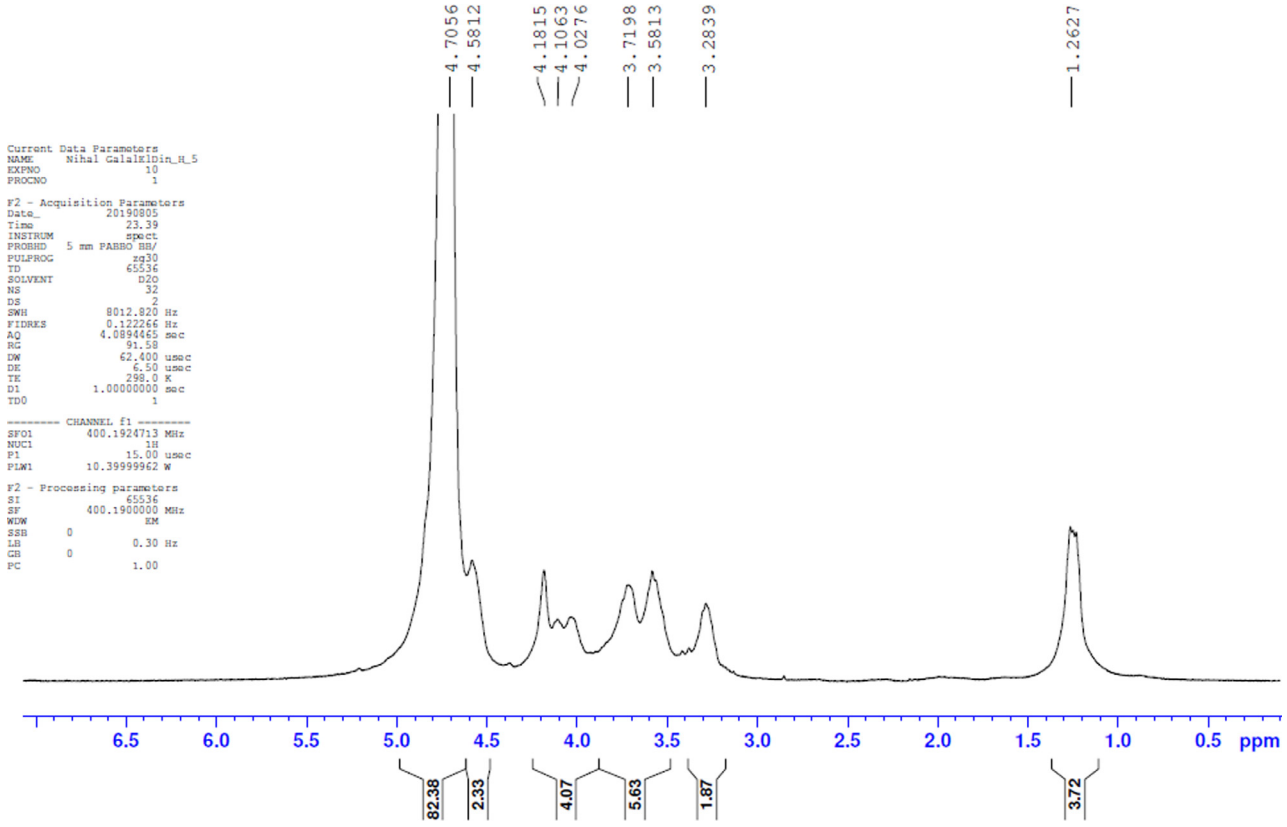


**Figure 2** $^{1}$H NMR spectrum of $F_{HCl}$NaOH extract.

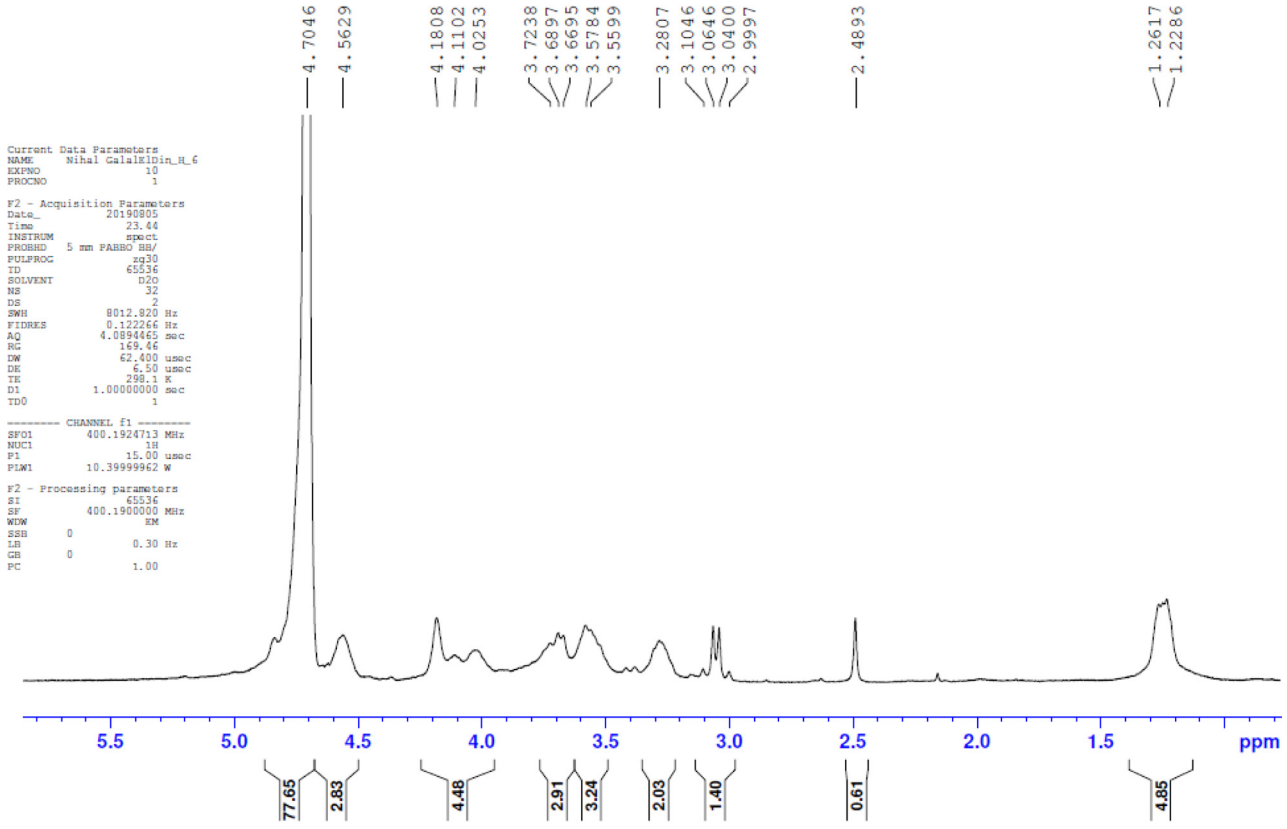


**Figure 3** $^{1}$H NMR spectrum of $F_{EDTA}$NaOH extract.

**Table 2** Assignments of infrared bands to the corresponding functional groups in different fractions of *Ulva fasciata*.

| Fractions | | Functional groups | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $cm^{-1}$ | | | | | | |
| | | O-H | C=O | C=O | C-O-S | C-O-C | Pyranose Ring &S=O | C-H |
| F-I | Water | 3197 | 1672 | - | - | 1054 | 981 | 603-427 |
| | HCl | 3399-3526 | 1620 | 1493-1451 | - | 1109 | 752 | 696-668-598 |
| | EDTA | 3335 | 1617 | 1397 | - | 1081 | - | 614-492 |
| F-II | Water | 3331 | 1669 | - | - | 1075 | - | 616-488 |
| | HCl | 3351 | 1629 | 1421 | 1217 | 1033 | 847-789 | 592 |
| | EDTA | 3347 | 1668 | - | - | 1080 | - | 634-611-497 |

