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Article

Bioactive Polysaccharides from Djiboutian Brown Algae: Antioxidant and Anti-Aging Potential for Cosmetic Applications

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Abstract

Brown macroalgae are a valuable source of bioactive polysaccharides, particularly alginates and fucoidans, with significant potential for cosmetic and pharmaceutical applications. In this study, polysaccharides were extracted from four species (*Padina pavonica*, *Sargassum ilicifolium*, *S. latifolium*, and *Turbinaria decurrens*) collected along the Djibouti coastline. Structural characterization by FT-IR and ^{1}H-NMR revealed pronounced interspecific variability. Alginates displayed distinct mannuronate/guluronate (M/G) ratios, with *Sargassum latifolium* showing the highest and *T. decurrens* the lowest, reflecting differences in polymer composition and structural flexibility. Fucoidan spectra exhibited characteristic sulfate bands at 1217–1220 and 840 cm^{-1}, with lower transmission values for *T. decurrens* indicating a relatively higher degree of sulfation. Biological assays demonstrated that alginate extracts exhibited moderate antioxidant activity, whereas fucoidans showed significantly stronger radical scavenging (DPPH) and ferric reducing (FRAP) capacities, in some cases comparable to vitamin C. Fucoidans also displayed potent inhibition of skin-aging enzymes, with elastase inhibition reaching 62.1% for *P. pavonica* and tyrosinase inhibition peaking at 63% for *S. ilicifolium* at 0.5 mg/mL. These results highlight the critical role of structural features, particularly sulfation patterns, in determining biological activity. Overall, Djiboutian brown algal polysaccharides combine antioxidant and enzyme inhibitory properties, confirming their potential as multifunctional and sustainable marine-derived ingredients for cosmetic formulations.

Keywords: brown algae; fucoidans; alginates; antioxidant activity; anti-aging enzymes

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1. Introduction

Skin, as the largest organ of the human body, fulfills essential physiological roles and serves as the primary barrier against external environmental aggressions [1]. Due to its continuous exposure, it is particularly vulnerable to various harmful factors such as ultraviolet (UV) radiation, chemical pollutants, fine particulate matter, and microbial invasion [2,3]. These environmental constraints trigger a cascade of detrimental effects on the skin, including oxidative stress, inflammatory reactions, disruption of the skin barrier, melanin overproduction, and degradation of collagen fibers, all of which accelerate skin

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aging and compromise skin health [4,5]. Tyrosinase is a key enzyme involved in melanogenesis, the biological process responsible for melanin synthesis and skin pigmentation [6,7]. In addition, increased levels of reactive oxygen species (ROS) enhance elastase activity, leading to elastin degradation, reduced skin elasticity, and the formation of wrinkles [8]. Therefore, tyrosinase and elastase inhibitors are considered promising targets for the prevention of hyperpigmentation and skin aging in cosmeceutical applications [9,10]. To address these challenges, a variety of therapeutic and preventive approaches have been explored. Among them, bioactive compounds extracted from marine algae, including polyphenols and polysaccharides, are gaining significant attention due to their perceived safety and excellent skin compatibility [11,12]. This growing interest is driving the demand for natural ingredients in cosmetic formulations designed to protect, repair, and enhance skin health.

Marine seaweed polysaccharides have attracted increasing interest in pharmaceutical and cosmeceutical research due to their structural diversity and broad spectrum of biological activities [13]. Representing nearly 60% of the bioactive metabolites found in seaweed, these macromolecules are composed of monosaccharide units linked by glycosidic bonds to form complex carbohydrate chains [14]. Among them, alginates and sulfated polysaccharides such as fucoidans and galactans have been extensively investigated for their antioxidant, anti-inflammatory, antimicrobial, anticoagulant, immunomodulatory, and photoprotective properties [14–18]. In cosmeceutical formulations, alginates are widely employed as gelling and thickening agents, emulsion stabilizers, and film-forming materials, providing enhanced skin adhesion and protective effects, particularly in creams, lotions, and dermatological preparations [19–21]. In parallel, sulfated polysaccharides have demonstrated significant bioactivities relevant to skin health, including free radical scavenging, UV protection, inhibition of tyrosinase activity, and prevention of skin aging and hyperpigmentation [22,23]. Among skin-aging-related enzymes, elastase and tyrosinase are considered key biological targets in cosmetic research. Elastase is responsible for the degradation of elastic fibers, leading to reduced skin firmness [24], while tyrosinase catalyzes melanin biosynthesis and is associated with hyperpigmentation disorders [25]. Therefore, inhibition of these enzymes is widely used to assess the anti-aging and depigmenting potential of natural cosmetic ingredients, including marine-derived polysaccharides.

The structural characterization of brown algal polysaccharides, particularly alginates and fucoidans, relies heavily on advanced analytical techniques such as Fourier Transform Infrared Spectroscopy (FT-IR) and Proton Nuclear Magnetic Resonance (^{1}H-NMR). These methods are essential for determining key structural parameters, including the mannuronic/guluronic acid (M/G) ratio in alginates and the degree of sulfation in fucoidans [26,27], both of which significantly influence their biological activities. FT-IR analysis enables the identification of functional groups, with characteristic absorption bands corresponding to the polysaccharide backbone and sulfate ester groups in fucoidans, typically observed as broad peaks in specific wavelength regions [11,28]. Complementarily, ^{1}H-NMR provides detailed insights into the monomeric composition and sequential arrangement of uronic acids within alginate chains [26]. Together, FT-IR and NMR spectroscopy are indispensable tools for elucidating the structure–activity relationships of these bioactive marine polysaccharides.

Despite the considerable amount of research conducted on macroalgae from various global regions, the brown algal flora of the Red Sea and adjacent coasts, including Djibouti, remains largely underexplored, particularly regarding its biotechnological potential. The Djiboutian marine environment is characterized by extreme conditions, including high salinity, elevated temperatures, and unique hydrodynamic features, which may drive the biosynthesis of polysaccharides with distinctive structural adaptations and potentially enhanced bioactivities [29,30]. Nevertheless, comprehensive studies investigating the biofunctional properties of polysaccharides from these local species are still lacking. In this context, the present study focused on four brown macroalgae *Padina pavonica*, *Sargassum ilicifolium*, *Sargassum latifolium*, and *Turbinaria decurrens*, collected along the Djiboutian coastline. Structural characterization was carried out using FT-IR and ^{1}H-NMR spectroscopies to determine key parameters such as the mannuronic/guluronic acid (M/G) ratio and sulfation degree. In parallel, the biological activities of fucoidan-rich extracts were assessed through antioxidant assays (DPPH and FRAP) and enzyme inhibition tests (tyrosinase and elastase). These analyses aimed to establish structure–activity relationships and to highlight the potential of Djiboutian brown algal polysaccharides as promising bioactive ingredients for marine-derived cosmetic applications.

