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Skin Anti-Aging Potential of Sulfated Polysaccharides from *Cladophora vagabunda* Green Seaweed

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Abstract

Sulfated polysaccharides (SPs) from green seaweed species have been scarcely studied for the development of novel pharmaceutical, cosmetic or nutraceutical products. The present study aimed to investigate the physico-chemical characteristics of the sulfated polysaccharidic fractions isolated from *Cladophora vagabunda* green seaweed and to evaluate their anti-aging properties in vitro. SPF1 and SPF2 fractions were separated from the purified polysaccharidic extract by size exclusion chromatography. The content of neutral carbohydrates, uronic acids and sulfate was assessed, while Fourier transform infrared spectroscopy (FT-IR) analysis confirmed the presence of a functional group characteristic for sulfated polysaccharides. Capillary zone electrophoresis indicated the monosaccharides profile and the presence of bioactive fucose and uronic acids. The two fractions differed in sulfate content (22.59% and 29.44%). SF2 showed stronger collagenase inhibition (95.69%), whereas SF1 exhibited greater elastase inhibition (84.2%) in comparison with EGCG. Both fractions exhibited antioxidant, anti-collagenase and anti-elastase activities and also a good biocompatibility and capacity to modulate the cell cycle progression in human dermal fibroblast culture. They showed anti-inflammatory potential by inhibition of interleukin-1 beta (IL-1β), tumor necrosis factor-α (TNF-α) and nitric oxide (NO) production in lipopolysaccharide (LPS)-inflamed THP-1-derived macrophages. Also, the level of matrix metalloproteinase-1 (MMP-1) and MMP-9 secretion was reduced after treatment with *C. vagabunda* fractions with MMP-1 reduced by ~95% in both fractions and MMP-9 reduced by ~79% in SF2 compared with the control. Both fractions stimulated the growth of probiotic cultures Lactobacillus acidophilus and L. rhamnosus. All these results demonstrated, for the first time, the anti-aging potential of sulfated polysaccharides isolated from *C. vagabunda* green seaweed.

Keywords: anti-aging; marine algae; sulfated polysaccharides; inflammation; metalloproteinases; prebiotic activity

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1. Introduction

Skin aging is a natural phenomenon that unfolds under the action of intrinsic- and extrinsic-type factors. Intrinsic aging, also known as chronological aging, is defined as the

natural aging process, depending on normal biological processes influenced by genetic and hormonal factors. The primary cause of intrinsic aging is the destruction of the antioxidant defence system and damage accumulation, at the cellular and tissular level, due to reactive oxygen species (ROS). Moreover, aging leads to the disintegration of type I collagen, the main structural protein of the dermal layer, due to overexpression of matrix metalloproteinases (MMPs), resulting in loss of skin functions, mechanical properties, and wrinkle formation [1–3]. Extrinsic aging, also known as photoaging, is a process caused by exposure to ultraviolet (UV) radiation, which can be partially controlled [4]. The mechanisms of skin aging processes are currently under investigation, together with searching for novel natural anti-aging agents [1,5].

Algal polysaccharides are complex, negatively charged macromolecules present in the cell wall [6]. They consist of monosaccharide repetitive units linked by glycosidic bonds, but exhibit great variation of the constituent unit type, nature of glycosidic bond, molecular weight (MW), degree of polymerisation and conformation [7]. Algal polysaccharides have been reported to exhibit numerous health benefits, in particular in preventing skin aging, through antioxidant, anti-inflammatory and photoprotective properties, and extracellular matrix (ECM) degradation through MMP expression control [8,9]. Thus, polysaccharides from *Sargassum* spp. brown alga have exerted UVB-protective effect in skin cells by reducing collagenase and elastase activity, and ROS and MMP expression.

Sulfated polysaccharides (SPs) are bioactive compounds isolated from marine seaweed, raising great interest for the formulation of novel skin anti-aging cosmetic products, besides their use in nutraceuticals and biomedicines [10]. Sulfated polysaccharides isolated from the red alga *Delesseria sanguinea* exhibited anti-inflammatory and skin anti-aging potential [11], while *Ulva* spp. polysaccharides enhanced the synthesis of ECM constituents, like collagen type I and glycosaminoglycans [12]. The polysaccharidic extracts with high sulfate content isolated from *Chnoospora minima* and *Sargassum polycystum* brown algae showed antiradical scavenging and anti-inflammatory activity, but also the capacity to inhibit collagenase and elastase activity [13]. In addition, sulfated polysaccharides derived from *S. binderi* and *S. fusiforme* have exhibited anti-inflammatory properties by decreasing the expression level of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in lipopolysaccharide (LPS)-stimulated human RAW264.7 cells and of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in UVB-stimulated human HaCaT keratinocytes [8]. Various antioxidant extracts isolated from macroalgae protected skin against loss of elasticity, wrinkling, uneven pigmentation and even changes of cutaneous microbiota [14].

Algae represent 76% of the marine ingredients used in commercialised anti-aging cosmetics, but only 3% are green algae [10]. In vivo studies demonstrated skin anti-aging effects of *Ulva lactuca* oligosaccharides in mice with induced senescence [15], while *Cladophora* spp. polysaccharides exerted antityrosinase activity [16]. Marine green seaweed from the *Cladophora* genus have a worldwide distribution, ranging from arctic to temperate and tropical waters, being characterised by filamentous branches, with multinucleate cells and cellulose cell walls that adhere to the protoplasm, having no mucilaginous sheaths [17,18]. It was reported that *Cladophora* sp. contained several biological active compounds, such as carbohydrates, phenolic compounds, sterols, terpenoids, fatty acids, minerals, vitamins, amino acids and proteins [19,20], which have demonstrated strong antioxidant, anticancer, immunomodulatory and antibacterial activity [17,21,22]. *Cladophora vagabunda* green seaweed is found in abundance in the waters of the Romanian Black Sea coast. It was investigated for chemical constituents, in terms of volatiles, fatty acids, terpenoids, sterols, phenols, carbohydrates, vitamins and minerals and valorised for biodiesel production and the development of new therapeutics [23–27]. However, studies on *Cladophora* species are still scarce and the algal biomass of *C. vagabunda* remains an under-exploited resource. In

particular, the polysaccharidic extract of Cladophora sp. had not been investigated until the present study.

The aim of this study was to investigate the preliminary chemical composition and the functional groups of two sulfated polysaccharidic fractions isolated from *C. vagabunda* green seaweed and to characterize their antioxidant activity and capacity to inhibit ECM-degrading enzymes. To the best of our knowledge, these sulfated polysaccharidic fractions have not previously been isolated from *C. vagabunda*.

In addition, their effect on specific markers of inflammation and MMP production in THP-1-derived macrophages culture, and the prebiotic effect in Lactobacilli culture, were evaluated as main processes involved in the complex process of skin aging.

2. Materials and Methods

2.1. Materials

C. vagabunda green seaweed (500 g) was collected from the Romanian Black Sea coast between 2 May and August 2022, in the Vama Veche area, and identified at the Faculty of Pharmacy, University "Ovidius" of Constanta, Romania (voucher specimen no. 002). Human dermal fibroblasts and human monocytic leukemia THP-1 cell lines were purchased from Cell Applications, Inc, and the American Type Culture Collection (ATCC), respectively. *Lactobacillus acidophilus* (DSM 20079) and *Lactobacillus rhamnosus* (DSM 6594) cultures were purchased from the German Collection of Microorganisms and Cell Cultures GmbH. All used chemicals were of analytical purity and purchased from Sigma-Aldrich (Darmstadt, Germany), unless otherwise specified.

