International Journal of Biological Macromolecules 366 (2026) 152553

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Ultrasound-assisted valorization of *Posidonia oceanica* polysaccharides: Discovery of novel monosaccharides and enhanced multifunctional bioactivity

Karima Malaoui [a], Asma Ben Haj Mbarek [b], Saida Karouche [c], Mariem Itaimi Dammak [b], Hatem Majdoub [d], Didier Le Cerf [e], Hamdi Bendif [f,*], Tarek H. Taha [f], Walid Elfalleh [f], Mohamed A.M. Ali [f], Ilyas Yildiz [g], Lamjed Bouslama [h], Chawki Bensouici [i], Yacine Laichi [j], Stefania Garzoli [k,*], Aicha Laouani [l,m]

[a] *Laboratory of Natural Substances, Biomolecules, and Biotechnological Applications, Department of Natural and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi, 04000, Algeria*
[b] *Laboratory of Interfaces and Advanced Materials, Faculty of Sciences of Monastir University of Monastir, Monastir, 5000, Tunisia*
[c] *Laboratory of Ecology and Animal Physiology, Department of Natural and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi, 04000, Algeria*
[d] *Department of Chemistry, College of Science, Qassim University, Buraidah, 51452, Saudi Arabia*
[e] *Normandie University, INSA Rouen, CNRS, PBS Laboratory, Rouen, 76000, France*
[f] *Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, 11623, Saudi Arabia*
[g] *Department of Molecular Biology and Genetics, Department of Biotechnology, Institute of Science, Tokat Gaziosmanpaşa University, Tokat, Türkiye*
[h] *Laboratory of Bioactive Substances, Center of Biotechnology, Technopole Borj Cédria, BP 901, Hammam-Lif, 2050, Tunisia*
[i] *Biotechnology Research Center, Ali Mendjli New City UV 03, BP E73, Constantine, 25016, Algeria*
[j] *Scientific and Technical Research Center in Physico- Chemical Analysis (CRAPC), BP384, Bou-Ismail, 42004, Algeria*
[k] *Department of Chemistry and Technologies of Drug, Sapienza University, P. le Aldo Moro, 5, 00185 Rome, Italy*
[l] *University of Sousse, Faculty of Medicine, Laboratory of Metabolic Biophysics and Applied Pharmacology, Tunisia*
[m] *University of Sousse, Faculty of Medicine, USCR Analytical Platform UHPLCMS &Research in Medicine and Biology, Tunisia*

ARTICLE INFO

Keywords:
Polysaccharide
Posidonia oceanica
Ultrasound-assisted extraction
Antioxidant activity
Photoprotective activity (SPF)
Antiviral activity
HSV-2
GC–MS

ABSTRACT

Posidonia oceanica, a Mediterranean seagrass, represents a promising marine resource for the production of high-value polysaccharides with potential industrial and biomedical applications. In this study, ultrasound-assisted extraction (UAE) was deliberately applied under non-optimized conditions to investigate its impact on the structure of polysaccharides obtained from *P. oceanica* leaves (MKF) and rhizomes (MKR), yielding 0.63% and 0.43% (*w*/w), respectively. GC–MS analysis of MKF revealed a diverse monosaccharide profile, including xylose (22.7%), arabinose (21.7%), glucose (16.3%), rhamnose (13.2%), and, for the first time in *Posidonia oceanica*, tentatively identified talose (7.2%), lyxose (7.7%), and fucose (4.0%). Molecular weight determination *via* size-exclusion Chromatography showed 394,000 g·mol^{-1} for MKF and 180,000 g·mol^{-1} for MKR. Leaf-derived polysaccharides exhibited significantly stronger antioxidant activity (DPPH IC_{50} = 10.91 ± 0.70 μg/mL; ABTS IC_{50} = 6.78 ± 0.07 μg/mL) compared to rhizome extracts. Both extracts demonstrated high photoprotective potential (SPF = 42.3 and 39.3 for MKF and MKR, respectively) and substantial antiviral potency toward type 2 herpes simplex virus, with selectivity indices of 750–850 and no detectable cytotoxicity up to 2500 μg/mL. These results suggest that ultrasound-induced structural modifications enhance the bioactivity of *P. oceanica* polysaccharides, positioning them as multifunctional biomolecules with potential applications in pharmaceuticals, cosmeceuticals, and other industrial sectors.

1. Introduction

Posidonia oceanica, a prominent seagrass species endemic to the Mediterranean Sea, plays a pivotal role in marine ecosystems, contributing significantly to biodiversity, coastal protection, and carbon sequestration [1]. Beyond its ecological importance, *P. oceanica* has

* Corresponding authors.
E-mail addresses: hlbendif@imamu.edu.sa (H. Bendif), stefania.garzoli@uniroma1.it (S. Garzoli).

https://doi.org/10.1016/j.ijbiomac.2026.152553
Received 25 March 2026; Received in revised form 12 May 2026; Accepted 14 May 2026
Available online 14 May 2026

garnered increasing scientific interest owing to its diverse and abundant phytochemical composition, which includes a heterogenous spectrum of biologically active metabolites, notably phenolic compounds, flavonoids, and polysaccharides [2,3]. Polysaccharides, in particular, are complex carbohydrates with immense structural and functional diversity, exhibiting a broad spectrum of biological activities encompassing antioxidant, anti-inflammatory, immunomodulatory, and anticoagulant properties [4,5]. These macromolecules are integral components of plant cell walls, providing structural integrity and mediating various physiological processes. The unique marine environment in which *P. oceanica* thrives often leads to the production of novel polysaccharide structures with distinct chemical and biological characteristics compared to terrestrial counterparts [6].

Ultrasound-assisted extraction (UAE) has gained considerable attention as a green and efficient technology for the extraction of natural products, including polysaccharides, due to its advantages in reducing extraction time, solvent consumption, and enhancing yields [7]. However, while UAE is widely adopted for its efficiency, its impact on the structural integrity and molecular characteristics of complex biopolymers like polysaccharides is a critical consideration that often receives less attention. The underlying principle involves acoustic cavitation, which generates microjets and shockwaves, leading to cell wall disruption and improved mass transfer. While the primary goal of UAE is typically to maximize yield and preserve the native structure of target compounds, the intense mechanical forces and cavitation effects inherent in sonication can also induce structural modifications, such as depolymerization or changes in glycosidic linkages, which may inadvertently alter the biological properties of the extracted compounds [8,9]. This study, therefore, deliberately employs non-optimized UAE conditions to investigate these structural modifications and their functional implications, rather than solely focusing on maximizing extraction yield.

To assess the functional relevance of these ultrasound-induced structural alterations, the extracted polysaccharides were further evaluated for antioxidant activity, photoprotective potential related to the sun protection factor (SPF), antiviral activity, and cytotoxicity assays to determine their safety and effects on cell viability.

This study investigates the structural and functional implications of ultrasound-assisted extraction (UAE) on polysaccharides derived from *P. oceanica* leaves and rhizomes. The deliberate application of non-optimized UAE conditions, chosen for its ability to induce structural changes, led to notable deviations in spectroscopic (^{1}H and ^{13}C NMR, FTIR) and chromatographic (SEC, GC–MS) profiles. These deviations strongly suggest structural modifications or partial depolymerization of the polysaccharides. Rather than viewing these changes as limitations, this research explores how such UAE conditions may generate novel polysaccharide structures with potentially enhanced biological activities. By characterizing these alterations, the study seeks to elucidate the functional significance of processing-induced changes in shaping biofunctional properties and to support future valorization strategies for *P. oceanica* polysaccharides.

2. Materials and methods

2.1. Plant material

Specimens of *P. oceanica* were collected in July 2022 from the Mediterranean Sea, off the eastern coast of Algeria, specifically from La Caroube, Annaba (36°54′N, 7°46′E; Fig. 1), at a depth of 8–10 m. The plant material was carefully separated into rhizomes and leaves, thoroughly washed with distilled water to eliminate epiphytes and adhering debris, and then, the cleaned plant sample was left to dry at room temperature until it was complete dehydration. Once dried, it was pulverized into a fine powder with a laboratory milling apparatus (IKA A11 Basic, Germany). The powdered material was subsequently preserved in hermetically sealed containers at 4 °C until further analysis."

2.2. Chemicals and reagents

All reagents and chemical materials utilized in this study were of analytical-grade specification. Deuterium oxide (D_2O, 99.9% D) for NMR spectroscopy was purchased from Sigma-Aldrich (Merck). All other solvents and reagents were procured from Sigma-Aldrich (Merck).

2.3. Polysaccharide aqueous extraction (ultrasound-assisted)

P. oceanica rhizome and leaves powder (100 g) was subjected to the preparation of alcohol-insoluble solids (AIS) by undergoing two successive washes with boiling 70% (*v*/v) ethanol solution (600 mL, 10 min each), followed by a final wash with absolute ethanol (600 mL, 10 min) at ambient temperature, after which the purified pellets were collected. Polysaccharides were subsequently extracted from the powdered *P. oceanica* rhizomes and leaves following the method of Benito-González et al. (2019) [10], wherein 35 g of the purified pellet (rhizome or leaves) was suspended in 700 mL of distilled water in a beaker. The aqueous mixture was then sonicated using an ultrasonic bath with a steady ultrasonic power (Elmasonic S 40H, Singen, Germany) operating at 50 kHz. The extraction was performed at a constant temperature of

Fig. 1. Geographic location of the *Posidonia oceanica* sampling site along the eastern Algerian coast. The map identifies the Annaba coastal area and indicates the precise sampling point. The inset illustrates *in situ* collection conducted by a diver at depths of 8–10 m (original photograph).

60 °C for a total sonication time of 30 min, applied in cycles of a 5 min sonication followed by a 5 min rest. After sonication, the resultant mixture was centrifuged at 4500 rpm for 15 min to separate the supernatant. The supernatant, containing the crude polysaccharide extract, was then precipitated by adding 2 volumes of absolute ethanol and left overnight at 4 °C. The pellets were harvested following a centrifugation. After Sevag-based deproteinization, the crude polysaccharide extract underwent a 72-h dialysis in deionized water through tubing with a molecular-weight exclusion limit of 30 kDa, and then lyophilized to obtain the dried polysaccharide extracts. The polysaccharide extract from leaves was labeled as MKF, and that from rhizome as MKR.

2.4. Nuclear magnetic resonance (NMR) spectroscopy

One-dimensional ^{1}H and ^{13}C nuclear magnetic resonance spectra were acquired using a Bruker Avance III HD 400 MHz spectrometer equipped with a BBO probe for liquids (55 mm bore). Approximately 10–15 mg of each lyophilized polysaccharide extract (MKF and MKR) was dispersed in 0.6 mL of D_2O and transferred into a 5 mm borosilicate NMR tube before acquisition. Spectral calibration was performed relative to the residual HDO resonance at 4.79 ppm for ^{1}H NMR and to external 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (TSP) at 0.00 ppm for ^{13}C NMR. For ^{1}H NMR, spectra were acquired using the pulse program zg30 with the following parameters: TD = 65,536; spectral width (SWH) = 20 ppm; relaxation delay (D1) = 1 s; dummy scans (DS) = 2; number of scans (NS) = 64; pulse width (P1) = 9 μs; power level (PLW) = 18.86 W. For ^{13}C NMR, proton-decoupled spectra were recorded using the pulse program zgpg30 with the following acquisition parameters: TD = 65,536; SWH = 238.8967 ppm; D1 = 1 s; DS = 2; NS = 3096; P1 = 9 μs; PLW = 97.72 W. All NMR data were processed and analyzed using TopSpin (Bruker).

2.5. Size exclusion chromatography (SEC)

Size-exclusion chromatographic analysis was employed to characterize the molecular-weight distribution of the isolated polysaccharides using a multi-angle light-scattering detector (HELEOS II, Wyatt Technology, Santa Barbara, CA, USA). The chromatographic setup consisted of a Shimadzu LC-10 Ai pump operating with 0.1 mol/L lithium nitrate as the mobile phase and a RID-10 A refractive-index detector (Shimadzu, Tokyo, Japan). Polysaccharide samples (MKF and MKR) were prepared at 2% (*w/v*) and stirred for 24 h to ensure complete dissolution in 0.1 mol/L $LiNO_3$, followed by filtration through a 0.45 μm syringe membrane before injection. Data acquisition and processing were carried out using the Astra 6.1 software package. Molecular-weight parameters were calculated using the Zimm or linear models based on scattering angles between 34.8° and 142.8°, assuming a refractive-index increment (dn/dc) of 0.15 mL/g. Hydrodynamic radii (R_h) were estimated from viscometric data using the Einstein–Simha relationship [11].

2.6. Fourier-transform infrared (FTIR) spectroscopy

Fourier-transform infrared spectra of the lyophilized polysaccharide fractions (MKF and MKR) were obtained using a PerkinElmer BX FTIR spectrometer equipped with an attenuated total reflectance (ATR) module. Each spectrum represented the average of 32 scans collected at a resolution of 4 cm^{-1} across the 4000–400 cm^{-1} wavenumber range. Three independent spectral replicates were recorded for each sample. Spectral interpretation and processing were performed using the Omnic 7.2 software.

2.7. Monosaccharide composition

The monosaccharide profile of the polysaccharides extracted under optimized conditions was determined following a modified protocol adapted from Erbing et al. (1995) [12]. Lyophilized MKF (0.20 mg) was hydrolyzed with 0.5 mL of 6 mol/L HCl at 70 °C for 2 h in sealed nitrogen-flushed tubes. After hydrolysis, 10 μL of D-myoinositol (internal standard, 1300 μg/mL in deionized water) was added. The liberated monosaccharides were converted to their trimethylsilyl derivatives by adding 100 μL each of anhydrous pyridine and BSTFA to the dried hydrolysate, following the procedure of Guentas et al. (2001) [13]. The derivatized residue was dissolved in 100 μL dichloromethane and stored at 4 °C until GC–MS analysis (Agilent 5975C inert MSD with Triple-Axis Detector). A 1 μL aliquot was injected in split mode (250 °C, 20:1 ratio, 1.8 mL/min flow) into an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm). The oven program initiated at 150 °C (1 min), increased to 180 °C at 1 °C/min, then to 250 °C at 2 °C/min, and finally to 300 °C at 15 °C/min (held for 10 min). Mass-spectrometric conditions included an ion-source temperature of 250 °C (electron impact, 70 eV), with the quadrupole at 150 °C, source at 230 °C, and transfer line at 250 °C. Quantification was performed in TIC mode using calibration curves based on concentration-to-peak-area ratios relative to D-myoinositol.

