Structural Characterization of Ulvan Polysaccharide from Cultivated and Collected *Ulva fasciata* (Chlorophyta)

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**Abstract**

Ulvan is a sulfated heteropolysaccharide present in the cell wall of *Ulva* species with unique structural properties and technological potential. Here we characterized by FTIR and NMR analysis the structure of ulvan from *Ulva fasciata* collected in natural environment (SEA) and after *in vitro* biomass cultivation in nutrient enriched water (CULT). FTIR spectrum of CULT ulvan presented stronger signals of sulfate groups than SEA. ¹H and ¹³C NMR showed that both ulvan are composed mainly of ulvanobiuronic acid 3-sulfate type A and type B. SEA ulvan presented signals characteristics of xylose, suggesting the presence of ulvanobiose in its structure, while CULT presented most signals of type A disaccharide. The cultivation of *Ulva* could be an alternative to suffice the emerging demand for ulvan meeting requirements of quality and quantity.

**Keywords**

Sulfated Polysaccharide, Aquaculture, FTIR Analysis, NMR Analysis, Biotechnology

1. Introduction

Marine ecosystems represent a rich source of macromolecules with unique physico-chemical characteristics [1]. In this sense, polysaccharides extracted from marine macroalgae are receiving increasing attention due to their diversity, biocompatibility and structural features not found in any other organism [2].
Species of the genus *Ulva* are the most abundant and cosmopolitan macroalgae in the Chlorophyta Division being able to adapt across diverse geo-climatic conditions with high productivity and opportunistic growth. *Ulva* cultivation is increasing worldwide due to its potential as functional foods, feed and biofuel [3] [4]. Although polysaccharides from the red (carrageenan and agar) and brown (alginate) macroalgae have been used in the food industry, polysaccharides from green macroalgae remain largely unexploited. The main polysaccharide of *Ulva* species is ulvan, corresponding to 29% of dry weight with a promising technological application.

Ulvan is homogeneously distributed within the intercellular space and in the fibrillar wall [5] [6] being responsible for maintaining the osmolar stability and protecting the thallus from marine bacterial attack [2] [7]. Ulvan is composed of variable amounts of rhamnose, glucuronic acid, iduronic acid, xylose and sulfate [8] [9]. This sulfated heteropolysaccharide is built on sequences of two major repeating disaccharides units designated as ulvanobiuronic acid 3-sulfate type A (A3s) \( \rightarrow(4)\-\beta-D-GlcA-(1 \rightarrow 4)\-\alpha-L-Rha 3S-(1\rightarrow) \) and type B (B3s) \( \rightarrow(4)\-\alpha-L-IdoA-(1 \rightarrow 4)\-\alpha-L-Rha 3S(1\rightarrow) \) [10] [11]. Minor sulfated residues with xylose (O-2 sulfated or not) denominated ulvanobiose (U3s) \( \rightarrow(4)\-\beta-d-GlcA-(1-2)-\alpha-D-Xyl-(1\rightarrow) \) can also occur in place of uronic acids [12].

With the growing interest in novel and renewable polymers, ulvan has drawing attention with studies showing its potential as antioxidant [13] [14], antiviral [15] [16], anticancer [17] [18] among others [19] [20] [21]. The presence of the remarkable rare sugars, rhamnose and iduronic acid, similar to mammalian glycosaminoglycans [4] [9] single out ulvan from other algal polysaccharides. L-rhamnose used in a variety of anti-aging cosmetics [21] [22] is specifically recognized by a number of mammalians lectins [6]. Iduronic acid, which has never been identified in algal polysaccharides [5] [23] is required in the synthesis of heparin analogs being used against respiratory syncytial virus infection and antithrombotic activities [24] [25]. Currently, this substance is obtained through several steps that could be avoided using ulvan [5] [25].

Although the biotechnological applications of ulvan are promising, structural variations may occur due to ecophysiological factors acting on *Ulva* [26] [27]. The commercial use of polysaccharides requires ulvan with predictive structure and functional properties, which could be obtained by the controlled cultivation of *Ulva*. To determine the potential of cultivated *Ulva* for ulvan production in this work we characterized (FTIR and NMR) the ulvan extracts from *Ulva fasciata* Delile (Chlorophyta) after in vitro biomass cultivation in nutrient enriched water and compared it against ulvan from biomass collected in an oligotrophic natural environment, to enhance potential structural differences.

