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Extraction and Characterization of Fucoidan Derived from *Sargassum ilicifolium* and Its Biomedical Potential with In Silico Molecular Docking

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Abstract: Fucoidan, a polymer derived from seaweed, poses a broad range of biological applications, and its potential medicinal benefits have been widely studied over the past decade. In this study, fucoidan was extracted from marine macroalga *Sargassum ilicifolium* and its bioactive potential for in silico molecular docking was investigated. Additionally, the computational in silico docking studies were applied on the fucoidan against anticancer and antioxidant target proteins by using Glide ligand docking, Schrodinger software. The FT-IR analysis revealed that fucoidan mainly consisted of the fucose residues (59.1%) and a few monosaccharides, such as uronic acid (11.7%) and sulphate (18.3%). The in vitro tests revealed that fucoidan possessed various antioxidative properties and anticoagulant activities. Fucoidans played an inhibitory role in the colony formation of HepG2 cells. The NADPH oxidase (−7.169 Kcal/mol) and cellular tumor antigen p53 protein (−6.205 Kcal/mol) exhibited the highest antioxidant and anticancer proteins, respectively. Overall, the present study results provide a theoretical foundation for broadening the application of fucoidan from *S. ilicifolium* as a pharmaceutical ingredient.

Keywords: fucoidan; *Sargassum ilicifolium*; characterization; antioxidant anticoagulant; anticancer activities; in silico docking

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1. Introduction

Seaweeds, also known as marine macroalgae, significantly contribute to global biodiversity. They are rich in nutrients, such as proteins, amino acids, carbohydrates, fats, and bioactive substances such as carotenoids, dietary fiber, vitamins, minerals, trace elements and iodine, and bromine, which can be consumed directly [1,2]. So far, several bioactive compounds have been detected in seaweeds. Green seaweeds can generate more secondary metabolites than red seaweeds due to their secondary-metabolite-producing capacity [3,4]. Among these various seaweeds, brown seaweeds contain chemopreventive and chemotherapeutic chemicals, such as phylorotannin, fucoxanthin, and fucoidan. Sulfated polysaccharides are extensively used as a thickening, suspending, stabilizing, gelling, and emulsifying agents in the cosmetics, fertilizer, textiles, and paper industries [5,6]. Similarly, marine algae contain many polysaccharides, including fucoidans, laminarins, and

alginates. Of these, fucoidans mostly contain fucose and sulfate groups [7,8]. In particular, fucoidans are more physiologically active elements in terms of structural qualifications [9]. Fucose backbones are mostly found in brown seaweeds [10]. However, species, habitat, and collecting season significantly affect fucoidan composition. The molecular weight and extraction methods of fucoidan are inter-linked based on the functional groups [11]. The bioactivity of fucoidan is determined by its extraction process, content, and structure. Additionally, environmental changes affect both nutritional and non-nutritional molecules, including antioxidants [12]. However, no standard extraction technique for fucoidan has been reported so far [13]. Recently, fucoidan has been isolated from brown algae such as *Sargassum filipendula* [14], *Sargassum wightii*, *Turbinaria ornata*, and *Padina tetrastromatica* [15].

Fucoidan is non-toxic with therapeutic potential for cancer, cardiovascular disease, atherosclerosis, inflammation, and aging [16]. In living organisms, antioxidants act as free radical scavengers, inhibiting ROS, lipid peroxidation, protein breakdown, and DNA damage [17,18]. The brown algae extracts exert antioxidant activity in the presence of high phenolic chemicals [19]. Processed meals often contain artificial antioxidants that can be harmful to humans [20]. Several natural antioxidants have been shown to reduce hyperlipidemia [21]. Additionally, many aquatic macroalgae species have been reported to have natural antioxidant properties that may protect from free radicals and prevent the occurrence of cardiovascular disease and diabetes [22,23].

