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# Isolation, characterization and antimicrobial potential of Ulvan from abundant green marine macroalgae, Egypt

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## Abstract

Ulvan, a sulfated polysaccharide with distinctive structural properties and promising biological applications, was extracted from various abundant marine macroalgae (*Ulva fasciata*, *Caulerpa racemosa*, and *Halimeda tuna*) which were collected from Port Said on the Mediterranean Sea and Hurghada on the Red Sea, Egypt. The ultrasonic assisted extraction (UAE) method proved to be the most efficient method for ulvan extraction compared to acid extraction (AE) and hot water extraction (HWE) methods. *U. fasciata* exhibited the highest ulvan yield, as well as the highest molecular weight ($120x10^5$ g/mol). Ash content was relatively low for all species, but there was a notable difference in moisture content and pH. Principal component analysis (PCA) revealed that ulvan showed a positive correlation with pH and ash content. Ulvan obtained from *U. fasciata* possessed superior physical properties, including higher specific and reduced viscosity (0.57 and 4.43 dL/g, respectively). FTIR and TGA analysis confirmed the presence of characteristic functional groups associated with ulvan. Moreover, the extracted ulvan demonstrated significant antimicrobial activity against several pathogenic microorganisms, highlighting its potential for various applications in the pharmaceutical and food industries.

**Keywords:** Ulvan; Green macroalgae; Extraction; FTIR; TGA; viscosity; molecular weight; Antimicrobial activity

## 1. Introduction

The potential of green macroalgae as a source for biomaterials and chemicals is being realized through ongoing advancements in bioprocessing [1]. The unique chemical composition of green seaweeds offers immense potential for developing environmentally sustainable chemical and fuel industries [2] .

Ulvan is a promising complex polysaccharide obtained from marine green algae, primarily belonging to Ulvales [3]. This vital part of the algae's cell wall provides essential structural support and protection [4]. It typically accounts for 9–36% of the dry weight of *Ulva* biomass [5]. In recent years, it can be developed as a marine resource for innovative materials. Ulvan acquired attractive physicochemical properties and biological activities, making it an adaptable substance with various applications [6].

[7] emphasize ulvan's superior structural complexity compared to other algal polysaccharides, attributing this to its diverse sugar units, unique glycosidic bonding patterns, and various molecular modifications. The chemical structure of Ulvan is significantly impacted by the *Ulva* species used as a source, the time of year it is harvested, and the specific techniques employed for extraction and purification, leading to a complex and diverse molecular structure. [5] reported in the literature a method for extracting ulvan from green seaweed. [8, 9] comprehensively evaluated ulvan extraction methods from green seaweed, characterized the extracted ulvan, and explored its biologic activities. They highlighted the critical influence of pH during extraction, which significantly impacts ulvan yield and the solubility of co-extracted

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macromolecules, ultimately affecting the final product's biological properties [9]. Moreover, the extraction conditions were shown to alter Ulvan's structural, thermal, and antioxidant characteristics [10].

Ulvan as a flexible biopolymer holds significant potential in various fields. It is a unique composition, rich in rhamnose, uronic acids, and xylose [9]. Its applications could span wound dressings, tissue engineering, and the pharmaceutical and biomedical realms, driven by its antioxidant properties, biological activities, and distinctive molecular structural design [3, 11]. The explorations on the composition, structure, physicochemical, functional, and gelling properties of ulvan are necessary to investigate the functions of ulvan [12, 13]. In the present study, we investigated the extraction and characterization of sulfated polysaccharides; ulvan from abundant marine macroalgal species. Also, the physicochemical properties and antimicrobial activity of the extracted ulvan were evaluated.

## 2. Material and methods

### 2.1. Collection of green macroalgal species

Abundant green marine macroalgal samples were collected from the intertidal zone of two study sites during the winter and spring of 2022 and 2023. The first site (I) was located at the Hunting Club, Port Said, on the Mediterranean Sea (31.27° N, 32.31° E). The second site (II) was situated in the El-Ahyaa region, Hurghada, on the Red Sea, Egypt (27°17′ N, 33°46′ E) (Fig., 1).