### 2. Material and Methods

#### 2.1. Algal Material

Healthy thalli of *Ulva fasciata* were collected in the intertidal zone at Prainha...
Beach, Arraial do Cabo/RJ Brazil (22°57′40″S/042°01′13″W) rinsed with local seawater and transported to laboratory inside coolers. Individuals were cleaned with distilled water for further removal of sediment and macroscopic epibionts. The species-level identification as *U. fasciata* was determined by molecular studies (barcoding using tufA markers) [28]. Voucher specimens were deposited in the Institute of Bioscience Herbarium, at the University of São Paulo, Brazil (SPF-57877). Part of the fresh biomass (SEA) was oven dried at 50°C until constant weight and stored in desiccator until ulvan extraction. The remaining material was used in the cultivation experiment (CULT). In sequence biomass from natural environment and cultivation experiment (SEA and CULT, respectively) were used for ulvan extraction (Figure 1).

**2.2. Ulva Cultivation**

To ensure that all individuals presented comparable initial physiological conditions, *U. fasciata* thalli underwent a seven-day acclimatization period to the laboratory conditions followed by a three-day starvation period prior to the cultivation. For acclimatization, individuals were kept in sterilized seawater enriched with von Stosch culture medium [28] in a temperature-controlled room at 24.0 ± 1.0°C, 70 μmol photons m⁻² s⁻¹ photosynthetically active radiation (PAR) and 12 hours light photoperiod. For starvation, individuals weighing approximately 3.5 g were placed in 2.8 L Erlenmeyer flasks filled UV-sterilized natural seawater (nutrient concentration—0.5 μM NH₄, 0.03 μM NO₂, 0.41 μM NO₃ and 0.09 μM PO₄³⁻).

For the cultivation, starved individuals weighting 3.0 ± 0.11 g were cultivated in 3 liters Erlenmeyer flasks (n = 4) filled with seawater enriched with 200 μM of ammonium (NH₄Cl), 8 μM of nitrate (NaNO₃), 12 μM of phosphate (Na₂HPO₄·12H₂O),

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**Figure 1.** Schematic representation of steps to ulvan extraction and characterization.
salts and vitamins [29]. The nutrient concentrations were chosen with reference to the mean maximum nutrient concentration after a five years monitoring of one of the points of an important Brazilian bay [30] with potential for Ulva cultivation. The same light, photoperiod and temperature conditions of the acclimatization and starvation period were maintained. The experiment lasted five days.

2.3. Ulvan Extraction

After the cultivation, individuals were washed with distilled water to remove salts and oven dried at 50°C until constant weight. The polysaccharide was extracted according to method described by [31]. Dried algal biomass (SEA and CULT) were grinded into a powder, suspended in ultrapure water (Milli-q®) (100 ml/10g) and autoclaved at 120°C for 40 min. The supernatant was centrifuged at 10,000 g and 4°C for 10 min (Eppendorf centrifuge 5810 r). Ulvan was precipitated with three volumes of ultrapure ethanol (Merck®), cooled at –20°C for 48 hours and further centrifuged at 3500 g at 4°C for 5 min. The recovered pellet (ulvan) was freeze-dried. Ulvan extraction yield was 16.29% ± 0.93% calculated using formula proposed by [32]:

\[
\text{Ulvan yield (\%)} = \left( \frac{W_e}{W_f} \right) \times 100
\]  

where, \(W_e\) is the dry ulvan weight extracted and \(W_f\) is the macroalgae dry weight.

2.4. Fourier-Transform Infrared Spectroscopy (FT-IR) Analysis

Ulvan (SEA and CULT) infrared spectra with Fourier transform (FT-IR) were recorded on a spectrophotometer (IR Prestige_21, Shimadzu) at room temperature. The FT-IR spectra were obtained in the transmission mode at 400-4000 cm\(^{-1}\). The transmission spectra were recorded using KBr (Merck®) pellets containing 2.5 mg of ulvan powder.

