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PHYSICOCHEMICAL PROFILE AND BIOACTIVITY OF OLIGO-ULVAN FROM *Ulva ohnoi* SEAWEED DEPOLYMERIZED BY DIFFERENT PHYSICAL METHODS

Caddoumy Zahrel Ben[1], Safrina Dyah Hardiningtyas[1]*, Wahyu Ramadhan[1,2]
[1]Department of Aquatic Products Technology, Faculty of Fisheries and Marine Sciences, IPB University Dramaga, Bogor, West Java, 16680 Indonesia
[2]Center for Coastal and Marine Resources Studies (PKSPL), International Research Institute for Maritime, Ocean and Fisheries (i-MAR), IPB University, Bogor, 16127

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*Correspondence: safrina_dyah@apps.ac.id

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Abstract

Ulva ohnoi is a fast-growing green seaweed with high biomass productivity but remains underutilized beyond food and agricultural uses. Their main bioactive compound, ulvan, a sulfated polysaccharide, exhibits en-hanced antioxidant and therapeutic activities when depolymerized into lower-molecular-weight forms. This study aimed to determine the effects of three physical depolymerization methods–ultrasound, ultraviolet (UV) radiation, and microwaves–on the physicochemical properties and bioactivity of ulvan extracted from *Ulva ohnoi*. Ulvan was extracted using hot maceration at 90°C for 2 h. The extracted ulvan was subjected to three treatments: ultrasonication for 60, 120, and 180 min; UV exposure for 60, 120, and 180 min; and microwave irradiation for 5, 10, and 15 min. Each treatment was performed in triplicates using a completely randomized design. The resulting oligo-ulvan was analyzed for molecular weight, viscosity, and bioactivities, including anti-oxidant activity (DPPH assay), α-glucosidase inhibition, and antiproliferative effects on fibroblast cells. Among the treatments, ultrasonication for 180 min produced oligo-ulvan with the lowest viscosity (1.41±0.06 cP) and molecular weight (78.2±2.5 kDa), along with significantly improved antioxidant activity (IC_{50}: 303.33 ppm), α-glucosidase inhibition (IC_{50}: 111.63±1.47 ppm), and antiproliferative effects (45.73±7.83% at 125 µg/mL). These findings highlight ultrasound-assisted depolymerization as a promising and environmentally friendly method for enhancing the therapeutic potential of ulvan for biomedical applications.

Keywords: antidiabetic, cell antiproliferative, microwave, ultrasonication, ultraviolet

Profil Fisikokimia dan Bioaktivitas Oligo-Ulvan dari Rumput Laut *Ulva ohnoi* yang Didepolimerisasi dengan Berbagai Metode

Abstrak

Ulva ohnoi merupakan rumput laut hijau dengan pertumbuhan cepat dan menghasilkan biomassa yang tinggi, namun belum dimanfaatkan secara optimal di luar sektor pangan dan pertanian. Senyawa bioaktif utama dari Ulva, yaitu polisakarida ulvan menunjukkan peningkatan aktivitas antioksidan ketika depolimerisasi menjadi bentuk dengan berat molekul yang lebih rendah. Penelitian ini bertujuan untuk menentukan pengaruh tiga metode depolimerisasi fisik, yaitu *ultrasound*, ultraviolet (UV), dan *microwave*, terhadap sifat fisikokimia dan bioaktivitas ulvan yang diekstrak dari *Ulva ohnoi*. Ekstraksi ulvan dilakukan terlebih dahulu dengan metode maserasi panas pada suhu 90°C selama 2 jam. Ekstrak ulvan diberikan perlakuan ultrasonik selama 60, 120, dan 180 menit; paparan UV selama 60, 120, dan 180 menit; serta iradiasi *microwave* selama 5, 10, dan 15 menit. Setiap perlakuan dilakukan tiga ulangan dengan rancangan acak lengkap. Oligoulvan yang dihasilkan dianalisis terhadap berat molekul, viskositas, serta bioaktivitasnya yang meliputi aktivitas antioksidan (metode DPPH), penghambatan enzim α-glukosidase,

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dan aktivitas antiproliferatif terhadap sel fibroblas. Dari ketiga metode, perlakuan ultrasonik selama 180 menit menghasilkan oligoulvan dengan viskositas terendah (1,41±0,06 cP) dan berat molekul terendah (78,2±2,5 kDa), serta menunjukkan peningkatan signifikan dalam aktivitas antioksidan (IC_{50}: 303,33 ppm), penghambatan α-glukosidase (IC_{50}: 111,63±1,47 ppm), dan efek antiproliferatif (45,73±7,83% pada konsentrasi 125 µg/mL). Hasil ini menunjukkan bahwa depolimerisasi menggunakan metode ultrasonik merupakan pendekatan yang menjanjikan dan ramah lingkungan untuk meningkatkan potensi terapeutik ulvan dalam aplikasi biomedis.

Kata kunci: antidiabetes, antiproliferasi sel, gelombang mikro, ultrasonikasi, ultraungu

INTRODUCTION

Ulva species are widely distributed green seaweeds known for their rapid growth rates, which often surpass those of cultivated red and brown seaweeds. The daily growth rate (DGR) of *Ulva* seaweeds can reach up to 24±1.6%, compared to species like *Gracilaria* and *Kappaphycus* with the rate of 3–8% for, and 4–16% for Sargassum (Cirik *et al.,* 2010; Trivedi *et al.,* 2013; Aaron-Amper *et al.,* 2020; Yan *et al.,* 2022). This rapid growth directly contributes to high biomass yield, making *Ulva* seaweed a promising resource for bioenergy production, with a potential productivity of 45 t DW ha^{-1} $year^{-1}$. This number is 2–20 times greater than that of many terrestrial plants, such as straw, willow, and corn, and approximately three times higher than that of brown seaweed (Bruhn *et al.,* 2011). Despite its abundance and nutritional richness, including carbohydrates, proteins, and various minerals, *Ulva* spp. remains underutilized. Current applications of *Ulva* spp. include food, agricultural fertilizer, and livestock feed, highlighting its potential in the agriculture, animal husbandry, and health sectors (Jatmiko *et al.,* 2019; Hamouda *et al.,* 2022; Widyartini *et al.,* 2023). Among these applications, particular attention has been given to ulvan, a sulfated polysaccharide with unique bioactivity.

Ulva spp. have attracted the attention of researchers from various scientific fields worldwide in recent years because of their uniqueness. The uniqueness of *Ulva* spp. lies in their bioactive content, specifically the sulfated polysaccharide, ulvan. Ulvan is a complex molecule with a structure that differs from that of other polysaccharides (Ramadhan *et al.,* 2024a, Pari *et al.,* 2025). It is a sulfated polysaccharide that forms part of the cell wall and constitutes approximately 9–36% of the dry biomass of *Ulva* spp. (Ramadhan *et al.,* 2024a). The main components of ulvan are rhamnose sulfate, uronic acids (glucuronate and iduronate), and xylose (Kidgell *et al.,* 2019). Amor *et al.* (2021) found molecular weights of 1,153–1,841 kDa, which falls into the high molecular weight category. The molecular weight of polysaccharides affects the bioactivity of a compound (Zeng *et al.,* 2023, Pang *et al.,* 2024), which also applies to ulvan. Increased bioactivity shows that ulvan exhibits significantly enhanced antioxidant bioactivity when it has a low molecular weight (Qi *et al.,* 2005; Yaich *et al.,* 2017). One method for reducing molecular weight is depolymerization. Therefore, controlling the molecular weight of ulvan through depolymerization is a promising strategy for enhancing its functional properties.

The depolymerization process can be carried out using different physical and biochemical methods. Biochemical methods are more commonly used in this process; however, they have several drawbacks, such as low yield, high cost, environmental concerns due to waste, and the need for additional purification processes because of the use of chemicals (Omar *et al.,* 2024, Muzzarelli *et al.,* 1999). An alternative is the use of physical methods. To date, many studies have been conducted on polysaccharide depolymerization; however, very few articles have discussed the results of depolymerization using physical methods. Physical depolymerization methods offer several advantages over enzymatic methods, including being more cost-effective and environmentally friendly, as there are no chemical or enzymatic residues that need to be purified or discarded (Chen *et al.,* 2023). However, the potential of physical depolymerization methods for ulvan has not been systematically investigated.