2.2. Extraction of Polysaccharides

The algal material was transported on ice to the institute, washed in cold distilled water, dried at room temperature, in the dark, and finely ground in a glass mortar. The powder was stored in sealed paper bags, in a dry place. The extraction of polysaccharides (SPs) from *C. vagabunda* powder was performed, as previously described [28]. Briefly, the algal powder was extracted three times with acetone (1:5, *w*/*v*), to remove chlorophyll and lipids, and three times with methanol (1:5, *w*/*v*), to remove polyphenols. The vegetal residue was then extracted in 150 mL of water (1:10, *w*/*v*), at 100 °C, for 3 h, in Soxhlet equipment. The obtained polysaccharidic extract was filtered, concentrated using a rotary evaporator (Heidolph, Schwabach, Germany), at 60 °C, to 1/4 volume, and purified by precipitation with cold ethanol at a final concentration of 75% (*v*/*v*), at 4 °C, overnight. The precipitate was collected after centrifugation at 6000 rpm, for 30 min, redissolved in distilled water and treated with Sevag reagent ($CHCl_3$: n-C_4H_9OH = 4:1, *v*/*v*), in a ratio of 2:1, in order to remove free proteins. After vigorous vortexing at room temperature, for 30 min, the mixture was centrifuged at 10,000× *g*, for 10 min. The supernatant was re-precipitated with 75% ethanol and dialysed against distilled water in cellulose tubes (cut-off MW 10,000 Da, Sigma-Aldrich, Darmstadt, Germany) for 24 h. Finally, the polysaccharidic extract was lyophilised by freezing at −35 °C, then incubation at 0 °C under vacuum, and drying at +30 °C, for 48 h, in Martin Christ (Niedersachsen, Germany) equipment. The yield of polysaccharides extraction was calculated on a dry weight (d.w.) basis using the following equation:

$$\text{Extraction yield (\%)} = (\text{final weight}_{\text{extract}} / \text{initial weight}_{\text{seaweed}}) \times 100 \quad (1)$$

2.3. Size Exclusion Chromatography

The polysaccharidic extract (2.5 mg) was dissolved in distilled water (1 mL) and subjected to size exclusion chromatography on a Sepharose CL-6B column (230 × 15 mm) [29]. After sample entered the gel, distilled water was used as an eluent. The elution rate was

0.3 mL/min and fractions of 2 mL were collected. A mixture of dextrans of different molecular weights (Dextran Blue, dextran 500, 250, 100, 40 and 20) were also separated on a Sepharose CL-6B column. Total carbohydrates content was analysed, in each fraction, by phenol-sulfuric acid method [30]. Major peaks, corresponding to polysaccharidic fractions SPF1 and SPF2, were pooled, concentrated and lyophilised. A calibration curve was built from the elution volume vs. the natural logarithm of dextrans molecular weight (MW) and served to determine the average MW of algal polysaccharidic fractions.

2.4. Preliminary Characterisation

The neutral carbohydrates content of the polysaccharidic fractions was analysed by phenol-sulfuric acid method [30] using glucose as standard. Uronic acids content was determined by orcinol assay [31] using galacturonic acid as standard. Sulfate content was evaluated by barium chloride assay [32] using potassium sulfate as standard. Total phenolic content was assessed using Folin–Ciocalteu reagent and gallic acid as standard. All results were expressed as a percentage based on the sample's d.w.

2.5. FT-IR Analysis

The samples were mixed with KBr in a mass ratio of 1:100, homogenised and pressed into a tablet. FT-IR spectra were recorded at room temperature using an IRTracer-100 spectrometer (Shimadzu, Kyoto, Japan). The analyses were conducted in transmission mode over the 4000–500 cm^{-1} region, with a scan speed of 2.8, at a resolution of 4 cm^{-1} and 45 scans. A background spectrum of KBr was recorded, under the same conditions, before sample measurement. The recorded spectra were processed using the BioLabSolution IR v2.30 software for atmospheric compensation, baseline correction and peak evaluation.

2.6. Determination of Monosaccharides Profile by Capillary Zone Electrophoresis

The analysis of the monosaccharides profile of SPF1 and SPF2 fractions from *C. vagabunda* was carried out, according to a previously described assay [33]. Briefly, the samples (20 mg) were hydrolysed in 1.5 M H_2SO_4 (2 mL), at 100 °C, for 5 h and neutralised with 6 M NaOH solution. The samples were then subjected to derivatisation with 1-phenyl-3-methyl-5-pyrazolone and the separation was performed using an Agilent CE instrument provided with ChemStation C.01.10 software and a diode array detector. The process took place in 90 mM boric acid, pH 9.9, at a voltage of 30 kV. The compound detection was measured at 245 nm. Monosaccharide standards were similarly processed.

2.7. ABTS Assay

The antioxidant activity of polysaccharidic fractions was evaluated using the ABTS radical scavenging assay, as previously described [34]. The ABTS radical was produced by mixing 7 mM 2,2'-azino-bis (3-ethyl-benzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) with 2.45 mM potassium persulfate (1:1, *v*/*v*) and placed in the dark for 12–16 h, at room temperature. The solution was then diluted with distilled water, to obtain an absorbance value of 0.700 at 734 nm. For experiments, a volume of 100 µL polysaccharide fractions of different concentrations (1000–5000 µg/mL) and ascorbic acid (25 µg/mL), used as positive control, was added to 1 mL diluted ABTS reagent and allowed to stand at room temperature, for 6 min. The absorbance (Abs) of the solution was measured at 734 nm using a UV/VIS V650 spectrophotometer (Jasco, Tokyo, Japan). The antioxidant activity was expressed as ABTS free radical scavenging percentage using the following equation:

$$\text{free ABTS scavenging activity (\%)} = [(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{blank}}] \times 100 \quad (2)$$

2.8. Reducing Power Assay

The reducing power of polysaccharidic fractions was determined, according to Yang et al. [35], with minor modification. Briefly, different concentrations of samples (1000–5000 μg/mL) and ascorbic acid (25 μg/mL), used as positive control, were mixed with 200 μL phosphate buffer (0.2 M, pH 6.5) and 200 μL potassium ferricyanide (0.1%). The mixture was incubated at 50 °C, for 20 min. A volume of 250 μL 10% trichloroacetic acid (TCA) was added and then, the mixture was centrifuged at 6000 rpm, for 10 min. The supernatant (500 μL) was mixed with 100 μL of 0.1% ferric chloride, and incubated at room temperature, for 10 min. The Abs of samples was measured at 700 nm using a Sunrise microplate reader (Tecan, Grödig, Austria).

2.9. Determination of Collagenase and Elastase Inhibition

The anti-collagenase potential of polysaccharidic fractions was evaluated using *N*-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) substrate, according to Thring et al. [36]. Collagenase from *Clostridium histolyticum* (0.8 units/mL) dissolved in Tricine buffer (50 mM, pH 7.5) containing 400 mM NaCl, and 10 mM $CaCl_2$ was mixed with different concentrations of polysaccharidic samples and incubated at 37 °C, for 15 min. Then, the substrate (1 mM) dissolved in the same buffer was added to the mixture. The decrease in absorbance was recorded at 340 nm, for 15 min, by a UV/VIS V650 spectrophotometer (Jasco, Tokyo, Japan) thermostated at 37 °C using a Peltier unit. The mixture containing the same volume of buffer instead of polysaccharidic sample served as negative control, while the solution using epigallocatechin gallate (EGCG) instead of polysaccharidic sample served as positive control. Collagenase inhibition (%) was calculated using the following equation:

$$\text{Inhibition (\%)} = [(\Delta\text{Abs/min}_{\text{control}} - \Delta\text{Abs/min}_{\text{sample}})/\Delta\text{Abs}_{\text{control}}] \times 100 \quad (3)$$

The anti-elastase potential was evaluated according to the Thring et al. [35] assay, using elastase from porcine pancreas and N-succinyl-Ala-Ala-Val-nitroanilide as substrate. Different concentrations of polysaccharidic samples were mixed with elastase (1 μg/mL) in 0.1 M Tris-HCl buffer, pH 8 and incubated at room temperature, for 15 min. Then, the substrate (1.6 mM) dissolved in the same buffer was added to the mixture. The decrease in absorbance was recorded at 410 nm, for 10 min, by a UV/VIS V650 spectrophotometer (Jasco, Tokyo, Japan) thermostated at 37 °C using a Peltier unit. The mixture containing buffer instead of polysaccharidic sample served as negative control, while the solution using EGCG instead of polysaccharidic sample served as positive control. Elastase inhibition (%) was calculated using Equation (3).

2.10. In Vitro Cytocompatibility Testing

2.10.1. Cell Culture and Treatment

Human dermal fibroblasts (Cell Applications, Inc—106-05a) were grown in T75 flasks in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% of penicillin/streptomycin/neomycin (PSN) solution, in a humidified incubator under 5% CO_2 atmosphere, at 37 °C. For experiments, cells were seeded in 96-well plates at a density of 5×10^4 cells/mL and grown in the same culture medium, in standard conditions, for 24 h. Then, the culture medium was replaced with a culture medium containing different concentrations (100–3000 μg/mL) of polysaccharidic fractions and the plates were incubated in standard conditions, for 24 and 48 h.