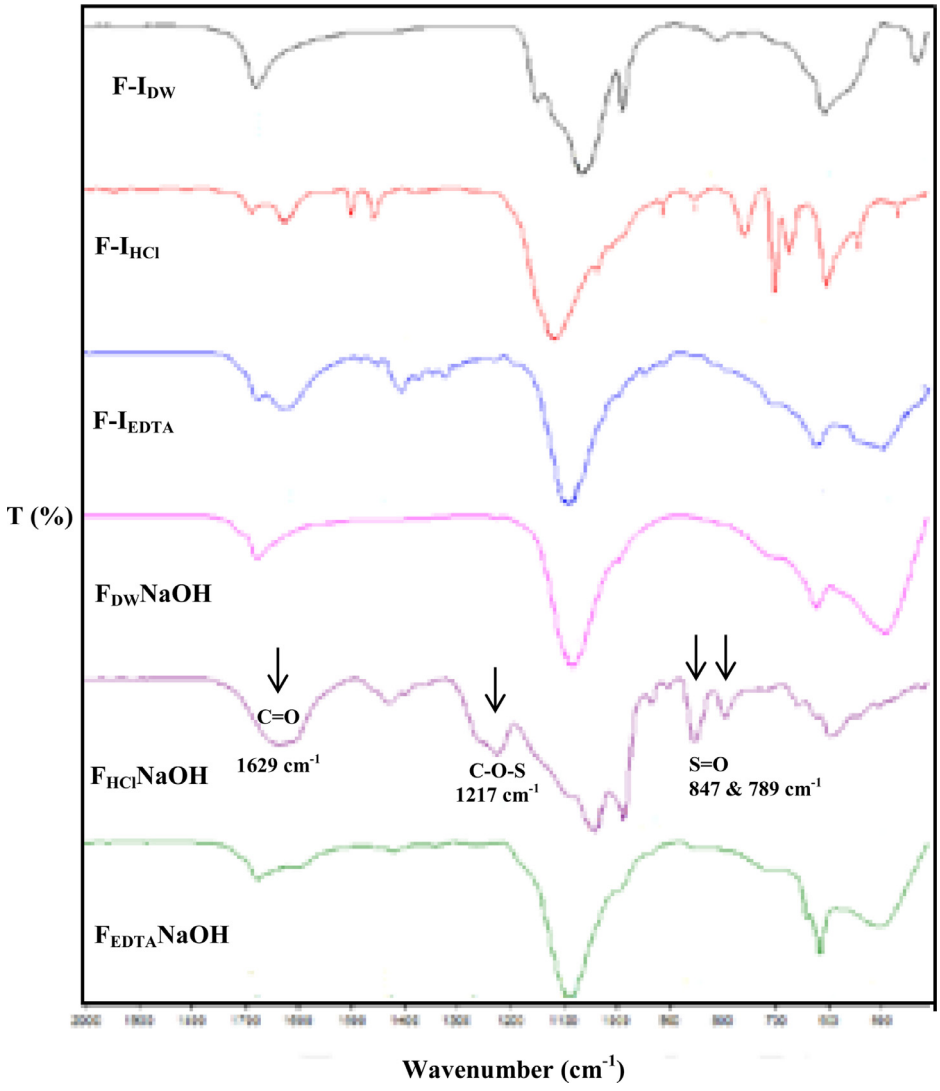


**Figure 4** FITR spectra in different fractions (F-I and F-II) using different extraction media.

### 3.3. FT-IR spectra for fractions I and II

The infrared spectra for F-I and F-II showed nearly similar signals, except for F-$I_{HCl}$ and $F_{HCl}$NaOH which were more complex (Table 2 and Figure 4). The broad signals in the range of 3526 to 3197 $cm^{-1}$ were attributed to the stretching vibrations of OH groups. The stretching bands that appeared at 1672 to 1617 $cm^{-1}$ were assigned to the carboxylate group (C=O). The other weak stretching bands of carboxylate group (C=O) appeared for F-$I_{HCl}$(1493—1451 $cm^{-1}$), F-$I_{EDTA}$ (1397 $cm^{-1}$), and $F_{HCl}$NaOH (1421 $cm^{-1}$) (Robic et al., 2009a; Yaich et al., 2017). The signal band shown at 1217 $cm^{-1}$ was attributed to the stretching band of the sulfate ester (C-O-S) in $F_{HCl}$NaOH (Wahlström et al. 2020). Two additional signals at 849 and 789 $cm^{-1}$ were pointed to sulfates (S=O) in the polysaccha-

ride (Hernández-Garibay et al., 2010; Pengzhan et al., 2003; Ray and Lahaye, 1995; Robic et al., 2009a).