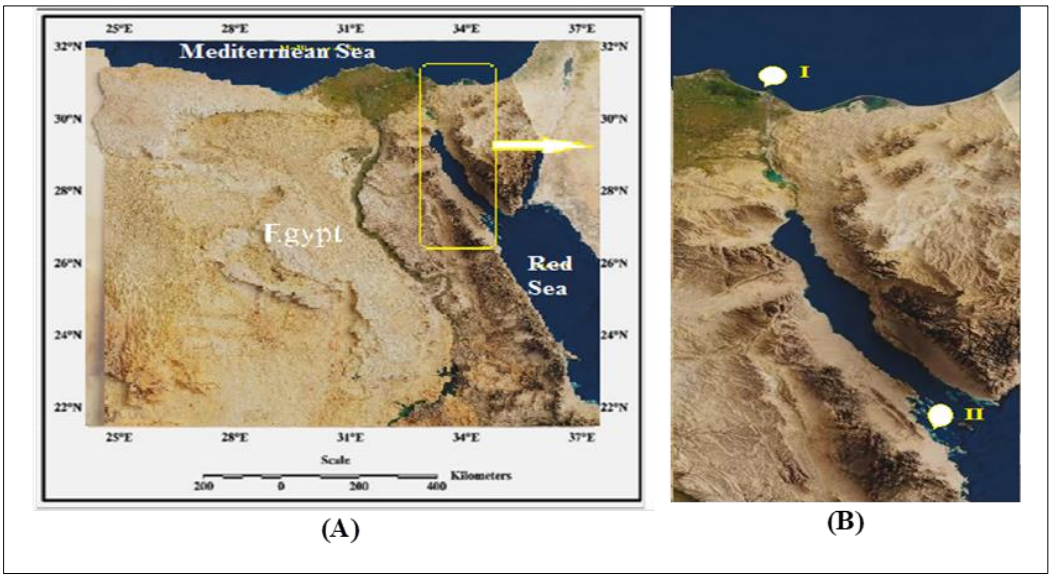



**Figure 1** A map of Egypt (A) showing the study area site I and site II (B)

### 2.2. Identification and preparation of the selected samples

Healthy macroalgae were collected manually and meticulously cleaned in seawater to remove attached debris. The samples were then thoroughly rinsed with distilled water to eliminate excess salt. The purified macroalgae were air-dried in shade at room temperature. Morphological identification of the samples was performed using taxonomic keys by **[14-17]**. Three abundant marine green macroalgae species were identified as exhibiting the highest biomass: *Ulva fasciata* Delile from site I, *Caulerpa racemosa* (Forsskål) J. Agardh, and *Halimeda tuna* (J. Ellis & Solander) J.V. Lamouroux from site II (Figure 2). Dried samples were homogenized using an electric mixer and stored at 4°C for subsequent analysis.

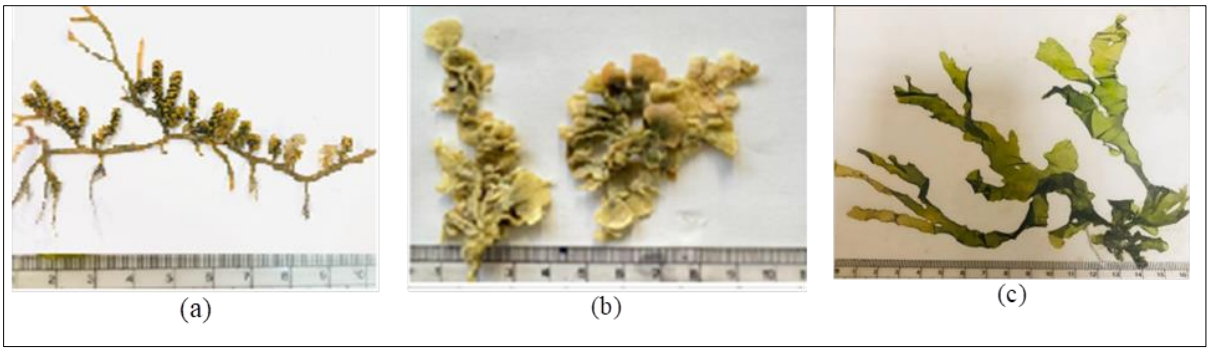



**Figure 2** The selected green macroalgal species: (a) *Caulerpa racemosa* (b) *Halimeda tuna* and (c) *Ulva fasciata*

### 2.3. Extraction of Ulvan

Ulvan was extracted from the dried green macroalgae using three methods: Acid extraction (AE), hot water extraction (HWE), and ultrasonic-assisted extraction (UAE). The HWE and UAE methods followed [18], where 15 g of the macroalgal powder was heated in 300 ml of water at 90 °C for 3 hours. In the ultrasonic-assisted method, ultrasound was applied for 2.5 hours at 90°C. AE method was acclimatized to [19], involved heating 15 g of the macroalgal powder in 250 ml of HCl solution (pH 1.5 and pH 2) at 90 °C for 3 hours. The mixture was cooled, centrifuged, filtered, precipitated with ethanol, and left to dry for 24 hours at 60 °C. All tests were operated in triplicate, and values are stated as the average ± standard deviation. The ulvan extraction yield was calculated as follows:

$$\text{Yield of ulvan (\% w/w)} = \frac{weight\ of\ the\ \ extracted\ ulvan}{\text{Initial dry weight of seaweeds}} \times 100\ \%$$

## 2.4. 2.3. Characterization of the extracted ulvan

*2.4.1. Physiochemical Properties of the highest yielded ulvan*

One gram of extracted ulvan from UAE method was dissolved in 100 ml of distilled water. pH was measured at room temperature with a Jenway Model 550 pH meter [20]. The color was visually evaluated on a white and dark lighting background. Moisture content was assessed through drying [20]. The ash concentration was estimated using weight loss following drying and calcination at 550 °C [21].

*2.4.2. Fourier Transform Infra-Red Spectroscopy (FTIR)*

The isolated *ulva*'s functional groups were detected using the Fourier Transform Infra-Red Spectroscopy (FTIR) approach described by **[22]**. The dried ulvan was crushed into a fine powder before recording the infrared spectra with a Bruker Alpha ATR-FTIR spectrometer. The spectra were averaged from two independent measurements ranging from 4000 to 400 $cm^{-1}$ with 128 scans at a resolution of 2 $cm^{-1}$.