Monosaccharide identification was performed by GC–MS through comparison of the obtained mass spectra with those available in the NIST spectral library (NIST MS Library 2.0). The relative composition of each monosaccharide was calculated based on peak area normalization, with total sugars considered as 100%. While the GC–MS analysis provides strong evidence for the presence of these monosaccharides based on fragmentation patterns and NIST library matches, definitive confirmation would require the use of authentic standards. Nevertheless, their detection, particularly talose and lyxose, represents a novel finding for *P. oceanica* and warrants further investigation.

2.8. In vitro functional and biological assays

This part addresses both functional assays (antioxidant and photoprotective activities) and biological assays (cytotoxicity and antiviral activity) conducted *in vitro*.

2.8.1. Antioxidant activity assays

2.8.1.1. DPPH radical scavenging activity. The antioxidant capacity of the methanolic extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay, following a modified version of the method described by Blois (1958) [14]. A DPPH working solution was prepared by dissolving 4 mg of DPPH in 100 mL methanol to obtain an absorbance of 0.5 at 517 nm. Subsequently, 40 μL of each *P. oceanica* sample, prepared in distilled water at varying concentrations, was mixed with 160 μL of the DPPH solution in a 96-well microplate. After incubation in the dark at room temperature for 30 min, absorbance was measured at 517 nm using a microplate reader (Perkin Elmer, EnSpire, Singapore). BHT served as the positive control. The measured absorbance was then converted into a percentage of inhibition relative to the absorbance of the control solution using the following equation:

$$\%\text{of Free Radical Scavenging} = \left[\left(\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{DPPH}}\right] \times 100 \quad (1)$$

The IC_{50} value (μg/mL), corresponding to the concentration required to inhibit 50% of the DPPH radical, was determined graphically from the relationship between the percentage of inhibition and the extract concentration. All measurements were performed in triplicate to ensure the reproducibility and accuracy of the results [14].

2.8.1.2. ABTS radical scavenging activity. The antioxidant potential of *P. oceanica* extracts was assessed using the ABTS radical cation decolorization method, adapted from Re et al. (1999) [15]. A 7 mM ABTS solution was combined in equal volume with 2.45 mM potassium persulfate and incubated in the dark at ambient temperature for 12–16 h to generate $ABTS^{\bullet+}$. Before analysis, the solution was diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm. For each test, 40 μL of

extract (prepared at varying concentrations in distilled water) was added to 160 µL of the ABTS•$^{+}$ solution in a 96-well plate. After 10 min of incubation in the dark at room temperature, absorbance was recorded at 734 nm. Butylated hydroxytoluene (BHT) served as the reference antioxidant. ABTS scavenging activity was calculated analogously to the DPPH method. Moreover, radical inhibition was expressed as IC_{50} values.

2.8.1.3. Reducing power assay (FRAP). The ferric reducing antioxidant power of *P. oceanica* extracts was evaluated following a modified protocol based on Oyaizu (1986) [16]. This method is based on the ability of antioxidant compounds to reduce the ferricyanide–ferric complex to ferrocyanide, which subsequently forms a colored complex measurable by spectrophotometry, reflecting the reducing power of the sample. Each reaction consisted of 10 µL of sample (dissolved in distilled water), 50 µL of 1% potassium ferricyanide, and 40 µL of phosphate buffer (pH 6.6). The mixture was incubated at 50 °C for 20 min. Subsequently, 50 µL of 10% trichloroacetic acid, 10 µL of 0.1% $FeCl_3$, and 40 µL of distilled water were added. Absorbance was measured at 700 nm. Antioxidant standards included BHA, BHT, α-tocopherol, and ascorbic acid. The concentration corresponding to an absorbance of 0.50 ($A_{0.5}$) was determined from the absorbance curve obtained at different extract concentrations. All measurements were performed in triplicate ($n = 3$) [16].

2.8.1.4. Phenanthroline assay. The chelating activity of *P. oceanica* extracts was determined using a modified phenanthroline assay based on Szydlowska-Czerniak et al. (2008). The principle of this method is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by the antioxidant compounds present in the extract. In a 96-well plate, 10 µL of sample was mixed with 50 µL of 0.02% $FeCl_3$, 30 µL of 0.5% 1,10-phenanthroline in methanol, and 110 µL of methanol to reach a final volume of 200 µL. The mixture was incubated for 20 min at 30 °C in the dark. Absorbance was measured at 510 nm. The concentration corresponding to an absorbance of 0.50 ($A_{0.50}$) was calculated using the same method described previously. BHA and ascorbic acid were used as standards [17].

2.8.1.5. Silver nanoparticle assay. Antioxidant activity *via* silver nanoparticle stabilization was assessed following the method of Ozyurek et al. (2012) [18]. The principle is based on the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) in the presence of antioxidant compounds, resulting in a color change measurable by spectrophotometry, which allows to estimate of the reducing capacity of the sample. SNPs were synthesized by mixing 50 mL of 1 mM $AgNO_3$ with 1 mL of 1% trisodium citrate, added gradually until a pale-yellow color appeared. For testing, 20 µL of *P. oceanica* extract was combined with 130 µL of SNP solution and 50 µL of distilled water in a microplate. After a 30 min incubation at 25 °C, absorbance was recorded at 423 nm. Activity was expressed as Abs_{423} (µg/mL), the concentration required to reach 0.50 absorbance. Reference antioxidants included BHA, BHT, Trolox, and ascorbic acid [18].

2.8.2. Photoprotective activity assay (SPF)

The UV-protective capacity of *P. oceanica* extracts was quantified using an *in vitro* spectrophotometric method adapted from Mansur et al. (1986) [18] and Reis Mansur et al. (2016) [19]. Extracts were dissolved in ethanol (2 mg/mL) and sonicated for 2 min. Absorbance was measured across 290–320 nm using a PerkinElmer EnSpire® microplate spectrophotometer [19,20]. SPF values were calculated using the equation:

Where $CF = 10$ (correction factor), EE(λ) = erythemal effect spectrum, I(λ) = solar intensity spectrum,

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

and Abs(λ) = absorbance at each wavelength. EE(λ) × I(λ) constants were adopted from Sayre et al. (1979) [21]. SPF values were interpreted according to the European Commission recommendations (Official Journal of the European Union, 2006). SPF represents the ratio between the minimal erythemal dose (MED) required for protected skin (2 mg/cm^2) and that for unprotected skin exposed to standardized UV radiation (FDA, 2001). This method is widely used for *in vitro* SPF estimation of natural extracts [22].

2.8.3. In vitro cytotoxicity assay

2.8.3.1. Cell line. Cytotoxicity was assessed using Vero cells, derived from African green monkey kidney fibroblasts.

2.8.3.2. Cell culture. Vero cells were cultured in RPMI-1640 medium supplemented with 5% fetal bovine serum (FBS) and standard 1× mixture of antibiotics and antifungal agents to prevent contamination. Cultures were maintained at 37 °C in a humidified 5% CO_2 incubator. Upon confluence, cells were detached with trypsin-EDTA, resuspended in RPMI-1640 with 2% FBS, and seeded into 96-well plates.

2.8.3.3. MTT-based cytotoxicity assay. The assay was performed using the MTT reagent [23], which is metabolized by mitochondrial succinate dehydrogenase in viable cells to form purple formazan. A series of two-fold dilutions of 100 µL of extract (starting at 2.5 mg/mL, ethanol 3.75%) were applied to confluent Vero cells in 96-well plates. After 72 h, media were replaced with 100 µL MTT solution and incubated for 3 h. Formazan was solubilized with DMSO (15 min), and absorbance was measured at 630 nm. Cytotoxicity was expressed as CC_{50}, the concentration causing a 50% reduction in cell viability.

2.8.4. Antiviral activity assay

Antiviral activity against HSV-2 and CVB-3 was evaluated using plaque reduction Kanekiyo et al. (2007) [24] and MTT Guo et al. 2006 assays [25], respectively. Serial two-fold dilutions of extract (starting at $CC_{50}/2$) were incubated with viral inocula (200 PFU for HSV-2; 50 $CCID_{50}$ for CVB-3) on confluent monolayers. After 1 h: (i) for HSV-2, the medium replaced with MEM containing 1% methylcellulose treated with the corresponding sample concentrations; (ii) for CVB-3, the medium replaced with MEM adjusted to contain the same concentration levels of the extract. Infected cells without the addition of the sample were utilized as the positive control.

After 48 h of incubation at 37 °C: (i) for HSV-2, the medium was eliminated, and the cells underwent staining with a 0.2% crystal violet solution (ethanol/water: 1/4), and the plaques were counted under a stereomicroscope; (ii) for CVB-3, the medium was replaced with an MTT solution (1 mg/mL in PBS), incubated for 3 h, then replaced with DMSO (100 µL) to dissolve the formazan, and absorbance was then measured using a microplate reader at 540 nm.

IC_{50} values were calculated by regression analysis. Selectivity index (SI) was defined as CC_{50}/IC_{50}. Extracts with SI ≥ 10 considered active.

2.9. Statistical analysis

All biological experiments were expressed as mean ± standard deviation (SD) from triplicate experiments. IC_{50}, CC_{50}, and SI values were derived *via* linear regression. ANOVA was performed using XLSTAT (v2021.2.1.1203), with Tukey's *post hoc* test for multiple comparisons. Statistical significance was set at $p < 0.001$.

3. Results

3.1. Polysaccharide yields and general characteristics

Ultrasound-assisted aqueous extraction (UAE) of *P. oceanica* yielded

polysaccharide-rich extracts from both leaves (MKF) and rhizomes (MKR). The lyophilized extracts appeared light brown powders and were readily soluble in distilled water, indicating their hydrophilic nature. The extraction yields were 0.63% (*w*/w) for leaves and 0.43% (w/w) for rhizomes.

These results indicate a higher polysaccharide yield from leaves compared to rhizomes under the applied extraction conditions. However, the employed UAE conditions were not optimized for maximum yield, but rather to investigate the structural and functional implications of the method itself. Therefore, a direct comparison of extraction efficiency with other methods was not the primary focus of this study. The observed difference may be related to variations in tissue composition and structural characteristics between leaves and rhizomes.

3.2. Fourier-transform infrared (FTIR) spectroscopy results

The FTIR spectra of polysaccharides extracted from *P. oceanica* leaves (MKF) and rhizomes (MKR) are shown in Fig. 2A and Fig. 2B, respectively. Both MKF and MKR spectra showed broad absorption bands around 3279–3281 cm^{-1}, indicative of O—H stretching vibrations from hydroxyl groups abundant in polysaccharides. Bands at 2927–2941 cm^{-1} and 2851–2862 cm^{-1} confirm the presence of C—H

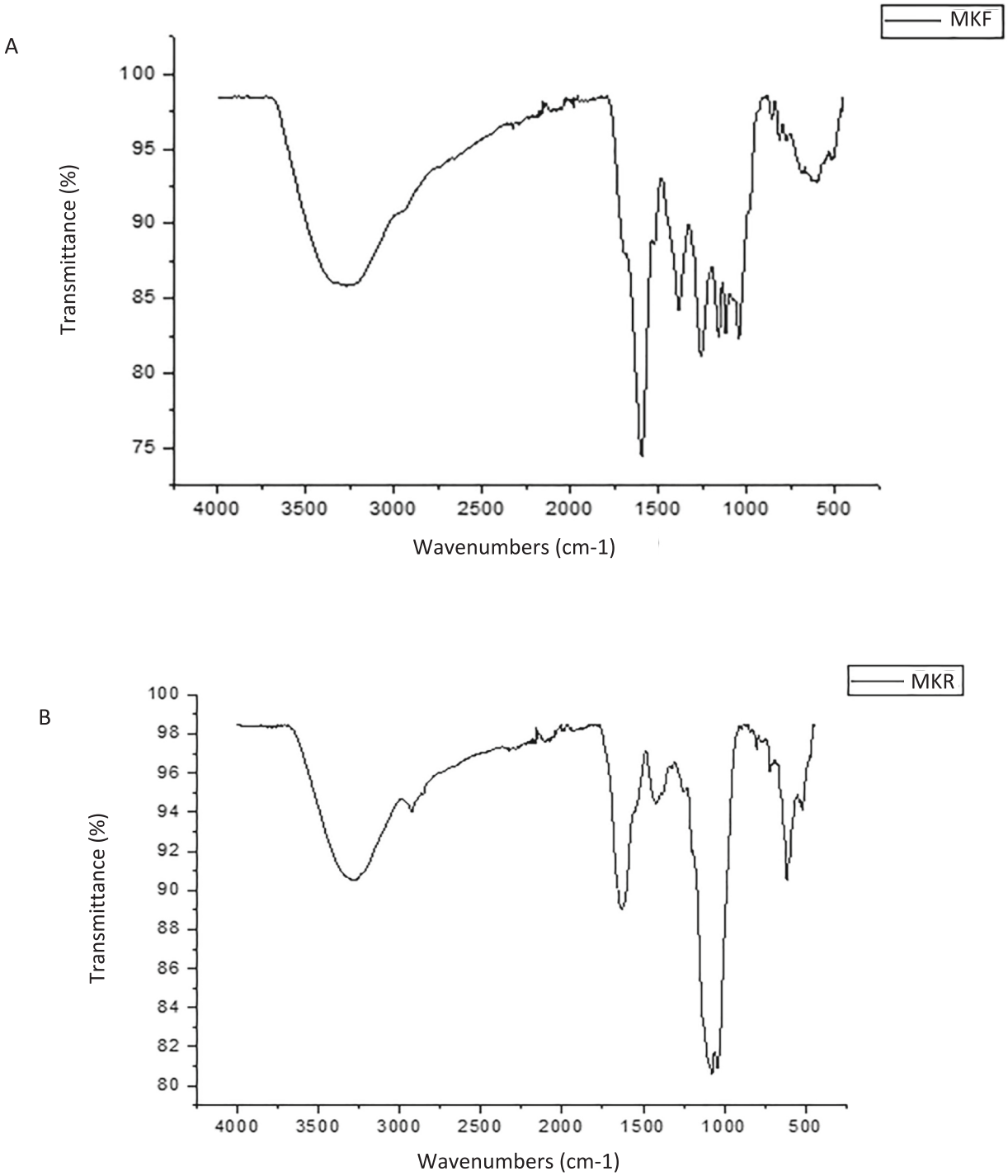

Fig. 2. FTIR spectra of polysaccharides extracted from *P. oceanica* leaves (A) and rhizomes (B).

stretching vibrations. A strong band at 1040–1044 cm^{-1} was observed, characteristic of the C—O stretching of the glycosidic linkages. Notably, bands at 1387 cm^{-1} (MKF) and 1416 cm^{-1} (MKR) are attributed to the C-O-C stretching of carboxylate groups, strongly suggesting the presence of pectins. Furthermore, bands at 1595 cm^{-1} (MKF) and 1635 cm^{-1} (MKR) are assigned to the carboxyl stretching in amide groups, with a noticeable difference in intensity between the leaf and rhizome extracts. Bands in the region of 763–855 cm^{-1} provided insights into the anomeric configurations (α and β) of the sugar units. While these general polysaccharide features were observed, differences in peak intensities, shifts, or the appearance/disappearance of minor bands between the MKF and MKR spectra could indicate structural modifications induced by the ultrasound-assisted extraction. These results are consistent with those of reported in literature [10].