2. Materials and Methods

2.1. Collection of Brown Algae Samples

Four species of brown macroalgae, namely *Padina pavonica* (Linnaeus) Thivy, *Sargassum ilicifolium* (Turner) C. Agardh, *Sargassum latifolium* (Turner) C. Agardh and *Turbinaria decurrens* were collected during the summer of 2022 from the coastal sites of Heron, Douda, Moucha Island and Khor-Ambado, located along the Tadjourah Gulf in Djibouti (Figure S1). Sampling was conducted within the intertidal zone on rocky substrates adjacent to fringing coral reefs, which harbor a diverse assemblage of macroalgae. The geographical coordinates of the sampling sites are approximately between 11°35′ N and 43°05′ E. The algal specimens were hand-collected at low tide, thoroughly rinsed with seawater on-site to remove adhering sediments and epiphytes, and immediately transported to the laboratory in ice-cooled containers for further processing. The collected brown algal species were identified based on their macroscopic and morphological characteristics using standard taxonomic keys and reference literature [31–33]. Representative voucher specimens were prepared and preserved as herbarium material at the Laboratory of Marine Sciences, University of Brest, under the supervision of Valérie Stiger-Pouvreau. The scientific names and taxonomic status of all algal species investigated in this study were verified and validated using the AlgaeBase (www.algaebase.org, accessed on 5 December 2025) [34].

2.2. Materials

All chemicals and reagents used in this study were of analytical grade unless otherwise specified. Deuterium oxide (D_2O) and sodium 3-(trimethylsilyl)propionate (TSP) used for ^{1}H-NMR analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). For antioxidant activity assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride ($FeCl_3 \cdot 6H_2O$), sodium acetate, acetic acid, and hydrochloric acid were obtained from Sigma-Aldrich (St, Louis MO, USA). Ascorbic acid (vitamin C) and butylated hydroxyanisole (BHA) were used as positive controls. For enzyme inhibition assays, porcine pancreatic elastase, *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (Suc-Ala_3-*p*NA), mushroom tyrosinase, L-tyrosine, epigallocatechin gallate (EGCG), and kojic acid were purchased from Sigma-Aldrich (St, Louis MO, USA). Potassium bromide (KBr) of spectroscopic grade was used for FT-IR analysis. All aqueous solutions were prepared using distilled water. Buffers and other reagents not explicitly listed were prepared according to standard protocols described in the corresponding methodological sections.

2.3. Polysaccharide Extraction and Fractionation

The extraction and fractionation procedures for polysaccharides were carried out according to the method initially described by Carvalho et al. [35], with specific modifications aimed at enhancing the removal of phlorotannins (by introducing an acetone-water extraction step at the beginning) and reducing the overall duration of the protocol by optimizing certain steps. A total of 4 g of algal powder was subjected to an initial dephlorotannination by maceration in 150 mL of acetone-water mixture (50:50, v/v), assisted by ultrasound treatment for 15 min at 4 °C. This step was followed by static maceration for 2 h at 40 °C in fresh 50% acetone. The mixture was then centrifuged at 4000 rpm for 10 min, and the supernatant was collected. The solid residue was re-extracted under identical conditions, and the supernatants from both extractions (2 × 150 mL) were combined and concentrated under reduced pressure at 40 °C using a rotary evaporator (Laborota 4000 efficient, Heidolph, Germany) to obtain a phlorotannin-enriched extract (not used here). The remaining insoluble residue was subsequently used for sequential polysaccharide extraction. The residue was subjected to two successive extractions, each lasting 2 h, with 150 mL of 0.01 N hydrochloric acid (HCl) solution supplemented with 2% calcium chloride ($CaCl_2$), assisted by ultrasonication. After centrifugation at 4000 rpm for 10 min, the soluble phase containing fucoidans was separated from the insoluble fraction. The collected supernatant (total volume of 300 mL) was concentrated under vacuum at 60 °C until the volume was reduced to approximately 50–100 mL. Finally, the insoluble residue was subjected to two additional alkaline extractions, each for 2 h, using 150 mL of 1.5% sodium carbonate (Na_2CO_3) solution at 50 °C, assisted by ultrasound. The soluble fractions containing alginates were separated by centrifugation and subsequently concentrated by rotary evaporation at 60 °C to a final volume of 50–100 mL. The extraction yield of fucoidan and sodium alginate was calculated based on the dry weight of the extracted polysaccharide relative to the initial dry weight of the algal biomass. The yield was expressed as a percentage (%) according to the following formula:

$$\text{Extraction yield (\%)} = (\text{Weight of extracted polysaccharide (g)}/\text{Initial dry weight of algae (g)}) \times 100$$

All yields were calculated from dried extracts after purification and drying to constant weight.

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

The structural characterization of the extracted sodium alginate and fucoidan fractions was performed using Fourier Transform Infrared (FT-IR) spectroscopy to identify their specific functional groups as described [36]. The dried polysaccharide samples were finely ground (<1 mm particle size) and thoroughly mixed with spectroscopic-grade potassium bromide (KBr) to form homogeneous pellets by compression under vacuum. The infrared spectra were recorded using a Bruker Alpha FT-IR spectrometer (Bruker Optics, Ettlingen, Germany) equipped with an attenuated total reflectance (ATR) accessory and operated with OPUS software (version 8.5). Spectra acquisition was performed over a wavenumber range of 4000–400 cm^{-1}, with a resolution of 2 cm^{-1}, averaging 128 scans per sample to ensure high signal-to-noise ratio and spectral accuracy. All measurements were conducted at room temperature, and each sample was analyzed in duplicate to ensure reproducibility. The characteristic absorption bands corresponding to functional groups such as hydroxyl (–OH), carboxylate (COO–), sulfate esters (S=O), and glycosidic linkages (C–O–C) were identified to confirm the presence and structural integrity of alginates and fucoidans.

2.5. Proton Nuclear Magnetic Resonance (^{1}H-NMR) Analysis of Alginates

Sodium alginate samples were dissolved in deuterium oxide (D_2O, 6 g/L) and heated to 80 °C to ensure complete solubilization prior to analysis. The molecular composition was determined by ^{1}H-NMR spectroscopy using a Bruker AVANCE 400 MHz spectrometer (UBO NMR platform), according to Rashedy et al. [37]. Spectra were acquired with a relaxation delay of 2 s, a 30° pulse angle, and 16–128 scans, using TSP as an external chemical shift reference. The anomeric proton signals of β-D-mannuronic acid (H-1, 4.7–4.9 ppm) and α-L-guluronic acid (H-1, 5.0–5.2 ppm) were identified, and the M/G ratio was calculated from the relative integration of these peaks, assuming one proton per monomer unit. NMR spectroscopy analysis was carried out following established methodologies previously reported in the literature [27,37–39]. The analyses were verified by comparison with the following standards: Alginic acid sodium salt from brown algae (Ref. A7128 labeled "high viscosity", SIGMA ALDRICH), Alginic acid sodium salt (Ref. A180947 labeled Sodium alginic acid, SIGMA ALDRICH). FT-IR analysis provided indicative information supporting M/G distribution, while accurate M/G ratios were derived from ^{1}H-NMR data.