*2.4.3. Viscosity and molecular weight determination*

The viscosity of ulvan extracted from different species using UAE method was measured in 0.1 M NaCl solution to reduce electrostatic interactions. The specific viscosity ($\eta_{sp}$) was assessed with an Ubbelohde viscometer in a thermostatic water bath at 25°C for different ulvan concentrations. The reduced viscosity ($\eta_{red}$) was calculated by dividing $\eta_{sp}$ by the concentration. Plotting $\eta_{red}$ against concentration and extrapolating to zero concentration yields the intrinsic viscosity [η], which is then used to derive the molecular weight. The average ulvan molecular weight was determined using the Mark-Houwink-Sakurada equation (Eq. 1), with k value of $2.1\times10^{-4}$ dL/g and a value of 0.76, to establish an empirical link between intrinsic viscosity and average molecular weight **[23, 24]**

$$[\eta] = kM_w^a \text{ (Eq. 1)}$$

*2.4.4. Thermogravimetric analysis (TGA)*

The thermal steadiness and composition of the extracted ulvan with the highest yield from *U. fasciata* by UAE method were determined using thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DTG). A thermogravimetry-differential thermal analysis (TG-DTA) instrument (DTG-60 SHIMADZU) was used to assess thermal properties by heating the sample from 10 to 1000°C at a rate of 20°C/min.

*2.4.5. Antimicrobial activity of ulvan*

The antimicrobial efficacy of ulvan extract from *U. fasciata* by UAE method was assessed against a panel of microorganisms using the disc diffusion method. The tested pathogenic microorganisms included *Escherichia coli* (ATCC 8739) and *Salmonella typhi* (ATCC 6539) as Gram-negative bacteria, *Staphylococcus aureus* (ATCC 6538) and Bacillus subtilis (ATCC 6633) as Gram-positive bacteria, and the fungi *Candida albicans* (ATCC 10231) and *Aspergillus fumigatus*. Bacterial cultures were grown in nutrient broth at 37°C for 24 hrs. and adjusted to a 0.5 McFarland standard (approximately $1.5x10^8$ CFU/ml) **[25, 26]**. Fungal cells were harvested, washed, and resuspended in sterile saline. Microbial suspensions were spread on Muller Hinton agar plates, followed by the placement of filter paper discs impregnated with 20 µg of ulvan extract. Plates were protected at 37°C for 24 hrs., and inhibition zones were determined to determine antimicrobial activity **[27]**.

*2.4.6. Statistical Analysis*

The results were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 10.0 software (SPSS, Richmond, VA, USA). A one-way analysis of variance (ANOVA) was used to assess the significance of differences between values, with a threshold of $P < 0.05$. Principal Component Analysis (PCA) plots were generated using PAST: Paleontological Statistics (Version 2.17).

## 3. Results and Discussion

### 3.1. Extraction Yield of ulvan

The ulvan extraction yield produced from selected abundant green macroalgae with three extraction methods (UAE, HWE and AE) are shown in Fig. 3. The highest yield was obtained using ultrasonic assisted extraction (UAE) for all species. The yield was 27.3±0.1, 26.3±0.2 and 20.6±0.5% for *U. fasciata*, *C. racemosa* and *H. tuna*, respectively, followed by HWE with yield of (14 ±0.3, 11.3±0.2 and 10±0.2% for *U. fasciata*, *C. racemosa* and *H. tuna* correspondingly). Statistical analysis using one-way ANOVA confirmed a significant difference in extraction yields between methods ($F=25.07$, $df=3.62$, $\rho \leq 0.008$), with UAE significantly outperforming the others. This is similar with prior research, such as [18], which encountered that ultrasonic increases cell wall breakdown, allowing for larger release of polysaccharides such as ulvan. UAE improves mass transfer efficiency, resulting in higher yield. UAE treatment increases yield by breaking down algal cell walls, allowing for more efficient ulvan release [28]. This method's success demonstrates its potential for scale-up, with higher efficiency and lower energy requirements than existing processes [9]. In contrast, the AE method proved less effective with yield (9.3±0.3, 7.3±0.2 and 6.6±0.3% for *U. fasciata*, *C. racemosa* and *H. tuna*, respectively). The lowered AE efficiency could be attributed to the degradation of some ulvan [29]. While acid extraction is effective for isolating certain polysaccharides, it may damage the polymer backbone for other polysaccharides and decrease the total yield. The findings are consistent with [23], who noted that harsher extraction conditions (e.g., strong acids) can cause polysaccharide breakdown, particularly of sensitive sulfated groups.

*U. fasciata* species demonstrated the greatest overall yield by all extraction methods. This is consistent with the findings of [30, 31], who discovered that ulvan from Ulvales species, including *U. fasciata*, has a high sulfate concentration (ranging from 14.3% to 23.2%), which may contribute to its increased extractability. Ulvan is more likely to be released during the extraction process due to its higher sulfate concentration and favorable molecular structure [6]. The study showed that the variation in ulvan yields is heavily impacted by the extraction process and specific circumstances such as temperature, pH, and length. This is reinforced by [9, 28], who underline the need of optimizing these factors for maximum yield. As [23] point out, particle size is very important; smaller algae particles increase surface area, improving extraction efficiency.