The fundamental theory of physical depolymerization of polysaccharides, particularly those from algae, involves breaking long polymer chains into smaller oligosaccharides or monosaccharides without the use of chemical reagents or enzymes. This process mainly relies on physical treatments such as thermal, ultrasonic, hydrothermal, microwave, ultraviolet, and gamma irradiation, as well as photolysis, which induce the cleavage of glycosidic bonds or disrupt polymer aggregates. Among these, hydrothermal treatment employs high temperature and pressure, including supercritical fluids, to hydrolyze glycosidic linkages in polysaccharides such as alginate and fucoidan, selectively cleaving bonds such as M-M, M-G, and G-G block in alginate while primarily disrupting non-covalent aggregates without significantly altering sugar composition. Ultrasonication applies acoustic cavitation to generate strong mechanical forces that fragment polymer chains, whereas irradiation methods, such as UV and gamma irradiation, act through free radicals or excited states that physically cleave glycosidic bonds. These approaches generally result in partial size reduction, often producing fragments of approximately 50 kDa, and may require combination with other methods to achieve smaller molecular fractions (Perumal *et al*., 2023). In contrast, microwave-assisted depolymerization uses microwave radiation to rapidly heat polysaccharide solutions, accelerating bond cleavage and enhancing oligosaccharide yield (Bounanti *et al*., 2020). In the case of ulvan, a sulfated polysaccharide from green algae, enzymatic depolymerization by ulvan lyases has been extensively studied; however, physical methods can also play an important role as pre-treatments (Gajanayaka *et al*., 2025). In addition, physical depolymerization involves the disruption of non-covalent interactions, such as hydrogen bonding and aggregation, as well as the cleavage of covalent glycosidic bonds, depending on the type and intensity of the applied energy (Beaumont *et al*., 2021).

The depolymerization process aims to produce oligosaccharides that enhance bioactivity. Research on polysaccharide depolymerization has shown a significant increase in antioxidant activity, which protects biomacromolecules from oxidation (Chaouch *et al.*, 2015). This suggests that reducing the molecular weight can enhance the antioxidant activity of other polysaccharides, such as ulvan. Polysaccharides also have the potential to affect cell proliferation, as oligosaccharides exert antiproliferative effects on cells (Ngo *et al.,* 2019). These antiproliferative properties of oligosaccharides underscore their potential as anticancer agents by inducing apoptosis and suppressing COX-2 expression. Several studies have explored the bioactivity of ulvan, including its antidiabetic (Zhang *et al.,* 2022), antioxidant (Al-Badaani *et al.,* 2025; Jacoeb *et al.,* 2024), functional prebiotic (Ramadhan *et al.,* 2024b), and fibroblast cell proliferation properties (Pari *et al.,* 2024). However, the effects of depolymerization using physical methods on the bioactive potential of the resulting oligoulvan remain to be studied. Therefore, this study aimed to determine the effects of three physical depolymerization methods, ultrasound, ultraviolet (UV) radiation, and microwaves, on the physicochemical properties and bioactivity of ulvan extracted from *Ulva ohnoi*.

MATERIALS AND METHODS

Ulva Seaweed Preparation

Green seaweed *Ulva ohnoi* samples were obtained from PT Razindo Global Nusantara, Lombok, Indonesia. The samples were prepared by washing and soaking them in clean water to separate the seaweed from impurities, such as sand, rocks, and plastics, that were still present in the raw material. This preparation process was repeated three times, and the samples were dried at 50°C for 6 h using a dehydrator (GETRA). The samples were leveled to ensure that the ulvan dried evenly. Once dried, the samples formed a thin, light-green layer. The dried samples were stored in zipper bags and kept in a refrigerator until further use.

DNA Identification

DNA identification of *Ulva* samples followed the procedure described by Mutizabal-Aros *et al*. (2024) and was carried

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out using a molecular approach based on the tufA gene. DNA was extracted from specimens collected at Algarrobo Bay, and partial sequences of the tufA gene, approximately 463 bp, were amplified by PCR using specific primers. The resulting sequences were aligned and compared with those available in the GenBank database using BLAST, allowing the identification of the closest species matches. To strengthen the analysis, previously validated tufA sequences from GenBank were included as references. Phylogenetic analyses conducted using both Bayesian and Maximum Likelihood methods incorporated the new sequences alongside reference data, providing strong node support for species-level resolution. This combined approach confirmed the identity of the samples as *Ulva ohnoi*, supported by a close match with AphiaID 376562. This study highlights that, despite morphological plasticity, the tufA gene provides a reliable marker for discriminating among *Ulva* species because of its variability and the availability of validated reference sequences.

Ulvan Extraction

Ulvan extraction followed the method of Kidgell *et al.* (2019), with modifications in temperature, extraction time, and the use of extraction aids during the process. Ulvan extraction was conducted by heating *Ulva ohnoi* in 2 liters of distilled water in a saucepan at a 1:30 (w/v) ratio, maintaining the stove temperature at 80–90°C with periodic manual stirring for 2 h. The suspension was filtered using a nylon filter 200 mesh and allowed to cool to room temperature. Once cooled, 99% isopropyl alcohol was added at a ratio of 1:2 (v/v), and the mixture was left to stand for 24 h to allow the formation of precipitates. The precipitate was then dried using a dehydrator at 50°C (GETRA). The crude ulvan extract was analyzed for its yield, protein, ash, and sulfate contents, and functional groups.

Ulvan Depolymerization

Assessing the depolymerization process of ulvan is crucial for understanding the breakdown of polysaccharide chains and the resulting molecular weight reduction. This process significantly influences the structural integrity and functional properties of ulvan, thereby affecting its potential applications. To investigate the effectiveness of different depolymerization techniques, three treatments, namely ultrasonication, ultraviolet (UV) irradiation, and microwave exposure, were applied to ulvan, as illustrated in Fig. 1.

The depolymerization procedure was performed using three different procedures: ultrasonic (Branson 1510, 150 W 220 V 42 kHz), ultraviolet (UV) light radiation (Phillips, 220 V 5 Hz), and microwave radiation (Duvo

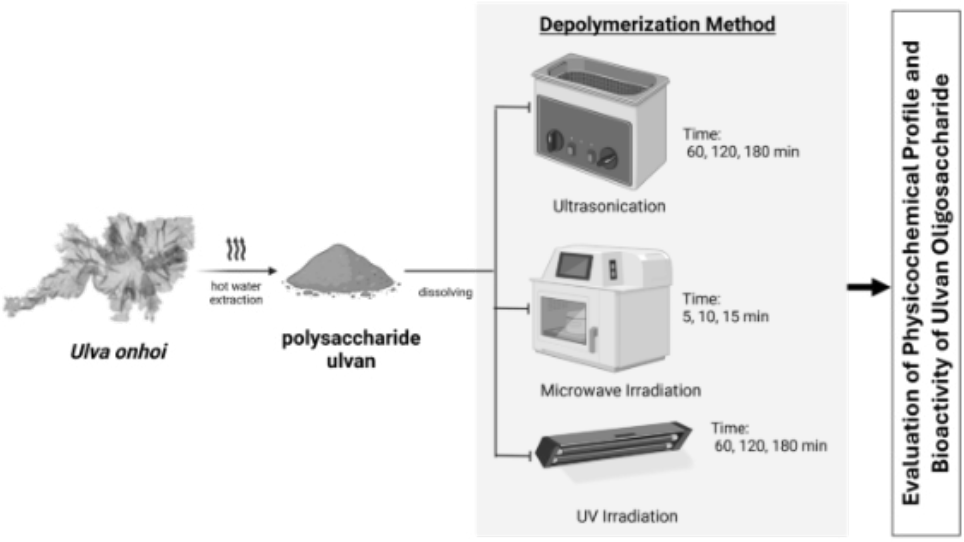

Figure 1 Schematic illustration of ulvan extraction and depolymerization using different method

DV-800). Ultrasonic depolymerization, based on the method described by Tecson *et al.* (2020) with modifications, was conducted by dissolving ulvan at a 2% concentration using 400 mL of distilled water. The sample was placed in an ultrasonic batch device (Branson 1510, 150 W 220 V) set to an output frequency of 40 kHz at a temperature of 40–50°C, maintained using a thermometer. The ulvan solution was depolymerized for 60, 120, and 180 min. The resulting oligoulvan characteristics were analyzed and dried using a freeze-dryer (Eylea).