The spectra showed strong signal bands at 1054, 1109, 1081, 1075, 1033, and 1080 $cm^{-1}$ in F-$1_{DW}$, F-$I_{HCl}$, F-$I_{EDTA}$, $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH, respectively, this may be due to the overlapping of C-OH side group and C-O-C glycosidic bond as well as the sugar ring.

The other bands in the range of 1000—750 $cm^{-1}$ related to sugar cycles (pyranose ring). The stretching bands (700—400 $cm^{-1}$) were assigned to C-H group in all fractions.

$F_{HCl}$NaOH showed better defined spectrum than those of other fractions (Figure 4). This may be due to the stretching bands of the sulfate ester (C-O-S) and sulfate groups (S=O) in the polysaccharide (Hernández-Garibay et al., 2010; Robic et al., 2009a) which indicates the presence of sulfated polysaccharides, typical for ulvan in this fraction (Li et al., 2018). Extraction at pH less than pKa plays a significant role in the selectivity of ulvan over other macromolecules. Moreover, the addition of HCl solution (0.1N) to the *U. fasciata* promotes ulvan release in the sequential extraction using NaOH. Similar observation was recorded by Hernández-Garibay et al. (2010).

In our study, sulfated polysaccharides consist mainly of rhamnose, sulfate groups, and uronic acid in $F_{HCl}$NaOH. Method of extraction, geographical distribution, maturity, environmental condition, and seasonality, may affect the amount of each monosaccharide residue or the arrangement of the polysaccharide, resulting in different ulvan structures (Alves et al., 2013; Lahaye and Robic, 2007).

### 3.4. Antimicrobial activity of polysaccharides

The results of the antimicrobial activity of polysaccharides showed significant differences based on the media of extraction (F-$I_{DW}$, F-$I_{HCl}$, F-$I_{EDTA}$, $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH) (Table 3 and 4). Currently, the microbial species *V. damsela* was the most susceptible to $F_{DW}$NaOH, with inhibition zone (30 mm) at a concentration of 112 and 150 mg/ml, followed by *A. hydrophila* and *V. fluvialis* both with inhibition zone (22 mm) at 150 mg/ml (Table 3). This was supported by the results of statistical analysis, where the effect of $F_{DW}$NaOH on *V. damsela*, *A. hydrophila*, and *V. fluvialis* showed the mean of 14.83, 14.61 and 7.83, respectively (Table 5). The efficiency of $F_{DW}$NaOH as solvent was supported by the mean of 12.42, whereas the mean of the ascending concentrations for all fractions (35, 50, 65, 75, 112 and 150 mg/ml) showed ascending increase mean from 1.72 to 2.88, 4.50, 10.33, 13.0 and 15.64, respectively (Table 5). The antibacterial activity of polysaccharides was promising, where all the fractions inhibited the three tested bacteria with a different degree. The fraction of $F_{DW}$NaOH was the most effective one for *V. damsela* followed by *A. hydrophila* and *V. fluvialis*. In contrast, Paulert et al. (2007) reported that polysaccharides extracted from *U. fasciata* did not show any antibacterial activity against different bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cerus*, *Micrococcus luteus*, *Xanthomonas campestris*, and *Erwinia carotovora*). This difference may be attributed to the different method for polysaccharides extraction as well as different bacterial strain. Despite of the same macroalgae species *U. fasciata*, the discrepancies between the results may be explained on