2.6. Antioxidant Activities and Anti-Aging Activities of Extracts

2.6.1. DPPH Radical Scavenging Activity Assay

The antioxidant capacity of the polysaccharides (alginate and fucoidan fractions) extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, following a modified protocol based on [10,40]. Briefly, 22 µL of the sample solution (polysaccharide extracts or controls) were mixed with 200 µL of a freshly prepared DPPH methanolic solution at a concentration of 25 mg/L. The reaction mixtures were incubated in the dark at room temperature for 60 min to allow for the completion of the radical scavenging reaction. Following incubation, the absorbance of the mixtures was measured at 540 nm. Distilled water served as a negative control, while ascorbic acid (vitamin C) and 2,3-tert-butyl-4-hydroxyanisole (BHA) were employed as positive antioxidant references. For antioxidant activity assays, fucoidan and alginate extracts obtained from the four brown seaweed species (*Sargassum latifolium*, *S. ilicifolium*, *Turbinaria decurrens* and *Padina pavonica*) were evaluated at a final concentration of 0.5 mg/mL. The scavenging activity was expressed as IC_{50} values (mg/mL), representing the sample concentration required to inhibit 50% of the DPPH radical activity. Lower IC_{50} values indicate stronger antioxidant potential. All assays were conducted in triplicate, and the results were reported as mean ± standard deviation (SD).

2.6.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of the polysaccharide extracts was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, based on their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) through electron donation following a modified protocol based on [10,40]. In this method, 25 µL of sodium phosphate buffer (0.2 mM, pH 6.6) and 25 µL of 1% potassium ferricyanide solution were added to 25 µL of sample (polysaccharide extract, fraction, or control) in a 96-well microplate. The mixtures were homogenized and incubated at 50 °C for 20 min to allow for the redox reaction to proceed. The reaction was halted by placing the plate on ice. Subsequently, 25 µL of 10% trichloroacetic acid and 100 µL of distilled water were added to each well. An initial absorbance reading was recorded at 620 nm. Then, 20 µL of 0.1% ferric chloride solution was added to each well, and after an additional 10-min incubation at room temperature, the final absorbance was measured at 620 nm. Ascorbic acid (vitamin C), α-tocopherol (vitamin E), and 2,3-tert-butyl-4-hydroxyanisole (BHA) served as positive controls. The antioxidant reducing capacity of the samples was expressed as EC_{50} (mg/mL), defined as the concentration at which 50% of the maximum reducing activity was achieved. All measurements were conducted in triplicate, and results were reported as mean ± standard deviation (SD).

2.6.3. Anti-Elastase Activity of Fucoidan Polysaccharide Extracts

The elastase inhibitory activity of the fucoidan extracts was assessed using a colorimetric assay conducted in 96-well microplates, adapted from previously described methods [9,10]. The assay is based on the hydrolysis of the synthetic substrate *N*-succinyl-Ala-

Ala-Ala-p-nitroanilide (Suc-Ala_3-pNA) by porcine pancreatic elastase, leading to the release of p-nitroaniline, which was quantified spectrophotometrically. A Tris–HCl buffer (179 mM, pH 8.0) was used to prepare both the substrate solution (Suc-Ala_3-pNA, 0.75 mg/mL) and the elastase enzyme solution (0.34 U/mL; Sigma-Aldrich, St. Louis, MO, USA). Epigallocatechin gallate (EGCG) served as positive control and was prepared at concentrations ranging from 0.05 to 1 mg/mL, while distilled water was used as a negative control. For each assay, 13 µL of polysaccharide extract (1 mg/mL) or control solution was combined with 93 µL of Tris–HCl buffer and 52 µL of the substrate solution in each well. After gentle mixing, the plate was incubated at 25 °C for 5 min, after which 42 µL of the elastase enzyme solution was added to initiate the reaction. The increase in absorbance was recorded at 410 nm every 48 s for 5 min using a microplate reader. Control wells containing the enzyme without inhibitors were included under identical conditions. The initial reaction velocity (Vi) was determined from the linear portion of the absorbance–time curves using GEN5™ software (version 3.02, BioTek Santa Clara, CA, USA). Elastase inhibition was expressed as a percentage relative to the control using the following equation:

$$\text{PI (\%)} = [(\text{Vi\_control} - \text{Vi\_sample})/\text{Vi\_control}] \times 100$$

All measurements were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD), with a comparison with standards (positive controls) to validate our results.

2.6.4. Tyrosinase Inhibitory Activity of Fucoidan Polysaccharide Extracts

The tyrosinase inhibitory activity of fucoidan extracts was assessed to evaluate their potential depigmenting and anti-aging effects using a spectrophotometric assay adapted from previously described methods [9,10]. This assay is based on the inhibition of the tyrosinase-mediated oxidation of L-tyrosine into dopachrome, which is monitored spectrophotometrically. A phosphate buffer (0.1 M, pH 6.5) was prepared by dissolving disodium phosphate (Na_2HPO_4, 4.117 g) and monosodium phosphate (NaH_2PO_4, 8.639 g) in distilled water. The substrate solution was prepared by dissolving L-tyrosine in distilled water to obtain a final concentration of 1.694 mM. The working substrate reagent was obtained by mixing 2.5 mL of the L-tyrosine solution with 2.5 mL of phosphate buffer and 2.25 mL of distilled water. Mushroom tyrosinase (Sigma-Aldrich, St, Louis, MO, USA) was freshly prepared at a concentration of 317.71 U/mL. In a 96-well microplate, 20 µL of fucoidan extract (1 mg/mL) or control solution was added to 243 µL of the L-tyrosine working reagent. After pre-incubation at 25 °C for 5 min to allow interaction between the extract and the substrate, the enzymatic reaction was initiated by adding 42 µL of the tyrosinase solution. The formation of dopachrome was monitored by measuring absorbance at 475 nm in kinetic mode for 5 min at 25 °C using a microplate reader. Blank wells were prepared for each sample by replacing the L-tyrosine reagent with phosphate buffer to correct for background absorbance. Kojic acid was used as positive control, while wells containing enzymes without inhibitors served as negative controls. Tyrosinase inhibition was expressed as a percentage relative to the control according to the following equation:

$$\text{Inhibition (\%)} = [(\text{Vi\_control} - \text{Vi\_sample})/\text{Vi\_control}] \times 100$$

where Vi represents the initial reaction velocity. All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD), with a comparison with standards (positive controls) to validate our results.