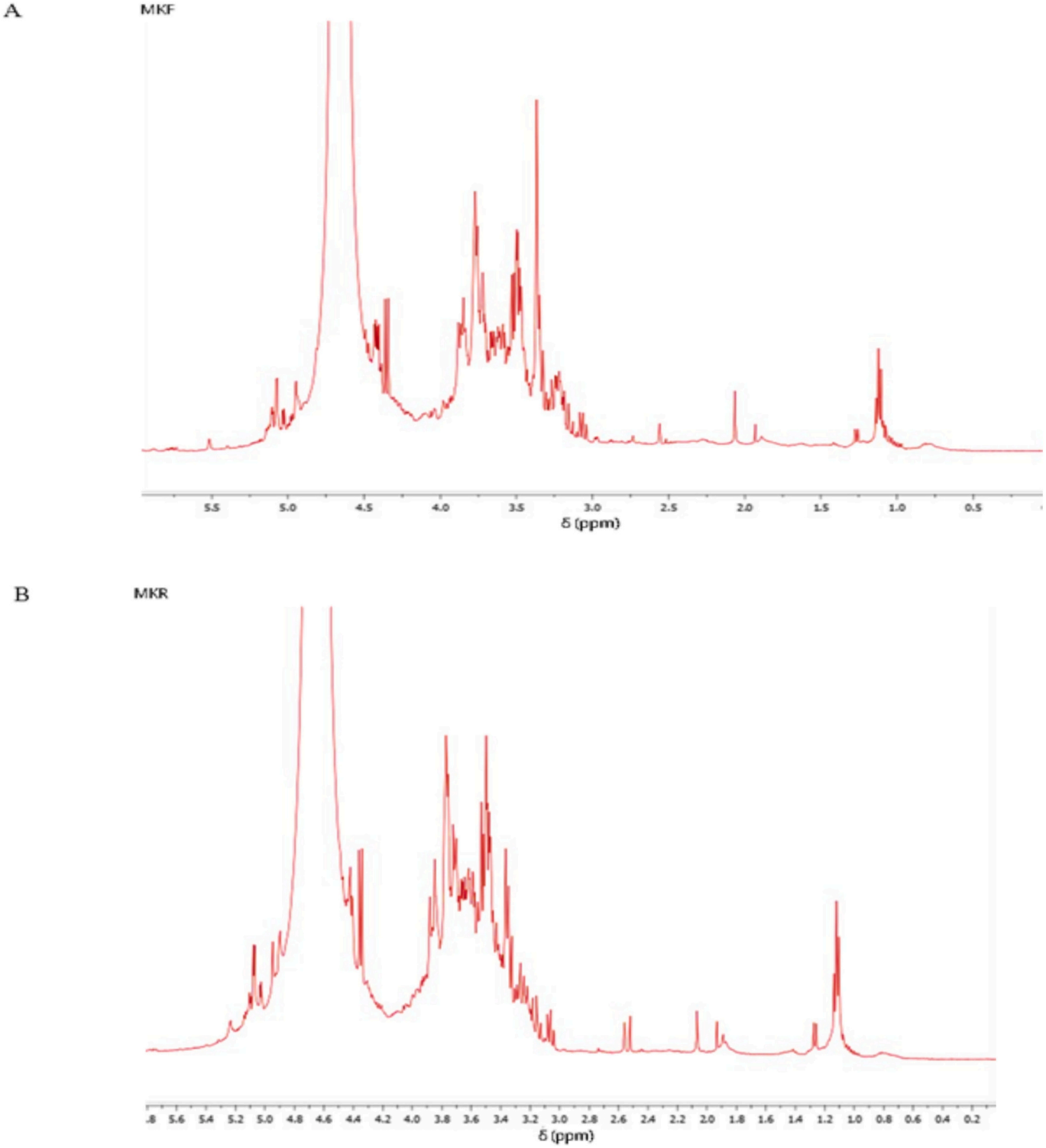

Fig. 3. ^{1}H NMR spectra of polysaccharides extracted from *P. oceanica* leaves (A) and rhizomes (B).

3.3. Nuclear magnetic resonance (NMR) spectroscopy results

3.3.1. ^{1}H NMR spectroscopy

The ^{1}H NMR spectra of polysaccharides extracted from *P. oceanica* leaves (MKF) and rhizomes (MKR) are presented in Fig. 3A and Fig. 3B, respectively. Both spectra exhibited characteristic broad and overlapping signals in the carbohydrate region, typically ranging from 3.0 to 5.5 ppm, which is indicative of complex polysaccharide mixtures. The most intense signals were observed between 3.0 and 4.5 ppm, corresponding to the ring protons (H-2 to H-6) of various sugar residues. A prominent signal around 4.79 ppm is attributed to residual HDO from the D_2O solvent. The anomeric proton region (4.8–5.5 ppm) showed

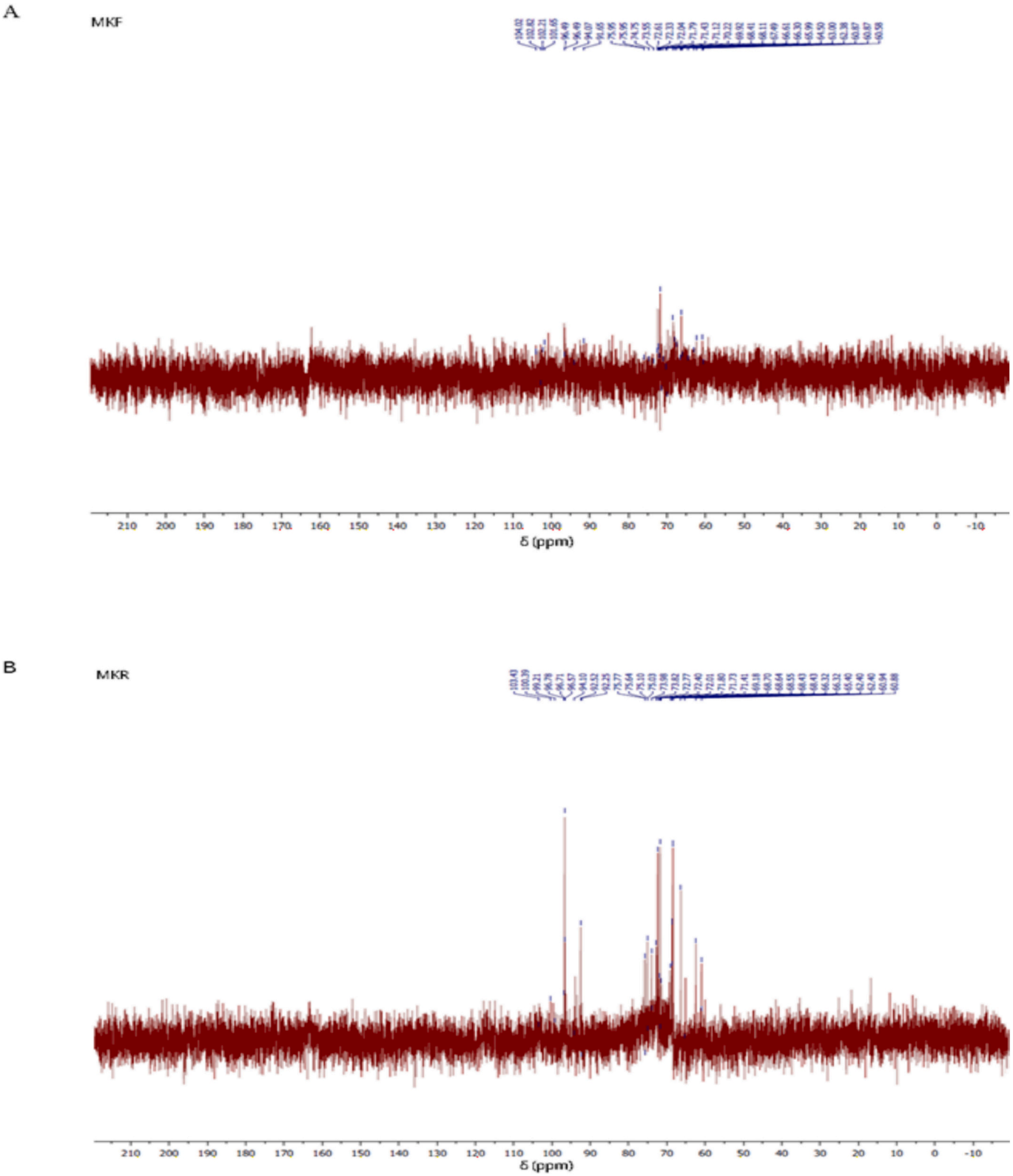

Fig. 4. ^{13}C NMR spectra of polysaccharides extracted from *P. oceanica* leaves (A) and rhizomes (B).

multiple signals, suggesting the presence of different glycosidic linkages and anomeric configurations within the polysaccharide samples. While the overall spectral patterns for MKF and MKR were broadly similar, subtle yet notable differences in peak intensities and fine structures were discernible, particularly when compared to typical ^{1}H NMR spectra of native, undegraded polysaccharides reported in the literature [10,26]. These variations, observed under our non-optimized UAE conditions, strongly suggest potential structural alterations or degradation induced by the ultrasound treatment, which will be further discussed.

3.3.2. ^{13}C NMR spectroscopy

The ^{13}C NMR spectra of polysaccharides extracted from *P. oceanica* leaves (MKF) and rhizomes (MKR) are shown in Fig. 4A and Fig. 4B, respectively. These spectra provide more resolved signals compared to ^{1}H NMR, allowing for a more detailed characterization of the carbon backbone of the polysaccharides. The main signals were observed in the carbohydrate region, typically between 60 and 105 ppm. Both spectra displayed characteristic signals for anomeric carbons (C-1) in the region of 95–105 ppm, indicating the presence of various glycosidic linkages (α-1,4/α-1,6). Signals corresponding to the ring carbons (C-2, C-3, C-5, C-6) were observed between 60 and 85 ppm, while C-6 carbons of hexose units typically resonated around 60–67 ppm free and substitute (α-1,6). Similarly to the ^{1}H NMR, while the general features align with polysaccharide structures, certain peak broadenings, shifts, or changes in relative intensities, particularly in the anomeric and ring carbon regions, were evident. These observations, arising from the non-optimized UAE, indicate structural modifications that will be critically examined in the discussion section. These results are consistent with finding reported in the literature [27].