The ultraviolet depolymerization method followed the procedure described by Pari *et al.* (2022), with modifications to the sample. A 2% (w/v) ulvan solution was prepared by dissolving 8 g of ulvan obtained from 400 mL of distilled water and stirring it with a hot plate at 50°C for 10 min until homogeneous. The solution was then depolymerized by exposure to ultraviolet radiation. The resulting oligo-ulvan was analyzed and dried using a freeze-dryer.

The depolymerization of ulvan polysaccharides using the microwave method followed the procedure described by Bounanti *et al.* (2020). The process began by dissolving ulvan at a concentration of 2% in 400 mL distilled water. The sample was poured into 100 mL glass vials and depolymerized using a microwave (Duvo DV-800 MS) at 800 W for 10, 30, and 60 min. The samples in the vials were then dialyzed using a Visking tube (molecular weight cut-off 12–14 kDa) to separate polysaccharides and oligosaccharides with distilled water. The samples were then lyophilized for further analysis.

Yield

The yield of the extracted ulvan was calculated by weighing the dried extract, following the procedure described by Ramadhan *et al.* (2024b). Ulvan yield was calculated as the ratio of the weight of the extracted ulvan to the weight of the *Ulva ohnoi* seaweed before extraction.

$$\text{Yield (\%)} = \frac{\text{ulvan weight (g)}}{\textit{Ulva} \text{ weight (g)}} \times 100$$

Protein

Protein content was assessed using the Bradford method (1976). The standard protein used was bovine serum albumin (BSA) at a concentration of 0.1–1.0 mg/mL, and it was dissolved in distilled water. A total of 20 µL BSA was added to 1 mL of Bradford reagent (HiMedia). The solution was vortexed and incubated for 5 min. The absorbance of the solution was measured at 595 nm. A total of 20 µL of the sample was added to 1 mL of Bradford reagent. The solution was then homogenized using a vortex mixer and incubated for 5 min at room temperature. The absorbance was then measured at 595 nm using a spectrophotometer (SP-UV1000).

Ash

The ash content was determined according to the AOAC (2005) protocol. The porcelain crucible was cleaned and dried in an oven at approximately 105°C for 30 min, cooled in a desiccator for 30 min, and then weighed. Five grams of ulvan sample were placed in the crucible, then incinerated at 600°C until fully burned. The crucible was cooled in a desiccator and weighed. The ash content was calculated using the following equation:

$$\text{Ash (\%)} = \frac{A-B}{C} \times 100$$

where:
A = sample and dish weight (g)
B = empty dish weight (g)
C = initial sample weight (g)

Sulfate

The sulfate content test was conducted following the AOAC (1995) method by placing 1 g of ulvan into an Erlenmeyer flask. Subsequently, 50 mL of 0.2 N HCl (Sigma-Aldrich) was added, and the mixture was refluxed for 1 h. Then, 25 mL of 10% H_2O_2 (Sigma-Aldrich) solution was added to the sample, and the resulting mixture was refluxed again for 5 h. Once the solution became clear, 10 mL of $BaCl_2$ (HiMedia) solution was added and heated for 2 h. The solution was then filtered using filter paper. The filter paper (Whatman No. 41) was washed with distilled water until free of sulfate, as indicated by

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no color change upon the addition of 0.1 N $AgNO_3$ (HiMedia) for three drops. The filter paper along with the sulfate-free precipitate was incinerated in a crucible at 1,000°C for 5 h and then weighed. The sulfate content was calculated based on the weighing results using the following equation:

$$\text{Sulfate (\%)} = \frac{A-B}{C} \times 100$$

where:
A = sample and dish weight (g)
B = empty dish weight (g)
C = initial sample weight (g)

Viscosity and Molecular Weight

Viscosity testing was performed by preparing a 0.75% ulvan solution. Ulvan (1.5 g) was added to preheated distilled water until the volume reached 200 mL. The solution was then stirred using a magnetic stirrer at 70°C until homogeneous and allowed to cool to room temperature. Once cooled, the viscosity of the solution was measured using a Brookfield Viscometer calibrated with water, with a torque percentage >20.7, using spindle code S02 at a speed of 100 rpm. Molecular weight analysis was performed to determine the changes in the molecular weight of ulvan polysaccharides after depolymerization. The molecular weight of ulvan was measured based on intrinsic viscosity, as described by Montes *et al.* (2021), with modifications. The intrinsic viscosity was obtained by calculating the specific viscosity using the Huggins equation (1942):

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]c$$

where:
ηsp = specific viscosity
[η] = intrinsic viscosity
kH = huggins coefficient (0.3)
c = concentration of ulvan

The relationship between the viscosity and molecular weight of ulvan follows the Mark-Houwink equation. The exponent a and the constant k_{MH} depend on the polymer type and selected solvent. The constant and exponent values used were obtained from Houwink (1940):

$$MW = \sqrt[\frac{1}{a}]{\frac{\eta}{k_{MH}}}$$

where:
MW = molecular weight (kDa)
[η] = intrinsic viscosity
kMH = Mark-Houwink coefficient (1.69×10^{-5})
a = solvent coefficient (2.03)

Functional Groups

Functional group analysis was conducted based on the method described by Tako *et al.* (2015) to characterize ulvan and oligoulvan with a Thermo Scientific Nicoleti S-10. One gram of the sample was placed on the plate until it covered the crystal surface. The metal tip was slid and rotated until it was very close to the sample surface. The sample was scanned 16 times, starting from a wavenumber of 4,000 to 440 cm^{-1}. The scan results produced a percentage transmittance.

Monosaccharide Composition

The monosaccharide composition of each ulvan was determined by high-performance liquid chromatography (HPLC) according to the method described by Baltrusch *et al.* (2024). The ulvan samples were dissolved at 2 mg/mL in aquadest. After dissolution, samples were added to 4% H_2SO_4 and incubated in an autoclave at 121°C for 15 min. Once the incubation process was completed, the samples were filtered using a 0.22 µm syringe filter and stored in glass bottles. An Aminex HPX-87H was used, operating at 60°C, with a mobile phase of 4 M trifluoroacetic acid (TFA) at 5 mL for each 13 mg sample. The HPLC instrument (Shimadzu LC2030C 3D) was equipped with a refractive index detector (RID), and the samples were measured in triplicate.

Antioxidant Activity

The DPPH antioxidant assay of oligo-ulvan was performed as described by Peng *et al.* (2015). A 0.2 µM DPPH solution was freshly prepared by dissolving DPPH crystals (Merck) in methanol immediately before use. Ulvan samples were prepared at a concentration of 300 ppm and diluted to five solutions with concentrations of 300, 240, 180, 120, and 60 ppm. The blank was prepared by mixing

methanol with the DPPH stock solution. The DPPH stock was mixed with the diluted ulvan solution at a ratio of 1:3, using 60 µL of DPPH and 180 µL ulvan. The mixture was then homogenized and incubated at 37°C for 30 min. Absorbance was measured using an Epoch™ Microplate Spectrophotometer at 517 nm. The percentage of free radical scavenging activity (% inhibition) was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

The concentration value that inhibited 50% of the antioxidant activity, or IC_{50}, was calculated using the linear regression equation y=ax+b. The IC_{50} value was obtained by substituting y=50 into the equation, along with the known values of a and b.