**Table 3** Inhibition zone (mm) of microbial growth by different concentrations of algal extracts from *Ulva fasciata*.

| Fractions | | *Aeromonas hydrophila* | | | | | | *Vibrio fluvialis* | | | | | | *Vibrio damsela* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fraction concentration (mg/ml) | | | | | | Fraction concentration (mg/ml) | | | | | | Fraction concentration (mg/ml) | | | | | |
| | | 35 | 50 | 65 | 75 | 112 | 150 | 35 | 50 | 65 | 75 | 112 | 150 | 35 | 50 | 65 | 75 | 112 | 150 |
| F-I | Water | 10 | 10 | 11 | 12 | 13 | 13 | 0 | 0 | 0 | 15 | 17 | 18 | 0 | 0 | 0 | 10 | 12 | 13 |
| | HCl | 0 | 10 | 10 | 12 | 13 | 14 | 0 | 0 | 12 | 9 | 9 | 12 | 0 | 0 | 0 | 14 | 15 | 15 |
| | EDTA | 0 | 0 | 0 | 13 | 15 | 20 | 0 | 0 | 0 | 13 | 14 | 15 | 0 | 0 | 12 | 13 | 14 | 15 |
| F-II | Water | 12 | 13 | 14 | 15 | 15 | 22 | 0 | 0 | 0 | 12 | 13 | 22 | 0 | 9 | 10 | 10 | 30 | 30 |
| | HCl | 9 | 10 | 12 | 13 | 13 | 14 | 0 | 0 | 0 | 13 | 15 | 17 | 0 | 0 | 0 | 0 | 14 | 15 |
| | EDTA | 0 | 0 | 0 | 12 | 12 | 12 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 9 |

**Table 4** Mean squares of antimicrobial activity of the extracted Ulvan in different media as affected by type of fraction, concentration, microbial species, and their interactions.

| Source of variations | Degrees of freedom (df) | Mean squares |
|---|---|---|
| Replica | 2 | 110.206 |
| Microbial species (A) | 2 | 434.410** |
| Error of A | 4 | 3.095 |
| Concentration (B) | 5 | 1800.588** |
| Interaction between A and B | 10 | 60.254** |
| Error of B | 30 | 3.910** |
| Fraction (C) | 5 | 484.943** |
| Interaction between A and C | 10 | 80.610** |
| Interaction between B and C | 25 | 40.761** |
| Interaction between A, B, and C | 50 | 52.361** |
| Interaction between E and C | 180 | 1.236 |

** The value is highly significant at 0.01 probability level.

**Table 5** Means of antimicrobial activity of the extracted Ulvan in different media as affected by type of fraction, concentration, microbial species, and their interactions.

| Microbial species | Conc. | Fractions | | | | | | Microbial *Conc. | Microbial | Conc. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F-I DW | F-I HCl | F-I EDTA | F-II DW | F-II HCl | F-II EDTA | | | |
