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Determining the Chain Conformation, Microstructure, and Antioxidant Activity of Fucoidan from *Dictyota dichotoma* Growing in the Sea of Cortez, Mexico

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Abstract

Brown algae, such as *Dictyota dichotoma*, contain fucoidan, whose bioactivity is linked to its molecular characteristics, which are in turn defined by environmental conditions. *D. dichotoma* fucoidan from the Sea of Cortez in Mexico (DDFSC) has not been previously investigated. This study aimed to extract DDFSC and investigate its chain conformation, microstructure, and antioxidant activity. The extraction yield was 3.6% (w DDFSC/w lyophilized seaweed). Fourier-transform infrared spectroscopy recorded bands characteristic of fucoidan. Fucose represented the main monosaccharide in DDFSC (49.52% *w*/*w*). The sulfate content was 3.40% (*w*/*w*). Size-exclusion chromatography with multi-angle light scattering determined the molecular weight, intrinsic viscosity, radius of gyration, and hydrodynamic radius as 880 kDa, 260 mL/g, 34 nm, and 32 nm, respectively. The characteristic ratio (C∞) and the persistence length (q) were 4.5 and 1.5 nm, respectively, suggesting a branched random coil conformation. Dynamic light scattering determined polysaccharide diameters of 27.6 and 173.1 nm. Scanning electron microscopy and atomic force microscopy revealed an irregular morphology and aggregates with rough topography, respectively. The IC_{50} values for $ABTS^+$ and DPPH radical scavenging assays were 4.21 and 5.64, respectively. The present study constitutes the first insight into DDFSC characterization and establishes a starting point for the development of sustainable future applications using this polysaccharide.

Keywords: brown algae; sulfated polysaccharides; chain flexibility; morphology; bioactivity

Academic Editors: Adrián Fuente-Ballesteros and Marcello Locatelli

Received: 25 May 2026
Revised: 10 August 2026
Accepted: 27 August 2026
Published: 2 September 2026

1. Introduction

As photosynthetic organisms, seaweeds play a pivotal role in regulating the marine ecosystem by producing oxygen and absorbing carbon dioxide. They are classified as

green, red, and brown seaweeds [1,2]. These organisms generate metabolites with diverse bioactive properties, which give them excellent potential for application in fields such as biomedicine, pharmaceuticals, food, and cosmetics [3,4].

The brown alga *Dictyota dichotoma* belongs to the Dictyotaceae family and can be found on various rocky substrates. *D. dichotoma* has excellent research potential due to its bioactive components [4]. This alga is abundant in the Sea of Cortez, a section of the Pacific Ocean in northwestern Mexico. However, until now, it has received little attention, even though it could become a model organism of great importance for studies in the public health and food industry sectors.

Fucoidans, fucose-rich polysaccharides with sulfate groups in their structure, are bioactive compounds in brown algae. These sulfated polysaccharides show a wide variety of biological activities, such as antimicrobial, anti-inflammatory, and antioxidant effects. These polysaccharides are accepted by the Food and Drug Administration (FDA) and are considered Generally Recognized As Safe (GRAS) [5].

Fucoidans from brown macroalgae consist of a sulfated L-fucopyranoside backbone connected by α-(1→3) and α-(1→4) linkages. Other neutral sugars, including galactose, glucose, mannose, and xylose, can also be found in fucoidans. Sulfate residues are present at the C-2 and C-4 positions and seldom at the C-3 position of fucose [5,6] (Figure 1).

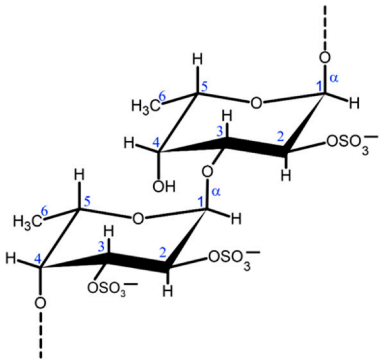


Figure 1. General representation of the chemical structure of fucoidan from brown algae.

The characterization of DDFSC in this work contributes to expanding the knowledge of the diversity of these polysaccharides and their possible relationships with bioactivities relevant to biomedical and biotechnological applications, as limited information is available on fucoidan from other marine regions. Several studies have shown that the structural characteristics and biological activities of sulfated polysaccharides from algae depend on factors such as seasonal variation, geographic location, seawater quality, and harvesting and extraction procedures. Furthermore, the bioactivity of these macromolecules is linked to various structural characteristics, like sulfate content and molecular distribution, as well as molecular size [1–4].

Previous reports on the characteristics and bioactivity of fucoidans from *D. dichotoma* are limited to algae collected from the Sea of Japan, near Russia [7]; Bahia Bustamante, Argentina [8]; and the Red Sea, Egypt [9]. To our knowledge, the present study is the first to report the extraction and characterization of fucoidan from *D. dichotoma* growing under the unique environmental conditions of the Sea of Cortez, Mexico.

The Sea of Cortez, also known as the Gulf of California or, less commonly, the Vermilion Sea, is considered a relevant area of global marine conservation. For this reason, the Sea of Cortez has been recognized as a UNESCO World Heritage Site since 2005 [10]. In this respect, the Sea of Cortez presents exceptional biodiversity and ecological significance and constitutes a vital area for research and conservation efforts. Given its unique environmental conditions, characterizing the fucoidans obtained from this specific marine ecosystem

is of great interest, as these factors likely contribute to variations in the properties of the polysaccharides.

Therefore, the present study provides critical insights and cutting-edge knowledge on DDFSC, comprehensively characterizing the chain conformation, microstructure, and antioxidant activity of this polysaccharide.

2. Materials and Methods

Dictyota dichotoma (Figure 2a) was collected at coordinates 26°35′57″ N, 109°21′30″ W in the Sea of Cortez (Figure 2b), Northwest Mexico. The taxonomic identification was verified by Dr. Anselmo Miranda-Baeza, a marine biologist from the Laboratory of Cultivation Technologies of Marine Aquatics, State University of Sonora. Seawater was analyzed considering temperature and dissolved oxygen concentration (YSI model 55 digital oximeter, Yellow Springs, OH, USA); pH (pH-10 digital potentiometer, Denver, CO, USA); salinity (ATAGO Refractometer, ATAGO Co., Bellevue, WA, USA); total suspended solids, NH_3, NO_3, and NO_2 (Hach DR 2800 spectrophotometer, HACH Co., Loveland, CO, USA); and chlorophyll a (AquaFluor® Handheld Fluorometer, San Jose, CA, USA). The American Public Health Association standard methods were used [11]. All chemicals used in this study were analytical-grade reagents (Sigma-Aldrich Chemical Co., St. Louis, MO, USA).