Inhibition of α-Glucosidase

The α-glucosidase inhibition assay of oligo-ulvan was performed according to Hsu *et al.* (2013). A concentration variation was prepared for the oligosaccharide, starting from 4 mg/100 µL. A total of 10 µL of oligosaccharide was added to 500 µL of phosphate buffer (pH 7), 250 µL of PNPG at a concentration of 0.625 mM, and 250 µL of α-glucosidase enzyme at a concentration of 0.2 U/mL. The mixture was incubated for 30 min at 37 °C. Then, 2,000 µL of 200 mM sodium carbonate was added to stop enzymatic reaction. The absorbance of the sample was measured using a spectrophotometer at a wavelength of 400.5 nm. The α-glucosidase inhibitory activity could be observed from the color of the solution, where a clearer solution indicated higher enzyme inhibition activity. The inhibitory activity of oligoulvan against α-glucosidase was measured by absorbance at 400.5 nm, and the % inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{(A-B) - (C-B)}{A-B} \times 100$$

where:

A = $A_{control}$

B = A_{blank}

C = A_{sample}

NIH/3T3 Cell Proliferation

The NIH/3T3 cell proliferation test of oligo-ulvan followed the method of Ho *et al.* (2009) with slightly modification. The in vitro cell proliferation test of oligoulvan was evaluated using NIH3T3 cell lines. Oligoulvan was dissolved in Opti-MEM at various concentrations of 125, 75, 50, and 25 µg/mL. NIH3T3 cells were seeded at a density of 5,000 cells in a 96-well plate and incubated for 24 hours. The medium was then replaced with the oligoulvan solution and incubated for another 24 hours. The samples were removed, and the cells were washed with D-PBS, followed by the addition of 100 µL of WST-8 cell counting solution to the NIH3T3 cells, then incubated for 3 hours. The absorbance produced by WST-8 was recorded using a microplate reader at a wavelength of 450 nm. The control was cells without oligoulvan treatment (0 µg/mL) incubated under the same conditions. Relative cell growth was measured using the following equation:

$$\text{Growth rate (\%)} = \frac{A_{sample}}{A_{blank}} \times 100$$

Statistical Analysis

The data analysis used a completely randomized design (CRD) with treatments based on different depolymerization times for each depolymerization method: ultrasonic (60, 120, and 180 min), ultraviolet (60, 120, and 180 min), and microwave (5, 10, and 15 min). Each treatment was performed in triplicate.

The Kolmogorov-Smirnov normality test was performed to analyze the particle size, polydispersity index, and count rate data. The normality test determines whether the data errors follow a normal distribution, which is required for parametric statistical analysis. If the p-value ≥α (0.05), the data were normally distributed. For each depolymerization method, an analysis of variance (ANOVA) was conducted to determine whether the depolymerization time affected the viscosity and molecular weight of ulvan, and the best treatment from each depolymerization method was selected, meeting the p-value<α (0.05). If the analysis indicates significant

effects, the least significant difference (LSD) test will be used to determine the differences between the factor levels. If p-value<α (0.05), the treatments are significantly different, and the selected oligouvan will be chosen. The selected oligoulvan from each method will undergo further analysis of variance and the LSD test to determine the best oligoulvan based on molecular weight, antioxidant activity, and α-glucosidase enzyme inhibition. If p-value<α (0.05), the three methods were significantly different, and the oligoulvan was selected based on the best value. The selected oligoulvan was subjected to a final test for NIH/3T3 cell proliferation. Data analysis will be performed using Microsoft Excel and IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

Ulva ohnoi DNA Identification

DNA identification was performed using the tufA gene marker. The analysis was performed to confirm the species identity of *Ulva* samples collected in this study by comparing their sequences with validated references. Identification was based on query cover, percentage identity, and BLAST matches to GenBank entries, supported by phylogenetic analysis for greater accuracy. The results of DNA identification are presented in Table 1.

A high query cover and high percentage identity are key indicators of reliable DNA-based species identification. A query cover close to 100% indicates that almost the entire DNA sequence is represented in the alignment, whereas a percentage identity approaching 100% indicates that the query sequence is nearly identical to the reference. When these two metrics are combined, they provide strong confidence in species assignment, as low coverage or partial identity often leads to errors in the algal barcoding (Bast *et al*., 2015). Algal DNA barcoding studies usually apply a threshold of 97–99% sequence identity for species-level identification, whereas values below this range may suggest different species or unresolved taxonomy (Bringloe *et al*., 2017). This is especially important for the genus *Ulva*, which is highly prone to misidentification because of its phenotypic plasticity, where a single species can appear very different under varying environmental conditions than other species. Such variation has caused widespread errors in genetic databases, with more than 20% of *Ulva* entries mislabeled, and rates reaching up to 65% in *U. lactuca* (Fort *et al*., 2022). To reduce these risks, BLAST searches against curated databases, together with phylogenetic analysis, provide stronger evidence by prioritizing molecular data over morphological data. Recent studies have confirmed that this combined approach is highly effective in resolving *Ulva* species identities (Ng & Huang, 2024).

Ulvan Composition

The ulvan obtained through an extraction process underwent characteristic analysis. The results of the characterization are shown in Fig. 2. The yield of ulvan obtained from extraction fell within the range reported in previous studies. Variations in ulvan yield are largely influenced by the extraction method, including filtration, precipitation, and purification processes (Alves *et al.,* 2013; Lin *et al.,* 2022). The sulfate content of the extracted ulvan was 31.83%, indicating a high presence of sulfate groups in its structure. As a sulfated polysaccharide, ulvan primarily consists of L-Rhamnose-3-sulfate and Xylose-2-sulfate monomers, which are linked to glucuronic acid and iduronic acid in a repeating sequence. Another study reported that *Ulva papenfussii*

Table 1 DNA identification of *Ulva ohnoi*

Parameters	Results
Sample ID	Algae
Query Cover (%)	81
Percentage Identity (%)	96.30
Species Identified (%)	*Ulva ohnoi*

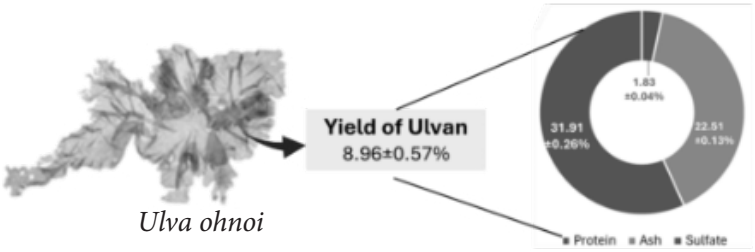

Figure 2 Profile of ulvan extract derived from *Ulva ohnoi*

contains 13% sulfate using a similar extraction method (Tran *et al.,* 2023). Another study reported a sulfate content of 20% in *Ulva fasciata* based on the total ulvan content. Ulvan was obtained using a closed reflux extraction method (Barakat *et al.,* 2022). Even higher values have been reported for *Ulva clathrata*, with a range of 27.87–35.8% (Hernandez-Garibay *et al.,* 2011). These comparisons show that the variability in sulfate content depends on factors such as the extraction method and environmental conditions. However, the consistently high sulfate levels in various *Ulva* species highlight ulvan's strong potential for pharmaceutical and industrial use.

Protein and ash contents in ulvan are key indicators of its purity. Proteins in ulvan, which originates from *Ulva* seaweed and is carried through the extraction process, are considered impurities due to their water-soluble nature (Kidgell *et al.,* 2019). In this study, the extracted ulvan contained 1.83% protein, which is lower than the 7.8% reported by Adrien *et al.* (2017), indicating a relatively high polysaccharide purity level. Similarly, ash content is another measure of ulvan purity, as it represents the presence of inorganic mineral residue. The 22.51% ash content found in this study is consistent with previous findings ranging from 23.3% to 28.5% (Glasson *et al.,* 2017; Shao *et al.,* 2015), but remains lower than the 29.4% reported by Qi and Sun (2015). High ash content suggests the presence of mineral impurities, such as sand, iron, calcium, and sodium, which originate from sediments accumulated on seaweed due to strong ocean currents (Sari-Chmayssem *et al.,* 2019).