Fucoidan research using brown seaweeds has broken through over the last four decades, due to its potential diverse pharmacological and biological applications such as antiviral, antimicrobial, antifungal, antioxidant [24,25], anticoagulant, anti-thromobotic, antidiabetic [26], anti-inflammatory activity [27] and anti-cancer [28]. The amount of biologically active compounds in algae varies depending on the geographical origins, reproductive phase (sterile versus fertile), environmental stressors, and the collecting season. So far, many cancer cells have been examined to determine the anticancer properties of fucoidan [29]. Fucoidan could significantly inhibit the development of cancer tumors and increase the overall survival rate of cancer patients [30]. Therefore, the present study aimed to identify the proteins that may act as chemotherapeutic drugs in hepatocellular carcinoma using molecular docking. The antioxidant capacity of the cell could maintain redox balance and decrease oxidative stress. Numerous enzymes, including cytochrome P450 (CP450), lipoxygenase (LO), myeloperoxidase (MP), NADPH oxidase (NO), and xanthine oxidase, could induce ROS generation during the metabolism of arachidonic acid (XO). Subsequently, ROS generation could reduce oxidative stress, and maintain redox equilibrium by inhibiting these enzymes [31]. Increased oxidative stress by ROS is associated with various diseases, including cancer. Fucoidan is a possible precautionary and therapeutic agent for cancer without any harmful effects on the liver [32]. Therefore, fucoidan is very promising as a natural anticancer agent. Herein, fucoidan was used as an antioxidant and cancer-preventing agent through computational and in vitro methods. The present study focuses on the extraction and purification of fucoidan from *S. ilifollium*, and evaluation of its anticoagulant, antioxidant, and anticancer activity in human hepatoma (HepG2) cells under in vitro conditions.

2. Materials and Methods

2.1. Collection of Seaweed Samples

The marine brown algae *S. ilicifolium* C. Agardh (1820) was obtained by hand picking from the intertidal area of Keelakarai coast (9°13′56.568″ N 78°47′5.3268″ E), GoMBR, Tamil Nadu, India. At the location, the seaweed species were extensively rinsed with saltwater, clung to calcareous sand particles, and immediately transported to the laboratory to remove the epiphytes. The herbaria were deposited at the Algal Biotechnology Lab, Department of Botany, Periyar University, Salem-636 011, with the voucher no. PU-BOT-SH-BS0110-2017. Later, the sample was cleaned with distilled water and dried at an ambient temperature in the shade. The shade-dried samples were crushed and cut into little bits. The powder sample was used for further testing.

2.2. Extraction of Polysaccharides

About 125 g of seaweed dried material of *S. ilicifolium* was soaked in an organic solvent (acetone: methanol in a 7:3 proportion) and left undisturbed in an orbital shaker for two days. The extraction was performed twice to obtain all the phenolic compounds fully from the seaweed powder. The seaweed materials were immersed in 0.1 M HCl for one day at room temperature before centrifuging at 10,000 rpm for 25 min. The pellet was again centrifuged, then the supernatant was collected and combined at a ratio of 1:1 with 100% ethanol and stored for precipitation. The impulsive was dissolved in sterile deionized water using a membrane filter and incubated for two days at 4 °C. Later, the solution was freeze-dried, and the mixture of sulfate-based polysaccharides (SPs) was subjected to further processing [33].

2.3. Fucoidan Purification by Chromatography

The extracted polysaccharides were mixed with 0.2 M Na_3PO_4 buffer and then added to a column Q sepharose rapid flow, where the stepwise elution procedure with 0.2 M Na_3PO_4 buffer and then NaCl solution separation were initiated at a flow rate of 60 mL/h. The eluent was collected at a 5 mL/tube ratio.

2.4. Chemical Analysis

The sugar substance of the sample extract was measured using the standard technique of phenol-sulfuric acid protocol [34]. The sulfate content present in the sample extract was determined by the standard protocol [35]. The total uronic acid was determined using the m-phenyl phenol method [36,37].

2.5. Monosaccharides Composition

The sugar content of the SPs was measured for the monosaccharide. In the round-bottom flask, 5 mg of samples were combined with trifluoroacetic acid. The reaction mixtures were then held overnight for reflux. Later, the solutions were diluted with 80% TFA and left undisturbed for half an hour. The distilled water was then mixed with reflux mixture for washing before evaporation. This process was repeated until the hydrolysate was neutral, and the content was analyzed using HPLC.