(**a**) (**b**)

Figure 2. (**a**) *D. dichotoma* on coarse sand substrate, (**b**) collection site in the Sea of Cortez, Mexico.

2.1. Fucoidan Extraction

Dictyota dichotoma samples were washed manually with seawater, followed by tap water, and then transported to the laboratory on ice. The seaweed was frozen at −20 °C and lyophilized (−84 °C/0.008 mbar) in a Freezone 6 freeze dryer (Labconco, Kansas City, MO, USA). The dried seaweed was ground using a mortar and pestle (<0.5 mm) and then stored (−20 °C).

Fucoidan extraction was carried out as previously described, with minor modifications [12]. In brief, 20 g of milled lyophilized algae was mixed with 1 L of 85% (*v*/*v*) ethanol (12 h/25 °C) to eliminate residual pigments and proteins and then centrifuged (9000 rpm/20 min/25 °C). The precipitate was treated with acetone, centrifuged (9000 rpm/20 min/25 °C), dried at room temperature, and resuspended twice in distilled water (100 mL, 95 °C/1 h).

The mixed extract was centrifuged (9000 rpm/20 min/25 °C), and the supernatant was mixed with 1% (*w*/*v*) $CaCl_2$ and stored (4 °C/12 h) to precipitate alginic acid. The mixture was centrifuged (9000 rpm/20 min/25 °C), and absolute ethanol was added to the supernatant until a concentration of 30% (*v*/*v*) was reached and stored (4 °C/4 h). The extract was centrifuged (9000 rpm/20 min/25 °C), and absolute ethanol was added to the supernatant until a concentration of 70% (*v*/*v*) was reached; it was then kept at 4 °C/12 h. The precipitate was desiccated by a solvent exchange procedure using 80% and 90% (*v*/*v*)

ethanol, followed by absolute ethanol, and finally, acetone. The powder obtained was *D. dichotoma* fucoidan from the Sea of Cortez (DDFSC).

DDFSC was obtained through multiple extraction cycles from a single batch of marine algal biomass (16 kg fresh weight), collected under controlled, homogeneous conditions of harvesting, transport, storage, and processing. The extracts from each cycle were combined to form a representative composite sample (bulk sample), considered as a single average batch. This composite sample was used for all laboratory experiments performed in this study.

2.2. Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR spectrum of DDFSC was recorded on a Nicolet iS50 FTIR spectrometer (Nicolet Instrument Corp., Madison, WI, USA) using an iS50 ATR analysis in the 4000 to 400 cm^{-1} range [1].

2.3. Neutral Sugars by Gas Chromatography

The DDFSC neutral sugar composition was determined following a methodology previously described [13]. In brief, DDFSC was hydrolyzed with 4 M trifluoroacetic acid at 120 °C for 4 h and then derivatized into alditol acetates. The resulting derivatives were analyzed by gas chromatography (Agilent HP 6890 GC Series, Santa Clara, CA, USA).

A high-performance capillary column of 30 m × 0.32 mm i.d. and 0.15 µm film thickness (Elite 225, PerkinElmer Inc., Waltham, MA, USA) was used. Before injection, samples were filtered (0.45 µm, Millipore, Burlington, MA, USA).

2.4. Sulfate Content

Sulfate quantification in DDFSC was performed using a turbidimetric method based on the barium chloride–gelatin reagent with a UV-Vis spectrophotometer (Cary 60, Agilent Technologies, Penang, Malaysia), according to a previous report [14].

2.5. Protein Determination

The protein quantification in DDFSC was carried out following the Bradford method [15].

2.6. Size-Exclusion Chromatography (SEC) with Multi-Angle Light Scattering (MALS)

The values of weight-average molar mass (Mw), number-average molar mass (Mn), intrinsic viscosity ([η]), radius of gyration (RG), hydrodynamic radius (Rh), and polydispersity index (I = Mw/Mn) of DDFSC were determined from SEC-MALS analysis (DAWN HELOS-II 8 MALS detector, ViscoStar-II Viscometer, and Optilab T-REx refractive index; Wyatt Technology Corp., Santa Barbara, CA, USA).

DDFSC was dispersed at 5 mg/mL using 50 mM $NaNO_3$/0.02% (*w*/*v*) NaN_3 as a solvent (80 °C for one hour). This dispersion was centrifuged (15,000 rpm for 10 min at 20 °C), and the supernatant was filtered (0.45 µm, Millipore, Burlington, MA, USA).

The sample was analyzed using 50 mM $NaNO_3$/0.02% (*w*/*v*) NaN_3 as the mobile phase at a flow rate of 0.5 mL/min (HPLC system, G1310B Iso-Pump, G1329B autosampler, G1314F Variable Wavelength Detector, Agilent Technologies, Inc., Santa Clara, CA, USA). The columns and software used were Shodex OH-pak SBH-Q-804 and 805 (Shodex Showa Denco K.K., Tokyo, Japan) and ASTRA 6.1, respectively.

The specific refractive index increment (dn/dc) value used was 0.146 mL/g. The characteristic ratio (C∞) and persistence length (q) of DDFSC were calculated according to a previous report [16].

2.7. Dynamic Light Scattering Analysis (DLS)

The DDFSC particle size was measured at 25 °C using a Möbiuζ DLS instrument and the DYNAMICS 7.3.1.15 software (Wyatt Technology Corp., Santa Barbara, CA, USA) as previously described [16]. DDFSC was dispersed at a concentration of 0.1% (*w*/*v*) using distilled water as a solvent.

2.8. Scanning Electron Microscopy (SEM)

The SEM analysis of DDFSC was performed using a scanning electron microscope (JSM 5410LV, JEOL, Peabody, MA, USA) operated at 10 kV. Imaging was conducted at a magnification of 1500×, employing secondary and backscattered electron detection modes [17].

2.9. Atomic Force Microscopy (AFM)

DDFSC was dispersed at 0.5 mg/mL in ultrapure water (stirring 24 h/25 °C) and then sonicated (15 min/25 °C) (1510R-MT sonicator, Branson Ultrasonic Corporation, Danbury, CT, USA). Immediately after, a drop of dispersion was deposited on a glass mica and dried in Petri dishes (24 h/25 °C) [18].