2.7. Statistical Analysis

Statistical analyses were performed using R software version 4.5.2 (31 October 2025). Data are expressed as mean ± standard deviation (SD). Prior to analysis, the assumptions of normality and homogeneity of variances were assessed. When homogeneity of variances was confirmed, differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) or Tukey's post hoc test for multiple comparisons, as appropriate. In cases where the assumption of homogeneity was violated, a non-parametric *Kruskal–Wallis* test was applied at a 95% confidence level. When significant differences were detected, appropriate non-parametric multiple comparison tests were subsequently conducted. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Polysaccharide Yields (Alginate and Fucoidan Fractions)

The extraction yields of sodium alginate and fucoidans from the four studied brown seaweed species are presented in Figure 1. Statistical analysis revealed significant differences in alginate yield among the species (ANOVA, $p < 0.05$). The highest alginate yield was observed in *Turbinaria decurrens* (*Td*), with an average of 29.3% of dry weight (DW), followed by *Sargassum ilicifolium* (*Si*), which exhibited an average yield of 22.17% DW. Conversely, *Padina pavonica* (*Pp*) showed the lowest alginate content, with a mean yield of 12.4% DW. Regarding fucoidan fractions, yields were consistent across the tested species, as indicated by the absence of significant differences (one-way ANOVA, $p = 0.439$). The highest fucoidan content was recorded in *P. pavonica*, with an average yield of 1.81% DW, followed by *T. decurrens* at 1.43% DW. The two *Sargassum* species (*S. ilicifolium* and *S. latifolium*) exhibited similar fucoidan yields, averaging 1.0% DW. Overall, these results indicate that, while fucoidan yields are comparable among species, alginate yields vary significantly, highlighting *T. decurrens* as the most productive species for alginate extraction under the tested conditions.

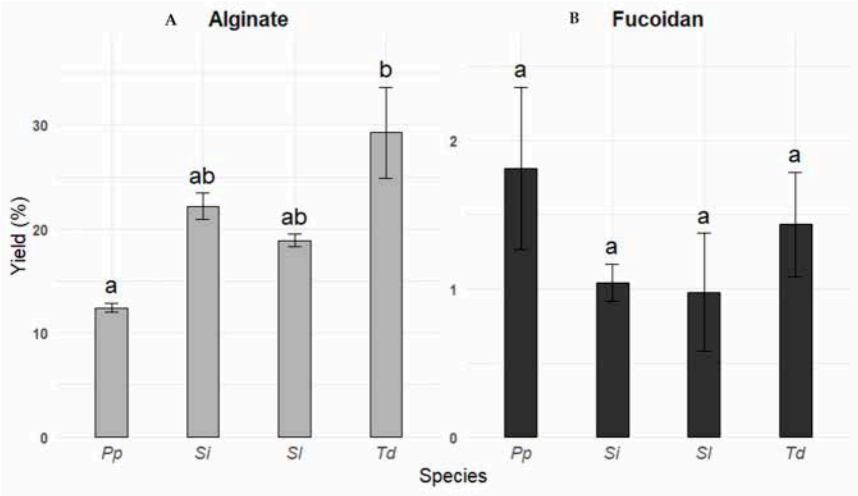

Figure 1. Extraction mass yields of polysaccharide extracts from selected brown seaweeds. (**A**) Alginate yields (% DW) from *Padina pavonica* (*Pp*), *Sargassum ilicifolium* (*Si*), *Sargassum latifolium* (*Sl*), and *Turbinaria decurrens* (*Td*). (**B**) Fucoidan yields (% DW) from the same species. Letters indicate significant differences between species according to the test ($p < 0.05$).

3.2. FT-IR Analysis of Polysaccharide Extracts

FT-IR analysis provided qualitative information on the structural features of alginates and fucoidans extracted from the four brown algal species studied in Table 1. For alginates, characteristic absorption bands associated with hydroxyl groups (O–H stretching) were observed in the range of 3262–3342 cm^{-1}, while the asymmetric and symmetric stretching vibrations of carboxylate (COO^{-}) groups appeared at approximately 1591–1599 cm^{-1} and 1408–1416 cm^{-1}, respectively. Bands attributed to mannuronic acid units were detected at 1024–1029 cm^{-1} (Figure S2), with transmission values ranging from 61% to 94%, whereas guluronic acid units were observed at 1080–1084 cm^{-1}, showing transmission values between 74% and 97%. Notably, *Turbinaria decurrens* exhibited markedly lower transmission values at both mannuronic and guluronic bands compared to the other species, indicating stronger absorption in this region. Additional diagnostic bands assigned to β-D-mannuronic acid (872–889 cm^{-1}) and α-L-guluronic acid (943–945 cm^{-1}) were consistently observed in all alginate samples, and their relative intensities indicated an M/G ratio greater than 1.0, reflecting a predominance of mannuronic acid units. For fucoidans, two characteristic sulfate-related bands were identified, including the asymmetric S=O stretching vibration at 1217–1220 cm^{-1} and the band around 840 cm^{-1} attributed to sulfate substitution at the C4 position of fucose residues (Figure S3). Transmission values at these bands ranged from 83% to 98%, with slightly higher transmission observed for fucoidans from *Padina pavonica* and *Sargassum ilicifolium* compared to *S. latifolium* and *T. decurrens*, indicating interspecific variability in fucoidan sulfation patterns.

Table 1. Characteristic FT-IR bands of alginates and fucoidans extracted from four brown macroalgae species.

Polysaccharide	**Vibrational Assignment**	**Expected Spectral Region (cm^{-1})**	***P. pavonica***	***S. ilicifolium***	***S. latifolium***	***T. decurrens***
Alginates	O–H stretching (hydroxyl groups)	3400	3298	3262	3334	3342
	COO^{-} asymmetric stretch	1600–1620	1599	1594	1596	1591
	COO^{-} symmetric stretch	1410–1425	1416	1411	1408	1411
	Mannuronic units	1025–1030	1029 (91%T)	1024 (92%T)	1025 (94%T)	1026 (61%T)
	Guluronic units	1080–1090	1084 (94%T)	1081 (95%T)	1080 (97%T)	1081 (74%T)
	β-D-mannuronic acid	890–900	872	817	873	889
	α-L-guluronic acid	950–960	945	944	943	943
Fucoidans	Asymmetric S=O stretching (sulfates)	1217–1220	1217 (95%)	1220 (95%)	1218 (83%)	1217 (83%)
	Sulfate groups at C4 of fucose residues	840	821.9 (98%)	822.7 (98%)	821 (97%)	822.6 (97%)

3.3. Analysis of Sodium Alginate Extracts and M/G Ratio Determination Using ^{1}H-NMR and FT-IR Spectroscopic Analysis

The ^{1}H-NMR spectra of sodium alginate extracted from the five seaweed species revealed characteristic signals corresponding to the anomeric protons of mannuronic (M) and guluronic (G) acid residues. Three key resonances were identified: the anomeric protons of guluronic acid appeared between 5.1 and 5.2 ppm, the anomeric protons of mannuronic acid were observed between 4.7 and 4.9 ppm, and the H-5 protons of guluronic acid residues resonated around 4.5 ppm. These spectral features confirm the presence of both M and G units in the extracted polysaccharides and are consistent with previously reported ^{1}H-NMR profiles of alginates (Figure 2).