2.6. Physiochemical Properties of Fucoidan

2.6.1. Molecular Weight

The molecular weight of the extracted fucoidan was identified using gel filtration chromatography. Standard dextrans were used to calibrate the column, and the column flow rate was set at 0.6 mL/min. after collected fractions were checked by phenol sulfuric acid [34].

2.6.2. Solubility

Approximately 1 mg/mL of the sample was collected and dissolved in several solvents before analysis to determine the solubility of pure dextran and fucoidan. Water, dimethyl sulfoxide, acetonitrile, chloroform, hexane, and sodium hydroxide were used at the concentrations ranging from 0.1 to 4 M. The maximum solubility concentration of the sample was evaluated using a known constant volume of solvent to saturation. The reaction mixture was stirred overnight before the samples were centrifuged, and the remaining waste and leftovers were air-dried and weighed.

2.6.3. Thermal Property of Fucoidan

Thermogravimetric analysis (TGA) tests were performed on a Netzsch DSC 204 with continuous nitrogen flushing to determine the maximum saturation and melting point of pure fucoidan. The temperature estimate was investigated by heating 1.71 mg of the samples at room temperature up to 900 °C at a heat rate of 20 °C/min. The thermograms were recorded with increased temperature.

2.6.4. Fourier Transform Infrared Spectroscopy (FT-IR)

The sample extracts were combined with 90 parts of dried KBr pellets. The mixture was crushed to form a 3 mm diameter salt disc. Then, the FT-IR spectrophotometer was used to measure the absorbance at 4000–400 cm^{-1}.

2.6.5. Nuclear Magnetic Resonance (NMR) Spectroscopy

A sophisticated spectrophotometer was used to conduct the 1H-NMR studies on extracted and purified fucoidan (400 MHz Bruker model, Bruker, Billerica, MA, USA). In the NMR test tube, the fucoidan sample was combined with NMR quality double distilled water. In the NMR spectrum, the chemical shift of the proton was presented in parts per million (ppm).

2.6.6. SEM Analysis

The lyophilized samples were observed under scanning electron microscope (Sophisticated Test & Instrumentation Centre, Cochin University, Cochin, India).

2.6.7. Atomic Force Microscopy (AFM)

The AFM examinations of isolated and purified fucoidan samples were carried out by combining the sample solutions with 0.3 M of sodium hydroxide at 200 µg/mL. The produced fucoidan extract was allowed to equilibrate with sodium hydroxide for 60 min before being neutralized. After alkali treatment, the purified fucoidan sample partly dissociated the helices formation by charging the hydroxyl groups and lowering the H-bonding between the chains on the laminarian backbone. The fucoidan residue solutions were then dialyzed against excess water for three days to eliminate the bulk of NaCl. The chemical solutions were then placed on a 1 cm^2 freshly cleaned glass plate surface through a 0.22 µm filter. The samples were air-dried at room temperature for 16 h. The microscopic images of the isolated and purified fucoidan samples were taken within 24 h of fucoidan deposition to prevent sample contamination. It should be noted that fucoidan formed comparable patterns with less order when the solutions were not treated with NaOH, dialyzed, and filtered before deposition onto the glass. Atomic force microscopy (AFM; Sri Ramakrishna Engineering, Coimbatore, India) was used to observe the structures of pure fucoidan.

2.7. Antioxidant Properties of Polysaccharide

2.7.1. DPPH Assay

The antioxidant activity of the isolated and purified fucoidan was tested using the standard technique [38]. Approximately 100 µL of the extracted sample was added with 3 µL of DPPH solution per doses. The antioxidant properties of the sample were measured at 517 nm, followed by a 30 min incubation at 30 °C in the dark. After incubation, the discolorations were detected at 517 nm with a suitable blank. All tests were performed in triplicate to prevent standard error.

$$\text{Scavenging } \% = A_{Control} - A_{Sample}/A_{control} \times 100$$

2.7.2. Hydroxyl Radicals Scavenging Activity

The hydroxyl radicals scavenging activity of isolated and purified polysaccharides was investigated using Fenton's reaction [39]. The percentage rate obtained from the hydroxyl radical scavenging test was expressed as inhibition rate. The hydroxyl radicals were prepared using 3 mL of Na_3PO_4 buffer and various polysaccharides. The solutions were incubated for 1 h, and the existence of hydroxyl radical was determined by measuring the absorbance at 510 nm. BHA and L. Ascorbic acid were used as control.