2.10.2. Cellular Viability Assay

To test the cellular viability in the presence of polysaccharidic samples, MTT assay was carried out, as previously described [37]. Briefly, after each incubation period, the culture

medium was removed and a stock MTT solution was added, to reach a final concentration of 0.25 mg/mL. After 3 h of incubation, at 37 °C, the formazan crystals were dissolved using dimethyl sulfoxide (DMSO). The absorbance of the samples was recorded at 570 nm using a SPECTROstar Nano microplate reader (BMG Labtech, Ortenberg, Germany). The results were expressed as percentage of cell viability reported to the negative control (untreated cells), considered 100% viable.

2.10.3. Cell Cycle Analysis by Flow Cytometry

Human dermal fibroblasts were seeded into 6-well plates, at a density of 1×10^5 cell/mL, in DMEM medium containing 10% FCS and incubated in atmosphere with 5% CO_2, at 37 °C, for 24 h. Then, the culture medium was replaced with fresh medium containing different concentrations (100–3000 µg/mL) of polysaccharidic fractions and the plates were further incubated in standard conditions, for 48 h. Cell cycle analysis was performed according to previously described protocol [38] with minor modifications. The cells were collected by trypsinisation, resuspended in PBS and 70% cold ethanol, at 4 °C, for 24 h. After centrifugation, the cells were mixed with PBS containing 0.2 mg/mL RNase and incubated at room temperature, for 30 min. Then, 20 µg/mL of propidium iodide was added and the cells were incubated in the dark, at room temperature, for 45 min. Cell cycle analysis was performed on an LSR II flow cytometer (BD Biosciences, Paramus, NJ, USA) and the results were processed using MODFIT^{TM} LT 3.0 software.

2.11. In Vitro Evaluation of Proinflammatory Cytokines, Nitric Oxide and MMP Secretion Level

2.11.1. Cell Culture and Treatment

The anti-inflammatory activity of *C. vagabunda* polysaccharidic fractions was analysed in human monocytic leukemia THP-1 cell line (American Type Culture Collection—ATCC, TIB-202), according to a previously described protocol [39]. For experiments, THP-1 cells were seeded in 24-well culture plates, at a cell density of 1×10^6 cells/mL, and grown in Roswell Park Memorial Institute (RPMI) 1640 medium with high glucose content (4.50 g/L), supplemented with 10% FCS and 1% PSN antibiotic mixture. The differentiation of THP-1 into macrophages was achieved by treatment of cells with 100 ng/mL phorbol 12-myristate 13-acetate (PMA), for 72 h. Then, the cells were inflamed by incubation with 10 ng/mL bacterial lipopolysaccharide (LPS) and exposed to different concentrations of polysaccharidic fractions (100–3000 µg/mL), for 18 h. The culture media were collected, centrifuged at 400× *g*, for 10 min, and the supernatants were kept at −18 °C, until analysis.

2.11.2. Determination of Inflammation Markers

Quantification of the proinflammatory cytokines IL-1β and TNF-α production was carried out using specific ELISA kits (Invitrogen, Carlsbad, CA, USA). The secretion level of MMP-1 and MMP-9 was determined by specific sandwich ELISA (Invitrogen, Carlsbad, CA, USA), while the level of nitric oxide (NO) was assessed using a total nitric oxide assay kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions.

2.12. Determination of Prebiotic Effect

Prebiotic activity was assessed as previously described [40]. Briefly, prior to seeding, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* cultures were cultivated in De Man, Rogosa and Sharpe broth (MRS) growth medium, overnight. Standardised inoculum, corresponding to 0.5 McFarland density, was seeded in the wells of a 96-well culture plate. Polysaccharidic samples were added to each well, to reach a final concentration of 3 mg/mL, and the plates were incubated at 37 °C, for 24 h. The inoculum cultivated in MRS medium without sample served as negative control. The absorbance was measured at

620 nm, at specific time points, using a Sunrise microplate reader (Tecan, Grödig, Austria). The values were proportional to the bacterial growth.

2.13. Statistical Analysis

Three experiments were performed in triplicate. The results were expressed as mean ± standard deviation (SD) (n = 9). Statistical analysis was performed on control-sample pairs of interest using Student t-test (Excel v.10) and the differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Isolation and Characterisation of C. vagabunda Polysaccharidic Fractions

A polysaccharidic extract was obtained, for the first time, and purified from *C. vagabunda* green seaweed with an extraction yield of 1.59 ± 0.10%, relative to dry matter. Higher percentages were recorded for *Codium decorticatum* (11%) and *Ulva prolifera* (20.3%), while *Cystoseira myriophylloides* showed the lowest yield, 0.87% [41,42]. These yield variations are likely due to differences in species, environmental conditions, and extraction methods [41].

The extract was then fractionated, according to MW, by size exclusion chromatography. The elution profile of the polysaccharidic extract is presented in Figure 1.

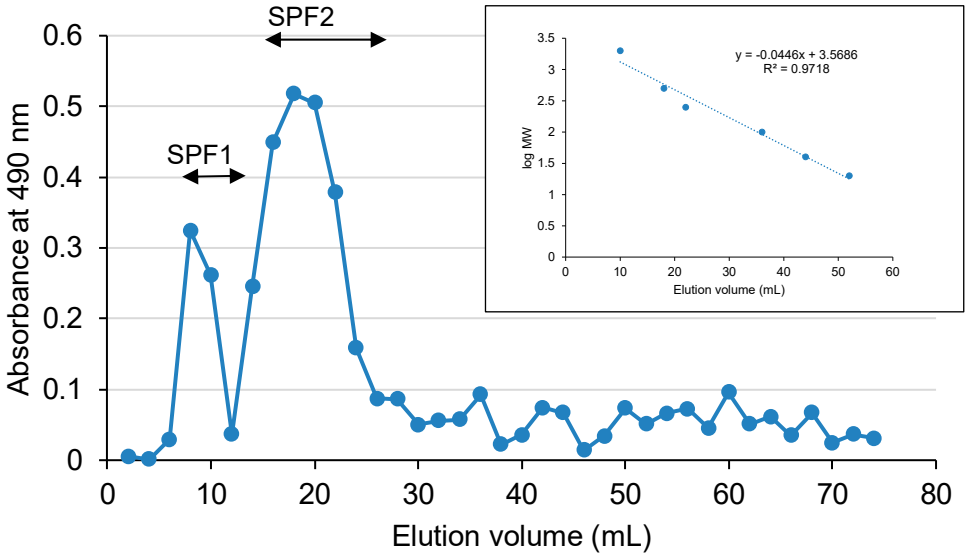


Figure 1. Elution curve of *C. vagabunda* polysaccharidic extract fractionated by size exclusion chromatography on Sepharose CL−6B, indicating separation of two major peaks, noted SPF1 and SPF2. The insert presents the standard curve built using dextrans of known MW.

Two fractions were separated, corresponding to the major peaks, denoted SPF1 and SPF2. Based on MW determination, according to dextrans standard curve, SPF1 consisted of high MW polysaccharides ranging between 1080 and 2000 kDa and an average value of 1628 kDa, while SPF2 comprised polysaccharides with MW values between 256 and 1080 kDa and an average value of 583 kDa. These are apparent values, taking into account that dextrans have a different hydrodynamic behaviour [43]. The values were in accordance with previous studies that reported values between 29 and 2173 kDa for polysaccharidic extracts isolated from *Caulerpa racemosa var peltata* [44] and *Codium decorticatum* [41] green algae species. The conditions and extraction methods applied in seaweed polysaccharide isolation influence their MW. Chi et al. [45] reported that using hot water and enzyme-assisted extractions of *Enteromorpha prolifera*, the isolated polysaccharides had an average

MW of 966 and 1327.5 kDa, respectively. In turn, polysaccharides with a lower MW (41.1 and 47.7 kDa) were isolated after acid and alkali extraction of *E. prolifera*. In another study, the MW of sulfated polysaccharides from *Ulva prolifera* decreased with higher temperature extraction conditions and increasing hydrochloric acid concentration [46].

The preliminary composition of *C. vagabunda* polysaccharidic fractions SPF1 and SPF2 was initially determined. The results are presented in Table 1.

Table 1. Total content of neutral carbohydrates, uronic acids, sulfate and phenolics in SPF1 and SPF2 polysaccharidic fractions isolated from *C. vagabunda* green seaweed. The results were expressed as mean ± SD (n = 9). * $p < 0.05$, compared to SPF1.