| *Aeromonas hydrophila* | 35 | 10 | 0 | 0 | 12 | 9 | 0 | $5.16^{e}$ | | |
| | 50 | 10 | 10 | 0 | 13 | 10 | 0 | $7.16^{d}$ | | |
| | 65 | 11 | 10 | 0 | 14 | 12 | 0 | $7.83^{d}$ | | |
| | 75 | 12 | 12 | 13 | 15 | 13 | 12 | $12.83^{b}$ | | |
| | 112 | 13 | 13 | 15 | 15 | 13 | 12 | $13.50^{b}$ | | |
| | 150 | 13 | 14 | 20 | $22^{b}$ | 14 | 12 | $15.27^{ab}$ | | |
| *Aeromonus** fraction | | $11.50^{b}$ | $9.83^{c}$ | $8.0^{ef}$ | $14.61^{a}$ | $11.83^{b}$ | $6.0^{h}$ | | $10.29^{a}$ | |
| *Vibrio fluvialis* | 35 | 0 | 0 | 0 | 0 | 0 | 0 | $0^{h}$ | | |
| | 50 | 0 | 0 | 0 | 0 | 0 | 0 | $0^{h}$ | | |
| | 65 | 0 | 12 | 0 | 0 | 0 | 0 | $2.0^{g}$ | | |
| | 75 | 15 | 9 | 13 | 12 | 13 | 0 | $10.33^{c}$ | | |
| | 112 | 17 | 9 | 14 | 13 | 15 | 0 | 11.33c | | |
| | 150 | 18 | 12 | 15 | $22^{b}$ | 17 | 9 | 15.5 | | |
| *Vibrio** fraction | | $8.33^{e}$ | $7.0^{g}$ | $7.0^{g}$ | $7.83^{f}$ | $7.50^{fg}$ | $1.50^{j}$ | | $6.52^{c}$ | |
| *Vibrio damsela* | 35 | 0 | 0 | 0 | 0 | 0 | 0 | $0^{h}$ | | |
| | 50 | 0 | 0 | 0 | 9 | 0 | 0 | $1.50^{g}$ | | |
| | 65 | 0 | 0 | 12 | 10 | 0 | 0 | $3.66^{f}$ | | |
| | 75 | 10 | 14 | 13 | 10 | 0 | 0 | $7.83^{d}$ | | |
| | 112 | 12 | 15 | 14 | $30^{a}$ | 14 | 0 | $14.16^{b}$ | | |
| | 150 | 13 | 15 | 15 | $30^{a}$ | 15 | 9 | $16.16^{a}$ | | |
| *Vibrio** fraction | | $5.83^{h}$ | $7.33^{g}$ | $9.0^{d}$ | $14.83^{a}$ | $4.83^{i}$ | $1.50^{j}$ | | $7.22^{b}$ | |
| Con* fraction | | | | | | | | | | |
| 35 | | $3.33^{j}$ | $0^{k}$ | $0^{k}$ | $4.0^{j}$ | $3.0^{j}$ | $0^{k}$ | | | $1.72^{f}$ |
| 50 | | $3.33^{j}$ | $3.33^{j}$ | $0^{k}$ | $7.33^{i}$ | $3.33^{j}$ | $0^{k}$ | | | $2.88^{e}$ |
| 65 | | $3.66^{j}$ | $7.33^{i}$ | $4.0^{j}$ | $8.0^{h}$ | $4.0^{j}$ | $0^{k}$ | | | $4.50^{d}$ |
| 75 | | $12.33^{f}$ | $11.66^{f}$ | $13.0^{ef}$ | $12.33^{f}$ | $8.66^{h}$ | $4.0^{j}$ | | | $10.33^{c}$ |
| 112 | | $14.0^{e}$ | $12.33^{f}$ | $14.33^{de}$ | $19.33^{b}$ | $14.0^{e}$ | $4.0^{j}$ | | | $13.0^{b}$ |
| 150 | | $14.66^{de}$ | $13.66^{e}$ | $16.66^{c}$ | $23.55^{a}$ | $15.33^{d}$ | $10.0^{g}$ | | | $15.64^{a}$ |
| Fraction | | $8.55^{b}$ | $8.05^{c}$ | $8.0^{c}$ | $12.42^{a}$ | $8.05^{c}$ | $3.0^{d}$ | | | |