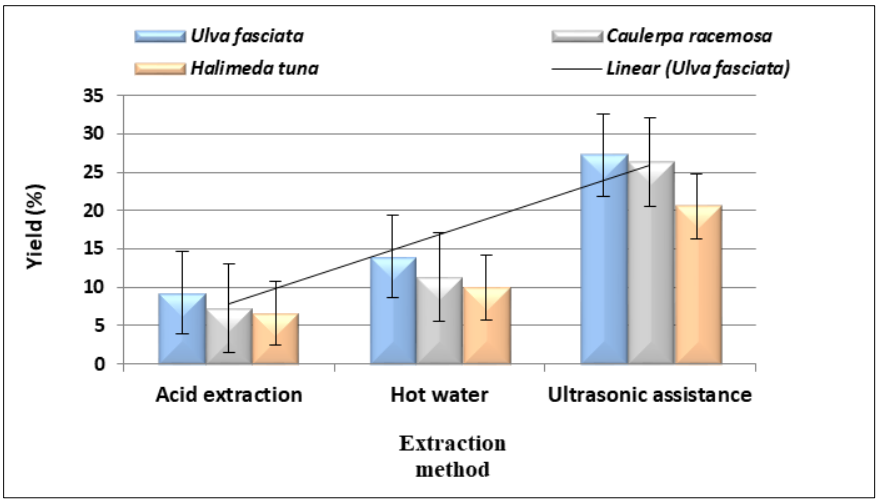
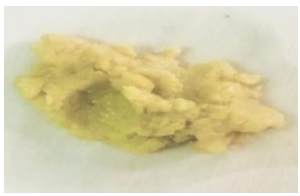
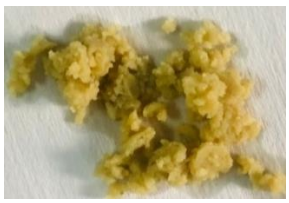


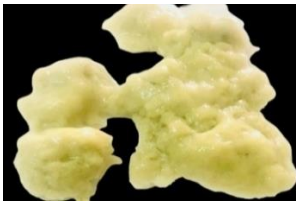

**Figure 3** Ulvan yield (%) of different extraction methods from various green macroalgal species with ±SD and linear of *Ulva fasciata*

The picture of ulvan retrieved from different green macroalgal species employing a different extraction method is summarized in Table 1. The UAE method is well known for increasing the production of polysaccharides, such as ulvan, from green macroalgae. The cavitation effect produced by ultrasonic improves cell wall disruption, resulting in the release of more bioactive chemicals. The photos demonstrate that *U. fasciata* and *C. racemosa* had somewhat fibrous structures after UAE extraction, but *H. tuna* remained more granular. UAE could thus be an effective approach for extracting ulvan from more fibrous macroalgae such as *U. fasciata*, where increased surface area exposure is required for maximum yield.

While hot water extraction (HWE) procedure most likely resulted in the breakdown or the dissolution of the additional heat-sensitive polysaccharides in the more rigid *U. fasciata* and *C. racemosa*, the structure of *H. tuna* remained intact. Previous research has indicated that HWE is often successful for extracting polysaccharides from algae, although it may result in some degradation [23, 32]. The AE approach is highly effective at solubilizing ulvan; however, its aggressive nature can occasionally destroy sensitive bioactive compounds. The visible structures imply a considerable loss of algal integrity, particularly in *U. fasciata*. UAE appears to be the most successful in retaining fibrous structures, making it excellent for *U. fasciata* and *C. racemosa. H. tuna* appears to be less damaged by all treatments. HWE offers a milder degradation of the algae's structure, making it suited for species with sensitive polysaccharides.

**Table 1** The extracted ulvan from selected green macroalgal species by using different extraction methods

| **Ulvan extraction method** | **Marine macroalgal species** | | |
|---|---|---|---|
| | ***Ulva fasciata*** | ***Caulerpa racemosa*** | ***Halimeda tuna*** |
| Ultrasonic Assistance Method |  |  |  |
| Hot water Extraction Method |  |  |  |

| | | | |
|---|---|---|---|
| Acid Extraction Method |  |  |  |

### 3.2. Characterization of the extracted ulvan

*3.2.1. Physicochemical properties of the extracted ulvan*

The extracted ulvan by ultrasonic assisted method was characterized by physicochemical properties such as pH value, which tended to alkalinity. Table (2) showed that pH values vary between the different species; *U. fasciata* had the highest pH value (10.54±0.3), followed by *C. racemosa* (9.74±0.05), whereas *H. tuna* has the lowest one (8.0±0.1). This may be due to the algae's different development conditions. As an instance, *U. fasciata*, which is widely found in the Mediterranean Sea, may have adapted to alkaline circumstances, affecting its biochemical composition [33]. The alkalinity of ulvan is important since it can increase its reactivity and solubility in a variety of applications, particularly in the food and pharmaceutical industries where pH stability is critical [34]. Moisture content was relatively low for all species. There was a noticeable difference in moisture content between *U. fasciata* and *C. racemosa* (10.1±0.1) compared to *H. tuna* (7.1±0.1) ($\rho \geq 0.05$). High moisture content can promote microbial growth, reducing the stability and shelf life of ulvan [35]. Moisture content is an important factor in determining the ultimate quality of ulvan, since excess moisture can affect the material's integrity and utility in a variety of applications, including food preservation and pharmaceuticals [35].