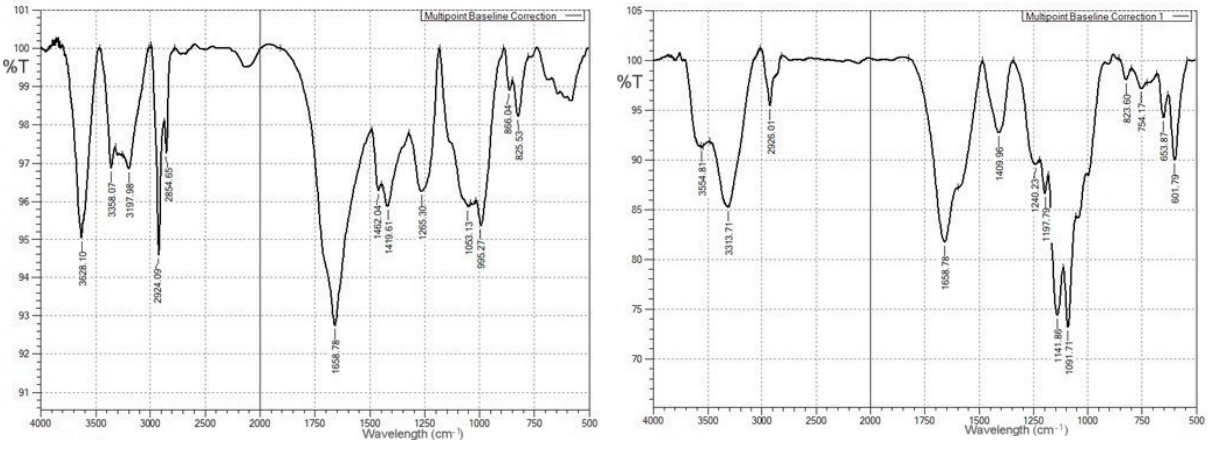
Analysis	SPF1	SPF2
Neutral carbohydrates (%)	41.64 ± 3.43	39.77 ± 1.55
Uronic acids (%)	32.42 ± 3.21	26.67 ± 2.96
Sulfate (%)	22.59 ± 0.29	29.44 ± 0.18 *
Total phenolic content (%)	1.77 ± 0.17	2.79 ± 0.15 *

SPF1—sulfated polysaccharides fraction 1; SPF2—sulfated polysaccharides fraction 2.

The data confirmed the high content of neutral carbohydrates and uronic acids, as the main chemical compounds present in both isolated fractions. SPF1 had higher values of neutral carbohydrates (41.64%) and uronic acids (32.42%), compared to SPF2 (39.77% and 26.67%, respectively), but not significantly different ($p > 0.05$). In turn, SPF2 presented a higher level of sulfate (29.44%), compared to that found in SPF1 (22.59%), indicating a significantly ($p < 0.05$) increased content of sulfated polysaccharides. Our results are consistent with the sulfate content reported for *Caulerpa racemosa* (25.8%), whereas lower sulfate content has been reported in other studies for the green seaweeds *Codium decorticatum* (13.21%) and *Enteromorpha prolifera* (16.2%) [41,44,45].

The phenolic compounds have been significantly removed, the samples showing values of 1.77% for SPF1 and 2.79% for SPF2. Some traces were still present, taking into account that polyphenols were strongly embedded in the seaweed cell wall matrix [47].

FT-IR spectra of the polysaccharidic fractions SPF1 and SPF2 obtained from *C. vagabunda* green seaweed is given in Figure 2.



(**A**) (**B**)

Figure 2. FT−IR spectra of the polysaccharidic fractions SPF1 (**A**) and SPF2 (**B**) isolated from *C. vagabunda* green seaweed, including sulfate esters linked to sugar units (bands at 825 and 823 cm^{-1}, respectively, confirming the presence of sulfated polysaccharides in both fractions.

The peaks in the 3700–3000 cm^{-1} region were attributed to O–H groups' stretching vibration. The absorption bands at 2924 cm^{-1} and 2855 cm^{-1} for SPF1 and 2928 cm^{-1} for SPF2 were assigned to the asymmetric and symmetric stretching vibration of C–H groups from the skeletal structure. The absorption band at 1658 cm^{-1} was present in both fractions and attributed to C = O stretching vibration, characteristic to uronic acids, while the bands in the 1141–1091 cm^{-1} region for SPF2 were attributed to C–O–C stretching vibration of the glycosidic bond [48]. The absorption bands at 1265 cm^{-1} for SPF1 and 1242 cm^{-1} for SPF2 were assigned to S = O vibration, characteristic for sulfated polysaccharides [49], while the absorption bands at 825 cm^{-1} for SPF1 and 823 cm^{-1} for SPF2 could be attributed to C–O–S bending vibration, indicating the presence of sulfation on galactose units [50,51]. FT-IR spectra confirmed the presence of sulfated polysaccharides in both fractions isolated from *C. vagabunda* green seaweed. Previous studies showed that sulfated galactans with different structures were found in *Cladophora* spp. [52].

The monosaccharides identification and content were determined for the polysaccharidic fractions SPF1 and SPF2 by capillary zone electrophoresis. The results are presented in Table 2.

Table 2. Monosaccharides content of the polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*, determined by capillary zone electrophoresis. The results were expressed as mean ± SD (n = 3). * $p < 0.05$, compared to SPF1.

Compound	Monosaccharides Content (mg/g d.w.)	
	SPF1	SPF2
Xylose	56.3 ± 1.6	67.2 ± 2.2 *
Arabinose	2.8 ± 0.5	1.9 ± 0.5
Glucose	314.8 ± 2.8	470.3 ± 4.2 *
Ribose	42.7 ± 1.8	59.1 ± 0.9 *
Rhamnose	15.1 ± 1.6	12.9 ± 0.9
Mannose	31.2 ± 1.7	33.2 ± 1.6
Fucose	26.0 ± 1.5	32.9 ± 2.4 *
Galactose	193.2 ± 0.9 *	144.4 ± 5.6
Galacturonic acid	10.2 ± 0.5	11.7 ± 1.5
Glucuronic acid	-	4.4 ± 0.6 *

SPF1—sulfated polysaccharides fraction 1; SPF2—sulfated polysaccharides fraction 2.

The data showed that the main monosaccharide found in both isolated fractions was glucose, with significant content in SPF1 (314.8 mg/g) and statistically increased ($p < 0.05$) content in SPF2 (470.3 mg/g). It was followed by galactose found at a significantly higher ($p < 0.05$) level in SPF1 (193.2 mg/g) than in SPF2 (144.4 mg/g). Important constituents identified in the polysaccharidic fractions were xylose (56.3–67.2 mg/g), ribose (42.7–59.1 mg/g), and fucose (26.0–32.9 mg/g), with significantly higher ($p < 0.05$) content in SPF2, compared to SPF1. Mannose was present in similar amounts in SPF1 (31.2 mg/g) and SPF2 (33.2 mg/g). In addition, uronic acids were present in both fractions with similar content of galacturonic acid (10.2–11.7 mg/g), while glucuronic acid was detected only in SPF2 (4.4 mg/g). These results indicated a molar ratio of galactose:xylose:glucose of 2.82:1.0:4.61 in SPF1 and 1.78:1.0:5.80 in SPF2.

The differences observed in the monosaccharide composition of SPF1 and SPF2, particularly the higher glucose content and the exclusive presence of glucuronic acid in SPF2, may reflect variations in the structural characteristics and heterogeneity of the extracted polysaccharide fractions. Similar findings have been reported by Wei et al. (2020) [53], who fractionated crude polysaccharides from *Sargassum fusiforme* using DEAE Sepharose Fast Flow chromatography with elution by water, 0.5 M NaCl and 2 M NaCl. Chemical characterisation showed that glucuronic acid was detected exclusively in one of the isolated

fractions, while the glucose content also differed among the three fractions. These results suggested that the fractionation of crude polysaccharide extracts could produce structurally distinct fractions with different monosaccharide compositions and uronic acid contents.

Previous studies reported that the polysaccharides isolated from *C. rupestris* consisted of galactose, arabinose, xylose, rhamnose and glucose, in a molar ratio of 2.8:3.7:1.0:0.4:0.2, while *C. socialis* presented a molar ratio of 4.5:3.0:1.0 between galactose, arabinose and xylose [54,55]. The most abundant monosaccharide found in *Caulerpa racemosa* var. *peltata* green seaweed was mannose [49], while in *Ulva pertusa* was rhamnose [56], and in *C. glomerata* was arabinose [17]. Pankiewicz et al. [57] isolated ulvan from the freshwater macroalga *C. glomerata* and the main identified component was 3-sulfated rhamnoglucuronan. Other studies revealed that the major water-soluble polysaccharides extracted from *C. falklandica* and *C. oligoclada* were sulfated xylogalactoarabinans and sulfated galactoarabinans [58]. Thus, similar composition, but high content variability, was observed for the polysaccharidic extracts isolated from different *Cladophora* sp.