The protein and ash contents of ulvan extracts play significant roles in determining their quality and potential applications. Lower protein content typically indicates a higher purity of the polysaccharide fraction, which is particularly important for structural characterization and biomedical applications, as it minimizes interference from protein contaminants and allows for clearer structure–function analysis (Kidgell *et al.,* 2019). Proteins can also influence the solubility of ulvan by enabling hydrogen bonding and hydrophobic interactions, as observed in studies involving ulvan–soy protein composites (Cao *et al.,* 2023). Additionally, increased protein levels can lead to higher viscosity in ulvan solutions due to stronger intermolecular interactions (Sari-Chmayssem *et al.,* 2019). Similarly, lower ash content, which reflects reduced inorganic mineral residue, is often associated with enhanced bioactivity and a higher proportion of organic matter, which is beneficial for applications in pharmaceuticals and cosmetics (Ashour *et al.,* 2025). Excessive ash content can disrupt polysaccharide chain interactions, affecting viscosity and overall functional behavior. Previous studies have also linked low ash content to improved water retention properties, likely because of decreased mineral interference (Sulastri *et al.,* 2021). Hence, minimizing both the protein and ash content in ulvan polysaccharides is essential for optimizing ulvan properties, particularly when preparing them for depolymerization in biomaterial development.

Effect of depolymerization on viscosity and molecular weight

The extracted and depolymerized ulvan polysaccharides were subjected to viscosity testing. The depolymerization methods employed were ultrasonication (60, 120, and

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180 min) and exposure to ultraviolet (UV) light (60, 120, and 180 min) and microwaves (5, 10, and 15 min). Viscosity measurements provide important information about polymer chain length and serve as indicators of molecular weight reduction (Dobrynin *et al.,* 2023). The results show that the duration of the depolymerization process affects the viscosity and molecular weight, as shown in Fig. 3.

Viscometer measurements demonstrated that depolymerization treatments significantly reduced the viscosity of ulvan (Fig. 3A) with a p-value<0.05.

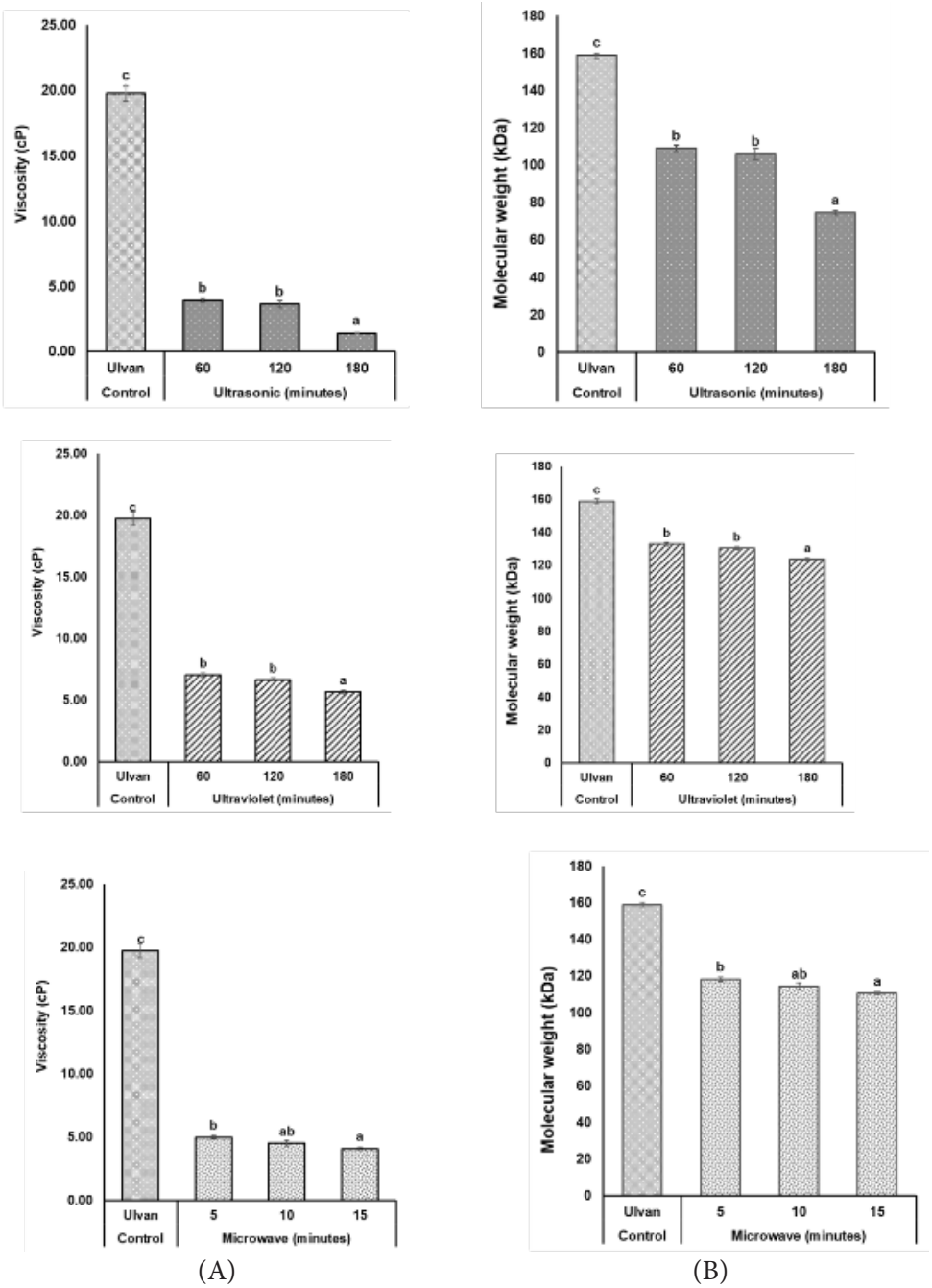

Figure 3 The viscosity (A) and molecular weight (B) of ulvan and oligoulvan obtained with the ultrasonication, exposure to ultraviolet light, and exposure to microwaves. Different letters above bars (a, b, c, d) indicate significant differences at α=0.05.

Among the methods tested, ultrasonication for 180 min resulted in the most pronounced reduction, yielding the lowest viscosity value of 1.41±0.06 cP. Ultraviolet (UV) exposure for 180 min produced a viscosity of 5.67±0.12 cP, while microwave treatment for 15 min yielded 4.07±0.07 cP. The viscosities of the three physical methods were significantly lower than those of the other methods ($p < 0.05$). These reductions are associated with the cleavage of the ulvan polymer backbone, leading to a decreased molecular weight and structural reconfiguration. Each method employs a distinct degradation mechanism: ultrasonic cavitation, UV-induced chain scission, and microwave dielectric heating, which disrupt hydrogen bonding, reduce chain entanglement, and lower the degree of polymerization. Among them, ultrasonication exhibited the strongest depolymerizing effect owing to its mechanical shear and cavitation, which alter polymer conformation and disrupt intermolecular interactions (Noguchi & Kobayashi, 2022). Ultrasonication reduces the intermolecular forces of hydocolloid polysaccharides such as ulvan, leading to decreased viscosity (Li *et al.,* 2022). Comparable reductions in viscosity due to ultrasonication have been reported for polysaccharides such as κ-carrageenan, agar, and alginate (Sanchez, 2013; Sandria *et al*., 2017; Khmelev *et al*., 2018).