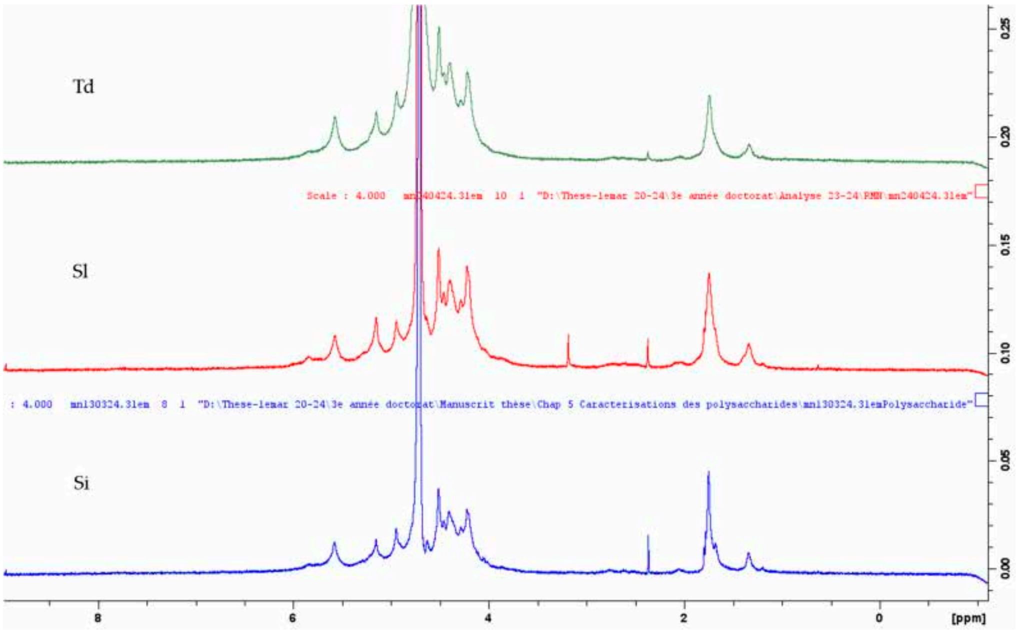

Figure 2. 1H-NMR spectra of the extracted alginate extract from *Sargassum ilicifolium* (**Si**), *S. latifolium* (**Sl**), *Turbinaria decurrens* (**Td**).

The M/G ratios of alginates varied among the studied species as determined by both ^{1}H-NMR and FT-IR analyses (Table 2).

Table 2. M/G ratio of alginates based on NMR and FT-IR analyses and studied in four brown macroalgae from Djibouti, ND (not data).

Species	M/G Ratio Using NMR	M/G Ratio Using FT-IR
Padina pavonica	ND	1.54
Sargassum ilicifolium	1.14	1.62
S. latifolium	1.98	2.07
Turbinaria decurrens	1.03	1.63

The highest M/G values were observed in *Sargassum latifolium*, indicating a predominance of mannuronic units, whereas *S. ilicifolium* and *Turbinaria decurrens* exhibited lower and more balanced M/G ratios; FT-IR-derived values were slightly higher than those obtained by ^{1}H-NMR.

3.4. Antioxidant Activities of Extracts

The antioxidant potential of polysaccharide extracts was assessed using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays, with vitamin C and BHA used as positive controls (Figure 3). Statistical analysis revealed significant interspecific differences for both assays ($p < 0.05$).

Alginate extracts exhibited moderate antioxidant activity in the DPPH assay, with IC_{50} values exceeding 1 mg/mL, which were substantially higher than those of the positive controls (0.20–0.30 mg/mL). Among alginates, *Sargassum latifolium* and *S. ilicifolium* displayed the lowest IC_{50} values (1.05 and 1.25 mg/mL, respectively), indicating slightly higher radical scavenging capacity compared to *Padina pavonica* and *Turbinaria decurrens*. In the FRAP assay, alginate extracts from *P. pavonica* and *T. decurrens* showed the lowest IC_{50} values (2.11 and 1.98 mg/mL, respectively), reflecting a moderate reducing power. In contrast, fucoidan extracts exhibited significantly stronger antioxidant activity than alginates in both assays ($p < 0.05$). In the DPPH assay, fucoidans from *S. ilicifolium*, *S. latifolium*, and *T. decurrens* displayed very low IC_{50} values (0.09, 0.11, and 0.10 mg/mL, respectively), comparable to those of the positive controls, indicating a strong free radical scavenging capacity. Similarly, in the FRAP assay, fucoidan extracts demonstrated high reducing power, with *T. decurrens* showing the lowest IC_{50} value (0.50 mg/mL), followed by *P. pavonica* (0.69 mg/mL), confirming the superior antioxidant performance of fucoidans relative to alginates.

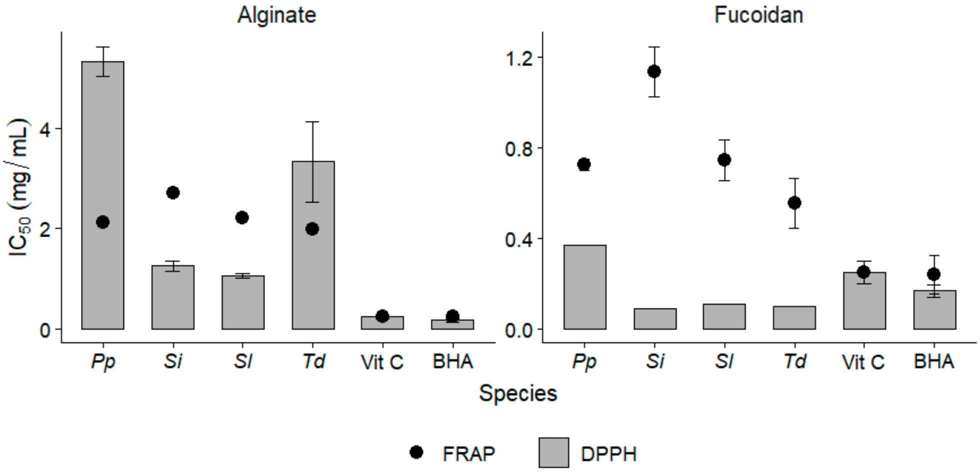

Figure 3. Radical scavenging (bars) and antioxidant (black point) activities (mean ± standard deviation, $n = 3$) of polysaccharide extracts from four macroalgae from Djibouti: *Padina pavonica* (**Pp**), *Sargassum ilicifolium* (**Si**), *Sargassum latifolium* (**Sl**), *Turbinaria decurrens* (**Td**), with Vit C and BHA as positive controls.