SP extracts isolated from different *Cladophora* spp. and other green seaweeds had high composition variability. Previous studies showed that the most abundant monosaccharide found in the green seaweed *C. glomerata* was arabinose [17], in *C. racemosa* var. peltata was mannose [49], in *U. pertusa* was rhamnose [56] and in *Ulva* spp. was glucose [59]. Also, insoluble cellulose as major SP of the algal cell walls and starch granules stored in the chloroplasts of *Cladophora* spp. could represent sources of glucose [60,61], taking into account that high temperature extraction of SPs was conducted in this study. *Cladophorales* also excrete a mucilage containing arabinose, galactose and xylose, forming structures of arabinopyranose 3-sulfate with branches of galactofuranose and galactopyranose [62]. In turn, the most common sequences found in the water-soluble SPs characteristic for the walls of Ulva spp., ulvans consist of repeating units of rhamnose-3-sulfate and glucuronic or iduronic acid, xylose or xylose 2-sulfate [63]. Galactose was also mentioned in studies on ulvan structure, but its binding mode was not deciphered [64].

To date, the identification of the SP sequence was not entirely possible, being hindered by their great variability and degradation during processing [65]. In the present study, FT-IR and capillary zone electrophoresis confirmed the presence of sulfated polysaccharides, including sulfate esters linked to sugar units of glucose, galactose, arabinose, rhamnose, xylose or fucose, as earlier found to be characteristic for green algae species [66]. The structural variety of algal polysaccharides could be due to different degrees of sulfation and the pattern distribution of sulfate groups and explain the wide range of pharmacological activities with beneficial modulatory effects on skin anti-aging processes [6]. It was previously reported that green seaweed ulvans with low MW and high sulfate and uronic acids content exhibited important biological activity [67]. In addition, the metal chelating activity of brown seaweed polysaccharides appeared positively correlated with sulfate/fucose ratio and MW [68]. In this context, the influence of SPF1 and SPF2 composition on their biological properties was further analysed in experimental models in vitro.

3.2. Antioxidant Activity of SP Fractions from C. vagabunda

The values of free ABTS radical scavenging capacity of the polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda* are presented in Table 3.

The results showed that the antioxidant activity varied between 16.86 and 48.07%, in a dose-dependent manner. It was observed that SPF2 displayed higher scavenging capacity, reaching significant increase ($p < 0.05$) of 1.5-fold and 1.8-fold over that of SPF1, at concentrations of 4000 and 5000 μg/mL, respectively. The values were significantly ($p < 0.05$) lower than that of a known antioxidant agent, ascorbic acid (46.12%), for all tested concentrations of algal SPF, excepting 5000 μg/mL SPF2 (48.07%). Similar values of

47.55–59.01% inhibition of free ABTS radicals were previously reported for the polysaccharidic fractions isolated from *Gracilaria rubra*, *Sarcodia ceylonensis*, *Ulva lactuca* L., *Gracilaria lemaneiformis* and *Durvillaea antarctica* green seaweed species [69,70].

Table 3. Antioxidant activity of *C. vagabunda* polysaccharidic fractions SPF1 and SPF2, determined by free ABTS radical scavenging capacity and reducing power assay. The results are expressed as mean ± SD (n = 9). * $p < 0.05$, compared to SPF1, # $p < 0.05$, compared to ascorbic acid.

Sample Concentration (µg/mL)	Free ABTS Radicals Scavenging Capacity (%)			Reducing Power (Absorbance at 700 nm)		
	SPF1	SPF2	Ascorbic Acid	SPF1	SPF2	Ascorbic Acid
25	-	-	46.12 ± 2.38	-	-	0.569 ± 0.03
1000	16.86 ± 1.04 #	20.33 ± 2.89 #		0.126 ± 0.01 #	0.132 ± 0.02 #	
2000	18.11 ± 2.35 #	23.16 ± 3.92 #		0.143 ± 0.02 #	0.157 ± 0.02 #	
3000	25.15 ± 3.63 #	30.75 ± 3.81 #		0.156 ± 0.03 #	0.169 ± 0.01 #	
4000	25.64 ± 2.89 #	37.64 ± 2.37 *,#		0.172 ± 0.02 #	0.186 ± 0.02 #	
5000	27.14 ± 3.71 #	48.07 ± 2.1 *		0.187 ± 0.01 #	0.202 ± 0.03 #	

SPF1—sulfated polysaccharides fraction 1; SPF2—sulfated polysaccharides fraction 2.

The values of the reducing power of *C. vagabunda* polysaccharidic fractions are presented in Table 3. Similar to the variation of free radical scavenging capacity, ascending values between 0.126 and 0.202 were observed at increasing concentrations of SPF1 and SPF2. In turn, although the values for SPF2 reducing power were slightly higher compared to SPF1, no significant differences were found ($p > 0.05$). All values were lower than that of ascorbic acid (0.569).

In the present study, the higher antioxidant activity of SPF2, in comparison to that of SPF1, could be due to lower MW and higher content of sulfated polysaccharides. In accordance, previous studies mentioned that the antioxidant potential of algal polysaccharides varied according to their structural characteristics, like glycosidic linkage, MW, degree of sulfation and monosaccharide composition [71]. Thus, MW decrease and chemical modifications by sulfation enhanced the antioxidant potential of polysaccharides due to the weakening of hydrogen bonds [72]. Also, polysaccharides containing abundantly specific sugar motifs, such as xylose and mannose, known to have the ability to donate electrons and to neutralize the free radicals, exhibited good antioxidant properties [73]. Using quantitative structure–activity relationship (QSAR) models for antioxidant activity, Li et al. [74] reported that arabinose and galacturonic acid were essential in determining the DPPH scavenging activity of polysaccharides, while uronic acids clearly influenced their hydroxyl radical scavenging capacity. In addition, a higher content of sulfate of seaweed polysaccharides appeared directly correlated with a stronger antioxidant activity [75].

3.3. The Capacity of SP Fractions from C. vagabunda to Modulate the ECM-Degrading Enzymes Activity

During aging, collagenase and elastase are the primary enzymes involved in the degradation of skin ECM constituents, such as collagen and elastin, leading to wrinkle formation. In this study, the effect of SPF1 and SPF2 isolated from *C. vagabunda* on the activity of collagenase and elastase was analysed using synthetic substrates. The results are presented in Figure 3.

The results showed that both polysaccharidic fractions inhibited the activity of collagenase in a dose-dependent manner. Thus, the inhibition values increased from 11.42 to 84.20% for SPF1 in the range of concentrations 100–250 µg/mL and from 13.81 to 95.69% for SPF2 in the same range of concentrations. At a concentration of 375 µg/mL, SPF2 had a significantly ($p < 0.05$) higher value than SPF1 and close to EGCG (84.37%), known as a potent collagenase inhibitor [76].

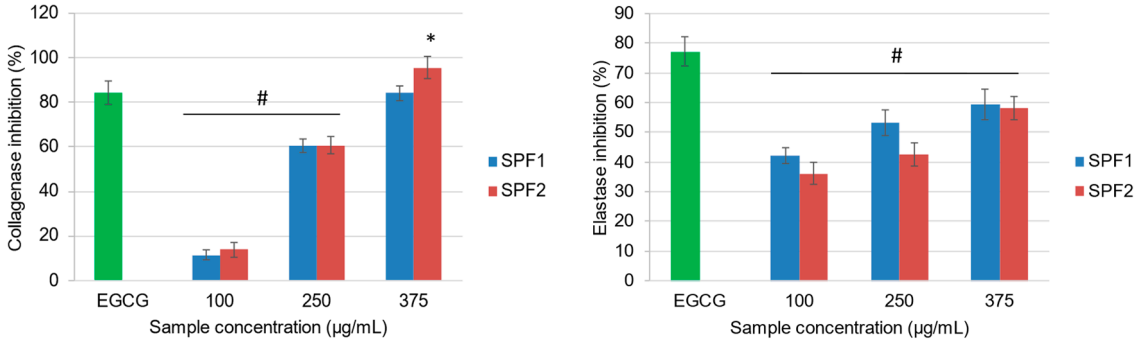


Figure 3. Inhibition of collagenase (**A**) and elastase (**B**) activity on specific substrates in the presence of different concentrations of polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*. Epigallocatechin gallate (EGCG) was used as positive control (50 µg/mL). The results are presented as mean ± SD (n = 9). * $p < 0.05$, compared to SPF1. # $p < 0.05$, compared to EGCG.