**Note:** $LSD_{0.05}$: between microbial spp. = 0.66; between fractions = 0.42; between concentrations = 0.77; between microbial spp. × fraction = 0.46; between microbial spp. × concentration = 1.34; between concentration × fraction = 1.03; between microbial spp. × fraction × concentration = 1.79

Means followed by the same letter (s) are statistically equal according to $LSD_{0.05}$ values.

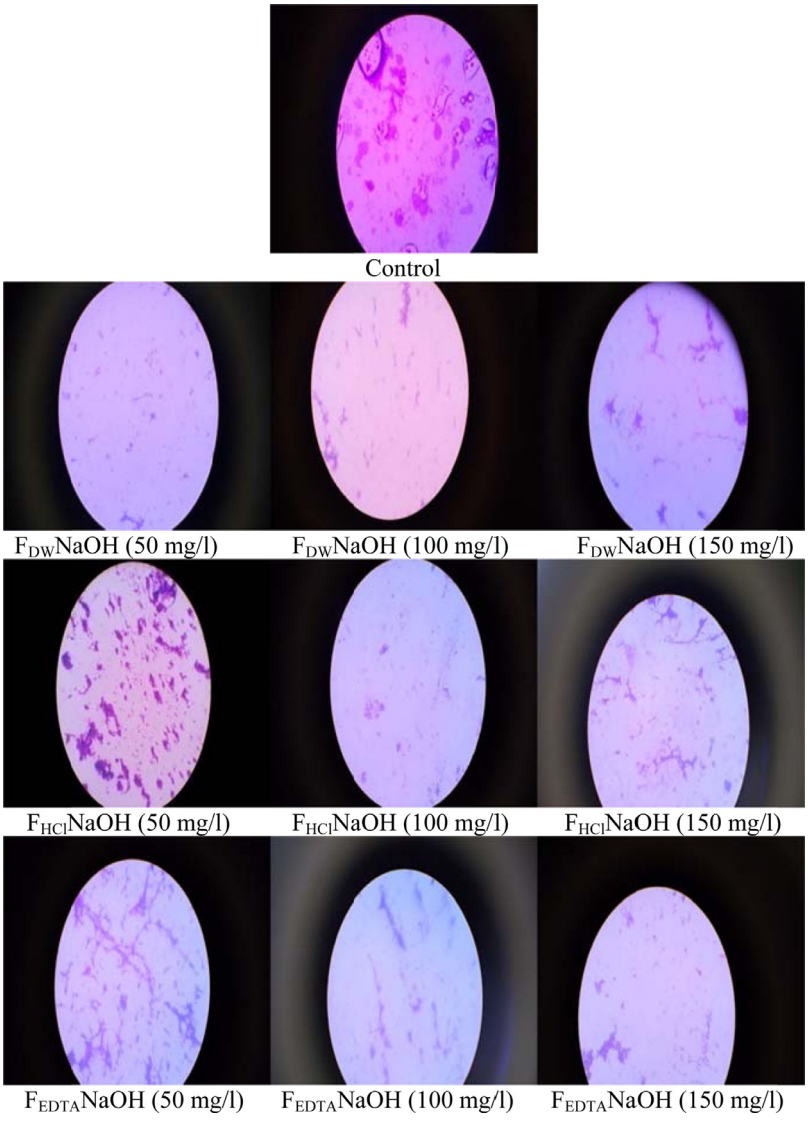


**Figure 5** Antifouling activity at different concentrations of polysaccharides for $F_{DW}$NaOH, $F_{HCl}$NaOH, and $F_{EDTA}$NaOH extracts using *Escherichia coli* as fouling microorganism.

the basis of the strong influence of environmental factors on different metabolites produced by algae (Selvin and Lipton, 2004). Species of Vibrios are pathogenic to human and marine organisms (micro flora, fish, shellfish, and penaeid shrimp). Vibrios also cause food borne diseases as well as the death of farmed fish species, leading to significant economic losses (Damsgard et al., 2004; FDA, 1992). Moreover, *A. hydrophila* is beta-haemolytic bacteria and produced cytotoxins (Scoglio et al., 2001).

### 3.5. Antifouling activity of polysaccharides

The results of polysaccharides as an antifouling agent showed that $F_{DW}$NaOH in descending concentration order (150, 100, 50 mg/l) was the most effective fraction in inhibiting the formation of a bacterial film on the cover glass, which is the first step of the biofouling process compared with the control. On the other hand, the other two fractions ($F_{HCl}$NaOH, and $F_{EDTA}$NaOH) showed negligible antifouling activity (Figure 5). Marine macroalgae could be an interesting antifouling agent, since they are an untapped source of bioactive compounds (Plouguerné et al., 2014), particularly polysaccharides (Arciola et al., 2003; Capo et al., 2009; Morra, 2005).

Gadenne et al. (2013) performed an adhesion assay by applying sulfated polysaccharides, ulvan against *Pseudomonas aeruginosa* which showed that immobilized polysaccharides on titanium surface strongly decreased by about 90% the adhesion of this microorganism. Furthermore, Gadenne et al. (2013) tested three pre-treated polysaccharides against *Staphyloccoccus aureus*. The better antifouling surface was that of a desulfated ulvan, showing that the absence of the sulfate group discouraged the bacterial adhesion. The current results agreed with these findings, since the $F_{DW}$NaOH was the most effective fraction in inhibiting the formation of a bacterial film which confirmed the previous mentioned antimicrobial activity. In general, the molecular weight, charge density, degree of sulfate content, properties of structure and conformation affect the antimicro-

**Table 6** Antioxidant capacity and anti-inflammatory activity of different algal extracts from *Ulva fasciata*.

| Fractions | | Antioxidant capacity (μM AAE/g) | Anti-inflammatory activity (%) | |
|---|---|---|---|---|
| | | | 25 mg/ml | 50 mg/ml |
| F-I | Water | 24.8 | 38.4 | 53.2 |
| | HCl | 63.3 | 71.4 | 86.3 |
| | EDTA | 34.4 | 66.3 | * |
| F-II | Water | 48.6 | 62.9 | 38.8 |
| | HCl | 27.5 | 85.4 | 88.5 |
| | EDTA | 26.5 | 94.0 | 91.4 |

bial activity of polysaccharides from seaweed (Silva et al., 2020).