3.5. Anti-Aging (Anti-Elastase and Anti-Tyrosinase Activities) Capacity of Fucoidan Extracts

Building upon the antioxidant activities previously demonstrated, the study was extended to assess the anti-ageing potential of fucoidan extracts from four brown algal species, focusing on their ability to inhibit two key enzymes involved in skin ageing: elastase and tyrosinase. A two-way ANOVA revealed a significant effect of species and concentration on anti-elastase activity, with a significant interaction between both factors ($p < 0.05$), indicating species-specific responses to increasing concentration. The results, illustrated in Figure 4, are expressed as mean ± standard deviation from three independent replicates ($n = 3$) at two tested concentrations (0.25 and 0.50 mg/mL).

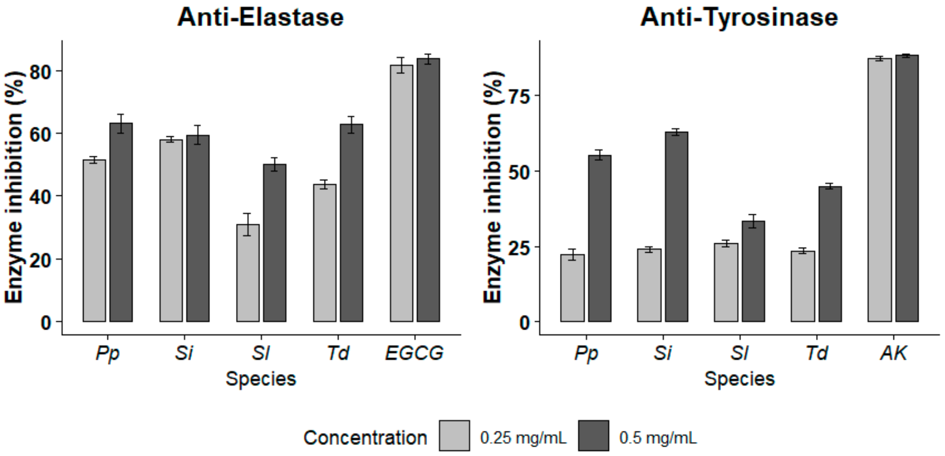

Figure 4. Inhibition of elastase by fucoidan extracts (0.25 and 0.50 mg/mL) from four brown macroalgae from Djibouti: *Padina pavonica* (**Pp**), *Sargassum ilicifolium* (**Si**), *Sargassum latifolium* (**Sl**), *Turbinaria decurrens* (**Td**) with EGCG and kojic acid (AK) as positive controls.

Overall, a clear dose-dependent trend was observed, with higher inhibition rates recorded at 0.50 mg/mL.

The anti-elastase activity of fucoidan extracts was markedly influenced by concentration (Figure 4). Overall, inhibition percentages were substantially higher at 0.5 mg·mL^{-1} than at 0.25 mg/mL, indicating a clear dose-dependent response. At the highest tested concentration (0.5 mg/mL), *Padina pavonica* and *Turbinaria decurrens* exhibited the strongest inhibitory effects, reaching approximately 62% inhibition, followed by *Sargassum ilicifolium*. In contrast, *Sargassum latifolium* showed the lowest anti-elastase activity, with only 32% inhibition observed at 0.25 mg/mL. These findings highlight pronounced interspecific variability and confirm the concentration-dependent anti-elastase potential of fucoidan extracts. The anti-tyrosinase activity varied among the tested species. At a concentration of 0.5 mg/mL, *Sargassum ilicifolium* exhibited the highest inhibition (63%), followed by *Padina pavonica* and *Turbinaria decurrens*, with inhibition rates of 56% and 44%, respectively. At the lower concentration of 0.25 mg/mL, all species showed substantially reduced inhibition, with values below 30%.

4. Discussion

This study provides novel insights into the structural composition and bioactive potential of polysaccharides extracted from four brown macroalgae (*Padina pavonica*, *Sargassum ilicifolium*, *Sargassum latifolium*, and *Turbinaria decurrens*) collected along the Djiboutian coast. The findings highlight a marked interspecific variability, reflecting both taxonomic diversity and the influence of local environmental conditions on polysaccharide biosynthesis. This heterogeneity is evident not only in extraction yields but also in structural features (M/G ratios of alginates, sulfation degree of fucoidans), which in turn translate into significant differences in biological activities, particularly antioxidant and anti-aging properties.

4.1. Polysaccharide Yields

The yields of alginates and fucoidans obtained in this study exhibited pronounced interspecific variability, highlighting the importance of taxonomic diversity in brown algal polysaccharide biosynthesis [41]. *Turbinaria decurrens* showed the highest alginate yield (29.3% DW), slightly exceeding the value previously reported by Kusumawati et al. [42] (22.7% DW). The alginate yield of *Sargassum ilicifolium* obtained in this study was consistent with previously reported values, which typically range between 14.26% and 28.2% of dry weight [43,44]. Similarly, the alginate yield of *Padina pavonica* was comparable to that reported for *Padina gymnospora* from Ghana [45]. Overall, the alginate yields obtained in this study fall within the ranges reported for other industrially exploited alginophytes, such as *Macrocystis pyrifera* (1–21% DW), *Ascophyllum nodosum* (15–20% DW), and *Saccharina japonica* (20–26% DW) [46], confirming the industrial relevance of the studied species. Regarding fucoidans, *P. pavonica* exhibited the highest content (1.81% DW), which is nearly sevenfold higher than the value reported by Usoltseva et al. [47] (0.26% DW) and comparable to that described by Fauziee et al. [48] (1.8% DW). These results are consistent with previous reports indicating that brown seaweeds accumulate substantial amounts of fucoidans, reaching up to 30% DW in *Fucus vesiculosus* and 25% DW in *Ascophyllum nodosum* [49,50]. Marked interspecific variations in fucoidan content have been documented within the genera *Padina* and *Sargassum*, with reported values ranging from 1% to 20% DW depending on species and environmental conditions [28,51]. This pronounced heterogeneity likely reflects the combined influence of phylogenetic traits and ecophysiological factors on sulfated polysaccharide biosynthesis, reinforcing the relevance of brown algae as valuable and sustainable sources of bioactive compounds for food, pharmaceutical, and cosmetic applications.