The inhibitory action of SPF1 and SPF2 fractions was also observed in the presence of elastase, at all tested concentrations. A potent inhibition of 60% was found at a concentration of 375 µg/mL polysaccharidic fractions, but significantly ($p < 0.05$) lower than that of EGCG (78%).

These results indicated that the collagenolytic inhibition could be correlated to the structural and compositional characteristics of the polysaccharidic fractions, being favored by lower MW and higher sulfate content. The present study has completed previous reports on brown algae antiwrinkle potential with two novel extracts from *C. vagabunda* green seaweed. Thus, it was earlier reported that 200 µg/mL sulfated polysaccharides isolated from *Hizikia fusiforme* brown alga had the capacity to act as protease inhibitors of the wrinkle-related enzymes collagenase and elastase, in a dose-dependent manner, reaching 49.77% and 56.53%, respectively [77]. Sulfated polysaccharides (100 µg/mL) obtained from *Ecklonia maxima* effectively inhibited the collagenase and elastase activity by 36.79 and 25.51%, respectively [9]. In addition, fucoidan, the sulfated polysaccharide isolated from *Chnoospora minima* and *Sargassum polycystum* brown algae, proved to suppress collagenase and elastase activity, in a dose-dependent manner, by activating specific inhibitors [13].

3.4. Cell Culture Testing

3.4.1. In Vitro Cytocompatibility Testing of SP Fractions from *C. vagabunda*

The cytocompatibility of *C. vagabunda* polysaccharidic fractions tested in human dermal fibroblast culture is presented in Figure 4. The results showed that the cell viability had values higher than 90%, indicating a high degree of cytocompatibility over a wide range of concentrations. The values were directly proportional with the number of viable cells, revealing the stimulation of fibroblast metabolism and cell proliferation. The highest values were found at concentrations of 750 µg/mL SPF2 (108%) and 1500 µg/mL SPF1 (112%). At higher concentrations, cell viability decreased, but remained at values close to that of the untreated culture (control, 100%), except for 3000 µg/mL SPF1 (90%).

3.4.2. Cell Cycle Analysis

Cell cycle analysis of SPF-treated cells was conducted to evaluate the effect of each fraction on cell viability. The obtained results showed that the polysaccharidic fractions SPF1 and SPF2 modulated the cell cycle progression (Figure 5A). Thus, the percentage of cells in the S phase increased, while the percentage of cells in G0/G1 and G2/M phases decreased after treatment with 1500 µg/mL SPF1 and 750 µg/mL SPF2, confirming the stimulation of cell proliferation observed by MTT test (Figure 5B). Similar results were

obtained for higher concentrations of polysaccharidic fractions, except for 3000 µg/mL SPF2, which determined lower cell viability, compared to the control, and induced cell accumulation in G0/G1 phase and cell percentage decrease in S and G2/M phases.

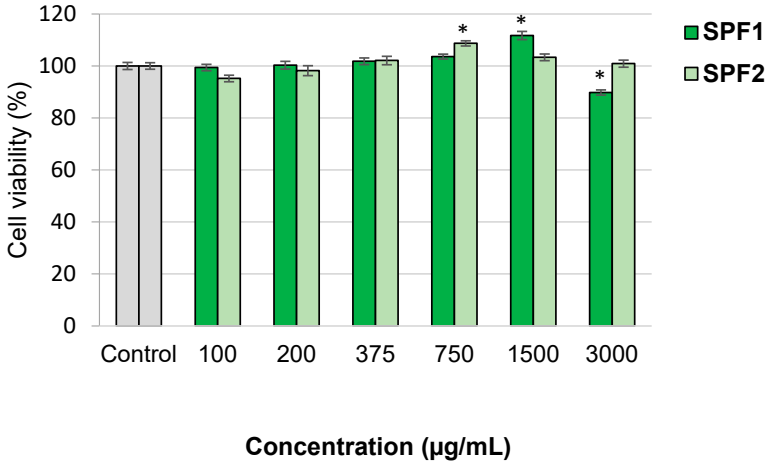
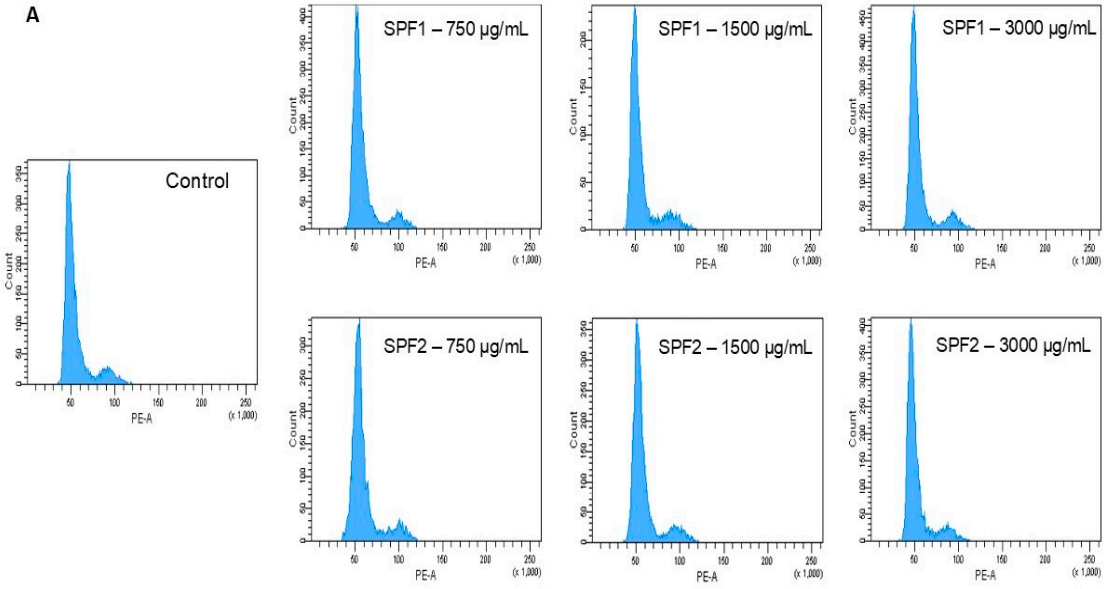


Figure 4. Cell viability of human dermal fibroblasts cultivated in the presence of different concentrations of polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*, for 24 h, determined by MTT assay. The results are presented as mean ± SD (n = 9). * $p < 0.05$, compared to control.



B

		Cell cycle distribution		
		G0/G1 (%)	S (%)	G2/M (%)
Control		83.05 ± 0.19	12.07 ± 1.06	4.87 ± 0.86
SPF1	750 µg/mL	82.62 ± 0.12	12.60 ± 0.41	3.27 ± 2.41
	1500 µg/mL	76.79 ± 2.10 *	19.21 ± 0.22 *	3.99 ± 2.32 *
	3000 µg/mL	88.35 ± 1.01 *	9.40 ± 2.68 *	2.24 ± 1.68 *
SPF2	750 µg/mL	81.69 ± 0.06	16.41 ± 0.15	1.90 ± 0.09
	1500 µg/mL	84.42 ± 0.07	14.38 ± 0.83	1.21 ± 0.92
	3000 µg/mL	85.87 ± 0.26	11.81 ± 0.07	2.31 ± 0.33

Figure 5. Flow cytometry analysis of cell cycle progression (**A**) and the percentage of cells in different phases of cell cycle (**B**) after treatment with different concentrations of polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*, for 48 h. The results are presented as mean ± SD (n = 9). * $p < 0.05$, compared to control.

3.4.3. Anti-Inflammatory Activity of SP Fractions from *C. vagabunda*

Specific markers of inflammation, such as pro-inflammatory cytokines IL-1β and TNF-α, and NO secretion were determined in the experimental model of inflamed macrophages cultivated in the presence of polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*. The results are presented in Figure 6A–C.