An AFM instrument (model Alpha300RA, WITec, Ulm, Germany) with a 20× objective lens in combination with an 8 nm tipped measuring needle was used. The probe presented a spring constant and a resonant frequency value of 42 N/m and 285 kHz, respectively. The analysis was performed on randomly selected areas of 25 × 25 µm. The images were then magnified by reconstruction of 5 × 5 µm areas, and their profiles were investigated using the WITec project FIVE v5.1 software in both 2D and 3D modes.

2.10. Antioxidant Activity

The DDFSC antioxidant activity was investigated using the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the 1,1-diphenyl-2-picryl-hidrazyl (DPPH) methods. For the ABTS method, a 7 mM $ABTS^+$ solution was diluted with ethanol–water (50/50 *v*/*v*) to reach an absorbance of 0.7 at 734 nm. The DDFSC (5 mg) was added to 3 mL of the diluted $ABTS^+$ reagent and incubated (2 min), and the absorbance at 734 nm was recorded after 14 and 21 min [18,19]. In the case of the DPPH method, 400 µL of DDFSC dispersion (1 mg/mL), 350 µL of methanol, and 750 µL of DPPH solution (91.3 µM) were mixed and incubated (35 min), and the absorbance was recorded at 515 nm after 40 and 60 min [20]. In both cases, the DDFSC antioxidant activity was reported as a percentage of radical inhibition.

3. Results

First, 16 kg (fresh weight) of *D. dichotoma* was collected manually from the supralittoral zone to the intertidal zone (2 m depth). Seawater conditions during the algae collection are shown in Table 1.

Table 1. Conditions in the Sea of Cortez water during brown algae (*D. dichotoma)* collection.

Parameter	Value
Salinity (ppt)	38.00
Total suspended solids (mg/L)	12.00
Chlorophyll **a** (µg/L)	7.97
pH	8.33
Dissolved oxygen (mg/L)	7.04
NO_2 (mg/L)	0.04
NO_3 (mg/L)	0.00
Temperature (°C)	22.2

Results are reported as mean of three replicates.

The extraction yield was 3.6% (*w*/*w*, DDFSC/lyophilized seaweed). DDFSC appeared as a fine, sandy-colored powder (Figure 3).

Figure 3. *D. dichotoma* fucoidan (DDFSC).

The FTIR spectrum of DDFSC (Figure 4) exhibited characteristic IR absorption bands at 3400 and 2920 cm^{-1}, related to O–H and C–H stretching, respectively. In the range 1750–500 cm^{-1}, the spectrum recorded representative bands for fucoidan associated with COO–, S=O, C–O–C, C–O–S, C–H, and deoxy sugars [21–25].

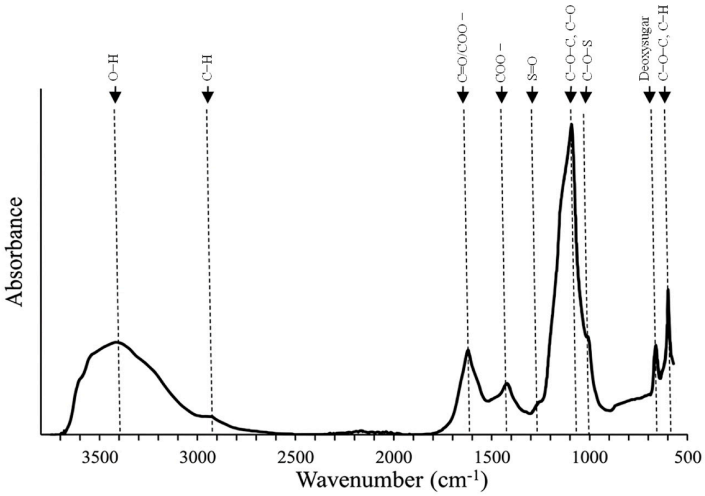


Figure 4. Fourier-transform infrared (FTIR) spectrum of DDFSC. The assignment of the main bands is indicated.

The neutral sugar composition of DDFSC revealed a heteropolysaccharide structure with fucose as the main component (49.52%) (Table 2). Other monosaccharides, including glucose, galactose, rhamnose, xylose, and mannose, were also identified, consistent with previous reports on the composition of this polysaccharide [6–8]. The sulfate and residual protein contents in the sample were 3.40 ± 0.01% and 0.57 ± 0.01%, respectively, which fell within the ranges reported for other brown algal fucoidans [12,21].

Table 2. Neutral sugar composition in *Dictyota dichotoma* fucoidan from the Sea of Cortez in Mexico (DDFSC).

Monosaccharides	Content (%)
Fucose	49.52 ± 1.35
Glucose	20.48 ± 0.34
Galactose	9.54 ± 0.57
Rhamnose	9.15 ± 0.18
Xylose	6.10 ± 0.51
Mannose	4.16 ± 0.20

Results are reported as mean ± standard deviation of three replicates.

The elution profile of DDFSC detected by differential refractive index (RI), light scattering (LS), and ultraviolet (UV) detection is shown in Figure 5a. The RI values reflect the polymer concentration at each data point. The LS detector measures the intensity of scattered light from the sample as it elutes from the SEC column. For DDFSC, the LS signal shows a shoulder on the left side before the central RI peak, suggesting polysaccharide macro-aggregates. A weak UV signal was also detected, possibly attributable to residual protein content in the sample. In Figure 5b, the chromatogram shows the RI, LS, and UV detector responses from 25.2 up to 27 min of elution time, where the main LS peak appears close to the central RI peak. It can be observed that the RI and LS values do not completely overlap, as the LS peak appears at 26.25 min, while the RI peak appears at 26.75 min. This small offset is related to the molecular weight dependence of light scattering; molecules eluting earlier are larger than those eluting later and thus scatter more light. Therefore, molecules eluting early, with low intensity in the RI trace, are low in concentration but high in molecular weight, resulting in high intensity in the LS trace [26]. The low UV signal followed a similar elution pattern, suggesting the presence of small amounts of free proteins or carbohydrate–protein complexes. The dotted line on the chromatogram shows the MM value of the molecule at each data point, representing the weight-average molar mass of the macromolecules eluting at that point. The MM value for the data point at an elution time of 25.3 min is 1000 kDa, and it decreases steadily with increasing elution time, reaching 680 kDa at 27 min. Using all the data points, the instrument generated the Mn and Mw values for the sample, which are reported in Table 3.