3.4. Size exclusion chromatography (SEC) results

Size exclusion chromatography (SEC) was employed to characterize polysaccharides extracted from *P. oceanica* leaves (MKF) and rhizomes (MKR), with profiles presented in Fig. 5A and B, respectively. Both chromatograms were analyzed. They exhibited broad elution profiles,

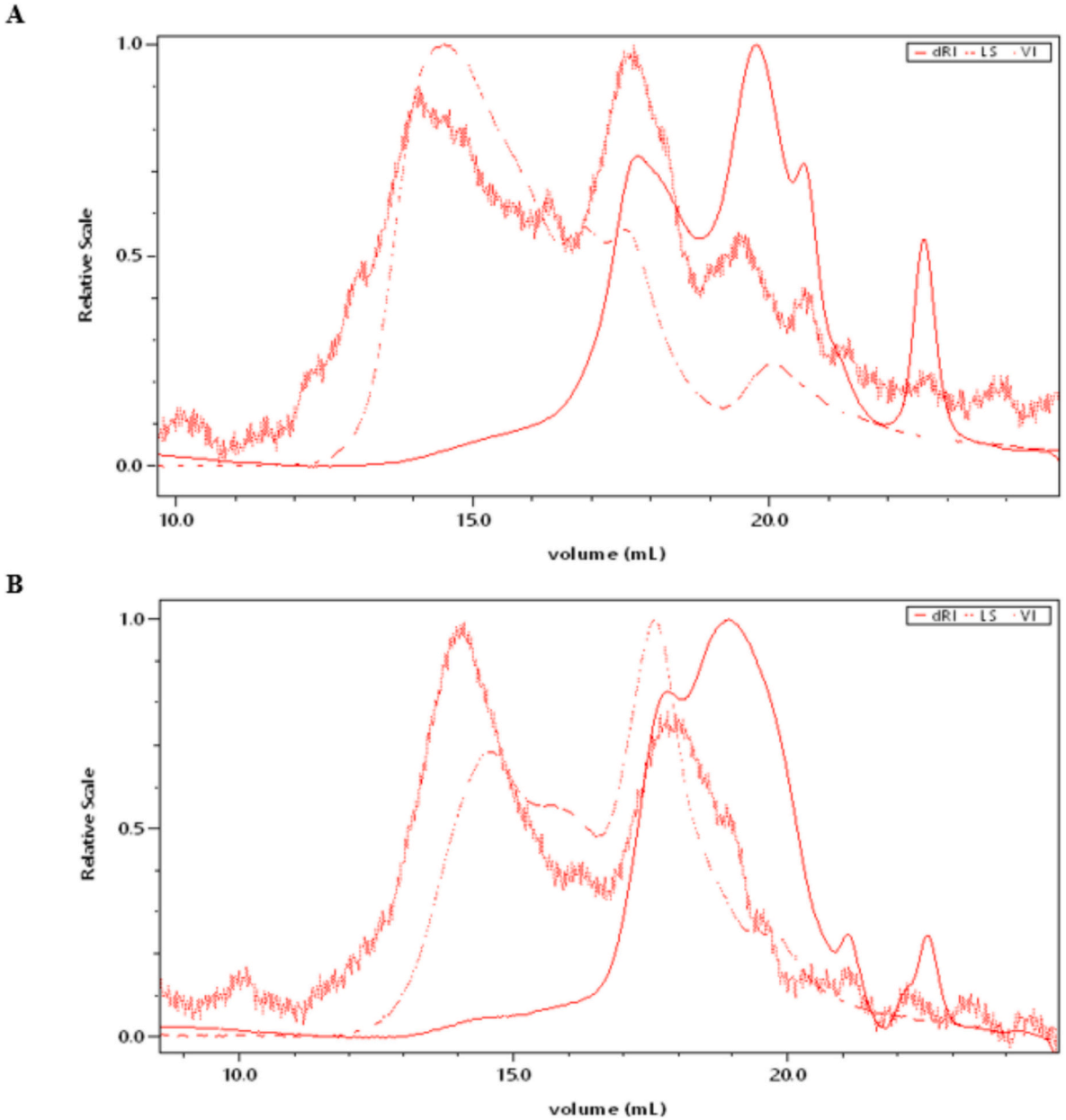

Fig. 5. Size exclusion chromatography profiles of polysaccharides extracted from *P. oceanica* leaves (A) and rhizomes (B).

indicative of heterogeneous polysaccharide mixtures spanning a wide range of molecular weights. The elution profiles detail the molecular weight distribution (circular markers), intrinsic viscosity (triangular markers), and detector responses for light scattering at 90° (dotted line), viscometry (solid line), and differential refractive index (DRI, dashed line).

For MKF (Fig. 5A), a substantial 69% of the injected mass eluted between 11.5 and 21.5 mL, indicating a high concentration of polymeric material. A distinct polymer-rich fraction, comprising 26.6% of the injected mass, eluted within the 15 to 18.5 mL range. This fraction exhibited a number-average molecular weight (Mn) of 105,000 $g{\cdot}mol^{-1}$ and a weight-average molecular weight (Mw) of 394,000 $g{\cdot}mol^{-1}$, yielding a dispersity index (Đ) of 3.8. Further characterization revealed a radius of gyration (Rgw) of 19.7 ± 0.6 nm and a hydrodynamic radius (Rh) of 9.3 ± 0.4 nm. The intrinsic viscosity ([η]w) of 25 ± 3 $mL{\cdot}g^{-1}$ suggests an expanded coil conformation in solution.

Conversely, the MKR chromatogram (Fig. 5B) showed a lower relative recovery (45%) within the same elution window compared to MKF. The polymeric fraction, eluting between 12 and 18.5 mL (17.2% of the injected mass), exhibited lower molecular weight parameters, with Mn and Mw values of 75,800 and 180,000 $g{\cdot}mol^{-1}$, respectively, and a dispersity index of 2.4.

MKR displayed lower molecular weight values than MKF, indicating differences in macromolecular distribution between leaf- and rhizome-derived polysaccharides. Although the Rgw value for MKR was slightly higher (20.6 ± 1.1 nm), the Rhw was lower (8.3 ± 0.4 nm), resulting in an increased Rg/Rh ratio. This suggests possible differences in the hydrodynamic behavior of the two polysaccharide fractions. However, in the absence of complementary structural analyses, detailed conformational interpretations remain tentative.

3.5. Monosaccharide composition of the Posidonia oceanica polysaccharide

The monosaccharide composition of the polysaccharides extracted from *P. oceanica* using ultrasound-assisted extraction was determined by GC–MS analysis of their trimethylsilyl (TMS) derivatives. The resulting chromatogram (Fig. 6) and quantitative data revealed a complex heteropolysaccharide structure, characterized by the presence of eleven distinct monosaccharide derivatives. The relative molar percentage of each constituent sugar, calculated from the peak area, is presented in Table 1.

This analysis was performed only for the leaf-derived polysaccharide (MKF), as the rhizome-derived polysaccharide (MKR) could not be analyzed due to an unexpected instrumental limitation encountered during GC–MS measurements.

Table 1
Monosaccharide Composition of the Polysaccharide Extracted from *P. oceanica* (MKF).

Monosaccharide	Derivative	Retention Time (RT, min)	Relative Molar Percentage (% area)
beta-Arabinopyranose	4TMS	14.027	8.31
beta-L-Rhamnose	4TMS	14.162	13.16
D-Arabinose	4TMS	14.418	13.40
alpha-D-(−)-Lyxopyranose	4TMS	14.691	7.66
beta-L-(−)-Fucopyranose	4TMS	14.954	3.99
beta-D-Xylopyranose (Peak 1)	4TMS	15.150	15.88
beta-D-Xylopyranose (Peak 2)	4TMS	15.562	1.02
D-Xylose	4TMS	15.728	5.77
beta-D-(+)-Talopyranose	5TMS	16.747	7.15
D-Glucose	5TMS	17.218	16.30
D-(+)-Galactopyranose	5TMS	17.973	2.39
Total			**95.03**

The analysis revealed that the extracted material was a heteropolysaccharide with a high degree of structural complexity. The major constituent monosaccharides, accounting for over 70% of the total sugar content, were xylose (22.67%), arabinose (21.71%), glucose (16.30%), and rhamnose (13.16%). The presence of multiple peaks for xylose (at 15.150 min, 15.562 min, and 15.728 min) is typical for GC–MS analysis of reducing sugars, representing different anomeric and/or ring forms (such as pyranose and furanose) of the same monosaccharide. These results are consistent with those documented in the current literature [10,28].

3.6. Functional and biological activities

3.6.1. Antioxidant activity

The antioxidant activity of the polysaccharide extracts from *P. oceanica* leaves (MKF) and rhizomes (MKR) are summarized in Table 1. In the DPPH assay, extract MKF exhibited strong radical scavenging activity with an IC_{50} of 10.91 ± 0.70 μg/mL, which is close to that of BHT (11.03 ± 0.08 μg/mL) but weaker than BHA (3.89 ± 0.09 μg/mL). In contrast, extract MKR showed a markedly lower activity (55.69 ± 0.77 μg/mL). In the ABTS assay, a similar trend was observed, with MKF (6.78 ± 0.07 μg/mL) displaying comparable potency to BHT (3.02 ± 0.03 μg/mL) and clearly greater activity than MKR (45.54 ± 1.25 μg/mL). Regarding the FRAP test, extract MKF (65.54 ± 3.20 μg/mL) demonstrated a moderate reducing power, while extract MKR (152.93

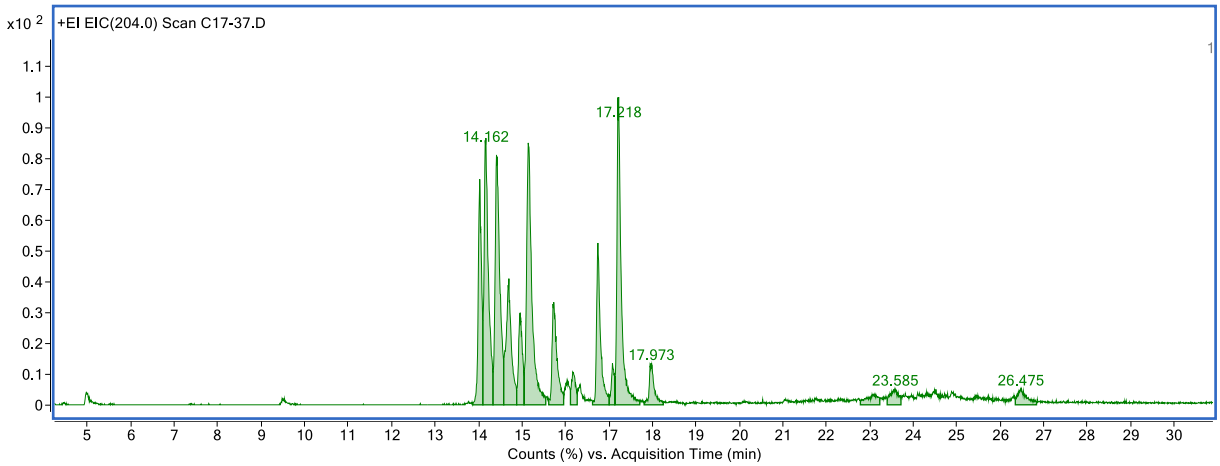

Fig. 6. GC–MS/MS chromatographic profile of monosaccharides identified in the *P. oceanica* leaf extract (MKF).

± 4.47 μg/mL) was substantially weaker. However, both extracts remained less effective compared to the references, particularly BHA (8.14 ± 0.05 μg/mL) and ascorbic acid (14.57 ± 0.40 μg/mL). In the phenanthroline assay, extract MKF (22.44 ± 0.38 μg/mL) showed higher reducing activity than MKR (43.22 ± 1.58 μg/mL), though both were weaker than ascorbic acid (5.88 ± 0.22 μg/mL). Finally, in the SNP assay, extract MKF (50.33 ± 2.25 μg/mL) exhibited significantly stronger nitric oxide scavenging activity compared to extract MKR (278.21 ± 5.33 μg/mL), with values approaching those of BHT (41.80 ± 0.39 μg/mL). Overall, the leaf extract (MKF) consistently exhibited superior antioxidant activity compared to the rhizome extract (MKR), with results in several assays being close to or comparable with standard antioxidants such as BHT, ascorbic acid, and, to a lesser extent, α-tocopherol. This difference may be related to their respective molecular weights and structural features. Despite the relatively low extraction yields (<1%), the observed antioxidant activities suggest that both fractions contain bioactive polysaccharide components of interest. However, their potential application should be considered in the context of high-value, low-dose bioactive uses rather than bulk production.

3.6.2. Photoprotective activity (SPF)

The evaluation of the photoprotective activity of *P. oceanica* polysaccharide extracts was evaluated using the *in vitro* spectrophotometric method, revealing remarkably high sun protection factor (SPF) values. As shown in Table 3, the leaf-derived extract (MKF) exhibited a calculated SPF value of 42.33 ± 0.58, whereas the rhizome-derived extract (MKR) showed a value of 39.28 ± 0.50. Both values fall within the "high protection" category according to international classification standards (SPF range: 30–49.9), corresponding to a labeled SPF of 30.

These results suggest that both extracts possess UV-absorbing properties, which may be related to their polysaccharide composition and structural characteristics. However, these SPF values were obtained using an *in vitro* method and therefore represent an estimation of photoprotective potential rather than direct *in vivo* efficacy. Despite this limitation, the results indicate that *P. oceanica* polysaccharides could be of interest for high-value cosmetic or cosmeceutical applications.

The cytotoxicity and antiviral activity results of *P. oceanica* extracts are presented in Table 2. The polysaccharide extracts MKR and MKF exhibited no cytotoxic effects on Vero cells at a concentration of 2500 μg/mL, indicating that their CC_{50} values exceed this concentration.

3.6.3. Cytotoxicity and antiviral activity against HSV-2 and CVB3

The results of the cytotoxicity and antiviral activity assays of *P. oceanica* extract are presented in Table 4. The polysaccharide extracts MKR and MKF did not exhibit cytotoxicity on Vero cells at a concentration of 2500 μg/mL, indicating that their CC_{50} values exceed this level. Regarding antiviral activity, neither extract showed activity against virus CVB-3. In contrast, both polysaccharide extracts demonstrated activity against virus HSV-2, with CI_{50} values of 3,32 ± 0,42 μg/mL for MKR and 2,85 ± 0,25 μg/mL for MKF, reflecting very high selectivity indices greater than 753 and 877, respectively. Both polysaccharide extracts exhibited remarkable potency and identical antiviral activity against HSV-2. These results suggest that both polysaccharide fractions possess selective antiviral activity against HSV-2 under *in vitro* conditions, with MKF showing a marginally stronger effect. However, further studies are required to confirm these findings and elucidate the underlying mechanisms of action.

Table 3
Sun protection factor (SPF) evaluation of *P. oceanica* polysaccharide extracts.

Extract	Calculated SPF	Measured SPF Range	Labeled SPF	Protection Category
MKF	42,33 ± 0,58	30–49,9	30	High protection
MKR	39,28 ± 0,50	30–49,9	30	High protection

Values are expressed as mean ± SD ($n = 3$); SPF values were determined using the Mansur spectrophotometric method.
The SPF range corresponds to standard European Commission classification.

Table 4
Cytotoxicity (CC_{50}), Antiviral Activity (CI_{50}), and Selectivity Indices (SI) of Polysaccharide Extracts from Leaves (MKF) and Rhizomes (MKR) of *P. oceanica* against HSV-2.