2.5. Nuclear Magnetic Resonance Spectroscopy

NMR analyses were performed using a Varian VNMRSYS 500 MHz spectrometer (Varian Inc., Palo Alto, CA, USA) at 37°C. Proton and carbon operating frequencies were 499.77 and 125.68 MHz, respectively. \(^1\)H NMR spectra were recorded with a 90 degree observe pulse width (pw = 90 µs), a 2.04 s acquisition time and a 1 s relaxation delay. A total of 32 scans were performed for each sample. For \(^13\)C NMR, a 90 degree pulse was used (pw = 90 µs), 1.04 s acquisition time, 2 s relaxation delay and a total of 114.624 scans were collected. Ulvan samples (2% w/v) were dissolved in D2O 99.99% (Sigma-Aldrich®). \(^1\)H and \(^13\)C NMR chemical shifts were expressed in parts per million (ppm).

3. Results and Discussion

FTIR and NMR spectroscopy are rapid and non-destructive analysis that provide
fundamental information on ulvan polysaccharide structure [26]. In this work, such techniques showed that polysaccharides from natural environment (SEA) and cultivated *U. fasciata* (CULT) are mainly constituted of rhamnose, iduronic and glucuronic acid, sulfate and, in the case of SEA, xylose.

IR Spectra of SEA and CULT ulvan are presented in Figure 2 with the signals assignment provided by comparison with published data [32] [33] [34] [35]. CULT and SEA spectra presented all the characteristics peaks described in literature [34] [36] [37], confirming that the extracted polysaccharides are ulvan. According to FTIR spectra, there was no visible difference between the two ulvan extracts and those reported in the literature [14] [17] [33].

The absorption band at around 3300 cm$^{-1}$ was attributed to a stretching of hydroxyl groups (O-H). Signal observed at approximately 2937 cm$^{-1}$ is due to C-H stretching vibration and is characteristic of polysaccharides [14]. Bands of carboxylate groups of uronic acid with similar intensities are present in both spectra at around 1651 and 1435 cm$^{-1}$. The absorptions between 1147 and 848 cm$^{-1}$ are known as the fingerprint region for ulvan, being the most important absorptions. At 983 cm$^{-1}$ signal is characteristic of the vibration of glycosidic bonds and at 848 cm$^{-1}$ corresponds to the bending vibration of C-O-S of sulfate in axial position. Peaks bellow 900 cm$^{-1}$ in both ulvan samples are characteristic of the presence of sulfate.

The presence, degree and distribution of the sulfate groups are important in determining the biological activity of ulvan [9]. CULT ulvan presented stronger signals of sulfate groups than SEA between 1159 and 625 cm$^{-1}$. Previous studies have found that the antioxidant activity and regulation of physiological stress by ulvan is related to it is sulfate content [38] [39]. This result suggests that CULT ulvan could have a higher antioxidant potential.

The $^{13}$C NMR spectra are shown in Figure 3, with typical signals of ulvan...
Figure 3. $^{13}$C NMR spectra (ppm) of ulvan from *Ulva fasciata* collected in natural environment (SEA) and cultivated *in vitro* (CULT). Residue A = rhamnose, B = glucuronic acid, C = iduronic acid and D = xylose.

Structure attributed by comparison with published data [11] [12] [40] [41]. Carbon of rhamnose and glucuronic acid that constitutes the type A ulvanobiuronic acid (A3s) and the chemical shifts attributed to rhamnose 3-sulfate linked to iduronic acid in type B ulvanobiuronicacid (B3s) were identified, confirming that the extracted polysaccharides are mainly composed of repeated sequences of these two disaccharides. Signals in the resonance region corresponding to carbon rings (70.06 - 80.28 ppm and 69.60 - 79.88 ppm in CULT and SEA, respectively), C-6 methyl group of rhamnose at around 19 ppm and carboxyl signal of uronic acid at approximately 177 ppm are present. Further assigned signals of anomeric carbons (99.28 - 104.62 ppm) were observed in SEA.