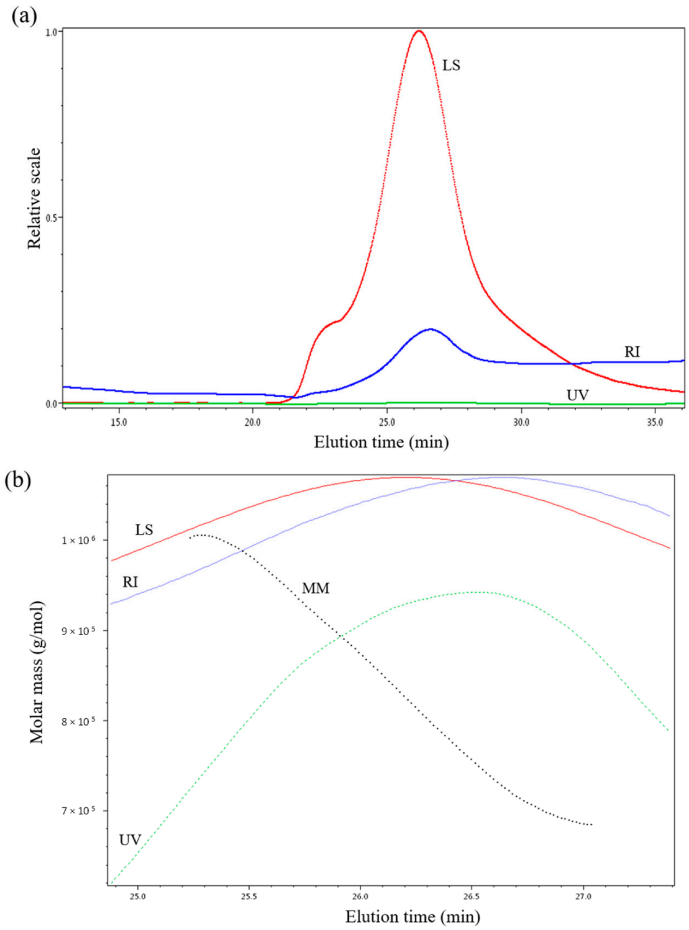


Figure 5. (**a**) Representative chromatogram of DDFSC using size-exclusion chromatography with multi-angle light scattering (SEC-MALS). (**b**) RI, LS and UV detector responses in the time range where the main LS peak corresponds to the central RI peak showing MM values. Light scattering (LS), ultraviolet (UV), differential refractive index (RI), and molar mass (MM) are indicated.

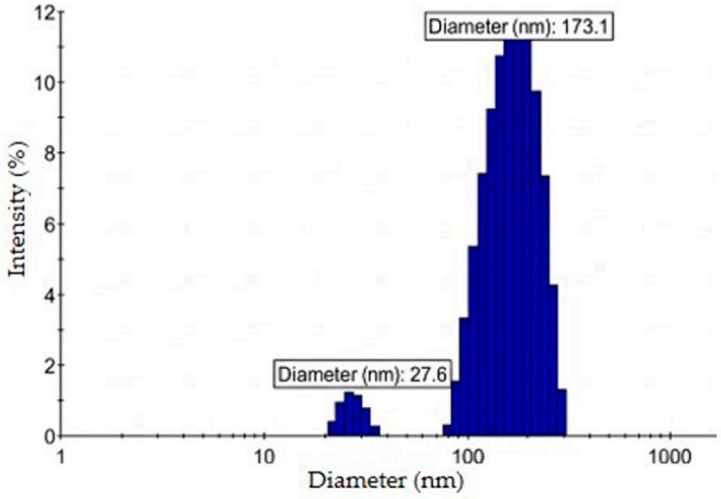
Table 3. DDFSC macromolecular characteristics determined by SEC-MALS.

Macromolecular Parameter	Value
*M*w (kDa)	880 ± 93
PI (*M*w/Mn)	1.01 ± 0.01
[η] (mL/g)	260 ± 18
RG (nm)	34 ± 3
Rh (nm)	32 ± 2
RG/Rh	1.04 ± 0.01
C∞	4.5 ± 0.40
q (nm)	1.50 ± 0.12

*M*w: weight-average molar mass; Mn: number-average molar mass; PI: polydispersity index; [η]: intrinsic viscosity; RG: radius of gyration; Rh: hydrodynamic radius; RG/Rh: radius of gyration/hydrodynamic radius; C∞: characteristic ratio; q: persistence length. Results are reported as mean ± standard deviation of three replicates.

The macromolecular characteristics of DDFSC are presented in Table 3. The weight-average molar mass (Mw), polydispersity index (PI), intrinsic viscosity ([η]), radius of gyration (RG), and hydrodynamic radius (Rh) recorded for DDFSC were in the ranges reported for fucoidans from other brown algae (Mw: 20–4354 kDa, PI: 1.3–3.9, [η]: 361–866 mL/g, RG: 23.7–111.4 nm, and Rh: 17.7–37.4 nm) [10,27–31].

Dynamic light scattering (DLS) measured the Brownian motion of DDFSC, allowing the determination of the polysaccharide diameter and size distribution. In Figure 6, the DDFSC populations and diameters are presented, showing that this sample is composed of two populations. The DLS analysis generates intensity-weighted results, where the intensity percentage depends on the contribution of each polysaccharide population detected.



Peak	Diameter (nm)	Pd index	Intensity (%)	Mass (%)	Number (%)
1	27.6	0.1	4.7	14.3	85
2	173.1	0.3	95.3	85.7	15

Figure 6. DLS size distribution of DDFSC and details of the individual populations and parameters.

The SEM analysis of DDFSC (Figure 7) revealed a microstructure characterized by an irregular distribution of particles. Similar microstructural features have been reported for several sulfated polysaccharides, including fucoidans.