4.2. Structural Properties of Polysaccharides (FT-IR and ^{1}H-NMR)

FT-IR spectroscopy provides valuable qualitative and semi-quantitative information on alginate composition and gelling-related properties through the relative abundance of mannuronic (M) and guluronic (G) acid units, while ^{1}H-NMR remains the most reliable technique for accurate M/G ratio determination [52–54] (See Figure 2). In the present study, FT-IR spectra confirmed the uronic acid nature and M/G superior to 1.0 of alginates extracted from Djiboutian brown algae and revealed clear interspecific variability in M/G ratios, which was generally consistent with trends obtained by ^{1}H-NMR analysis Table 2). According to the FT-IR-based semi-quantitative estimation, *Padina pavonica* exhibited an M/G ratio of 1.54, suggesting a predominance of mannuronic acid units. Mannuronate-rich alginates are known to confer greater chain flexibility and elasticity, properties that are particularly desirable in food, cosmetic, and pharmaceutical formulations [55,56]. Rashedy et al. [37] reported even higher M/G ratios for *P. pavonica*, reaching up to 5.12, highlighting the strong influence of geographical origin and environmental conditions on alginate composition [39]. For *Turbinaria decurrens*, the M/G ratio differed slightly between FT-IR (1.63) and ^{1}H-NMR (1.03) but remains superior to 1.0. The ^{1}H-NMR value close to unity indicates a relatively balanced distribution of mannuronic and guluronic acid units, rather than a clear predominance of either block type (Figure 5). Such a balanced M/G composition is known to confer intermediate gelling properties, combining both flexibility and gel strength, depending on ionic conditions [57,58]. *Sargassum* species showed intermediate M/G ratios, with *S. ilicifolium* presenting values of 1.14 (^{1}H-NMR) and 1.62 (FT-IR), and *S. latifolium* exhibiting the highest M/G ratios among the analyzed species (1.98 by ^{1}H-NMR and 2.07 by FT-IR). These results indicate a predominance of mannuronic units, in agreement with previous studies on *Sargassum* spp. alginates [52,59]. Similar M/G ratios ranging from 1.0 to 1.2 were reported for *Sargassum* and *Turbinaria* species collected along the Djibouti coastline [60]. The FT-IR spectral features supported these compositional trends, as the absorption band attributed to mannuronic units (1025 cm^{-1}) was generally more pronounced than that corresponding to guluronic units (1080 cm^{-1}), along with the consistent presence of a diagnostic band near 872 cm^{-1} characteristic of β-D-mannuronic acid residues [37,59]. Overall, the M/G ratios obtained in this study fall within the range reported for industrially relevant alginates and remain lower than those observed for highly mannuronate-rich species such as *Himanthalia elongata* (1.5–1.8), *Bifurcaria bifurcata* (2.1–2.4), and *Saccharina latissima* (1.4–1.6) [61], further underscoring the functional versatility of Djiboutian brown algal alginates. Accordingly, the lower transmission values recorded for *S. latifolium* and *Turbinaria decurrens* at both the asymmetric S=O stretching band (1217–1220 cm^{-1}) and the C4 sulfate substitution band (840 cm^{-1}) indicate a relatively higher degree of sulfation compared to *Padina pavonica* and *Sargassum ilicifolium*. These interspecific variations in sulfation patterns reflect the inherent structural heterogeneity of fucoidans and may partly account for the differences observed in their antioxidant and enzyme inhibitory activities [62,63]. These spectral features confirm interspecific differences in sulfation degree, a structural parameter strongly linked to the biological properties of fucoidans. Indeed, previous studies have highlighted that both the degree and position of sulfation critically influence antioxidant potential and enzyme inhibitory effects [61]. Although FTIR and ^{1}H-NMR provided reliable comparative structural information, additional analytical approaches such as HPLC, GPC and an acid hydrolysis of the polysaccharide would be valuable in future studies to further elucidate monosaccharide composition, sulfation patterns, and molecular weight distribution of the extracted polysaccharides.

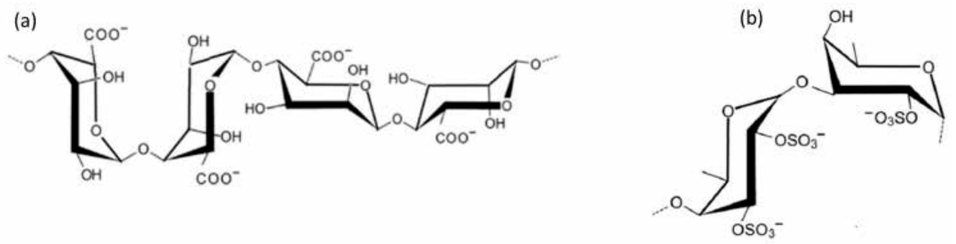

Figure 5. Chemical structure of polysaccharides, (**a**) units of alginic acid, (**b**) units of fucoidan.

4.3. Antioxidant Properties of Brown Algal Polysaccharides

The polysaccharide extracts obtained from the investigated brown algae exhibited distinct antioxidant profiles depending on their chemical nature, with fucoidans showing markedly higher activities than alginates, as demonstrated by both DPPH radical scavenging and FRAP assays (Figure 2). Among alginates, extracts from *Sargassum latifolium* and *S. ilicifolium* displayed slightly higher radical scavenging capacities, in agreement with previous reports highlighting the antioxidant potential of sodium alginates from *Sargassum* species [64,65]. Several studies have highlighted the notable antioxidant potential of alginates extracted from different macroalgae species within the Phaeophyceae class. For instance, alginate obtained from the brown Fucales *Ericaria crinita* exhibited significant antioxidant activity, with a DPPH assay IC50 value of 505 μg/mL, demonstrating its free radical scavenging capacity [66]. Similarly, alginate polymers extracted from *Sargassum polycystum* cultivated in Vietnam using various extraction methods showed high antioxidant activity [67]. In contrast, fucoidan extracts exhibited significantly stronger antioxidant activities, particularly those derived from *S. ilicifolium*, *Padina pavonica*, and *Turbinaria decurrens*. These results are consistent with earlier studies reporting pronounced antioxidant properties of fucoidans isolated from *Sargassum* and *Turbinaria* [15,68]. Notably, fucoidans from *T. decurrens* showed one of the highest antioxidant responses, corroborating the findings of Manikandan et al. [69], who described fucoidans from this species as potent radical scavengers capable of acting as primary antioxidants. Comparable antioxidant activities have also been documented for fucoidans from *Saccharina japonica* and *Undaria pinnatifida* [70–72], highlighting the broad antioxidant potential of sulfated fucans across diverse brown algal taxa. The superior antioxidant efficacy of fucoidans is closely associated with their highly heterogeneous structural features, which are influenced by species, developmental stage, environmental conditions, seasonal variation, and extraction procedures [73]. Among these structural parameters, the degree of sulfation is recognized as a key determinant of biological activity. In the present study, FT-IR analysis indicated a relatively higher sulfation level in fucoidans from *T. decurrens*, as evidenced by lower transmission values at the characteristic sulfate-related bands (1217–1220 cm^{-1} and ~840 cm^{-1}). This higher sulfate content likely enhances the electron-donating capacity and free radical scavenging efficiency of fucoidans, thereby contributing to their superior antioxidant performance compared to non-sulfated polysaccharides such as alginates [74,75]. Overall, these structure–activity relationships provide a mechanistic basis for the observed interspecific variability in antioxidant activities among the studied brown algae.