$^1$H NMR spectra are presented in Figure 4 and the signals associated to each hydrogen atom are assigned according to reference data [17] [32] [41] [42]. Anomeric protons of rhamnose and iduronic acid are present between 4.28 - 5.13 ppm. Proton chemical shifts of rhamnose are shown at 1.30 ppm. Glucuronic acid chemical shifts are between 3.36 and 3.79 ppm. Signals of xylose are indicated at 3.20 and 5.27 ppm at SEA ulvan.

Noise observed in the $^{13}$C NMR spectra is related to sample dilution increased by the high molecular weight of the polymer and solution viscosity. Ulvan molecular weight can vary from $1.8 \times 10^5$ to $2 \times 10^6$ depending on extraction methods, specie and polydispersity of the samples [32] [43]. According to [6] ulwan
extracted with temperatures between 80˚C - 90˚C, close to the used in this study, tend to present higher molecular weight.

In ¹³C and ¹H NMR spectra of SEA ulvan we could observe signals of xylose, suggesting the presence of ulvanobiose (U3s) in this ulvan structure. In CULT we could not detect U3s, but peak characteristic to C-1 of rhamnose in A3s disaccharide (4.82 ppm) was present [6]. According to [27] during the active growth of Ulva the macroalgae tends to synthesize more ulvanobiuronic acid type A, with the production of ulvanobiose being developmentally regulated. In this study, U. fasciata presented an average growth rate of 5.7% day⁻¹ and active nutrient uptake throughout the cultivation experiment (data not shown), an indication that individuals had not reach their growth plateau when collected.

In this study both ulvan presented similar global structure, but ulvan from cultivated U. fasciata presented stronger signals of sulfate and ulvan from natu-
ral environment had signals of xylose. Future studies with purification and sugar quantification procedures could help elucidate the fine structure of both ulvan samples and assess the efficacy of CULT ulvan for different applications such as antioxidant.

4. Conclusion

The production of ulvan with predictive structure and in necessary amounts is one of the hindrances for the ulvan market development. The results gathered here show that ulvan from cultivated *U. fasciata* is similar to those reported in literature and could be a source for obtaining this polysaccharide. By controlling abiotic conditions ulvan production could be maximized meeting commercial requirements.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Wang, H.M.D., Chen, C.C., Huynh, P. and Chang, J.S. (2015) Exploring the Potential of Using Algae in Cosmetics. *Bioresource Technology*, 184, 355-362. [https://doi.org/10.1016/j.biortech.2014.12.001](https://doi.org/10.1016/j.biortech.2014.12.001)

[2] Cunha, L. and Grenha, A. (2016) Sulfated Seaweed Polysaccharides as Multifunctional Materials in Drug Delivery Applications. *Marine Drugs*, 14, 42. [https://doi.org/10.3390/md14030042](https://doi.org/10.3390/md14030042)

[3] Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S., Helliwell, K.E., Smith, A.G., Camire, M.E. and Brawley, S.H. (2017) Algae as Nutritional and Functional Food Sources: Revisiting Our Understanding. *Journal of Applied Phycology*, 29, 949-982. [https://doi.org/10.1007/s10811-016-0974-5](https://doi.org/10.1007/s10811-016-0974-5)

[4] Holdt, S.L. and Kraan, S. (2011) Bioactive Compounds in Seaweeds: Functional Food Applications and Legislation. *Journal of Applied Phycology*, 23, 543-597. [https://doi.org/10.1007/s10811-010-9632-5](https://doi.org/10.1007/s10811-010-9632-5)

[5] Chiellini, F. and Morelli, A. (2011) A Versatile Platform of Biomaterials from Renewable Resources. *Biomaterials*, 75-98. [https://doi.org/10.5772/24901](https://doi.org/10.5772/24901)

[6] Lahaye, M. and Robic, A. (2007) Structure and Function Properties of Ulvan, A Polysaccharide from Green Seaweeds. *Biomacromolecules*, 8, 1765-1774. [https://doi.org/10.1021/bm061185q](https://doi.org/10.1021/bm061185q)

[7] Alves, A., Sousa, R.A. and Reis, R.L. (2013) A Practical Perspective on Ulvan Extracted from Green Algae. *Journal of Applied Phycology*, 25, 407-424.
[8] Tziveleka, L.A., Ioannou, E. and Roussis, V. (2019) Ulvan, a Bioactive Marine Sulphated Polysaccharide as a Key Constituent of Hybrid Biomaterials: A Review. Carbohydrate Polymers, 218, 355-370. https://doi.org/10.1016/j.carbpol.2019.04.074