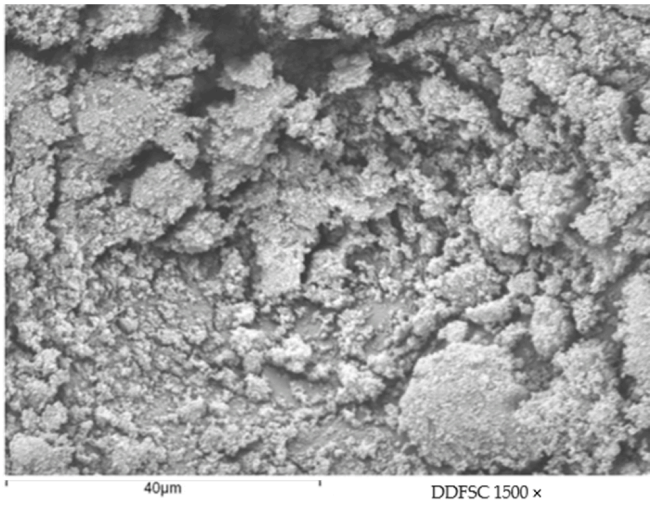
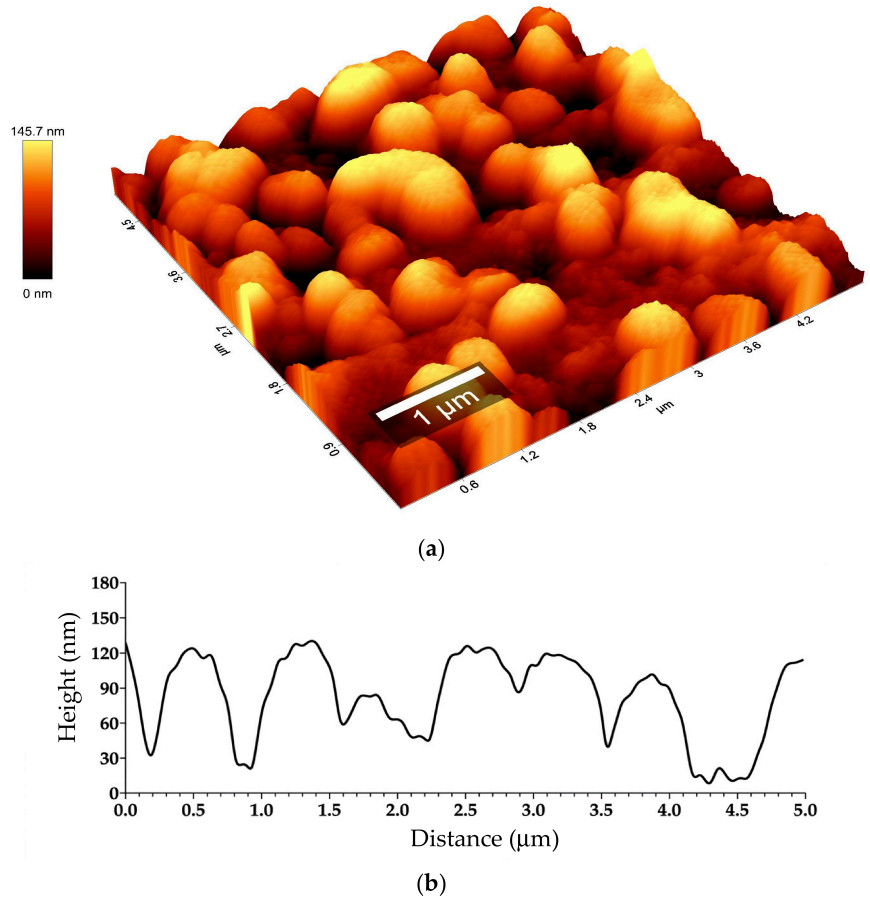


Figure 7. Scanning electron microscopy analysis of DDFSC (1500×).

The AFM analysis of DDFSC revealed a rough, irregular surface morphology characterized by nanometer- and micrometer-scale structures (Figure 8a). Topographical analysis indicated a height variation from 0 to approximately 125 nm (Figure 8b), suggesting significant roughness on the surface.



(**a**)

(**b**)

Figure 8. Atomic force microscopy analysis of DDFSC. 3D surface image (**a**) and height profile line (**b**). Area of 5 × 5 µm.

The antioxidant activity of DDFSC, as well as the IC_{50} values determined by the ABTS and DPPH assays, are presented in Table 4. Under the experimental conditions used, DDFSC showed noticeable antioxidant potential, which increased with increasing

concentration. The sample exhibited IC_{50} values of 5.64 and 4.21 mg/mL for the DPPH and ABTS methods, respectively. The IC_{50} value for DDFSC using the DPPH method was higher than that reported for fucoidan from Dictyota dichotoma collected from the Red Sea, Egypt (IC50 of 4.59 mg/mL) [9]. Nevertheless, using the ABTS method, DDFSC presented a lower IC50 compared to that reported in that study (8.55 mg/mL). Additionally, DDFSC and ascorbic acid at 5.35 mg/mL presented a radical inhibition capability of 94.68% and 47.68%, respectively, using the DPPH method and 98.48% and 65.40% using the ABTS method, respectively (Figure 9a,b).

Table 4. Antioxidant activity of DDFSC.

DDFSC Concentrations (mg/mL)	Inhibition (%)	Antioxidant Activity (mmol TEAC/kg)	IC_{50} (mg/mL)
DPPH radical scavenging activity			
0.67	25.32 ± 0.67	18.82 ± 0.91	5.64 ± 0.10
1.33	31.30 ± 0.66	26.98 ± 0.90	
2.67	42.53 ± 0.51	42.33 ± 0.69	
5.35	47.68 ± 0.46	49.35 ± 0.62	
10.7	71.21 ± 0.26	81.49 ± 0.35	
ABTS radical scavenging activity			
0.67	26.73 ± 0.77	26.44 ± 1.02	4.21 ± 0.19
1.33	35.06 ± 2.82	37.46 ± 3.73	
2.67	45.79 ± 2.59	51.52 ± 3.42	
5.35	65.40 ± 1.27	77.56 ± 1.68	
10.7	75.57 ± 0.79	91.02 ± 1.04	

Results are reported as mean ± standard deviation of three replicates.