Furthermore, UV-induced depolymerization occurs through polymer chain scission caused by ionizing radiation and oxidant atoms reacting with UV light, generating free radicals that randomly break glycosidic bonds and alter the polysaccharide structure, often transforming it from a crystalline to a more amorphous state (Najafabadi *et al.,* 2018). Studies on k-carrageenan and other polysaccharides have confirmed that UV exposure reduces intrinsic viscosity and promotes depolymerization, particularly at low concentrations (Prajapat & Gogate, 2019; Prasetyaningrum *et al.,* 2020; Zhu *et al.,* 2024). Microwave-assisted depolymerization utilizes rapid heating to accelerate hydrolysis reactions and cleave glycosidic bonds efficiently, decreasing molecular weight while preserving sulfate groups important for bioactivity (Prajapat & Gogate, 2015; Abeln *et al.,* 2019; Mission *et al.,* 2019; Bounanti *et al.,* 2020). This results in oligosaccharides with lower viscosity and improved functional properties, making both UV and microwave methods valuable for producing bioactive oligo-ulvans.

Molecular weight analysis confirmed that depolymerization treatments led to a substantial decrease in the molecular size of ulvan (Fig. 3B). The control ulvan exhibited a molecular weight of approximately 160 kDa, which was reduced to 78.2±2.5 kDa following 180 min of ultrasonication. UV treatment for the same duration resulted in a molecular weight of 126.8±1.7 kDa, and microwave treatment for 15 min yielded 118.5±2.1 kDa, with a p-value of less than 0.05 for each. These results demonstrate a direct correlation between the depolymerization time, molecular weight reduction, and viscosity decline. The significant reduction in molecular weight, especially during ultrasonic treatment, suggests that low-molecular-weight ulvan fragments, possibly in the form of oligosaccharides, were successfully generated. This is supported by previous findings, where ultrasonic depolymerization yielded oligosaccharides from other marine polysaccharides (Qiu *et al.,* 2019; Tarman *et al.,* 2023). Ulvans fragmented into oligosaccharide form, often referred to as oligoulvan, possess improved bioavailability and functional properties, making them suitable for applications in nutraceuticals, cosmetics, and drug delivery. Therefore, based on the viscosity and molecular weight data, this study confirms the successful production of oligoulvan, particularly under prolonged ultrasonication, offering a promising strategy for tailoring ulvan properties for high-value applications in the future.

Functional Groups

Functional group analysis was performed using FTIR. The analysis was conducted to determine the structural functional groups in untreated ulvan, compared to the best oligoulvan from each depolymerization method. The FTIR analysis results are shown in Fig. 4.

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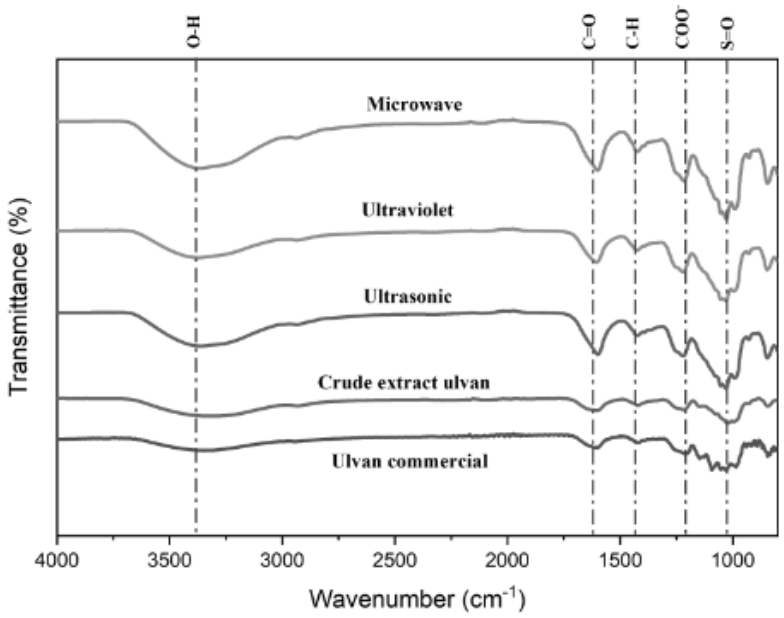

Figure 4 Functional groups of ulvan and oligoulvan in the samples of commercial ulvan, crude ulvan extract, oligoulvan ultrasonicated for 180 min, oligoulvan treated with ultraviolet light for 180 min, and oligoulvan microwaved for 15 min.

The FTIR spectra of ulvan and oligoulvan extracted from *Ulva ohnoi* showed several peaks. The absorption bands in the spectra were almost identical. This suggests that the crude ulvan extract obtained was indeed ulvan. This is evidenced by the identical absorption band B (crude ulvan extract) compared with A (standard ulvan). The absorption peaks exhibited similar functional group structures between oligoulvan and ulvan. The functional groups detected include hydroxyl (O-H), carboxylate (C=O), alkane (C-H), ester (COO–), and sulfate (S-O) groups. The peaks of the oligosaccharides were sharper and more intense than those of the crude ulvan extract. The sharpening and increased intensity indicate the amount of energy transmitted by the sample, which is closely related to the concentration of the functional groups (Krangkratok *et al.,* 2023). The distinguishing feature of oligoulvan compared to crude ulvan extract is the occurrence of peak shifting towards the right. Peaks that shifted to the right indicated absorption at smaller wavenumbers. This is due to the weakening of the polymer bonds resulting from the depolymerization process. Additionally, in the FT-IR spectral readings, peaks shifting to the right indicate the readings of functional group fragments. This causes intermolecular interactions to occur. A similar observation was reported by Lahaye and Robic (2007), who observed a rightward shift in the absorption bands, particularly in the sulfate groups (S=O).

The peaks detected in the spectra confirmed that depolymerized ulvan was successfully transformed into oligosaccharides while retaining the same ulvan characteristics. The wavenumber range of 3,600–3,500 cm^{-1} indicates the O-H stretching vibration of the functional group. The carboxylic acid functional group is indicated by the wavenumber range of 1,700–1,500 cm^{-1}. This value is consistent with the findings of Murphy *et al.* (2008), where the carboxylic acid in this range is an asymmetrical carboxylic acid. The ester group is shown at a wavenumber of 1,250 cm^{-1}, which originates from the sulfate ester present in the ulvan monomer (Tako *et al.,* 2015). The sulfate group is indicated in the wavenumber range of 1,200–900 cm^{-1}. This value was also reported by Crescencio *et al.* (2024) in the range of 1,099–1,136 cm^{-1}. Finally, a fingerprint was observed in the lower wavenumber region, which

served as a marker for ulvan polysaccharides. Wavenumbers below 1,000 cm^{-1} are indicative of ulvan, in which sulfate groups are present (Figueira *et al.,* 2020). The sulfate groups in polysaccharides, particularly ulvan, can enhance bioactivity, including antioxidant and antidiabetic properties (Suresh *et al.,* 2013, Tziveleka *et al.,* 2019).

Monosaccharide Composition Analysis of Oligo-ulvan

The Oligo-ulvan in this study was composed of glucose (Glu), xylose (Xyl), and rhamnose (Rha) sugars. The FTIR analysis results are shown in Fig. 5.

The results showed that depolymerization using ultrasonication and microwaves produced higher peaks than crude ulvan. The concentrations of glucose from ulvan, ultrasonic, microwave, and ultraviolet oligo-ulvan were 132.00, 163.56, 146.01, and 76.08 ppm, respectively. The xylose sugar contents of ulvan, ultrasonic, microwave, and ultraviolet oligo-ulvan were 127.14, 133.36, 144.367, and 60.18 ppm, respectively. Finally, the rhamnose sugar content of ulvan, ultrasonic oligo-ulvan, microwave oligo-ulvan, and ultraviolet oligo-ulvan was 137.01, 159.99, 147.35, and 79.12 ppm respectively. Ultraviolet depolymerization has the lowest sugar concentration compared to the other two depolymerization processes.