Extracts	CC_{50} (μg/mL)	IC_{50} (μg/mL)	IS = CC_{50}/IC_{50}
MKR	> 2500	3.32 ± 0.42	> 753
MKF	> 2500	2.85 ± 0.25	> 877

MKR: polysaccharides from rhizomes; MKF: polysaccharides from leaves; HSV-2: herpes simplex virus type 2; CC_{50}: 50% cytotoxic concentration; IC_{50}: 50% inhibitory concentration; SI: selectivity index; Values are expressed as mean ± standard deviation (SD) of three independent experiments ($n = 3$); CC_{50} values are reported as the highest concentration tested with no detectable cytotoxicity; SI values were calculated as CC_{50}/IC_{50}; All assays were performed on Vero cells using the MTT colorimetric method.

4. Discussion

This study provides a comprehensive characterization of polysaccharides extracted from the rhizome and leaves of *P. oceanica* using ultrasound-assisted extraction (UAE), integrating insights from ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier-Transform Infrared (FTIR) spectroscopy, and Size Exclusion Chromatography (SEC). Our findings revealed distinct polysaccharide profiles between the two plant parts and, more importantly, offered a critical assessment of the impact of ultrasound-assisted extraction on the structural integrity of these polysaccharides, laying the groundwork for future investigations into their altered biological activities.

4.1. Comparison of extraction methods and polysaccharide yields

The ultrasound-assisted extraction (UAE) method employed in this study yielded 0.63% (*w*/w) for leaves (MKF) and 0.43% (w/w) for rhizomes (MKR). These yields are generally lower than those reported in some previous studies utilizing alternative or optimized extraction techniques for *Posidonia oceanica* polysaccharide. For instance, Ben

Table 2
Antioxidant activity values (IC_{50} and $A_{0.5}$) of polysaccharide extracts from leaves (MKF) and rhizomes (MKR) of *P. oceanica*, compared with standard antioxidants.

Extracts	Yield (%)	IC_{50} μg/mL		$A_{0.5}$ μg/mL		
		DPPH	ABTS	FRAP	PHENANTHROLINE	SNP
MKF	0.63 ± 0.03	10.91 ± 0.70	6.78 ± 0.07	65.54 ± 3.20	22.44 ± 0.38	50.33 ± 2.25
MKR	0.43 ± 0.08	55.69 ± 0.77	45.54 ± 1.25	152.93 ± 4.47	43.22 ± 1.58	278.21 ± 5.33
BHA*	/	3.89 ± 0.09	1.25 ± 0.01	8.14 ± 0.05	2.22 ± 0.33	5.57 ± 0.04
BHT*	/	11.03 ± 0.08	3.02 ± 0.03	17.16 ± 0.33	0,93 ± 0,07	41.80 ± 0.39
Ascorbic acid *	/	5.22 ± 0.30	NT	14.57 ± 0.40	5.88 ± 0.22	10.26 ± 0.62
α-Tocopherol*	/	13.02 ± 5.17	NT	34.93 ± 2.38	NT	NT

Values are means ± SD of three measurements (n = 3). * The standards used. Values with different subscripts in the same column are significantly different ($p < 0.05$). NT: not tested.

salem et al. (2017) reported a polysaccharide yield of 2.55% from *P. oceanica* using microwave-assisted extraction under optimized conditions [26]. Similarly, Benito-González et al. (2019) compared UAE and hot water extraction (HWE) of *P. oceanica* waste-based, reporting yields ranging from 2.1% to 9.3% for hot water-based extracts, with HWE generally yielding higher amounts of carbohydrates, proteins, and polyphenols compared to UAE under their specific conditions [10].

The observed differences in yields can be attributed to several factors. Firstly, the raw material used (fresh leaves and rhizoms in our study *versus* waste biomass in some others) and its pre-treatment can significantly influence extraction efficiency. Secondly, the specific conditions of UAE (*e.g.*, sonication time, temperature, power, solvent-to-solid ratio) were not optimized for maximum yield in our study, but rather to investigate the structural and functional implications of the method itself. It is well established that UAE, while offering advantages in terms of reduced extraction time and solvent consumption, can lead to varying yields depending on the matrix and target compounds [7]. In the study by Benito-González et al. (2019), HWE extracts (E2 H2O) showed higher carbohydrate content than UAE extracts (E2 US), suggesting that prolonged thermal treatment might be more effective for solubilizing certain polysaccharide fractions [10]. Conversely, enzymatic extraction (EAE) has been shown to significantly enhance the extraction of bioactive compounds, including phenolics and flavonoids, from *P. oceanica*, with reported increases of up to ten-fold compared to conventioanal methods [6]. While our study focused on polysaccharides, the literature suggests that EAE could potentially offer higher yields of specific polysaccharide types by selectively breaking down cell wall components.

Our relatively lower yields, therefore, do not necessarily indicate an inefficient extraction but rather highlight the selective nature of the non-optimized UAE conditions employed, which may favor the extraction of specific polysaccharide fractions or induced structural modification that influence subsequent characterization and bioactivity. This aligns with the notion that different extraction methods, can lead to extracts with varying compositions and properties [11,12].

4.2. Interpretation of spectroscopic data: Ultrasound-induced structural changes

The ^{1}H and ^{13}C NMR spectra of *P. oceanica* polysaccharides from both leaves (MKF) and rhizomes (MKR) exhibited characteristic signals consistent with the presence of major polysaccharide classes, including cellulose and hemicelluloses. The ^{13}C NMR data, in particular, provided strong evidence for cellulose with characteristic C-1 (approx. 105 ppm), C-4 (approx. 89 and 84 ppm for crystalline and amorphous regions), and C-6 (approx. 63 ppm) signals [29,30]. The broad and complex signals in the 60–105 ppm region of the ^{13}C NMR, along with the corresponding ^{1}H NMR signals in the 3.0–5.5 ppm range, indicated the presence of diverse hemicelluloses [31,32]. Furthermore, the distinctive carboxyl carbon signals (170–180 ppm) in the ^{13}C NMR confirmed the presence of pectinaceous material [33]. However, a critical comparison of our NMR spectra with those reported for native, non-ultrasound extracted *P. oceanica* polysaccharides, or those obtained through milder extraction methods, revealed subtle yet significant differences. These included pronounced peak broadening, shifts, and changes in the relative intensities of certain signals, particularly in the anomeric (4.8–5.5 ppm for ^{13}CNMR) and ring carbon regions (3.0–4.5 ppm for ^{1}HNMR). For instance, increased peak broadening in the carbohydrate region of both ^{1}H and ^{13}C NMR spectra strongly suggest a higher degree of structural heterogeneity or a reduction in the average molecular size of the polysaccharides due to depolymerization [34]. Such changes are consistent with the known effects of high-energy ultrasound, where acoustic cavitation and mechanical shear can induce chain scission and alter the chemical environment of polysaccharide residues [35]. These observations provide compelling evidence that the ultrasound-assisted extraction process has indeed impacted the structural integrity of the extracted polysaccharides, leading to modifications that are clearly detectable at the molecular level.

4.2.1. FTIR spectroscopy: Confirming functional group alterations

FTIR spectroscopy provided complementary insights into the functional groups and structural characteristics of the extracted polysaccharides. The spectra of both leaf (MKF) and rhizome (MKR) extracts displayed typical polysaccharide absorption bands, including a broad O—H stretching band (3279–3281 cm^{-1}), C—H stretching vibrations (2920–2941 cm^{-1}), and the characteristic fingerprint region (1200–1000 cm^{-1}) associated with C—O and C—C stretching and C-O-H bending vibrations [36,37]. Crucially, subtle yet significant differences in the FTIR spectra between our UAE-extracted samples and those reported for conventionally extracted *P. oceanica* polysaccharides, or even between our leaf and rhizome extracts, further support the notion of ultrasound-induced structural changes. For example, the more intense band at 1595 cm^{-1} in the MKF(leaves) spectrum compared to a less intense band at 1635 cm^{-1} in the MKR (rhizome) spectrum, attributed to the stretching vibration of the carboxyl group in the amide group C(*O*) NH2 [34], could indicate variations in the degree of amidation or the presence of nitrogen-containing compounds. More importantly, changes in the intensity or position of bands within the fingerprint region (*e.g.*, 1040–1044 cm^{-1} for glycosidic linkages, and bands at 855/816 cm^{-1} for α-anomers and 802/763 cm^{-1} for β-anomers) [38] strongly signify alterations in glycosidic linkages, sugar ring conformations, or the relative proportions of different polysaccharide components due to sonication-induced depolymerization or rearrangement. These shifts and intensity changes, while requiring further in-depth analysis for definitive assignment, collectively reinforce the NMR and SEC observations regarding structural modifications caused by the UAE process.

4.3. Size exclusion chromatography: Evidence of depolymerization

The size exclusion chromatography (SEC) profiles for MKF (leaves) and MKR (rhizome) provided direct evidence of the molecular weight distribution of the extracted polysaccharides. Both chromatograms exhibited broad elution profiles, indicating a heterogeneous mixture of polysaccharides with a wide range of molecular weights. Significantly, the presence of substantially lower molecular weight fractions and increased polydispersity, particularly when compared to SEC profiles of native or mildly extracted polysaccharides from *P. oceanica* reported in the literature, provides direct and compelling evidence of depolymerization or chain scission induced by the ultrasound treatment. The mechanical forces and cavitation effects inherent to UAE are known to cause degradation of high molecular weight polymers, leading to a shift toward lower molecular weights and increased polydispersity [39,40]. The observed differences in the molecular weight distributions between the leaves and rhizome extracts (*e.g.*, a higher proportion of lower molecular weight components in MKR compared to MKF) could be attributed to variations in the initial polysaccharide structure, their susceptibility to ultrasound-induced degradation, or variations in the efficiency of the UAE process for plant part. This depolymerization, while reducing the average molecular weight, can also expose new functional groups or alter the three-dimensional conformation of the polysaccharides, which could have profound implications for their biological activities by increasing solubility and accessibility to biological tergets.

4.4. Structural implications of monosaccharide profile

The monosaccharide profile is highly indicative of a cell wall-derived polysaccharide, consistent with the known composition of seagrasses. The dominance of Xylose (22.67%) and Arabinose (21.71%) suggests that the polysaccharide is primarily composed of arabinoxylans or xyloglucans, which are major components of the non-cellulosic cell wall matrix in higher plants and seagrasses [26,28]. Specifically, the high Xylose content aligns with previous studies on *P. oceanica* fibers, where

Xylose was reported as the most abundant neutral sugar [41]. The substantial proportion of Rhamnose (13.16%) further points to the presence of pectic polysaccharides, such as rhamnogalacturonans, which are known to be structural components in the cell walls of aquatic plants [42].

The high relative molar percentage of D-Glucose (16.30%) is a significant finding. While glucose is the backbone of cellulose, which is typically insoluble under these extraction conditions, its presence here suggests either the co-extraction of a highly branched, soluble glucan or the partial hydrolysis and solubilization of xyloglucans or storage glucans during the ultrasound-assisted extraction process [43]. This high glucose content warrants further investigation to differentiate between structural and storage polysaccharide origins.

A key feature of this analysis is the detection of relatively rare monosaccharides, namely beta-D-(+)-talopyranose (7.15%), alpha-D-(−)-lyxopyranose (7.66%), and beta-L-(−)-fucopyranose (3.99%).

The presence of talose and lyxose, particularly talose, is highly unusual in terrestrial plants but has been reported in certain marine organisms and microalgae [44]. Their combined molar percentage of over 14% suggests that they are integral components of the polysaccharide structure rather than minor contaminants. This finding may represent a unique structural signature of the *P. oceanica* polysaccharide, potentially reflecting an evolutionary adaptation to the marine environment. The incorporation of these rare sugars into the backbone or side chains could confer distinct physicochemical properties, such as enhanced water-holding capacity or unique biological activities.

Fucose is a deoxy sugar commonly found in sulfated polysaccharides (fucoidans) of brown algae [45]. Its presence in a seagrass polysaccharide, albeit in a minor amount, suggests a structural motif shared with other marine-derived biopolymers.

The use of ultrasound-assisted extraction (UAE) is a critical factor influencing the final composition. UAE is known to enhance the extraction yield and potentially alter the structure of polysaccharides by causing cavitation and mechanical shear [10]. This process may lead to the extraction of more tightly bound or less soluble polysaccharide fractions, such as those rich in xylose and arabinose, which form the rigid cell wall matrix. Furthermore, the energy input from sonication may cause partial depolymerization or hydrolysis of the native polysaccharide, which could explain the detection of multiple anomeric forms (like the three xylose peaks) and potentially contribute to the high relative percentage of D-glucose if a portion of the cellulose or xyloglucan was solubilized.

4.5. Implications of ultrasound-induced structural changes for biological activity

The observed structural modifications in *P. oceanica* polysaccharides due to ultrasound-assisted extraction, as evidenced by NMR, FTIR, and SEC, are not merely analytical curiosities but hold significant implications for their biological activities. It is well-established that the biological functions of polysaccharides are highly dependent on their structural characteristics, including molecular weight, monosaccharide composition, glycosidic linkages, branching patterns, and conformation [5]. Therefore, any alteration to these features, even subtle ones, can lead to changes in bioactivity. This study proposes that the ultrasound-induced degradation or structural changes, rather than being a drawback, could potentially lead to novel or enhanced biological activities. For instance, depolymerization, as directly evidenced by SEC, can increase the solubility and bioavailability of polysaccharides, making them more accessible for interaction with biological systems [46]. Smaller polysaccharide fragments, often referred to as oligosaccharides, have been shown to possess distinct and sometimes superior biological activities (*e.g.*, antioxidant, prebiotic, immunomodulatory) compared to their high molecular weight counterparts [47]. The exposure of new reducing ends or specific functional groups due to chain scission, as suggested by NMR and FTIR changes, could also create novel binding sites or enhance existing interactions with cellular receptors or enzymes, thereby modulating their bioactivity. Furthermore, the observed differences in the extent of structural modification between leaves and rhizomes, as suggested by the comparative NMR, FTIR, and SEC data, imply that the polysaccharides resulting from each plant parts may exhibit distinct biological profiles. This opens avenues for exploring specific applications for polysaccharides derived from different *P. oceanica* organs, potentially leading to a more targeted and efficient valorization of this marine resource. The critical assessment of the UAE impact, transforming a perceived limitation into a research opportunity, allows for a deeper understanding of the structure-function relationship of *P. oceanica* polysaccharides and paves the way for optimizing extraction parameters to tailor polysaccharides for specific biological applications.