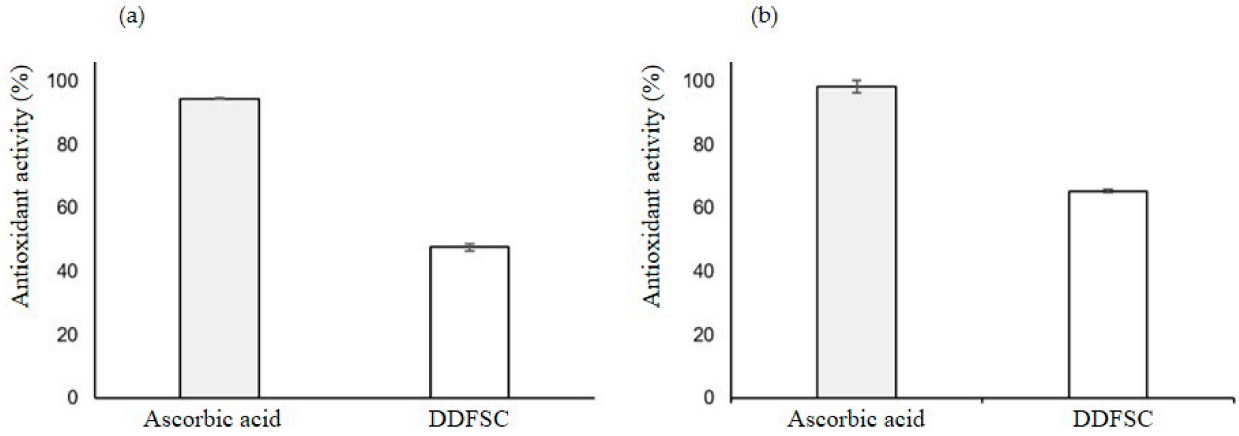


Figure 9. Antioxidant activity of DDFSC compared to ascorbic acid using the DPPH (**a**) and ABTS (**b**) methods.

4. Discussion

The DDFSC yield obtained in this study (3.6% *w*/*w*) was markedly higher than those reported for this polysaccharide recovered from the same alga in other marine regions, such as the Red Sea, Egypt (0.057% *w*/*w*) [9]; the Sea of Japan, Russia (0.4% *w*/*w*) [7]; and Bahia Bustamante, Argentina (2.2% *w*/*w*) [8]. These variations in extraction yield could be attributed to a combination of biological, environmental, and methodological factors. According to the literature, extraction parameters such as solvent type, temperature, and extraction time strongly influence the outcome. Concurrently, seasonal and environmental conditions—including temperature, salinity, nutrient availability, oxygenation, and sunlight

intensity—play a key role in shaping the metabolic pathways of different species, thereby affecting their sulfate grade and fucose content [32,33]. Therefore, the higher yield obtained for DDFSC could be associated with the unique environment of the Sea of Cortez, which likely favored fucoidan synthesis in the collected *Dictyota dichotoma*.

Fucoidans extracted from different species of brown algae harvested in the same marine region have distinct chemical structures, even when the eco-environmental conditions are similar. This structural diversity is due to each species having specific metabolic pathways that influence physicochemical characteristics such as monosaccharide composition, molecular weight, and degree of sulfation. These differences directly govern the solubility, conformation, viscosity, and rheological behavior of the sulfated polysaccharide. Hence, brown algae collected from the same geographic area exhibit diverse biological properties and activities [34].

The extraction method used for DDFSC is particularly suitable for fucoidan, as it is performed under mild conditions, without high temperatures or harsh chemicals. Beyond being simple and controllable, the method avoids the use of dangerous chemicals and facilitates the incorporation of fucoidan into composite materials for pharmaceutical, cosmetic, and biomedical applications. The absence of hydrolytic steps during extraction also helped to preserve biological activity in fucoidan. Overall, the extraction method used primarily involves physical processes, during which the glycosidic bonds in fucoidan are cleaved, and no new chemical products are generated. For these reasons, this method offers advantages over other extraction techniques that may be more complex or potentially detrimental to the structural integrity of sulfated polysaccharides [35–37].

In the FTIR analysis, the main functional groups of DDFSC were identified. The FTIR spectrum (Figure 4) presented bands at 3400 and 2920 cm^{-1}, which were assigned to O–H and C–H stretching, respectively, as previously reported for fucoidan [21–25]. The signals in the 1624−1630 cm^{-1} region are related to the C=O structure and COO- asymmetric stretching, whereas those in the 1417−1420 cm^{-1} region are attributed to the symmetric stretching of the COOH group [21–23]. These absorption bands suggest the presence of carbonyl groups that are part of the uronic acids in the molecule. It has been reported that uronic acids are present in fucoidan, with their amount depending on specific seaweed characteristics [23]. However, a limitation of this technique is that it does not allow for the elucidation of the fine structure of this molecule, such as the exact positions of the sulfate groups or variations associated with the marine region. Consequently, the data obtained do not provide a detailed description of the molecular architecture of DDFSC. Additional techniques such as nuclear magnetic resonance (NMR) and methylation analysis, coupled with gas chromatography–mass spectrometry (GC-MS), could be used in the future to elucidate the fine structure of DDFSC. NMR provides information about the environments of atoms in the molecule analyzed based on the principle that certain nuclei resonate in a magnetic field, while methylation analysis (GC-MS) reveals the glycosidic linkage patterns of polysaccharides [36,37].

The presence of sulfate in DDFSC was detected in the FTIR spectrum in the 1220–1230 cm^{-1} region (S=O vibration) and in the 820–856 cm^{-1} region (C–O–S signal) [24]. The high signal in the 1175–1140 cm^{-1} region is attributed to the stretching vibration of the C–O–C and C–O components in polysaccharide glycosidic links. The band at 667 cm^{-1} has been associated with deoxy sugars, such as fucose. Finally, the signal at 601 cm^{-1} corresponds to C–O–C bending and C–H out-of-plane vibrations in the molecule [25].

Fucose was the primary monosaccharide component in DDFSC (Table 2), with a percentage value close to that found for fucoidan obtained from *D. dichotoma* collected in the Sea of Japan, Russia (57.9% w/w) [7] and higher than the value reported in Bahia Bustamante, Argentina (36% w/w) [8], and in the Red Sea, Egypt (21.3 ± 1.30%) [9].

The sulfate content in DDFSC (3.40 ± 0.01% *w*/*w*) was lower than the level found for fucoidan from *D. dichotoma* growing in the Red Sea, Egypt (8.33% *w*/*w*) [9], but close to that found for this alga growing in Bahia Bustamante, Argentina (5% *w*/*w*) [8]. The low protein content (0.57% *w*/*w*) indicates that DDFSC is mainly free from associations with these macromolecules.