The ash concentration of the various macroalgal species reflects their differing mineral compositions, which are important in defining the possible applications of the extracted ulvan. *U. fasciata* had the greatest ash concentration (8.1±0.1%), followed by *C. racemosa* (7.4±0.3%) and *Halimeda tuna* with the lowest (7.1±0.0%). The higher ash percentage in *U. fasciata* indicates a stronger mineral presence, which may improve the structural qualities of ulvan, making it appropriate for applications requiring mechanical strength and thermal stability, such as bioplastic manufacture [36]. In contrast, the reduced ash concentration in *H. tuna* may imply a higher organic content, possibly making its ulvan more appropriate for biodegradable applications in the pharmaceutical or cosmetic industries, where lightweight materials are required [9].

Principal component analysis (PCA) of the green macroalgal species and their ulvan physicochemical parameters are shown in Fig. (4). The Figure shows that the moisture is positively correlated with pH and ash content. *U. fasciata* have the highest pH and ash content with 10.54±0.3 and 8.1±0.1, respectively. *C. racemosa* is positively correlated with moisture content. While *H. tuna* is negatively correlated with ash content %, pH and moisture content % and had the lowest value with 8.0±0.1, 7.1±0.0 and 7.1±0.1 respectively.

The increased ash and moisture content of *U. fasciata* suggests that it may be better suited for applications that require stable ulvan, such as bioplastic manufacturing, where mineral content can add to material strength and thermal stability [36]. In contrast, *H. tuna*, with its reduced ash and moisture content, may be more suitable for applications where lightweight and highly biodegradable materials are preferred, such as pharmaceuticals or cosmetics [37].

**Table 2** Ulvan physicochemical parameters of the tested green macroalgal species

| **Macroalgal** | **physicochemical parameters** | | |
|---|---|---|---|
| **Species** | **pH** | **Ash (%)** | **Moisture (%)** |
| *Ulva fasciata* | 10.54±0.3 | 8.1±0.1 | 10.1±0.1 |
| *Caulerpa racemosa* | 9.74±0.05 | 7.4±0.3 | 10±0.1 |
| *Halimeda tuna* | 8.0±0.1 | 7.1±0.0 | 7.1±0.1 |

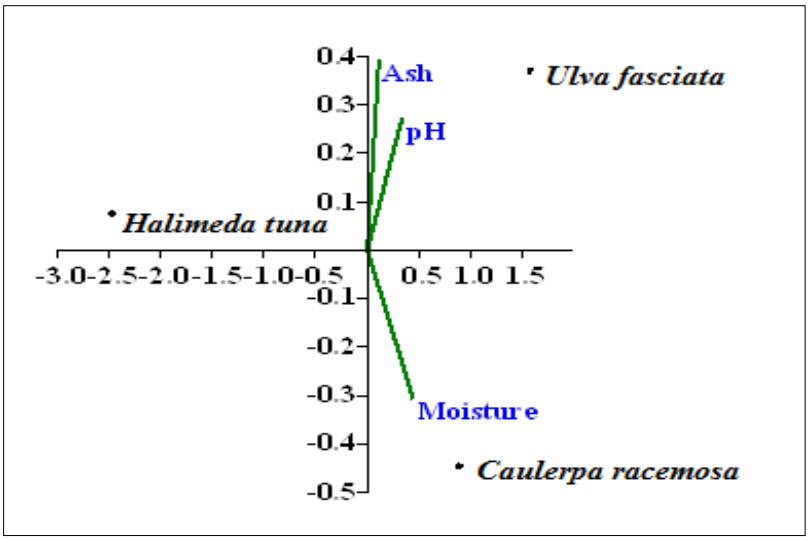



**Figure 4** Principal component analysis (PCA) plot of the selected green macroalgal species with the physicochemical parameters

*3.2.2. Determination of Viscosity and Molecular Weight*

Generally, both specific and reduced viscosity decreased from *U. fasciata* (0.57 and 4.43dL/g, respectively) to *H. tuna* (0.46 and 4.27dL/g, correspondingly). Consequently, *H. tuna* exhibited the lowest resistance to flow compared to the other two species. In contrast, molecular weight displayed a more variable pattern. *U. fasciata* possessed the highest molecular weight (120x105g/mol), while *H. tuna* had the lowest molecular weight (3.2x105 g/mol) as shown in table 3. Specific and reduced viscosity decreased from *U. fasciata* to H. tuna. This may be attributed to *H. tuna* having the lowest resistance to flow compared to the other two species. In contrast, *U. fasciata* displayed the highest molecular weight. This was confirmed that the higher molecular weight often leads to higher viscosity. Viscosity, representing the resistance between liquid molecules, is a crucial parameter in ulvan analysis [38]. Higher molecular weight Ulvan, such as that found in *U. fasciata*, is frequently associated with higher gel-forming capabilities and superior mechanical qualities, making it ideal for use in bioplastics or food packaging [36]. *H. tuna*, on the other hand, has a smaller molecular weight, indicating that it may dissolve more quickly, making it excellent for biodegradable applications that require earlier breakdown. The viscosity of ulvan solvents is impacted by several factors, including sample type, extraction process, measurement temperature, and extraction pH. The solution concentration, temperature, polymer chain length, and presence of polyvalent calcium ions can all influence the viscosity value [38]. Significant efforts have been made to assess the effect of the different parameters not only on the ulvan's extraction yield, but also on its structure, composition of monosaccharides and molecular weight [6].[7] mentioned that the high-value development and utilization of ulvan resources have been limited by their large molecular weight, poor solubility, and low bioavailability.