Marine polysaccharides are increasingly recognized as valuable bioactive molecules due to their strong antioxidant and antiviral properties. Their structural diversity, molecular weight distribution, and conformational features play a pivotal role in defining their biological activities [45]. Recent advances in extraction technologies, such as ultrasound-assisted extraction (UAE), have further enhanced the recovery of these macromolecules while occasionally modifying their molecular architecture in ways that may influence their physicochemical and biological properties [48].

Regarding antioxidant activities, our findings clearly demonstrate that polysaccharide extracts from *Posidonia oceanica* leaves exhibited significantly higher radical scavenging and reducing power compared to rhizome extracts. The leaf polysaccharides showed activities comparable to reference antioxidants such as BHT and ascorbic acid in several assays, indicating their strong potential as natural antioxidants. These results are consistent with earlier studies on the antioxidant potential of *P. oceanica* polysaccharides [10,49], and align with findings from other marine plants and algae where polysaccharides displayed potent free radical scavenging activities [50]. The comparatively lower activity observed in rhizome extracts may be linked to structural differences in polysaccharide composition, highlighting the tissue-specific distribution of bioactive compounds within *P. oceanica*.

Antioxidant activity is a complex phenomenon that cannot be adequately assessed by a single assay, since each method targets a distinct mechanism of action. Radical scavenging assays, such as DPPH and ABTS, primarily reflect the ability of compounds to donate hydrogen atoms or electrons, while reducing power assays such as FRAP and phenanthroline evaluate their electron-transfer capacity through metal ion reduction. In contrast, the SNP assay highlights the ability to neutralize reactive nitrogen species, particularly nitric oxide, which plays a crucial role in oxidative stress-induced cellular damage. Therefore, the use of multiple complementary assays ensures a more comprehensive and reliable evaluation of antioxidant potential [51]. The consistent performance of *P. oceanica* leaf extracts across these assays highlights their broad-spectrum antioxidant capacity, beyond the limitations of individual tests.

In addition to these observations, particular attention was devoted to evaluating the photoprotective properties (SPF) of the same extracts to broaden the understanding of their overall bioactive profile.

Prolonged exposure to ultraviolet radiation, especially UV-B (290–320 nm), is one of the major factors responsible for oxidative stress, premature skin aging, and various forms of cellular damage [52]. In this context, the search for natural, effective, and environmentally safe photoprotective filters represents a major scientific and industrial challenge. Marine-derived polysaccharides have recently attracted particular interest due to their antioxidant, moisturizing, and photoprotective properties, making them promising candidates for cosmetic and dermatological formulations [53,54].

The results obtained in this study showed that *P. oceanica* polysaccharide extracts exhibited high sun protection factor (SPF) values, reaching 49 for the leaf extract (MKF) and 39 for the rhizome extract (MKR). According to international classification standards, these values

fall within the "high protection" category, suggesting a significant capacity to absorb UV-B radiation and reduce erythemal effects induced by solar exposure.

These findings support the notable photoprotective potential of marine polysaccharides, which has already been reported for certain algal genera such as *Sargassum*, whose sulfated polysaccharides (fucoidan and carrageenan) have shown SPF values ranging from 25 to 45 [55]. The values obtained in our study for *P. oceanica* therefore fall within a comparable range, and in some cases are slightly higher, highlighting the relevance of this marine plant as an alternative source of natural photoprotective compounds.

The observed photoprotective activity may be attributed to the richness of these polysaccharides in functional groups capable of interacting with UV photons, such as hydroxyl and carboxyl groups, which may confer intrinsic photostabilizing capacity to these macromolecules. Moreover, the possible presence of trace phenolic compounds associated with the polysaccharide matrix could enhance this synergistic effect, contributing to improved radiation absorption and limiting oxidative damage [53,55].

To the best of our knowledge, no previous study has evaluated the photoprotective activity of *P. oceanica* polysaccharides. This originality adds substantial value to the present work and opens the way for the biotechnological valorization of marine phanerogams in the field of skin photoprotection. The results thus represent a promising first step, suggesting that these biomolecules could be incorporated into natural cosmetic formulations intended for UV protection.

Nevertheless, further investigations, particularly regarding photochemical stability, skin penetration, and *in vivo* efficacy, are required to confirm the application potential of *P. oceanica* polysaccharides as sustainable and eco-friendly photoprotective agents.

In this perspective, and considering the frequently observed link between antioxidant, photoprotective (SPF), and antiviral activities of many natural compounds, it appears relevant to also explore the antiviral potential of these extracts. This research direction is aligned with the global context marked by the urgent need to identify new bioactive molecules derived from marine resources.

The increasing prevalence of drug-resistant viral strains and the emergence of new viral infections have made the discovery of novel antiviral compounds an urgent global priority [56]. Natural products remain a cornerstone in antiviral drug discovery, providing chemically diverse scaffolds with unique mechanisms of action [57]. Marine organisms, in particular, have received growing attention as sources of bioactive metabolites, owing to their structural novelty and broad pharmacological potential [58]. Among marine-derived compounds, polysaccharides and sulfated polysaccharides stand out as some of the most promising antiviral agents, capable of inhibiting a wide range of enveloped viruses, including herpesviruses, retroviruses, and coronaviruses [51,59].

Despite these promising results, the majority of the studied marine polysaccharides present significant limitations: some do not combine high antiviral activity with a satisfactory cellular safety profile, while others have not undergone structural modifications to optimize their efficacy. In addition, the variability between enveloped and non-enveloped viruses, as well as the specificity of viral targets, highlights the need to evaluate extracts on a case-by-case basis for each type of virus. These observations emphasize the necessity of precisely characterizing new marine polysaccharides in order to identify those that combine antiviral potency, selectivity, and safety for host cells.

Herpes simplex virus type 2 (HSV-2) is one of the most widespread and persistent human pathogens worldwide. It is the primary cause of genital herpes, a chronic and recurrent infection mainly transmitted through sexual contact. According to estimates from the World Health Organization (WHO), approximately 520 million people aged 15 to 49 are infected with HSV-2, and nearly 205 million experienced at least one symptomatic episode in 2020, of which 92% were attributed to HSV-2.

Available antiviral treatments, such as acyclovir, valacyclovir, and famciclovir, only reduce the duration and severity of symptoms without eradicating the virus. Their effectiveness is limited by the ability of HSV-2 to establish latent infection in neuronal cells and by the emergence of resistant strains.

Recent research has explored the use of natural compounds and plant extracts as alternative antiviral agents. Several marine and terrestrial polysaccharides have demonstrated some antiviral activity *in vitro*. However, most of these extracts do not simultaneously exhibit high efficacy against HSV-2 and low cytotoxicity, nor do they possess specific structural modifications capable of significantly enhancing their antiviral activity Naithani et al., 2010.

In parallel, Coxsackievirus B3 (CVB3) is one of the most medically significant human enteroviruses due to its involvement in various acute and chronic diseases. It is particularly recognized as the leading viral cause of myocarditis and may progress to dilated cardiomyopathy, leading to severe heart failure. This infection primarily affects children, adolescents, and young adults and represents a significant cause of morbidity and mortality worldwide.

According to several epidemiological studies, enterovirus infections, including CVB3, are responsible for seasonal outbreaks and constitute a major public health concern. Despite their clinical impact, no vaccine or specific antiviral treatment is currently available for CVB3. Management mainly relies on symptomatic treatment and supportive care, highlighting the importance of developing new targeted therapeutic strategies.

Broad-spectrum antivirals tested against enteroviruses have shown limited efficacy, mainly due to the genetic variability of the virus, its ability to evade immune responses, and the lack of clearly defined therapeutic targets. Furthermore, oxidative stress and inflammation play a central role in the pathophysiology of CVB3 infection, contributing to cardiac tissue damage and progression toward chronic complications.

Thus, despite ongoing efforts, there remains an unmet medical need for the development of new therapeutic approaches based on natural polysaccharides that combine targeted antiviral activity against both HSV-2 and CVB3 with a high safety profile for host cells.

In this study, we evaluated the biological activity of polysaccharide extracts from the leaves and rhizomes of *P. oceanica*, a Mediterranean endemic seagrass. Two major outcomes emerged: (i) *P. oceanica* polysaccharides exhibited a potent inhibitory effect against herpes simplex virus type 2 (HSV-2) with exceptionally high selectivity indices (SI 750–850), and (ii) no inhibitory effect was detected against Coxsackievirus B3 (CVB-3), a non-enveloped RNA virus, under identical experimental conditions. Importantly, the extracts were non-cytotoxic to Vero cells even at concentrations above 2500 μg/mL, highlighting their favorable safety profile. These findings suggest that *P. oceanica* polysaccharides combine high potency, strong selectivity, and minimal toxicity, placing them among the most promising natural antiviral candidates reported to date.

The selective antiviral profile observed in this study—potent inhibition of HSV-2 but no activity against CVB-3—can be attributed to fundamental differences in viral entry mechanisms. HSV-2, an enveloped DNA virus, requires glycoprotein-mediated attachment and fusion with host cells, processes heavily dependent on interactions with heparan sulfate proteoglycans and viral glycoproteins gB, gC, and gD [60–62]. Sulfated and acidic polysaccharides are known to mimic heparan sulfate, thereby competitively blocking viral attachment and penetration [63]. Our findings strongly suggest that *P. oceanica* polysaccharides interfere with these early steps, consistent with the established mechanism of other marine polysaccharides.

In contrast, CVB-3 is a non-enveloped enterovirus that utilizes receptor-mediated endocytosis *via* the CAR receptor for cell entry [61,64]. Unlike HSV-2, its infection process does not rely on glycoprotein–heparan sulfate interactions, which likely explains the absence of inhibitory effects by *P. oceanica* polysaccharides. This discrepancy highlights the virus-specific nature of polysaccharide bioactivity and

reinforces the importance of structural determinants in defining antiviral specificity [62,63,65].

The extraordinarily high SI values (>700) obtained in this study surpass those reported for many other marine polysaccharides. For instance, sulfated polysaccharides from *Undaria pinnatifida*, *Splachnidium rugosum, Gigartina atropurpurea*, and *Plocamium cartilagineum* showed potent anti-HSV-2 activity with EC_{50} values ranging from 0.7 to 6.6 μg/mL [66]. Moreover, the sulfated polysaccharide PSSD demonstrated superior *in vivo* efficacy compared to acyclovir in alleviating genital herpes symptoms, acting at early infection stages by binding directly to HSV-2 particles and preventing their attachment and fusion [67]. By contrast, acyclovir acts later by inhibiting viral DNA polymerase, underscoring the mechanistic advantage of polysaccharides in targeting the initial steps of infection [62,68].

Other marine-derived polysaccharides further support this early-stage inhibitory paradigm. The sulfated polysaccharide SP2 from Sargassum patens was shown to block HSV-2 at the adsorption stage [69]. Kang et al. (2022) [70] have recently demonstrated that polysaccharides from *Hizikia fusiforme* and *Sargassum horneri* inhibited SARS-CoV-2 spread by interfering with early infection stages. Although not HSV-2-specific, these findings reinforce the hypothesis that sulfated polysaccharides generally target viral adsorption and entry.

Recent reviews consolidating this perspective emphasized that marine polysaccharides exhibit multifaceted antiviral mechanisms, including blocking adsorption, inhibiting replication, and modulating host immunity [63]. Likewise, Dong et al. (2025) highlighted their broad-spectrum potential against enveloped viruses, while Claus-Desbonnet et al. (2022) and Bai & Tuvikene (2021) summarized strong evidence for fucoidans, carrageenans and alginates in HSV inhibition [59,61,62]. Taken together, these reports converge on the view that marine polysaccharides act primarily at the earliest and most critical steps of infection.

Interestingly, *P. oceanica* polysaccharides did not show measurable antiviral activity against CVB-3 in our assays, a result that contrasts with reports of anti-CVB-3 effects from other marine organisms [71]. These discrepancies emphasize that structural features such as sulfation degree, molecular weight, and monosaccharide composition are critical determinants of antiviral specificity [72]. Future structural characterization of *P. oceanica* polysaccharides is therefore essential to understand their selective antiviral spectrum.

The dual antioxidant–antiviral properties of *P. oceanica* polysaccharides are of particular importance, given the role of oxidative stress in exacerbating viral infections, including HSV-2 [73]. By mitigating reactive oxygen and nitrogen species, *P. oceanica* extracts may offer complementary benefits that support both direct antiviral activity and host cell protection.

The absence of cytotoxicity at concentrations up to 2500 μg/mL represents a major strength, particularly given that toxicity is a frequent barrier in the clinical translation of natural antivirals. This excellent safety profile, coupled with SI values exceeding 700, positions *P. oceanica* polysaccharides among the most promising natural antiviral candidates described to date. Notably, their selectivity indices far surpass those of most algal polysaccharides, which typically range from 100 to 400 [74,75].

Taken together, these results consolidate the position of *P. oceanica* within the "blue medicine bank" of marine-derived therapeutic molecules, highlighting its unique potential as both an antioxidant and antiviral source.