The SEC-MALS analysis of DDFSC showed a Mw of 880 kDa, which is considerably higher than the molecular weights reported in the literature for brown algae such as *Sargassum hemiphyllum* (0.8 kDa), *Fucus vesiculosus* (20–190 kDa), *Laminaria japonica* (20–200 kDa), and *Undaria pinnatifida* (47–276 kDa) [38]. However, little information is available on fucoidan from *D. dichotoma* in other marine regions. To date, the few studies conducted on this same species indicate molecular weights of 48.6 kDa, 250–280 kDa, and 15 kDa for samples from the Red Sea, Egypt; the Sea of Japan, Russia; and Bahia Bustamante, Argentina, respectively [7–9]. In this regard, the high molecular weight of the fucoidan obtained from *D. dichotoma* in the Sea of Cortez could be influenced by the geographic origin, environmental conditions, and the extraction and characterization methods used. The SEC-MALS results show a single well-defined peak. This single elution peak is due to the homogeneous distribution of the molecular weight of DDFSC, as supported by the low polydispersity index (1.01). This absence of low-molecular-weight fractions in the SEC-MALS analysis suggests that the extraction method used in the present study allowed for the recovery of a *D. dichotoma* sulfated polysaccharide presenting a low Mw dispersity.

The Rh, RG and [η] values have not been previously reported in the literature for fucoidan from *D. dichotoma.* Rh represents the radius of a hard sphere that circulates at the same rapidity as the molecule investigated, and the value experimentally measured represents the size adopted by this molecule under solvation and moving conditions. RG is the average distance from the center of the polymer to each mass component. In the present study, the ratio of RG/Rh was 1.04, suggesting that DDFSC presents a spherical shape, which could be related to a highly branched structure as previously reported for fucoidans from other marine macroalgae. An RG/Rh value of 0.98 in fucoidan has been attributed to a densely branched chain and a spherical-like conformation [29].

Reports on the characteristic ratio (C∞) and persistence length (q) of fucoidans are scarce. Sulfated glucans have been reported to present C∞ and q values of 13.9 and 4.8 nm, respectively, indicating moderately extended flexible chains in dispersion [30]. The persistence length suggests that DDFSC molecules are moderately flexible, with directional changes along the chain that favor coiled configurations in an aqueous solution. Furthermore, the characteristic ratio of this polysaccharide indicates that it lacks a rigid conformation and instead exhibits mobility, adapting to different configurations. These results are consistent with an Rg/Rh ratio of 1.04, corresponding to a branched random coil with spherical geometry. Taken together, these findings suggest that DDFSC molecules can form coiled, compact, and highly hydrated chains in aqueous solution. Stability is maintained despite this flexibility due to electrostatic interactions associated with sulfate groups, hydrogen bonds involving the hydroxyl groups on the structure, and the high molecular weight, which allows for molecular associations between the chains, thereby contributing to the excellent stability of the sulfated polysaccharide in dispersion. The macromolecular characteristics of DDFSC suggest a branched, flexible chain conformation similar to that previously reported for fucoidans from other brown seaweeds [12,27,29]. The specific polysaccharide chain conformation mainly depends on the source and extraction method, and differences in the molecular structure can improve or reduce the biological activities of the polysaccharide [27], representing an exciting tool for diverse and advanced applications, including in the food and biomedical sectors.

For DDFSC, the presence of two well-defined polysaccharide peaks was detected via DLS analysis (Figure 6), with diameters of 27.6 nm and 173.1 nm and polydispersity (Pd) values of 0.1 and 0.3, respectively. The amount of light scattered by the DDFSC dispersion is dependent on the size of the molecules. In the current investigation, the two populations presented differences in intensity, mass, and number. Intensity and mass percentage were stronger for the large-diameter population (peak 2); however, the number of particles was greater in the small-diameter population (peak 1). Consequently, peak 2 contributes to higher signal intensity, whereas peak 1 consists of smaller particles at a higher numerical concentration. The bimodal distribution indicates that DDFSC exhibits two distinct structural states in an aqueous environment. The smaller population (peak 1) may be associated with individual fucoidan chains, while the larger population (peak 2) may be associated with aggregates formed by physical interactions of the same polysaccharide. The high molecular weight of DDFSC influences these two states of organization, as its chains are very large and promote cross-linking, leading to aggregated structures with larger hydrodynamic sizes. The formation of aggregates in the DDFSC molecule could hinder its antioxidant capacity by partially decreasing the accessibility of the sulfate or hydroxyl groups along the chain. However, ABTS and DPPH assays show that the functional groups responsible for neutralizing free radicals are sufficiently accessible to interact with radical species. These findings confirm that the antioxidant activity of DDFSC may depend on different parameters such as sulfate group content, distribution, and exposure, as well as spatial conformation and aggregation state, as previously suggested for other sulfated polysaccharides [28,39].

The SEM analysis revealed a rough morphology, suggesting the presence of intermolecular interactions, likely associated with hydrogen bonds and electrostatic forces (Figure 7). This morphology is not unique to sulfated polysaccharides; similar microstructural characteristics have been frequently reported in sources derived from other marine algae [1,16]. The observed high porosity could also influence the DDFSC solubility and behavior in aqueous environments, a critical factor in biomedical applications. Increased surface roughness may enhance interactions with cells and biopolymers, which can be particularly relevant for applications such as skin engineering and medication delivery.

The maximum AFM height found in the present study (125 nm) (Figure 8b) is greater than that reported for sulfated polysaccharides extracted from sea cucumber, which ranges between 50.1 and 100.1 nm [40]. This difference could be attributed to the high molecular weight of DDFSC (1449 kDa) and the greater polysaccharide concentration (0.5 mg/mL) used in the present study (both promoting increased polymer chain aggregation), compared to the values reported for sulfated polysaccharides from sea cucumber (180.8 kDa and 5 μg/mL, respectively) [40]. The rough morphology and the presence of aggregates of various sizes can be attributed to hydrogen bonding, which is primarily responsible for the macromolecular structure. Sulfated polysaccharides are enriched with hydroxyl (-OH) groups along their backbones, which form hydrogen bonds that generate cross-linking and proximity between the chains. This molecular arrangement promotes a more compact aggregation pattern, as detected by SEM and AFM [39]. Furthermore, the electrostatic interactions generated by the negative charges of the sulfate groups, along with the high molecular weight of DDFSC, increase the likelihood of physical cross-linking and self-association. These mechanisms lead to the formation of complex three-dimensional networks, aligning with the aggregate populations detected via DLS. Additionally, a homogeneous distribution of the aggregates was observed; their formation may be driven by the chemical structures of sulfate and hydroxyl groups, which could promote intermolecular interactions. The identified DDFSC surface roughness and morphology can be helpful in

determining polysaccharide functional properties, such as adsorption capacity, interaction with other charged molecules, and overall bioactivity.