**Table 3** Viscosity and Molecular weight of ulvan from the selected green macroalgal species

| Macroalgal Species | Spec. Viscosity (dL/g) | Red. Viscosity (dL/g) | Molecular weight (g/mol) |
|---|---|---|---|
| *Ulva fasciata* | 0.57 | 4.43 | 120×105 |
| *Caulerpa racemosa* | 0.50 | 4.36 | 33.5×105 |
| *Halimeda tuna* | 0.46 | 4.27 | 3.2 ×105 |

### 3.3. Fourier Transform Infrared (FTIR) analysis

Functional group analysis of ulvan extracted by ultrasonic assisted method from different species was conducted using Fourier Transform Infrared (FTIR) spectroscopy [39]. The FTIR spectra of ulvan extracted from *U. fasciata*, *C. racemosa* and *H. tuna* are shown on Fig. 5. The group of stretching peaks at 3370–3730 $cm^{-1}$ for ulvan samples indicating the presence of the stretching vibration of the hydroxyl group(O-H) of strongly adsorbed water molecules in the polysaccharide structure [40]. The subtle shoulder observed at 2930–2986 $cm^{-1}$ corresponds to the stretching vibration of the aliphatic C-H bond in methyl groups, a characteristic feature of polysaccharides. The band observed around 1607–

1640 $cm^{-1}$ was attributed to the stretching vibration of the (C=O) group and the asymmetric stretching of the carboxylic group.

The sulfated nature of the polysaccharide was determined by the absorption bands in the region 1213-1324 $cm^{-1}$, which is related to stretching vibration of the sulfate ester (S=O) group. The peaks at 980 and 850 $cm^{-1}$ are assigned to the C-O-S stretching of axial sulfate groups as reported for ulvan. These peaks appear as strong and sharp in ulvan extracted from *Ulva fasciata* and *Caulerpa racemosa* and devious shoulder in ulvan extracted from *Halimeda tuna*. This is agreement with [38] who mentioned that the C-O-S functional group is commonly recognized as a primary marker of ulvan. The absorption bands typically occur within the wave number region between 1000-1200 $cm^{-1}$ [41]. Absorption bands of the O-C-O functional group are detected across all extraction conditions, serving as markers for carboxyl and uronic acid groups in ulvan [42].

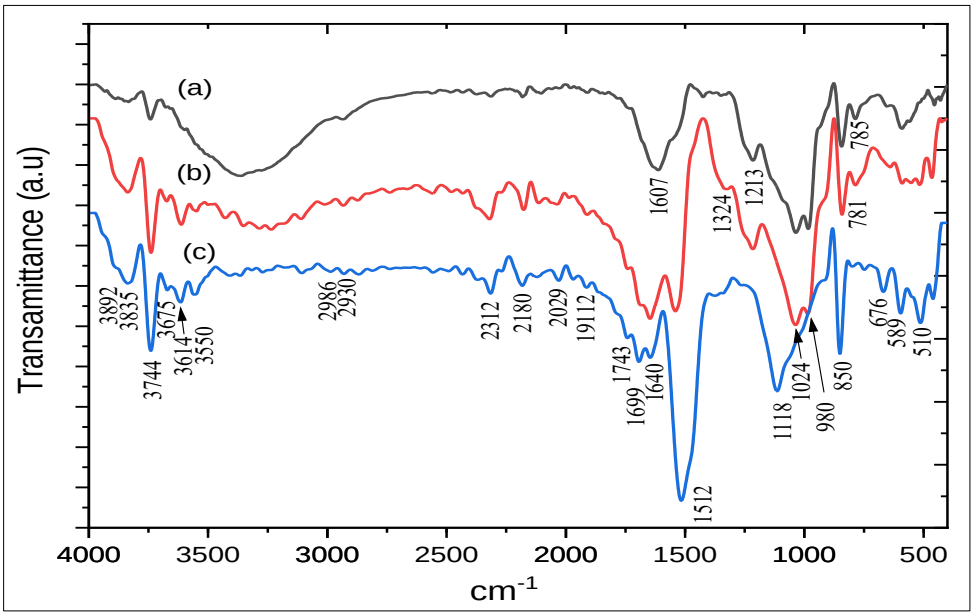



**Figure 5** ATR-FTIR spectra of ulvan extracted from: (a) *Ulva fasciata*, (b) *Caulerpa racemosa* and (c) *Halimeda tuna*