4.6. Cross-referencing and originality

This research integrates a multi-spectroscopic and chromatographic approach to critically evaluate the impact of ultrasound-assisted extraction on *Posidonia oceanica* polysaccharides. By systematically analyzing the NMR, FTIR, and SEC data, we provide compelling evidence for ultrasound-induced structural modifications, including depolymerization and subtle changes in functional groups. This detailed characterization, coupled with a comparative analysis between leaves and rhizomes, offers a unique perspective on the phytochemical diversity of *P. oceanica*.

Remarkably, these structural observations were directly confirmed by the obtained biological results. Polysaccharides extracted from both the leaves (MKF) and rhizomes (MKR) of *P. oceanica* demonstrated exceptional antiviral activity against herpes simplex virus type 2 (HSV-2), with high selectivity indices (SI) ranging from 750 to 877. This high level of efficacy, combined with a complete absence of cytotoxicity, highlights the beneficial influence of ultrasound-induced structural modifications, such as depolymerization and the exposure of new functional groups, on the biological activation of polysaccharides. These findings position ultrasound-assisted extraction as a promising strategy for producing innovative biomolecules with strong pharmaceutical potential.

The originality and significance of this work are underscored by:

- Reframing the Narrative: Rather than viewing ultrasound-induced changes as a negative outcome, this study reinterprets them as a potential source of novel bioactive polysaccharides, thereby transforming a methodological challenge into a unique research opportunity. This innovative perspective distinguishes our work from conventional polysaccharide characterization studies.
- Comprehensive Structural Elucidation of Modified Polysaccharides: We provide detailed spectroscopic and chromatographic characterization of polysaccharides that have undergone ultrasound-assisted extraction (UAE), offering insights into the specific types of structural changes (*e.g.*, depolymerization, altered functional groups) that occur during this process. This level of detail is crucial for understanding the structure-function relationships of these modified biopolymers.
- Comparative Organ-Specific Analysis: The comparative study between *P. oceanica* leaves and rhizomes, in the context of UAE-induced modifications, highlights organ-specific differences in polysaccharide susceptibility to sonication and the resulting structural profiles. This contributes to a more nuanced understanding of *P. oceanica* biochemistry.
- Foundation for Bioactivity Studies: By establishing the structural alterations, this study lays a robust foundation for subsequent investigations into the biological activities of these modified polysaccharides. It provides a rationale for exploring whether these changes lead to enhanced, altered, or novel bioactivities, thereby adding significant value to the research.

5. Conclusion

This study critically evaluated the impact of ultrasound-assisted extraction (UAE) on the structural integrity of polysaccharides derived from the leaves and rhizomes of *Posidonia oceanica*, employing a comprehensive set of analytical techniques including ^{1}H and ^{13}C NMR spectroscopy, FTIR spectroscopy, and Size Exclusion Chromatography (SEC). Our findings suggest that ultrasound-assisted extraction (UAE) is a suitable approach for obtaining bioactive polysaccharides. This study deliberately focused on investigating the structural and functional implications of UAE, rather than conducting a direct comparison with other extraction methods. The results provide compelling evidence that UAE induced significant structural modifications, including partial depolymerization, in the extracted polysaccharide. These changes are clearly reflected in the distinct variations observed in NMR and FTIR spectra, as well as a pronounced shift toward lower molecular weights and increased polydispersity in SEC profiles, when compared to typical polysaccharide structures reported in the literature for native or mildly extracted *P. oceanica* polysaccharides.

Importantly, these structural features may be associated with enhanced biological functionality. The extracted polysaccharides

exhibited notable antioxidant activity, demonstrating radical-scavenging capacity across multiple assays (DPPH, ABTS, FRAP and SNP). The samples also showed *in vitro* photoprotective potential, with high SPF values indicating effective UV-B absorption. In addition, promising antiviral activity against herpes simplex virus type 2 (HSV-2) was observed, with high selectivity indices and no detectable cytotoxicity up to 2500 μg/mL under the tested conditions.

These findings suggest that the observed structural characteristics may influence polysaccharide solubility, accessibility and interactions with biological targets, thereby contributing to their bioactivity. However, a more definitive and quantitative understanding of the specific role of ultrasound in inducing these structural modifications would benefit from a direct comparative study with non-ultrasound extraction methods.

Overall, this study provides a basis for future investigations into structure–activity relationships and the optimization of ultrasound-assisted extraction parameters. While the results highlight the potential of *Posidonia oceanica* as a source of multifunctional polysaccharides, further studies are required to validate these findings and explore their applicability in pharmaceutical and biotechnological contexts.

CRediT authorship contribution statement

Karima Malaoui: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Asma Ben Haj Mbarek:** Writing – original draft, Investigation, Formal analysis, Data curation. **Saida Karouche:** Supervision, Resources, Conceptualization. **Mariem Itaimi Dammak:** Methodology. **Hatem Majdoub:** Visualization, Supervision, Resources. **Didier Le Cerf:** Methodology. **Hamdi Bendif:** Project administration. **Tarek H. Taha:** Writing – review & editing, Funding acquisition. **Walid Elfalleh:** Writing – review & editing, Project administration. **Mohamed A.M. Ali:** Funding acquisition. **Ilyas Yildiz:** Methodology. **Lamjed Bouslama:** Methodology. **Chawki Bensouici:** Methodology. **Yacine Laichi:** Methodology. **Stefania Garzoli:** Writing – review & editing, Supervision. **Aicha Laouani:** Supervision, Resources.

Funding

This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2601).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] O.F. Restaino, C.V.L. Giosafatto, S.F. Mirpoor, M. Cammarota, S. Hejazi, L. Mariniello, C. Schiraldi, R. Porta, Sustainable exploitation of Posidonia oceanica sea balls (Egagropili): A review, Int. J. Mol. Sci. 24 (8) (2023), https://doi.org/10.3390/ijms24087301.

[2] M. Karima, K. Saida, O. Benslama, H. Bendif, S. Lekmine, R. Erenler, D. Mouna, L. Bouslama, S.A. Alsalamah, T.H. Taha, F. Boufahja, S. Garzoli, Metabolite profiling and bioactivity of Eastern Algerian, 2025, https://doi.org/10.1021/acsomega.5c08586.

[3] M. Vasarri, L. De Marchi, C. Pretti, Posidonia oceanica (L.) Delile in Focus : In Vitro and In Vivo Evidence for Biomedical Potential, 2026, pp. 1–22.

[4] M.I. Dammak, H. Majdoub, A review of the characterization and biological properties of polysaccharides from food waste, Chem. Afr. 6 (5) (2023) 2265–2279, https://doi.org/10.1007/s42250-023-00715-2.

[5] H. Ben Khelifa, M.I. Dammak, A. Msehli, R. Chebbi, R.B. Said, D. Le Cerf, H. Majdoub, A. Bouraoui, Physico-chemical characterization and evaluation of the antioxidant, anti-inflammatory and antinociceptive potential of fucoidan from the Mediterranean seaweed, *Sargassum vulgare*, Algal Res. (2026) 104667, https://doi.org/10.1016/j.algal.2026.104667.

[6] E.R. Oliveira, A. Nunes, F. de S. Dutra, G.Z. Azevedo, A.R. Schneider, B.R. dos Santos, D. Munaro, S. Moura, G.P.P. Lima, M. Maraschin, Marine and terrestrial biostimulant elicitors of tolerance to cold stress, Front. Plant Sci. 16 (April) (2025) 1–21, https://doi.org/10.3389/fpls.2025.1569516.

[7] N. Riyamol, J. Gada Chengaiyan, S.S. Rana, F. Ahmad, S. Haque, E. Capanoglu, Recent advances in the extraction of pectin from various sources and industrial applications, ACS Omega 8 (49) (2023) 46309–46324, https://doi.org/10.1021/acsomega.3c04010.

[8] X. Lu, Changes in the structure of polysaccharides under different extraction methods, EFood 4 (2) (2023) 1–7, https://doi.org/10.1002/efd2.82.

[9] H. Valisakkagari, C. Chaturvedi, H.P.V. Rupasinghe, Green extraction of phytochemicals from fresh vegetable waste and their potential application as cosmeceuticals for skin health, Processes 12 (4) (2024), https://doi.org/10.3390/pr12040742.

[10] I. Benito-González, A. López-Rubio, A. Martínez-Abad, A.R. Ballester, I. Falcó, L. González-Candelas, G. Sánchez, J. Lozano-Sánchez, I. Borrás-Linares, A. Segura-Carretero, M. Martínez-Sanz, In-depth characterization of bioactive extracts from posidonia oceanica waste biomass, Mar. Drugs 17 (7) (2019) 1–18, https://doi.org/10.3390/md17070409.

[11] G. Ali, C. Rihouey, V. Larreta-Garde, D. Le Cerf, L. Picton, Molecular size characterization and kinetics studies on hydrolysis of pullulan by pullulanase in an entangled alginate medium, Biomacromolecules 14 (7) (2013) 2234–2241, https://doi.org/10.1021/bm400371r.

[12] B. Erbing, P. Jansson, G. Widmalm, Structure of the capsular polysaccharide from the. 273 (1995) 197–205.

[13] L. Guentas, P. Pheulpin, P. Michaud, A. Heyraud, C. Gey, B. Courtois, Structure of a polysaccharide from a Rhizobium species containing 2-deoxy- b - D - arabino -hexuronic acid. 332 (2001) 167–173.

[14] M. Blois, Antioxidant determinations by the use of a stable free radical, Nature 181 (1958) 1199–1200, https://doi.org/10.1038/1811199a0.

[15] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (9) (1999) 1231–1237, https://doi.org/10.1016/S0891-5849(98)00315-3.

[16] M. Oyaizu, Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr. Diet. 44 (6) (1986) 307–315, https://doi.org/10.5264/eiyogakuzashi.44.307.

[17] A. Szydłowska-Czerniak, C. Dianoczki, K. Recseg, G. Karlovits, E. Szłyk, Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods, Talanta 76 (4) (2008) 899–905, https://doi.org/10.1016/j.talanta.2008.04.055.

[18] M. Özyürek, N. Güngör, S. Baki, K. Güçlü, R. Apak, Development of a silver nanoparticle-based method for the antioxidant capacity measurement of polyphenols, Anal. Chem. 84 (18) (2012) 8052–8059, https://doi.org/10.1021/ac301925b.

[19] J. de S. Mansur, M.N.R. Breder, M.C. d'Ascenção Mansur, R.D. Azulay, Determinação do fator de proteção solar por espectrofotometria, An. Bras. Dermatol. (1986) 121–124.

[20] M.C.P.P. Reis Mansur, S.G. Leitão, C. Cerqueira-Coutinho, A.B. Vermelho, R. S. Silva, O.A.F. Presgrave, Á.A.C. Leitão, G.G. Leitão, E. Ricci-Júnior, E.P. Santos, *In vitro* and *in vivo* evaluation of efficacy and safety of photoprotective formulations containing antioxidant extracts, Rev. Bras 26 (2) (2016) 251–258, https://doi.org/10.1016/j.bjp.2015.11.006.

[21] R.M. Sayre, P.P. Agin, G.J. LeVee, E. Marlowe, A comparison of *in vivo* and *in vitro* testing of Sunscreening formulas, Photochem. Photobiol. 29 (3) (1979) 559–566, https://doi.org/10.1111/j.1751-1097.1979.tb07090.x.

[22] B.H. More, S.N. Sakharwade, S.V. Tembhurne, D.M. Sakarkar, Evaluation of sunscreen activity of cream containing leaves extract of Butea monosperma for topical application, International Journal of Research in Cosmetic Science 3 (1) (2013) 1–6.

[23] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1) (1983) 55–63, https://doi.org/10.1016/0022-1759(83)90303-4.

[24] K. Kanekiyo, K. Hayashi, H. Takenaka, J.B. Lee, T. Hayashi, Anti-herpes simplex virus target of an acidic polysaccharide, nostoflan, from the edible blue-green alga Nostoc flagelliforme, Biol. Pharm. Bull. 30 (8) (2007) 1573–1575, https://doi.org/10.1248/bpb.30.1573.

[25] J.P. Guo, J. Pang, X.W. Wang, Z.Q. Shen, M. Jin, J.W. Li, *In vitro* screening of traditionally used medicinal plants in China against enteroviruses, World J. Gastroenterol. 12 (25) (2006) 4078–4081, https://doi.org/10.3748/wjg.v12.i25.4078.

[26] Y. Ben Salem, A. Abdelhamid, K.M. Hammi, D. Le Cerf, A. Bouraoui, H. Majdoub, Microwave-assisted extraction and pharmacological evaluation of polysaccharides from *Posidonia oceanica*, Biosci. Biotechnol. Biochem. 81 (10) (2017) 1917–1925, https://doi.org/10.1080/09168451.2017.1361808.

[27] A. Bruno, A.H. Velders, A. Biasone, M. Li Vigni, D. Mondelli, T. Miano, Chemical composition, biomolecular analysis, and nuclear magnetic resonance spectroscopic fingerprinting of Posidonia oceanica and Ascophyllum nodosum extracts, Metabolites 13 (2) (2023), https://doi.org/10.3390/metabo13020170.

[28] L. Pfeifer, "Neptune balls" polysaccharides: disentangling the wiry seagrass detritus, Polymers 13 (24) (2021), https://doi.org/10.3390/polym13244285.