The higher HPLC peaks observed in ulvan with ultrasonication and microwave treatments can be correlated to the changes in molecular weight and structural characteristics. Ultrasonication and microwave depolymerization mechanisms involve cleaving ulvan polysaccharides into smaller parts and cause a significantly low average molecular weight. These low molecular weights exhibit greater solubility and sharper and more intense peaks in HPLC (Zhou *et al*., 2024). In the case of ultrasonication treatment, induced physical bond cleavage and alteration of the side chains increase the proportion of oligosaccharides (Tecson *et al*., 2021). Microwave treatment, on the other hand, accelerates chain breakdown and can modify, but is not limited to, uronic

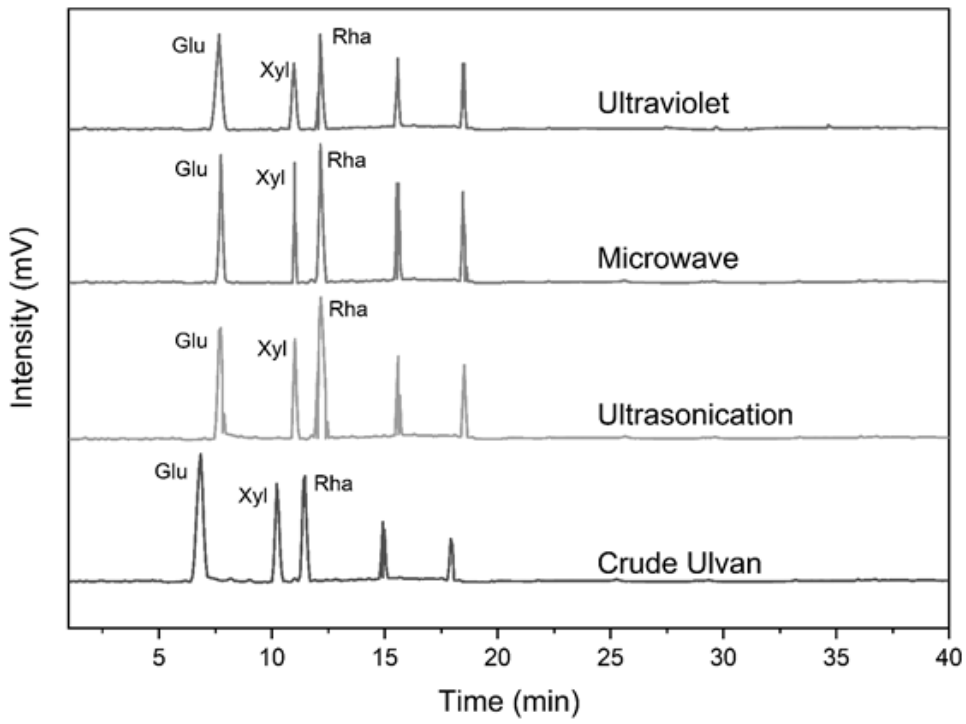

Figure 5 Monosaacharide composition of ulvan, oligoulvan ultrasonicated for 180 min, oligoulvan oligoulvan treated with ultraviolet light for 180 min, and oligoulvan microwaved for 15 min.

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acid or sulfated parts. This factor further affects the retention time and peak intensity of HPLC (André *et al*., 2023). Crude ulvan, in contrast, can reach high-molecular-weight sulfated polysaccharides from *Ulva* algae, which also showed slightly lower peaks than ultrasonication and microwaves (Kraithong *et al*., 2025).

However, ultraviolet treatment did not produce a peak as high as the other two depolymerization methods. Compared to ultrasonication and microwaves, UV irradiation has a different mechanism of degradation instead of the usual cleaving. UV exposure primarily initiates photochemical reactions that produce free radicals or reactive oxygen species (ROS), which promote the oxidative cleavage of ulvan and structural deterioration (Santunione *et al*., 2024). The oxidative pathway can damage both the polysaccharide backbone and ulvan's group, affecting random chain scission and even the loss of functional groups, such as sulfate groups in ulvan. This mechanism eventually reduces molecular integrity and yields fragments that are often less soluble (Araujo *et al*., 2022).

Antioxidant Activity

The IC_{50} antioxidant test indicated that the reduction in molecular weight affected the antioxidant bioactivity. The IC_{50} values of oligoulvan obtained from each depolymerization method showed that different depolymerization methods resulted in significant differences in the antioxidant activity of ulvan (p<0.05). The highest antioxidant activity of oligoulvan is shown in the treatment of ultrasonication for 180 minutes, followed by exposure to ultraviolet light for 180 minutes and to microwaves for 15 minutes. The ultrasonication factor yielded a significantly different IC_{50} compared to the ultraviolet and microwave factors. The same applies to the IC_{50} values for ultraviolet light, but the microwave factor did not show a significant difference compared to the control. This suggests that there was no improvement in the antioxidant bioactivity of ulvan depolymerized by microwave treatment (Fig. 6).

Previous studies have reported a correlation between the lower molecular weights of hydrocolloids and increased antioxidant activity. Research on polysaccharides derived from *Enteromorpha prolifera* and *Sargassum fusiforme* has shown that a reduced molecular weight enhances antioxidant properties. This increase indicates an improved scavenging ability of antioxidants against free radical compounds (Guo *et al.,* 2018; Que *et al.,* 2024). In addition, studies on other low-molecular-weight compounds have indicated that sulfated glucans exhibit better antioxidant properties and enhanced immunological activity (Lei *et al.,* 2015). This improvement is attributed not only to

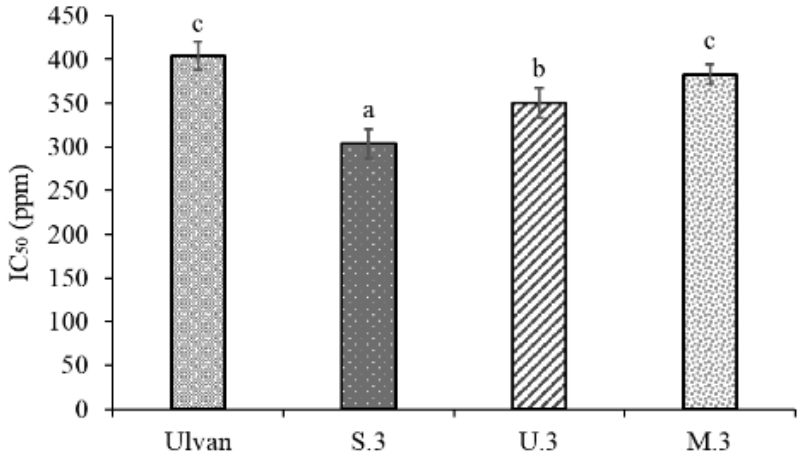

Figure 6 Antioxidant activity in the samples: ulvan polysaccharide, S.3=ultrasonication for 180 min, U.3=ultraviolet light for 180 min, M.3=microwaves for 15 min;
Different letters above bars (a, b, c) indicate significant differences at α=0.05

the low molecular weight but also to the absorption mechanisms in the body, which become significantly better. When the size of a component becomes very small, its absorption in the body, particularly in the small intestine, increases, aiding cells in inhibiting free radicals.

The enhanced antioxidant caused by the reduction in molecular weight only reached an IC_{50} value of 303.33 ppm, indicating a mild free radical scavenging potential for use as an antioxidant source. Similar results have been reported in several previous studies. The antioxidant value of the extracted *Ulva* sp. yielded an IC_{50} of 404.73 ppm (Widowaty *et al.,* 2020). Pradhan *et al.* (2023) also reported that ulvan extracted achieved an IC_{50} value ranging from 623.58–785.48 ppm, which relatively lower than the ulvan acquired in this study. The better IC_{50} value was attributed to the extraction method using an ethanol solution, which has polar properties. Another solvent used for ulvan extraction was n-hexane, resulting in an IC_{50} of 448.659 ppm (Kurniasih *et al.,* 2014). The results reported in previous studies indicate that ulvan polysaccharides have relatively mild antioxidant effects.