[9] Kidgell, J.T., Magnusson, M., de Nys, R. and Glasson, C.R.K. (2019) Ulvan: A Systematic Review of Extraction, Composition and Function. Algal Research, 39, 101422. https://doi.org/10.1016/j.algal.2019.101422

[10] Chi, Y., Li, H., Wang, P., Du, C., Ye, H., Zuo, S., Guan, H. and Wang, P. (2020) Structural Characterization of Ulvan Extracted from Ulva clathrata Assisted by an Ulvan Lyase. Carbohydrate Polymers, 229, Article No.: 115497. https://doi.org/10.1016/j.carbpol.2019.115497

[11] Lahaye, M. and Ray, B. (1996) Cell-Wall Polysaccharides from the Marine Green. Carbohydrate Research, 283, 161-173. https://doi.org/10.1016/0008-6215(95)00407-6

[12] Costa, C., Alves, A., Pinto, P.R., Sousa, R.A., Borges da Silva, E.A., Reis, R.I. and Rodrigues, A.E. (2012) Characterization of Ulvan Extracts to Assess the Effect of Different Steps in the Extraction Procedure. Carbohydrate Polymers, 88, 537-546. https://doi.org/10.1016/j.carbpol.2011.12.041

[13] Qi, H., Zhang, Q., Zhao, T. and Chen, R. (2005) Antioxidant Activity of Different Sulfate Content Derivatives of Polysaccharide Extracted from Ulva pertusa (Chlorophyta) in Vitro. International Journal of Biological Macromolecules, 37, 195-199. https://doi.org/10.1016/j.ijbiomac.2005.10.008

[14] Li, W., Jiang, N., Li, B., Wan, M., Chang, X., Liu, H., Zhang, L., Yin, S., Qi, H. and Liu, S. (2018) Antioxidant Activity of Purified Ulvan in Hyperlipidemic Mice. International Journal of Biological Macromolecules, 113, 971-975. https://doi.org/10.1016/j.ijbiomac.2018.02.104

[15] Hardouin, K., Bedoux, G., Burlot, A.S., Donnay-Moreno, C., Bergé, J.P., Nyvall-Collén, P. and Bourgougnon, N. (2016) Enzyme-Assisted Extraction (EAE) for the Production of Antiviral and Antioxidant Extracts from the Green Seaweed Ulva armoricana (Ulvales, Ulvophyceae). Algal Research, 16, 233-239. https://doi.org/10.1016/j.algal.2016.03.013

[16] Lopes, N., Ray, S., Espada, S.F., Bomfim, W.A., Ray, B., Faccin-Galhardi, L.C., Linhares, R.E.C. and Nozawa, C. (2017) Green Seaweed Enteromorpha compressa (Chlorophyta, Ulvaceae) Derived Sulphated Polysaccharides Inhibit Herpes Simplex Virus. International Journal of Biological Macromolecules, 102, 605-612. https://doi.org/10.1016/j.ijbiomac.2017.04.043

[17] Thanh, T.T.T., Quach, T.M.T., Nguyen, T.N., Vu Luong, D., Bui, M.L. and Van Tran, T.T. (2016) Structure and Cytotoxic Activity of Ulvan Extracted from Green Seaweed Ulva lactuca. International Journal of Biological Macromolecules, 93, 695-702. https://doi.org/10.1016/j.ijbiomac.2016.09.040

[18] Shao, P., Pei, Y., Fang, Z. and Sun, P. (2014) Effects of Partial Desulfation on Antioxidant and Inhibition of DLD Cancer Cell of Ulva fasciata Polysaccharide. International Journal of Biological Macromolecules, 65, 307-313. https://doi.org/10.1016/j.ijbiomac.2014.01.043