4.4. Enzyme Inhibitory Activities and Cosmetic Potential of Fucoidans

In the present study, enzyme inhibition assays were primarily focused on fucoidan extracts, as these fractions exhibited markedly stronger antioxidant activities than alginates. Fucoidans isolated from *Sargassum ilicifolium*, *Turbinaria decurrens*, and *Padina pavonica* showed significant inhibitory effects against both elastase and tyrosinase, highlighting their multifunctional bioactivity and strong relevance for cosmetic applications [73,76,77]. Inhibition rates ranging from approximately 45% to 65% at 0.5 mg/mL are comparable to those reported for fucoidans extracted from other brown algae species, such as in several genera: *Sargassum*, *Fucus*, and *Laminaria*, which are well recognized for their antioxidant and anti-aging-related enzyme inhibitory activities [78–80]. These similarities support the biological relevance of the activities observed in the present study and confirm that Djiboutian brown algae constitute a competitive source of bioactive marine polysaccharides [81,82]. The bioactivity of fucoidans is closely linked to their structural features, particularly the degree and pattern of sulfation. *Turbinaria decurrens*, which combines a relatively high sulfation level with pronounced antioxidant capacity, exhibited one of the strongest inhibitory effects against both elastase and tyrosinase, suggesting a synergistic relationship between radical scavenging ability and direct enzyme interaction [63,83]. Sulfate groups are known to enhance electrostatic interactions with enzyme active sites, notably through metal ion complexation (e.g., copper ions in the case of tyrosinase) and through stabilization of enzyme–inhibitor complexes [84]. Although *Padina pavonica* and *Sargassum ilicifolium* displayed comparatively lower overall sulfation levels, their fucoidan fractions still showed significant inhibition of both enzymes. This indicates that biological activity is not solely governed by the total degree of sulfation but also depends on the spatial distribution of sulfate groups, polymer conformation, and polysaccharide chain length [85,86]. This observation is consistent with previous studies reporting that moderately sulfated fucoidans can retain strong bioactivity when their structural organization is favorable. Overall, these results confirm that fucoidans from *P. pavonica*, *S. ilicifolium*, and particularly *T. decurrens* represent promising natural enzyme inhibitors, whose efficacy is closely related to both the degree and pattern of sulfation. The combined antioxidant and enzyme inhibitory activities observed in these fucoidans further enhance their relevance for anti-aging cosmetic applications, particularly in the prevention of skin elasticity loss and pigmentation disorders. Although FT-IR spectroscopy allows the identification of sulfate groups in fucoidans, it offers only semi-quantitative information on sulfation, highlighting the need for complementary quantitative approaches in future studies to strengthen structure–activity relationships.

Although alginates were not evaluated for enzyme inhibitory activities in this study, they exhibited moderate antioxidant properties, supporting their potential use as mild antioxidant materials in food, pharmaceutical, and cosmetic applications [21]. Nevertheless, their well-established gelling, thickening, and stabilizing properties make alginates particularly valuable as structuring agents in cosmetic formulations, such as creams and emulsions, where they can enhance texture, viscosity, and product stability while acting as biocompatible carriers for active ingredients [19,87,88].

5. Conclusions

This study provides a comprehensive evaluation of bioactive polysaccharides extracted from brown macroalgae collected along the Djiboutian coastline, highlighting their significant potential for cosmetic and biotechnological applications. Both alginates and fucoidans exhibited distinct structural and biological properties, with fucoidans consistently demonstrating superior antioxidant and enzyme inhibitory activities compared to alginates. The different spectra FT-IR and ^{1}H-NMR acquired in our study revealed marked interspecific variability in the M/G ratio of alginates and in the sulfation degree of fucoidans, which was closely associated with their functional performance. Fucoidans from *Padina pavonica*, *Sargassum ilicifolium*, and particularly *Turbinaria decurrens* displayed strong radical scavenging capacity, high reducing power, and significant inhibition of key skin-aging enzymes, namely elastase and tyrosinase. The results obtained in this study highlight the significant value of Djibouti's brown macroalgae as a promising source of bioactive polysaccharides for cosmetic applications. Polysaccharides extracted from these abundant marine resources exhibited notable antioxidant and enzyme inhibitory activities, supporting their potential use as natural cosmetic ingredients. Alginates demonstrated suitability as gelling and thickening agents for the formulation of cosmetic creams or milks, while fucoidans showed strong potential as marine-derived anti-aging active compounds. Collectively, these findings suggest that Djiboutian brown algae represent an underexploited and sustainable resource that could contribute to the development of innovative cosmetic products within the framework of blue economy valorization. This work not only contributes to a better understanding of the structure–activity relationships of brown algal polysaccharides but also opens new perspectives for the valorization of underexplored marine biodiversity in high-value cosmetic formulations. Future studies focusing on detailed molecular characterization, formulation stability, and in vitro or in vivo skin models would further support their industrial and commercial application. They will focus on cytocompatibility in human keratinocytes (HaCaTs) and the exploration of associated anti-ageing mechanisms to reinforce the cosmetic relevance and safety assessment of these compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics13020053/s1, Figure S1: Morphological representation of the brown macroalgae species studied. (a) *Padina pavonica*, photographed in its natural underwater habitat near Moucha Island; (b) *Sargassum ilicifolium*, harvested in Douda and processed in the laboratory; (c) *Turbinaria decurrens*, collected around Moucha Island and processed in the laboratory; (d) *Sargassum latifolium*, harvested in Héron and processed in the laboratory. Figure S2: FTIR spectrum of alginate isolated from Brown seaweed Djiboutian (*P. pavonica*; *T. decurrens*; *S. ilicifolium* and *S. latifolium).* Figure S3: FTIR spectrum of fucoidan isolated from Brown seaweed Djiboutian (*P. pavonica*; *T. decurrens*; *S. ilicifolium* and *S. latifolium).*

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