Inhibition of α-Glucosidase Activity

The α-glucosidase inhibitory activity analysis is expressed as IC_{50} values in ppm, which varied significantly across the different depolymerization treatments (Fig. 7). The lowest IC_{50} value was recorded for the ultrasonication treatment, with an IC_{50} of 111.63±1.47 ppm, indicating the highest inhibitory activity. This result was significantly different from those of the control, ultraviolet (UV) irradiation, and microwave treatment groups. In contrast, the highest IC_{50} value was observed for the microwave-depolymerized oligoulvan at 236.98±6.33 ppm, indicating the lowest inhibitory activity. These findings suggest that the depolymerization method not only influences the molecular weight but also significantly affects the antidiabetic activity.

Inhibition of α-glucosidase is a strategy for managing diabetes, as it slows carbohydrate digestion and reduces post-meal blood sugar spikes. The effectiveness of an inhibitor is often measured by its IC_{50} value, where a lower IC_{50} indicates stronger inhibition. An IC_{50} of 110 ppm in this study for α-glucosidase inhibition is considered a mild effect compared to standard antidiabetic drugs, which typically have much lower IC_{50} values. Standard antidiabetic drugs, such as acarbose, have reported IC_{50} values ranging from approximately 27 to 51 ppm (Sun *et al.,* 2017; Patil *et al.,* 2024). This is approximately 2–4 times lower than the capability of oligo-ulvan. This mild activity may be attributed to the structural characteristics of oligo-ulvan, such as its sulfated polysaccharide backbone and oligosaccharide chain length, both of which are known to influence enzyme interactions. For instance, lower molecular weights and specific monosaccharide

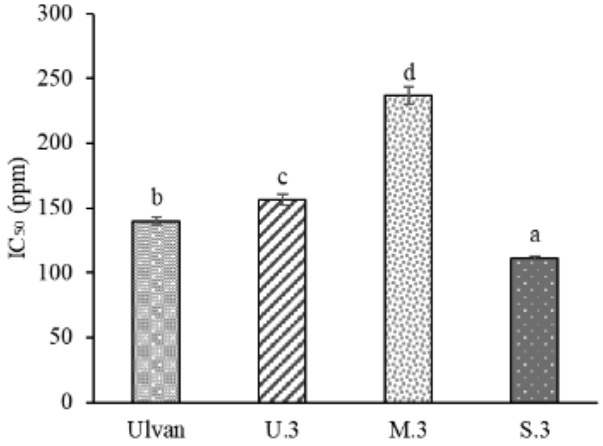

Figure 7 IC_{50} values for α-glucosidase inhibition in ulvan samples, S.3=ultrasonication for 180 min, U.3=ultraviolet light for 180 min, M.3=microwaves for 15 min;
Different letters above bards (a, b, c, d) indicate significant differences at α=0.05

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compositions correlate with stronger α-glucosidase inhibition. Additionally, sulfated polysaccharides from marine sources are known to interact with enzymes through competitive and non-competitive inhibition, possibly due to their negative charge and conformation (Long *et al.,* 2022; Gajanayaka *et al.,* 2024). While the IC_{50} value places oligo-ulvan in the category of modest inhibitors, its natural origin, potential biocompatibility, and additional bioactivities, such as antioxidant activity, may support its further development as a complementary or alternative antidiabetic agent. Further studies, including in vivo evaluations and mechanistic investigations, are required to confirm its efficacy and explore its full therapeutic potential.

NIH/3T3 Cell Proliferation on Sonicated Oligoulvan

The results of the NIH/3T3 cell testing indicated that oligoulvan S.3 (sonication treatment 3) inhibited the growth of these cells (Fig. 8). This inhibition is due to the antiproliferative properties of ulvan. At the highest concentration of 125 µg/mL, it inhibited cell growth by 45.73±7.83%.

At the highest tested concentration (125 µg/mL), oligo-ulvan S.3 inhibited cell growth by 45.73±7.83%, suggesting a notable antiproliferative activity. This antiproliferative effect also implies potential anticoagulant properties, as several known anticoagulants, such as heparin and warfarin, are recognized for their ability to inhibit cell proliferation. Previous studies have demonstrated that heparin inhibits the proliferation of NIH/3T3 fibroblast cells (Ramadhan *et al.,* 2019, Ramadhan *et al.,* 2020, Ling *et al.,* 2023). We hypothesized that oligo-ulvan exerts its inhibitory effect by arresting the cell cycle at the G1/S transition. This phase transition is critical for DNA replication and cell division in eukaryotes. By interfering with this checkpoint, oligo-ulvan may effectively reduce fibroblast proliferation. The antiproliferative activity of ulvan is largely attributed to the presence of sulfate groups in its polysaccharide structure. These functional groups are known to play a key role in modulating cellular responses, including the inhibition of fibroblast and smooth muscle cell growth (Garg *et al.,* 2005). Previous studies have confirmed that the degree of sulfation in polysaccharides correlates with increased antiproliferative potency in NIH/3T3 cells (Song *et al.,* 2019).

Although oligo-ulvan exhibits antiproliferative activity, its effectiveness appears to be more modest than that of well-established anticoagulants, such as heparin. For example, heparin has been reported to inhibit NIH/3T3 cell growth by approximately 45±3% at relatively low concentrations (Cavari *et al.,* 1993), whereas a similar level of inhibition by oligo-ulvan was observed only at a higher concentration of 125 µg/mL. This difference may be related to their distinct mechanisms of action. Oligo-ulvan

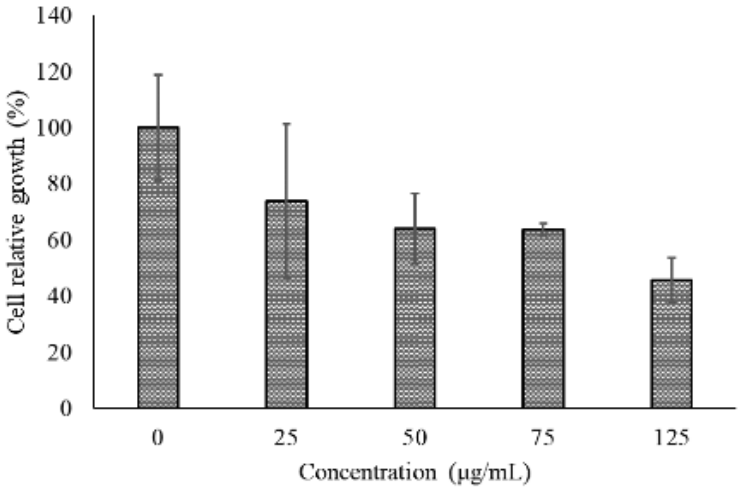

Figure 8 Effect of different concentration of oligo-ulvan ultrasonication 180 min (S.3) on NIH/3T3 cell relative growth)

primarily relies on its sulfate groups to exert biological effects, whereas heparin is known to act through additional pathways, such as inhibiting phosphoinositide-4-phosphate (PIP) kinase, which reduces intracellular levels of phosphatidylinositol 4,5-bisphosphate (PIP2), an important molecule involved in cell proliferation (Garg *et al.,* 2002). Nonetheless, the antiproliferative potential of oligo-ulvan remains promising, especially considering its natural origin and biocompatibility. Although it may be less potent than synthetic drugs in its current form, oligo-ulvan could play a valuable role in biomedical applications, particularly when incorporated into composite systems or formulations with other functional materials, such as gelatin or protein-based polymers. Furthermore, its ability to modulate cellular processes, including those related to growth factors, such as basic fibroblast growth factor (bFGF), highlights its potential as a supportive bioactive compound in therapeutic strategies.

CONCLUSION

This study demonstrated that different physical depolymerization methods, namely ultrasonication, ultraviolet irradiation, and microwave treatment, significantly influenced the physicochemical properties and bioactivity of ulvan extracted from *Ulva ohnoi*. Among the evaluated methods, ultrasonication was the most effective in reducing molecular weight and viscosity, which was associated with enhanced activity. Although the bioactivity of oligo-ulvan was slightly lower than that of the reference compound, it has promising potential for biomedical applications. These findings confirmed that depolymerization treatments play a critical role in determining the functional properties of ulvan. Further studies are recommended to improve product purity and to conduct additional analyses to support its safety and therapeutic potential.

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