### 3.6. Antioxidant activity of polysaccharides

The investigation of the antioxidant capacity of polysaccharide fractions showed that F-$I_{HCl}$ was the best one (63.3 μmole AAE/g), followed by $F_{DW}$NaOH being 48.6 μmole AAE/g, while F-$I_{DW}$ was the lowest one (24.8 μmole AAE/g) (Table 6). Previous studies have reported that the strong antioxidant activity of sulfated polysaccharides correlated to the degree substitution of sulfate groups along the polymeric backbone (Massironi et al., 2019). The antioxidant activity results agreed with those obtained by Huimin et al. (2005), who found that the high sulfate content of polysaccharides had a better antioxidant effect. F-$I_{HCl}$ and $F_{DW}$NaOH showed the best results which had the highest sulfate contents (21.38 and 15.03% for F-I and F-II, respectively) (Table 1). The least antioxidant capacity was noticed in F-$I_{DW}$ with respect to F-I as it had the least sulfate content (14.92%) (Table 1). Several studies concluded that the antioxidant activity was directly proportional to the reduction potential of polysaccharides that depends on the molecular weight, the type of sugar, the glycoside bond, the degree of sulfation and site of acetylation (Del Olmo et al., 2018; Guedes et al., 2013; Kellogg and Lila, 2013; Kosanić et al., 2015; Raja et al., 2016; Wang et al., 2008).

### 3.7. Anti-inflammatory activity of polysaccharides

The anti-inflammatory activity of the polysaccharides fractions showed great variations. The increase in the anti-inflammatory activity with the higher concentration was the general trend. The $F_{EDTA}$NaOH demonstrated the highest activity at both concentrations 25 and 50 mg/l being 94.0 and 91.40%, followed by $F_{HCl}$NaOH being 85.4 and 88.5%, respectively (Table 6). Null activity was observed in F-$I_{EDTA}$ at 50 mg/ml. The concentration of standard anti-inflammatory agent diclofenac sodium at 100 μg/ml was not effective, whereas anti-inflammatory activity at 250 and 500 μg/ml were equivalent to 75.71% and 88.97%, respectively. Inflammation is caused by the release of chemicals from tissues and cells that migrate throughout the organism (Faradila et al., 2020). Polysaccharides from macroalgae possess anti-inflammatory properties as reported by De Jesus Raposo et al. (2014). $F_{EDTA}$NaOH recorded the highest anti-inflammatory activities followed by $F_{HCl}$NaOH. The highest anti-inflammatory activity of $F_{EDTA}$NaOH may be due to the high content of protein, total carbohydrates, galactose, and glucosamine (Table 1).

## 4. Conclusion

This study is considered as updated information in Egypt on the isolation of sulfate polysaccharides from green alga *U. fasciata*. The spectrum of $^1$H NMR of (F-II) demonstrated the ring and methyl protons signals of polysaccharides. $F_{HCl}$NaOH showed well-defined spectrum than those of other fractions due to the stretching bands of the sulfate ester (C-O-S) and sulfate groups (S=O) which indicates the presence of sulfated polysaccharides, typical for ulvan. In our study, sulfated polysaccharides consist mainly of rhamnose, sulfate groups, and uronic acid in $F_{HCl}$NaOH. The microbial species *Vibrio damsela* was the most susceptible to $F_{DW}$NaOH, followed by *Aeromonas hydrophila* and *Vibrio fluvialis* with inhibition zones of 30, 22, 22 mm at 150 mg/ml, respectively. In addition, $F_{DW}$NaOH was the most effective fraction possessing antifouling activity. F-$I_{HCl}$ and $F_{DW}$NaOH showed the best results for antioxidant activity which had the highest sulfate contents (21.38 and 15.03% for F-I and F-II, respectively). The $F_{EDTA}$NaOH demonstrated the highest anti-inflammatory activity at both concentrations 25 and 50 mg/l being 94.0 and 91.40%, respectively.

## Acknowledgement

The authors are grateful to the Marine Environment Division and the National Institute of Oceanography and Fisheries for the support.

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