[29] B. Addison, M.C. Dickwella Widange, Y. Pu, A.J. Ragauskas, A.E. Harman-Ware, Solid-state NMR at natural isotopic abundance for bioenergy applications, Biotechnol. Biofuels Bioprod. 18 (1) (2025), https://doi.org/10.1186/s13068-025-02648-z.
[30] Y. Liu, L. Gao, Z. Yu, Revealing the complexity of polysaccharides: advances in NMR spectroscopy for structural elucidation and functional characterization, Appl. Sci. (Switz.) 15 (10) (2025), https://doi.org/10.3390/app15105246.
[31] I. Álvarez-Martínez, F. Pfrengle, On the structure, conformation and reactivity of β-1,4-linked plant cell wall glycans: why are xylan polysaccharides or furanosyl substituents easier to hydrolyze than cellulose? Cellulose 32 (4) (2025) 2145–2165, https://doi.org/10.1007/s10570-025-06424-y.
[32] R. Mnafki, R. Khiari, Y. Moussaoui, J. Labidi, Posidonia Oceanica: chemical composition, fractionation methods, and applications, Springer Nature Singapore. (2025), https://doi.org/10.1007/978-981-96-4546-6_4.
[33] X. Ye, Z. Xu, D. Xu, J. Cai, S. Dai, Y. Luo, L. Guo, Y. Wang, J. Su, J. Yang, Study on13C MultiCP/MAS ssNMR analysis of tobacco pectin, Curr. Anal. Chem. 20 (9) (2024) 686–696, https://doi.org/10.2174/0115734110315088240516072328.
[34] K. Ferchichi, N. Amdouni, Y. Chevalier, S. Hbaieb, Low-cost Posidonia oceanica bio-adsorbent for efficient removal of antibiotic oxytetracycline from water, Environ. Sci. Pollut. Res. 29 (55) (2022) 83112–83125, https://doi.org/10.1007/s11356-022-21647-3.
[35] Y. Hua, H. Zhang, Q. Fu, Y. Feng, Y. Duan, Modes on the Structure , Chain Conformation , and Immune, 2022.
[36] P. Camarena-Bononad, P.A.V. Freitas, A. Chiralt, M. Vargas, Subcritical water extraction for recovering cellulose fibres from *Posidonia oceanica* waste, Carbohydr. Polym. Technol. Appl. 8 (July) (2024) 100550, https://doi.org/10.1016/j.carpta.2024.100550.
[37] R. Mnafki, A. Morales, L. Sillero, R. Khiari, Y. Moussaoui, J. Labidi, Integral valorization of Posidonia oceanica balls: an abundant and potential biomass, Polymers 16 (1) (2024) 1–16, https://doi.org/10.3390/polym16010164.
[38] M.A. Chaouch, J. Hafsa, C. Rihouey, D. Le Cerf, H. Majdoub, Depolymerization of polysaccharides from Opuntia ficus indica: antioxidant and antiglycated activities, Int. J. Biol. Macromol. 79 (2015) 779–786, https://doi.org/10.1016/j.ijbiomac.2015.06.003.
[39] X. Chen, D. Fang, R. Zhao, J. Gao, B.M. Kimatu, Q. Hu, G. Chen, L. Zhao, Effects of ultrasound-assisted extraction on antioxidant activity and bidirectional immunomodulatory activity of Flammulina velutipes polysaccharide, Int. J. Biol. Macromol. 140 (2019) 505–514, https://doi.org/10.1016/j.ijbiomac.2019.08.163.
[40] Y. Pan, C. Liu, S. Jiang, L. Guan, X. Liu, L. Wen, Ultrasonic-assisted extraction of a low molecular weight polysaccharide from *Nostoc commune* Vaucher and its structural characterization and immunomodulatory activity, Ultrason. Sonochem. 108 (June) (2024) 106961.
[41] L. Pfeifer, B. Classen, The Cell Wall of Seagrasses : Fascinating , Peculiar and a Blank Canvas for Future, Research 11 (October) (2020), https://doi.org/10.3389/fpls.2020.588754.
[42] N. Yuvaraj, V. Arul, Sulfated polysaccharides of seagrass Halophila ovalis suppresses tumor necrosis factor-α-induced chemokine interleukin-8 secretion in HT-29 cell line, Indian J. Pharm. 50 (6) (2018) 336–343, https://doi.org/10.4103/ijp.IJP_202_18.
[43] C. Du, X. Liu, H. Algadi, Y. Hou, X. Fu, H. Li, J. Fan, Polysaccharide extraction optimization , monosaccharide composition , and antioxidant activity analysis of different varieties of Gastrodia elata Bl aerial parts, Biomass Convers. Biorefinery 14 (23) (2024) 29353–29365, https://doi.org/10.1007/s13399-023-05014-x.
[44] N. Ruocco, S. Costantini, S. Guariniello, M. Costantini, Polysaccharides from the marine environment with pharmacological, cosmeceutical and nutraceutical potential, Molecules 21 (5) (2016) 1–16, https://doi.org/10.3390/molecules21050551.
[45] A. Akter, M.K.A. Sobuj, M.S. Islam, K. Chakroborty, N. Tasnim, M.H. Ayon, M. F. Hossain, S.M. Rafiquzzaman, Seaweed polysaccharides: sources, structure and biomedical applications with special emphasis on antiviral potentials, Future Foods 10 (August) (2024) 100440, https://doi.org/10.1016/j.fufo.2024.100440.
[46] R. Zhang, T. Belwal, L. Li, X. Lin, Y. Xu, Z. Luo, Recent advances in polysaccharides stabilized emulsions for encapsulation and delivery of bioactive food ingredients: A review, Carbohydr. Polym. 242 (2020), https://doi.org/10.1016/j.carbpol.2020.116388.
[47] Y.-J. Chen, X. Sui, Y. Wang, Z.-H. Zhao, T.-H. Han, Y.-J. Liu, J.-N. Zhang, P. Zhou, K. Yang, Z.-H. Ye, Preparation, structural characterization, biological activity, and nutritional applications of oligosaccharides, Food Chem. X 22 (2024) 101289, https://doi.org/10.1016/j.fochx.2024.101289.
[48] A.G. Demesa, S. Saavala, M. Pöysä, T. Koiranen, Overview and toxicity assessment of ultrasound-assisted extraction of natural ingredients from plants, Foods 13 (19) (2024) 1–13, https://doi.org/10.3390/foods13193066.
[49] M. Orlando-Bonaca, A. Bonaca, D. Bonaca, A. Rotter, Seagrasses: a promising source of bioactive compounds for human health applications, Annales, Series Historia Naturalis 35 (1) (2025) 153–164, https://doi.org/10.19233/ASHN.2025.19.
[50] P. Bhuyar, S. Sundararaju, M.H.A. Rahim, Y. Unpaprom, G.P. Maniam, N. Govindan, Antioxidative study of polysaccharides extracted from red (Kappaphycus alvarezii), green (Kappaphycus striatus) and brown (Padina gymnospora) marine macroalgae/seaweed, SN Appl. Sci. 3 (4) (2021) 1–9, https://doi.org/10.1007/s42452-021-04477-9.
[51] L. Bai, D. Xu, Y.M. Zhou, Y.B. Zhang, H. Zhang, Y.B. Chen, Y.L. Cui, Antioxidant activities of natural polysaccharides and their derivatives for biomedical and medicinal applications, Antioxidants 11 (12) (2022), https://doi.org/10.3390/antiox11122491.
[52] J. D'Orazio, S. Jarrett, A. Amaro-Ortiz, T. Scott, UV radiation and the skin, Int. J. Mol. Sci. 14 (6) (2013) 12222–12248, https://doi.org/10.3390/ijms140612222.
[53] M. Bilal, H.M.N. Iqbal, Marine seaweed polysaccharides-based engineered cues for the modern biomedical sector, Mar. Drugs 18 (1) (2020), https://doi.org/10.3390/md18010007.
[54] A. Capart, Bénéfices et risques de l ' application d ' un produit solaire To cite this version : HAL Id : dumas-03622143, 2022.
[55] V. Jesumani, H. Du, P. Pei, M. Aslam, N. Huang, Comparative study on skin protection activity of polyphenol-rich extract and polysaccharide-rich extract from *Sargassum vachellianum*, PLoS One 15 (1) (2020) 1–17, https://doi.org/10.1371/journal.pone.0227308.
[56] S.I. Ali, W.M. Sheikh, M.A. Rather, V. Venkatesalu, S. Muzamil Bashir, S.U. Nabi, Medicinal plants: treasure for antiviral drug discovery, Phytother. Res. 35 (7) (2021) 3447–3483, https://doi.org/10.1002/ptr.7039.
[57] A.G. Atanasov, S.B. Zotchev, V.M. Dirsch, I.E. Orhan, M. Banach, J.M. Rollinger, D. Barreca, W. Weckwerth, R. Bauer, E.A. Bayer, M. Majeed, A. Bishayee, V. Bochkov, G.K. Bonn, N. Braidy, F. Bucar, A. Cifuentes, G. D'Onofrio, M. Bodkin, C.T. Supuran, Natural products in drug discovery: advances and opportunities, Nat. Rev. Drug Discov. 20 (3) (2021) 200–216, https://doi.org/10.1038/s41573-020-00114-z.
[58] A.R. Carroll, B.R. Copp, T. Grkovic, R.A. Keyzers, M.R. Prinsep, Marine natural products, Nat. Prod. Rep. 41 (2) (2024) 162–207, https://doi.org/10.1039/d3np00061c.
[59] X. Dong, Y. Qiu, N. Jia, Y. Wu, Q. Nie, J. Wen, C. Zhao, Y. Zhai, Recent advances of edible marine algae-derived sulfated polysaccharides in antiviral treatments: challenges vs. opportunities, Front. Nutr. 12 (March) (2025) 1–14, https://doi.org/10.3389/fnut.2025.1561119.
[60] A.M. Agelidis, D. Shukla, Cell entry mechanisms of HSV: what we have learned in recent years, Future Virol. 10 (10) (2015) 1145–1154, https://doi.org/10.2217/fvl.15.85.
[61] R.G. Bai, R. Tuvikene, Potential antiviral properties of industrially important marine algal polysaccharides and their significance in fighting a future viral pandemic, Viruses 13 (9) (2021), https://doi.org/10.3390/v13091817.
[62] H. Claus-Desbonnet, E. Nikly, V. Nalbantova, D. Karcheva-Bahchevanska, S. Ivanova, G. Pierre, N. Benbassat, P. Katsarov, P. Michaud, P. Lukova, C. Delattre, Polysaccharides and their derivatives as potential antiviral molecules, Viruses 14 (2) (2022) 1–26, https://doi.org/10.3390/v14020426.
[63] C. Hao, Z. Xu, C. Xu, R. Yao, Anti-Herpes simplex virus activities and mechanisms of marine derived compounds, Front. Cell. Infect. Microbiol. 13 (January) (2023) 1–16, https://doi.org/10.3389/fcimb.2023.1302096.
[64] A. Hazini, M. Pryshliak, V. Brückner, K. Klingel, M. Sauter, S. Pinkert, J. Kurreck, H. Fechner, Heparan sulfate binding Coxsackievirus B3 strain PD: A novel Avirulent oncolytic agent against human colorectal carcinoma, Hum. Gene Ther. 29 (11) (2018) 1301–1314, https://doi.org/10.1089/hum.2018.036.
[65] T. Handa, A. Saha, A. Narayanan, E. Ronzier, P. Kumar, J. Singla, S. Tomar, Structural virology: the key determinants in development of antiviral therapeutics, Viruses 17 (3) (2025), https://doi.org/10.3390/v17030417.
[66] E.A. Harden, R. Falshaw, S.M. Carnachan, E.R. Kern, N. Mark, Virucidal activity of polysaccharide extracts from four algal species against Herpes simplex virus, Antivir. Res. 83 (3) (2009) 282–289, https://doi.org/10.1016/j.antiviral.2009.06.007.Virucidal.
[67] H. Yan, J. Wang, J. Yang, Z. Xu, C. Li, C. Hao, S. Wang, W. Wang, Inhibition effects and mechanisms of marine polysaccharide PSSD against herpes simplex virus type 2, Mar. Drugs 21 (6) (2023) 1–12, https://doi.org/10.3390/md21060364.
[68] G. Ren, L. Xu, J. Zhao, Y. Shao, Y. Lin, L. Li, Q. Liu, T. Lu, Q. Zhang, Antiviral activity of crude polysaccharide derived from seaweed against IHNV and IPNV *in vitro*, Viruses 14 (9) (2022) 1–12, https://doi.org/10.3390/v14092080.
[69] W. Zhu, L.C.M. Chiu, V.E.C. Ooi, P.K.S. Chan, P.O. Ang, Antiviral property and mode of action of a sulphated polysaccharide from Sargassum patens against herpes simplex virus type 2, Int. J. Antimicrob. Agents 24 (3) (2004) 279–283, https://doi.org/10.1016/j.ijantimicag.2004.02.022.
[70] S.M. Kang, D. Tark, B.M. Song, G.H. Lee, J.H. Yang, H.J. Han, S.K. Yim, Evaluation of antiviral effect against SARS-CoV-2 propagation by crude polysaccharides from seaweed and abalone viscera *in vitro*, Mar. Drugs 20 (5) (2022), https://doi.org/10.3390/md20050296.
[71] M. Rabanal, N.M.A. Ponce, D.A. Navarro, R.M. Gómez, C.A. Stortz, The system of fucoidans from the brown seaweed Dictyota dichotoma: chemical analysis and antiviral activity, Carbohydr. Polym. 101 (1) (2014) 804–811, https://doi.org/10.1016/j.carbpol.2013.10.019.
[72] A.M. Elissawy, M. El-Shazly, S.S. Ebada, A.N.B. Singab, P. Proksch, Bioactive terpenes from marine-derived fungi, Mar. Drugs 13 (4) (2015) 1966–1992, https://doi.org/10.3390/md13041966.
[73] M.E.H. Kayesh, M. Kohara, K. Tsukiyama-Kohara, Effects of oxidative stress on viral infections: an overview, Npj Viruses 3 (1) (2025) 1–9, https://doi.org/10.1038/s44298-025-00110-3.
[74] J. Carrasqueira, S. Bernardino, R. Bernardino, C. Afonso, Marine-derived polysaccharides and their potential health benefits in nutraceutical applications, Mar. Drugs 23 (2) (2025) 1–32, https://doi.org/10.3390/md23020060.
[75] B. Liu, L. Zhang, T. Zhu, Y. Liu, J. Chu, N. Chen, Structural characterization of polysaccharides of marine origin: a review, Int. J. Biol. Macromol. 317 (2025) 144797 doi:10.1016/j.ijbiomac.2025.144797 doi:10.1016/j.ultsonch.2024.106961.