[19] del Rocio Quezada-Rodriguez, P. and Fajer-Ávila, E.J. (2017) The Dietary Effect of Ulvan from Ulva Clathrata on Hematological-Immunological Parameters and Growth of Tilapia (Oreochromis niloticus). Journal of Applied Phycology, 29, 423-431. https://doi.org/10.1007/s10811-016-0903-7
[20] Stadnik, M.J. (2014) Ulvan Effect on Conidial Germination and Appressoria Formation of Colletotrichum gloeosporioides. Phytoparasitica, 42, 631-640. https://doi.org/10.1007/s12600-014-0404-7

[21] Adrien, A., Bonnet, A., Dufour, D., Baudouin, S., Maugard, T. and Bridiau, N. (2017) Pilot Production of Ulvans from Ulva sp. and Their Effects on Hyaluronan and Collagen Production in Cultured Dermal Fibroblasts. Carbohydrate Polymers, 157, 1306-1314. https://doi.org/10.1016/j.carbpol.2016.11.014

[22] Pageon, H., Azouaoui, A., Zucchi, H., Ricois, S., Tran, C. and Asselineau, D. (2019) Potentially Beneficial Effects of Rhamnose on Skin Ageing: An in Vitro and in Vivo Study. International Journal of Cosmetic Science, 41, 213-220. https://doi.org/10.1111/ics.12523

[23] Alban, S., Schauerte, A. and Franz, G. (2002) Anticoagulant Sulfated Polysaccharides: Part I. Synthesis and Structure-Activity Relationships of New Pullulan Sulfates. Carbohydrate Polymers, 47, 267-276. https://doi.org/10.1016/S0144-8617(01)00178-3

[24] Hallak, L.K., Collins, P.L., Knudson, W. and Peeples, M.E. (2000) Iduronic Acid-Containing Glycosaminoglycans on Target Cells Are Required for Efficient Respiratory Syncytial Virus Infection. Virology, 271, 264-275. https://doi.org/10.1006/viro.2000.0293

[25] Bindschädler, P., Adibekian, A., Grünstein, D. and Seeberger, P.H. (2010) De Novo Synthesis of Differentially Protected L-Iduronic Acid Glycosylating Agents. Carbohydrate Research, 345, 948-955. https://doi.org/10.1016/j.carres.2010.02.004

[26] Robic, A., Rondeau-Mouro, C., Sassi, J. F., Lerat, Y. and Lahaye, M. (2009) Structure and Interactions of Ulvan in the Cell Wall of the Marine Green Algae Ulva rotundata (Ulvales, Chlorophyceae). Carbohydrate Polymers, 77, 206-216. https://doi.org/10.1016/j.carbpol.2008.12.023

[27] Robic, A., Sassi, J.F., Dion, P., Lerat, Y. and Lahaye, M. (2009) Seasonal Variability of Physicochemical and Rheological Properties of Ulvan in Two Ulva Species (Chlorophyta) from the Brittany Coast. Journal of Phycology, 45, 962-973. https://doi.org/10.1111/j.1529-8817.2009.00699.x

[28] Martins, N.T. (2016) Physiological Responses of Ulva fasciata Delile (Ulvales, Chlorophyta): Comparison of Two Populations from Thermally Distinct Sites from Brazilian Coast. Msc Dissertation, Federal University of São Paulo, São Paulo.

[29] Uursi, S. and Plastino, E.M. (2001) Crescimento in Vitro de Linhagens de Coloração Vermelha e Verde Clara de Gracilaria Birdiae (Gracilariales, Rhodophyta) em Dois Meios de Cultura: Análise de Diferentes Estágios Reprodutivos. Revista Brasileira de Botânica, 24, 587-594. https://doi.org/10.1590/S0100-84042001000500014

[30] Valentin, J.L., Paiva, P.C. and Solomon, P.S. (2018) A Baía de Guanabara: Passado, presente e futuro de um ecossistema ameaçado. CRV, Curitiba. https://doi.org/10.24824/9788544442800.9

[31] Reis, R.P., Carvalho Junior, A.A., Facchinei, A.P., Calheiros, A.C.S. and Castelar, B. (2018) Direct Effects of Ulvan and a Flour Produced from the Green Alga Ulva fasciata Delile on the Fungus Stemphylium solani Weber. Algal Research, 30, 23-27. https://doi.org/10.1016/j.algal.2017.12.007