Sulfated polysaccharides exhibit antioxidant activity by free radical scavenging. The sulfate groups present in the polysaccharide chain act as electron donors, resulting in free radical neutralization. In the case of DPPH, the free radical scavenging mechanism follows a hydrogen atom transfer, in which the sulfated polysaccharides can donate hydrogen atoms to neutralize DPPH free radicals, stopping the radical chain reaction. For $ABTS^{+}$, a single electron-transfer mechanism is followed, with sulfated polysaccharides donating a single electron to neutralize free radical species, such as the ABTS radical cation. It has been reported that a high sulfate concentration in these polysaccharides increases antioxidant activity, as these sulfate groups provide electron-donating capacity. In addition, functional groups such as hydroxyls or specific polymeric linkages may contribute to antioxidant activity in polysaccharides [41]. Sulfate groups are considered one of the main factors that enhance the antioxidant activity of sulfated polysaccharides, as an increased negative charge promotes electron or hydrogen atom transfer, thereby neutralizing free radicals. For this reason, a lower degree of sulfation is generally associated with lower antioxidant activity.

Concerning antioxidant activity, DDFSC was a more effective antioxidant in the ABTS assay (IC_{50} = 4.21 mg/mL) than in the DPPH assay (IC_{50} = 5.64 mg/mL), while *Dictyota dichotoma* from the Red Sea, Egypt, showed an IC_{50} = 4.59 mg/mL for DPPH and IC_{50} = 8.55 mg/mL for ABTS [9]. The sample from the Red Sea presented a molecular weight of 48.6 kDa, while DDFSC exhibited a higher molecular weight, approximately 30-fold greater (1449 kDa). It is possible that the high molecular weight of DDFSC decreases molecular mobility and limits access to sterically hindered radicals, such as DPPH. In contrast, the ABTS radical, being highly water-soluble, favors interaction with high molecular weight hydrophilic polysaccharides. Furthermore, the branched random coil conformation observed for DDFSC may help keep the sulfate and hydroxyl groups exposed, thereby facilitating the neutralization of the ABTS radical. However, the differences between the two studies may also be related to variations in the degree and pattern of sulfation, the composition of monosaccharides, and viscosity, among other factors that together contribute to the antioxidant activity of fucoidans. It has been shown that fucose-enriched polysaccharides exhibit remarkable antioxidant capacity by scavenging free radicals. This bioactivity is linked to the synergistic combination of sulfate and fucose groups present along the backbone of sulfated polysaccharides, as well as the molecular weight [42,43]. When compared to ascorbic acid, DDFSC exhibited moderate radical scavenging potential, particularly in the ABTS assay, where its antioxidant capacity was considerably higher than in the DPPH assay. This difference was expected due to the different chemical natures of the two compounds. While ascorbic acid is a low-molecular-weight molecule with a direct and highly efficient electron-transfer mechanism, DDFSC is a sulfated polysaccharide whose antioxidant activity depends on the accessibility of its functional groups and the three-dimensional architecture of the macromolecule [44,45]. Taken together, these findings demonstrate that the structure–function relationship of DDFSC is multifactorial.

The present study provides pioneering insights into DDFSC, particularly regarding its polysaccharide chain conformation, microstructure, and antioxidant activity. These findings support the potential of DDFSC as a natural antioxidant and provide evidence of a structure–function relationship, strengthening its possible applications in functional foods, nutraceuticals, controlled-release systems, and biomaterials with bioactive properties.

5. Conclusions

Fucoidan can be extracted from *D. dichotoma* growing in the Sea of Cortez. DDFSC exhibits a high molecular weight (880 kDa) and macromolecular characteristics that suggest a branched random coil conformation with chains displaying a semi-flexible spatial arrangement. DDFSC contains sulfate (3.4% *w*/*w*) and exhibits moderate antioxidant capacity. The polysaccharide morphology reveals a rough texture, irregular topography, and particle aggregation. The new knowledge generated in this study is essential for understanding the molecular characteristics, morphology, and bioactivity of DDFSC, contributing to the record and overall understanding of bioactive macromolecules in organisms from the Sea of Cortez. This information could play a pivotal role in the development of sustainable DDFSC applications across diverse areas, such as biomedicine, pharmaceuticals, and food technology.

Author Contributions: Conceptualization, E.C.-M. and E.G.-S.; methodology, E.C.-M., E.G.-S., J.M.-E., A.J.B.-E., F.B.-B., and K.G.M.-R.; software, J.M.-E., K.G.M.-R., A.J.B.-E., F.B.-B., and E.G.-S.; validation, E.C.-M., J.M.-E., and K.G.M.-R.; formal analysis, E.C.-M., A.M.-B., J.L.-M., A.R.-C., E.M.-B., A.J.B.-E., and F.B.-B.; investigation, E.C.-M., E.G.-S., A.M.-B., J.L.-M., A.R.-C., E.M.-B., A.J.B.-E., and F.B.-B.; resources, E.C.-M., A.M.-B., A.J.B.-E., and F.B.-B.; data curation, E.C.-M., E.G.-S., J.M.-E., and K.G.M.-R.; writing—original draft preparation, E.G.-S. and E.C.-M.; writing—review and editing, E.C.-M., E.G.-S., A.M.-B., J.L.-M., A.R.-C., E.M.-B., A.J.B.-E., F.B.-B., and A.C.-M.; visualization, E.G.-S. and E.C.-M.; supervision, E.C.-M., A.M.-B., J.L.-M., A.R.-C., E.M.-B., and A.C.-M.; project administration, E.C.-M. and A.C.-M.; funding acquisition, E.C.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CONAHCYT, grant number 319684, to E. Carvajal-Millan.

Data Availability Statement: Data are contained within the article.

Acknowledgments: E.G.-S. thanks SECIHTI for the Ph.D. scholarship provided.

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

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