### 3.4. Thermal Gravimetric Analysis (TGA)

The thermal stability of ulvan isolated from *U. fasciata* was investigated using thermogravimetric analysis (TGA) as shown in Fig. 6. The results showed that polysaccharides lose mass gradually when heated, as is characteristic of thermal breakdown. The initial mass loss of 13.88% measured between room temperature 26.19°C and 99.48°C is consistent with the loss of moisture and low molecular weight components [43]. A further mass loss of 17.99% was detected between 249.3 and 473°C, indicating the start of ulvan depolymerization and decomposition as the temperature rises. Such thermal properties are important for applications requiring materials to endure changing heat conditions, such as bioplastics or food packaging [44]. Data shows that ulvan has some thermal stability, although its low mass loss percentages indicate that it may not be suitable for high-temperature applications. However, its thermal profile may be advantageous for biodegradable applications because decreased thermal stability promotes disintegration under decomposing conditions [9].

Overall, the TGA/DTA results are significant for estimating the efficiency and suitability of ulvan derived from *U. fasciata* in a variety of industrial applications, particularly those requiring critical thermal properties.

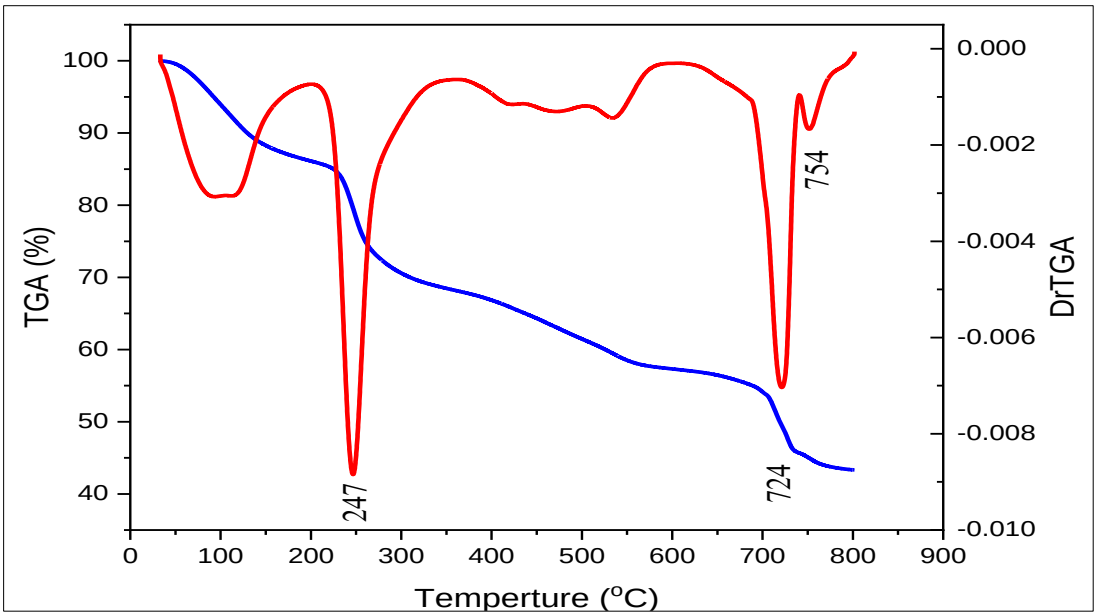



**Figure 6** A) TG-DTA analysis of ulvan from *Ulva fasciata*

### 3.5. Antimicrobial activity

The extracted ulvan from *U. fasciata* demonstrated significant antimicrobial activity compared to control against the tested pathogenic microorganisms, such as some Gram-positive and Gram-negative bacteria, yeast *C. albicans* and *A. fumigatous*. (Table, 4). The inhibition zone (mm) was compared to control (Fig., 7). The ulvan powder exhibited larger inhibition zone (28±0.2, 21±0.2, 26±0.1, 24±0.1 and 29±0.2 mm) compared to control (24±0.2, 17±0.2, 23±0.1, 21±0.2 and 27±0.2 mm) for *B. subtilis*, *E. coli*, *S. aureus*, *S. typhi* and *C. albicans* receptively. These results are inconsistent with others reported by [45], who found that the values of inhibition zones were 11 and 18 mm against *Escherichia coli* and *Staphylococcus aureus* correspondingly. [30] also showed that the extracted ulvan from *U. fasciata* had significant antimicrobial activity against human and fish pathogens. The ulvan powder did not register any inhibition zone against *A. fumigatous*.

**Table 4** Antimicrobial activity of the ulvan powder compared with control

| **Pathogenic microorganism** | | **Inhibition zone** | |
|---|---|---|---|
| | | **Control (mm)** | **Ulvan (mm)** |
| Pathogenic bacteria | *Bacillus subtilis* | 24±0.2 | 28±0.2 |
| | *Staphylococcus aureus* | 17±0.2 | 21±0.2 |
| | *Escherichia coli* | 23±0.1 | 26±0.1 |
| | *Salmonella typhi* | 21±0.2 | 24±0.1 |
| Pathogenic Fungi | *Candida albicans* | 27±0.2 | 29±0.2 |
| | *Aspergillus fumigatous* | 33±0.1 | NA |

*NA: No activity, *Control for bacteria was gentamycin and for fungi was fluconazole

Obtaining high purity of ulvan by purification played a key role in their structure and activity. In addition, the rich biological activities of ulvan make it potentially applicable in food, medicine, cosmetics, and other fields [46]. This study was highlighting to ulvan content in marine macroalgae potential for various pharmaceutical applications.