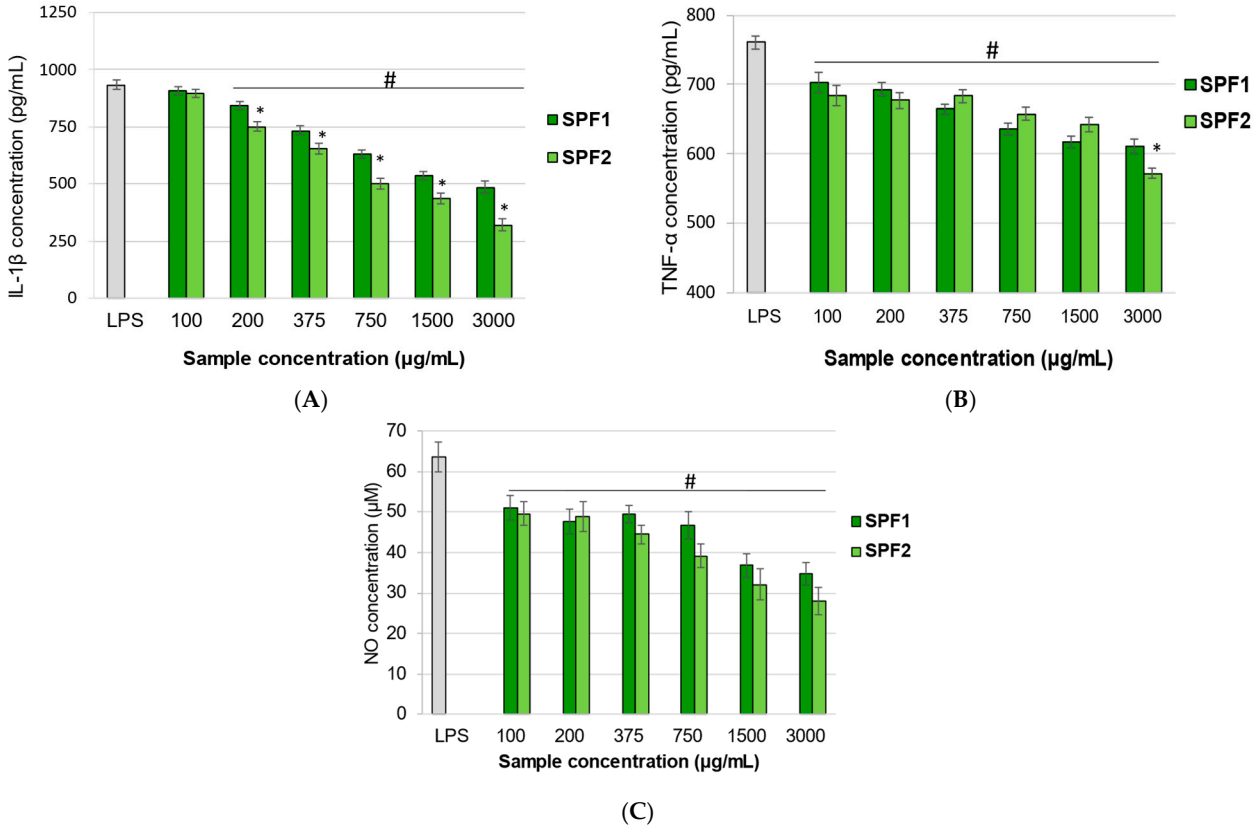


Figure 6. The level of the pro-inflammatory cytokines IL-1β (**A**) and TNF-α (**B**) and secretion of NO (**C**) in LPS-induced THP-1-derived macrophages in the presence of different concentrations of polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*, determined by ELISA. The results are presented as mean ± SD (n = 9). * $p < 0.05$, compared to SPF1. # $p < 0.05$, compared to LPS-induced control.

The data showed that both polysaccharidic fractions had the capacity to inhibit the production of IL-1β in a dose-dependent manner (Figure 6A). It was observed that SPF2 was significantly ($p < 0.05$) more effective than SPF1 at all tested concentrations, excepting 100 µg/mL. The most marked inhibition effect was observed at a concentration of 3000 µg/mL SPF2, which reduced IL-1β production by 65%, while SPF1 exerted 47% inhibition.

Regarding TNF-α production, a dose-dependent inhibition was observed for both polysaccharidic fractions (Figure 6B). At a concentration of 3000 µg/mL, SPF1 lowered the secretion of TNF-α by 24%, while SPF2 by 19%.

In terms of the inflammation marker NO, the level significantly ($p < 0.05$) decreased in SPF1- and SPF2-treated cells, in comparison to the LPS-inflamed control (Figure 6C). The results showed variation in a dose dependent-manner for both tested fractions. At 3000 µg/mL, SPF2 inhibited NO secretion by 10%, compared to SPF1, but no statistically significant differences ($p > 0.05$) were found.

The observations in the present study indicated that sulfated polysaccharidic fractions SPF1 and SPF 2 isolated from *C. vagabunda* green seaweed could serve as valuable anti-inflammatory agents. Previous studies have reported that sulfated polysaccharide

extract from *Sargassum hemiphyllum* brown alga and Spirogyra sp. green seaweed had similar activity and exerted an inhibition of the production of IL-1β, TNF-α and NO in LPS-stimulated murine RAW 264.7 cells [78,79]. It was shown that the process was mediated by down-regulation of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression [78,79].

In addition, the MW of algal sulfated polysaccharides could influence the bioactivity [80], such that lower MW compounds significantly increased the anti-inflammatory activity [81,82]. It was previously showed that, similar to the present study, polysaccharides with lower MW from *Laminaria japonica* green alga had the best anti-inflammatory effect and down-regulated the expression of TNF-α, IL-1β and IL-6 gene expression in LPS inflamed HaCaT cells [83].

Higher sulfation degree and sugar composition are other factors that could positively influence the anti-inflammatory activity. Sulfate groups provided negatively charged molecules that might interact with inflammatory mediators, while mannose, glucuronic acid and fucose could significantly contribute to the anti-inflammatory potential [73]. Further, sulfated polysaccharides isolated from *Sargassum horneri* containing high amounts of fucose and galactose exhibited anti-inflammatory properties by reducing the level of inflammatory cytokines and prostaglandin E2 in LPS-stimulated RAW 264.7 cells, also protecting zebrafish embryos from LPS-induced NO production [84]. In addition, Ye et al. [85] reported that the high content of fucose and galactose significantly improved the anti-inflammatory activity of a sulfated fucoidan fraction extracted from *Saccharina japonica* by blocking the pathways of nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), Janus kinase (JAK)-2, and signal transducer and activator of transcription (STAT)-1/3, in LPS-induced RAW 264.7 macrophage inflammation. In the same experimental model of inflammation, the production of MMP-1 and MMP-9 was evaluated after cell treatment with SPF1 and SPF2 polysaccharidic fractions. The results are presented in Figure 7A,B.

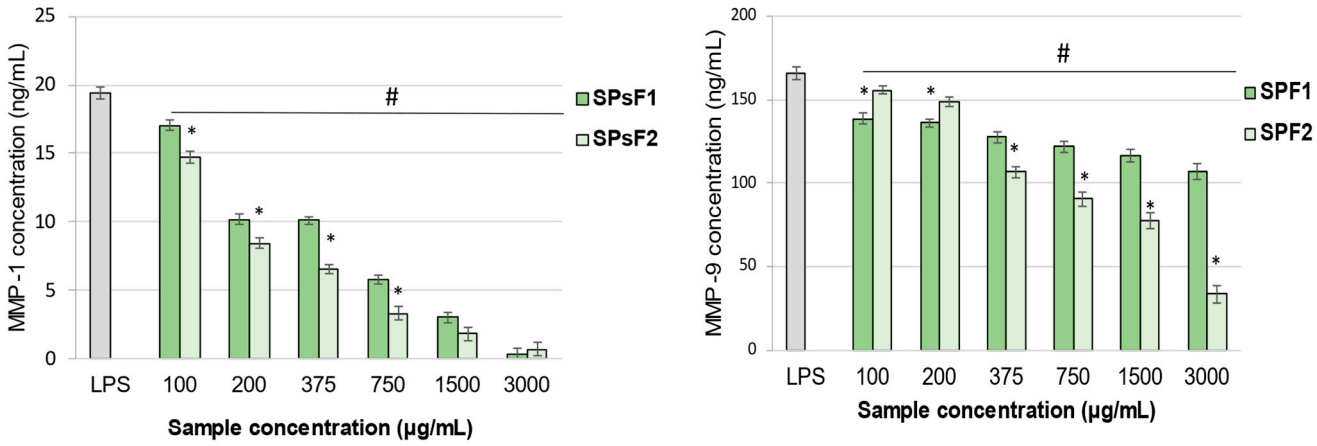


Figure 7. Production of MMP-1 (**A**) and MMP-9 (**B**) in LPS-induced THP-1-derived macrophages cultivated in the presence of different concentrations of polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*, determined by ELISA. The results are presented as mean ± SD (n = 9). * $p < 0.05$, compared to SPF1. # $p < 0.05$, compared to LPS-inflamed cells.