[32] Yaich, H., Ben Amira, A., Abbas, F., Bouaziz, M., Besbes, S., Richel, A., Blecker, C., Attia, H. and Garnha, H. (2017) Effect of Extraction Procedures on Structural, Thermal and Antioxidant Properties of Ulvan from Ulva lactuca Collected in Monastir Coast. International Journal of Biological Macromolecule, 105, 1430-1439. https://doi.org/10.1016/j.ijbiomac.2017.07.141
[33] Tako, M., Tamanaha, M., Tamashiro, Y. and Uechi, S. (2015) Structure of Ulvan Isolated from the Edible Green Seaweed, *Ulva pertusa*. *Advances in Bioscience and Biotechnology*, 6, 645-655. https://doi.org/10.4236/abb.2015.610068

[34] Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z. and Xing, R. (2005) Antioxidant Activity of Different Molecular Weight Sulfated Polysaccharides from *Ulva pertusa* *Kjellm* (Chlorophyta). *Journal of Applied Phycology*, 17, 527-534. https://doi.org/10.1007/s10811-005-9003-9

[35] Alves, A., Caridade, S.G., Mano, J.F., Sousa, R.A. and Reis, R.L. (2010) Extraction and Physico-Chemical Characterization of a Versatile Biodegradable Polysaccharide Obtained from Green Algae. *Carbohydrate Research*, 345, 2194-2200. https://doi.org/10.1016/j.carres.2010.07.039

[36] Robic, A., Bertrand, D., Sassi, J. and Lerat, Y. (2009) Determination of the Chemical Composition of Ulvan, a Cell Wall Polysaccharide from *Ulva* spp. (Ulvalve, Chlorophyta) by FT-IR and Chemometrics. *Journal of Applied Phycology*, 21, 451-456. https://doi.org/10.1007/s10811-008-9390-9

[37] Tabarsa, M., You, S. and Hashem, E. (2018) Water-Soluble Polysaccharides from *Ulva intestinalis*: Molecular Properties, Structural Elucidation and Immunomodulatory Activities. *Journal of Food and Drug Analysis*, 26, 599-608. https://doi.org/10.1016/j.jfda.2017.07.016

[38] Leiro, J.M., Castro, R., Arranz, J.A. and Lamas, J. (2007) Immunomodulating Activities of Acidic Sulphated Polysaccharides Obtained from the Seaweed *Ulva rigida* C. Agardh. *International Immunopharmacology*, 7, 879-888. https://doi.org/10.1016/j.intimp.2007.02.007

[39] Qi, H. and Sun, Y. (2015) Antioxidant Activity of High Sulfate Content Derivative of Ulvan in Hyperlipidemic Rats. *International Journal of Biological Macromolecules*, 76, 326-329. https://doi.org/10.1016/j.ijbiomac.2015.03.006

[40] Lahaye, M., Cimadevilla, E.A., Kuhlenkamp, R., Lognon, V. and Dion, P. (1999) Chemical Composition and 13C NMR Spectroscopic Characterization of Ulvans from *Ulva* (Ulvalve, Chlorophyta). *Journal of Applied Phycology*, 11, 1-7. https://doi.org/10.1023/A:1008063600071

[41] Lahaye, M. (1998) NMR Spectroscopic Characterization of Oligosaccharides from Two *Ulva Rigidula* Ulvan Samples (Ulvalve, Chlorophyta) Degraded by a Lyase. *Carbohydrate Research*, 314, 1-12. https://doi.org/10.1016/S0008-6215(98)00293-6

[42] Alves, A., Sousa, R.A. and Reis, R.L. (2013) *In Vitro* Cytotoxicity Assessment of Ulvan, a Polysaccharide Extracted from Green Algae. *Phytotherapy Research*, 27, 1143-1148. https://doi.org/10.1002/ptr.4843

[43] Van Tran, T.T., Truong, H.B., Tran, N.H.V., Quach, T.M.T., Nguyen, T.N., Bui, M.L., Yuguchi, Y. and Thanh, T.T.T. (2018) Structure, Conformation in Aqueous Solution and Antimicrobial Activity of Ulvan Extracted from Green Seaweed *Ulva reticulata*. *Natural Product Research*, 32, 2291-2296. https://doi.org/10.1080/14786419.2017.1408098