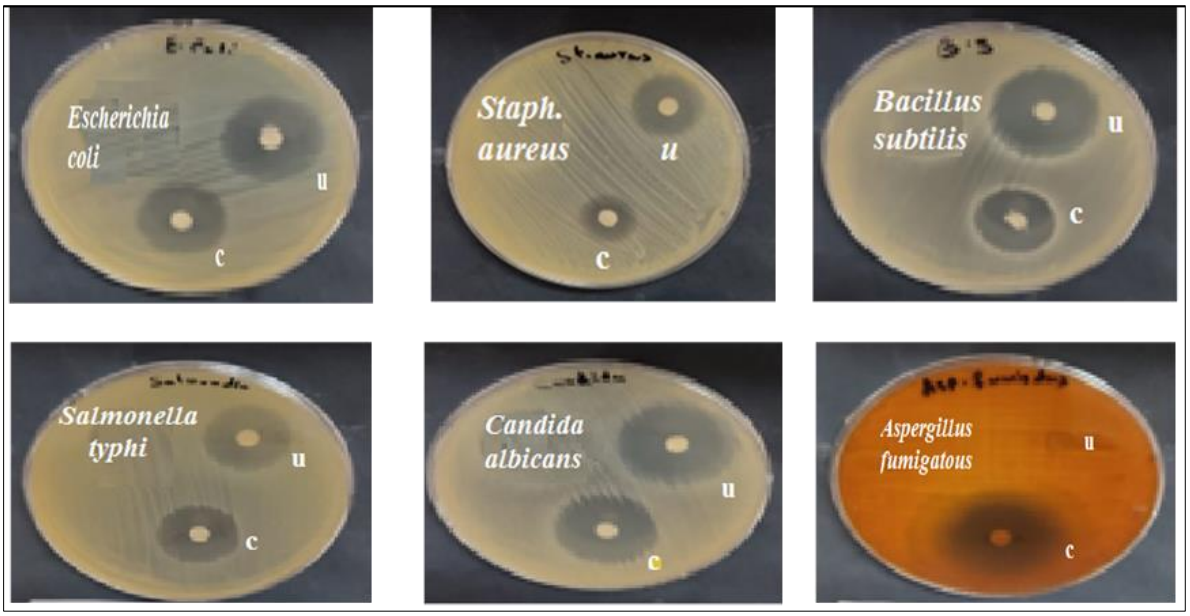



**Figure 7** Antimicrobial activity of: u) ulvan powder and c) control against the tested pathogenic microorganisms

## 4. Conclusion

Ulvan is a flexible biological polymer with huge potential in a variety of industries. The ultrasonic assisted extraction (UAE) method was shown to be the most efficient for extracting ulvan when compared to the acid extraction (AE) and hot water extraction (HWE) methods. UAE could thus be a useful method for extracting ulvan from more fibrous macroalgae like *U. fasciata*, where higher surface area exposure is necessary for optimal yield. *U. fasciata* had the highest overall extraction potential using the various extraction procedures and had an alkaline pH. *U. fasciata* possessed Higher molecular weight ulvan than *H. tuna* which has a smaller molecular weight, thus *U. fasciata* is ideal for use in bioplastics or food packaging, while *H. tuna* could be excellent for biodegradable applications that require earlier breakdown. FTIR, NMR, and TGA analysis of ulvan obtained from *U. fasciata* confirmed the unique structure of ulvan as sulfated polysaccharide. The primary sugar residues of ulvan are rhamnose (typically in the form of rhamnose-3-sulfat), xylose (often sulfated), glucuronic acid and iduronic acid. The last two residues are types of uronic acid that give ulvan the anionic characters. The degree and position of sulfation play a critical role in ulvan's properties, including solubility, gelling ability, and biological activities. Its structure is due to the presence of uncommon sugar residues and sulfate groups, making it a subject of interest for applications in biomedicine, agriculture, and materials science. Moreover, the extracted ulvan demonstrated significant antimicrobial activity against several pathogenic microorganisms, highlighting its potential for various applications in the pharmaceutical and food industries. Ulvan from green macroalgae have potential applications in food thickeners, stabilizers, and biomedical uses, with higher molecular weight ulvan being particularly valuable.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors declare that they have no competing financial interests or personal relationships that could have affected the work reported in this study.

There are no conflicts of interest to declare.

### *Authorship contribution statement*

Nourhan A. Ashour: Writing review and editing Writing (original draft), software, and data curation. Gihan A. El-Shoubaky: Writing-review & editing, Writing – original draft, Software, Data curation. Marwa M. Saleh: Writing-review & editing, Writing – original draft. Nasser Y. Mostafa: Writing- review & editing, Writing – original draft, Software.

*Data availability*

The article describes a study that used no data

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