The data showed that the production of MMP-1 was significantly ($p < 0.05$) inhibited by both polysaccharidic fractions (Figure 7A). A dose-dependent decrease in MMP-1 secretion was observed in SPF1-and SPF2-treated cells, compared to LPS-inflamed cells. The collagenolytic activity of MMP-1 was 90% inhibited by both polysaccharidic fractions

of *C. vagabunda*, at a concentration of 3000 µg/mL. At doses between 100 and 750 µg/mL, SPF2 fraction was significantly ($p < 0.05$) more effective in lowering MMP-1 secretion, compared to SPF1.

A similar trend was observed in the case of MMP-9 production with 16–35% inhibition in the case of SPF1 treatment and 6–79% inhibition from SPF2 at all tested concentrations. A marked activity was observed of SPF2 over SPF1 by inhibition of MMP-9 activity in the range of concentrations 375–3000 µg/mL (Figure 7B). The results indicated that both fractions acted as potent MMP inhibitors, which could favor the preservation of collagen and elastin fibres from skin.

No studies were found on the effect of SPs from *C. vagabunda* as regulators of the MMPs involved in skin aging. It is known that MMP-1 specifically degrades the main structural proteins of aged skin ECM, like the fibrillar collagens type I and III from dermis, while MMP-9 was found as a marker of the inflammatory state, which could degrade the denatured forms of collagen (gelatin) and elastin, being involved in ECM remodeling [86]. Skin aging and photoaging triggers an inflammatory response based on molecular mechanisms of mitogen-activated protein kinases (MAPK) stimulation, involved in the phosphorylation of transcription factor activator protein 1 (AP-1), which induces the up-regulation of certain MMPs [1]. Thus, the proinflammatory cytokine IL-1β could stimulate MMP-9 expression through the induction of AP-1 and NF-κB [87]. In the present study, both IL-1β and MMP-9 were clearly inhibited by *C. vagabunda* polysaccharidic fractions and to a higher extent by SPF2.

3.5. Prebiotic Effect of SP Fractions from *C. vagabunda*

Skin probiotics refer to beneficial bacteria that support a healthy skin microbiome, contribute to maintain its function as a natural barrier, modulate the immune system response and slow down the aging process [88–90]. In the present study, the growth of *L. acidophillus* and *L. rhamnosus* probiotics was investigated in the presence of *C. vagabunda* polysaccharidic fractions. The results are presented in Figure 8A,B.

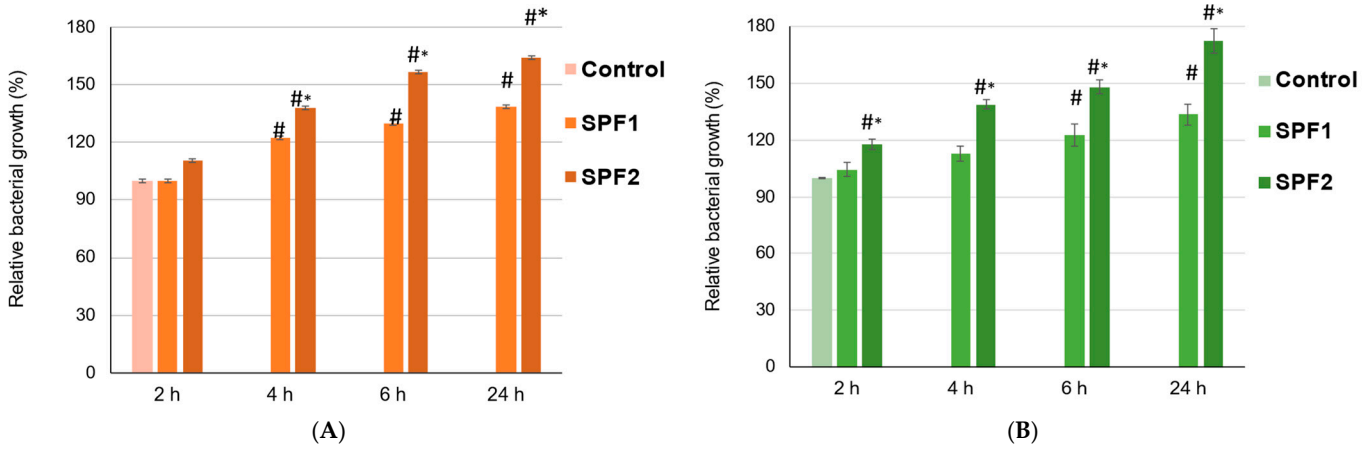


Figure 8. The growth of *L. acidophilus* (**A**) and *L. rhamnosus* (**B**) in the presence of polysaccharidic fractions SPF1 and SPF2 isolated from *C. vagabunda*. The results are presented as mean ± SD ($n = 9$). * $p < 0.05$, compared to SPF1. # $p < 0.05$, compared to the untreated culture (control).

The data showed that the growth of *L. acidophilus* was promoted in the presence of SPF1 and SPF2 fractions at all periods of time, except for 2 h of cultivation (Figure 8A). A similar trend was registered in the case of *L. rhamnosus* culture growth (Figure 8B). In both cultures, SPF2 induced a significantly ($p < 0.05$) faster bacterial growth, compared to SPF1, after 24 h of cultivation. These results indicated that SPF1 and SPF2 isolated from the green seaweed *C. vagabunda* could provide a nourishing environment for the stimulation of

the growth and activity of beneficial bacteria, and present great interest as ingredients for skincare formulations.

In accordance with the present study, it was previously reported the significant stimulation of *Lactobacillus* growth by low-MW SPs with high sulfate content [82]. Also, SPs isolated from the green seaweed *U. rigida*, containing high amounts of rhamnose, glucose and galactose, promoted the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. [91]. In a previous study, the prebiotic activity of ulvan was demonstrated by stimulation of *Bifidobacteria* and *Lactobacillus* growth, as well as the increase of lactate and acetate production observed using a microbial fermentability test [92]. Some bifidobacterial species have been identified in cheek skin bacterial microbiota [93], but they are also essential in maintaining a healthy intestinal flora. Skin disorders have been correlated with skin and gut microbiota dysbiosis [94]. Thus, the oral administration of *B. longum* and galacto-oligosaccharides appeared to act through the gut–skin axis, improving atopic dermatitis symptoms [95]. Probiotics have been proved to have anti-aging, skin whitening, anti-inflammatory, and photoprotective properties. They have been included as ingredients in new skin care products as ferment lysates or ferment extract [96]. On the other hand, the stimulating effect of seaweed polysaccharides on skin resident bifidobacteria growth could represent a viable alternative to improve skin conditions.

4. Conclusions

This study reports the isolation and purification of two water-soluble polysaccharidic fractions from *C. vagabunda* green seaweed, which, to the best of our knowledge, has not been previously described. The fractions contained significant amounts of polysaccharides made up of glucose and galactose, but also bioactive fucose and uronic acids. Polysaccharides with a higher content of sulfate and lower MW were found in SPF2, compared to SPF1. Cell culture studies showed good biocompatibility of both fractions and their capacity to positively modulate the cell cycle progression in human dermal fibroblasts. Both fractions presented anti-aging properties, being involved in modulation of oxidative stress, inflammation, and degradation of extracellular matrix processes at the cellular level. In addition, *C. vagabunda* polysaccharidic fractions stimulated the growth of *Lactobacillus* strains with beneficial effects on skin microbiome and aging. A more efficient biological activity was highlighted of SPF2, compared to SPF1, in correlation with higher sulfate content of specific sugars and lower MW. Future work will reveal their mechanisms of action and key molecular signalling pathways in cellular models of aging and in vivo validation, in view of their use as valuable natural bioactive ingredients.

Author Contributions: A.G.-P.: conceptualization, methodology, investigation, data analysis, writing—original draft, writing—review & editing, funding acquisition. A.-M.S.-G.: methodology, investigation, data analysis, writing—original draft, writing—review & editing. A.-M.P.: methodology, investigation, data analysis, writing—original draft. A.A.: methodology, investigation, data analysis, writing—original draft. F.G.: methodology, investigation, data analysis, writing—original draft. T.N.-P.: investigation, data analysis, writing—original draft. O.Z.: investigation, data analysis, writing—original draft, supervision. O.C.: conceptualization, data analysis, writing—original draft, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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