2.7.3. Superoxide Radical Scavenging Activity

The superoxide radical scavenging capacity of isolated and purified fucoidans was investigated [40]. The test tubes were pre-labeled, then 3 mL of a reaction mixture comprising superoxide radical solution was added into 1 mL of the sample. The blue color development was observed, and the absorbance was measured at 560 nm. The whole chemical reaction was carried out in complete darkness and the test materials were covered in a container with aluminum foil. In the dark, the identical tubes containing the reaction mixture acted as blanks. BHA and L. Ascorbic acid were used as control.

2.7.4. ABTS Scavenging Activity

The ABTS scavenging activity of isolated fucoidan samples was investigated using a standard protocol [41]. The process began with a 15 h reaction at room temperature of the ABTS solution with 25.5 mM $K_2S_2O_8$. The ABTS+ chemical solutions were then diluted with phosphate buffer saline. After mixing the ABTS solution with polysaccharide samples of different concentrations, L-ascorbic acid, BHA, and ABTS were added as standard solutions. The absorbance was measured to determine the kinetics of the process.

2.7.5. Reducing Power Assay

The total reducing power assay of isolated and purified fucoidan was calculated [42]. Briefly, 0.13 mL of pure polysaccharide sample was prepared with phosphate buffer and potassium ferricyanide and incubated at 50 °C (20 min). The chemical reaction was then stopped by mixing 1.25 mL of TCA (10% w/v) with the test chemical. The chemical combination was centrifuged at 3000× g rpm (15 min), and then the supernatant was separated. Approximately 1.5 mL (0.1 % w/v) of ferric chloride solution was added and the absorbance was measured at 700 nm.

2.8. Anticoagulant Properties

2.8.1. Activated Partial Thromboplastin Time (APTT)

The APPT of isolated fucoidan samples was determined by a standard methodology with some modifications [43]. The samples and solutions used in this technique were produced using an isotonic solution of 0.9% sodium chloride. The device was set to combine the platelet-depleted plasma with fucoidan sample. After incubating for 60 s at 37 °C, each combination was incubated for another 5 min (37 °C) with APPT reagent. The time for clot formation was then monitored upon the introduction of $CaCl_2$. The same steps were repeated thrice. Heparin and NaCl were used as positive and negative controls, respectively.

2.8.2. Prothrombin Time (PT)

The prothrombin time of purified fucoidan sample was measured using a standard procedure with minor modifications [44], and the experiment set up was similar to that of APTT estimation. Each test was carried out in triplicate to prevent standard errors. Heparin and sodium chloride (NaCl) were used as positive and negative controls, respectively.

2.9. Anticancer Properties

Cytotoxicity Effect on Hep G2 Cell Lines

The Hep G2 cell line was collected from the NCCS in Maharashtra, India. The cells were maintained at 37 °C in a 5% CO_2 atmosphere in the tissue culture lab. The MTT experiment was carried out according to a previously reported method [45]. In 24 multi-well plates, 5×10^3 cells were plated in DMEM medium containing 10% FBS. The cells were incubated at 37 °C for 12 h with 5% CO_2 and 95% O_2. The DMEM medium containing FBS was then withdrawn and replaced with serum-free media for 24 h. Following the removal of the media, the control plate received 0.01% DMSO medium, while the content treatment plates received 100–1000 µg/mL fucoidan-containing DMEM medium. The medium was withdrawn after 48 h of cell proliferation treatment and replaced with 0.5% MTT containing

DMEM media, which was kept at 37 °C for 4 h. After the media was withdrawn from the plate, DMSO was added. After 2 h, the sample was measured at 570 nm using an ELISA reader.

2.10. Computational Methods

2.10.1. Macromolecule and Small Molecule Preparation

The antioxidant target proteins were selected based on their properties and activities. The multitargeted proteins investigated the comprised lipoxygenase (PDB ID 1N8Q), cytochrome p450 (PDB ID 1OG5), NADPH oxidase (PDB ID 2CDU), xanthine dehydrogenase/oxidase (PDB ID 3NRZ), and NADPH oxidase (PDB ID 1DNU). The cellular tumor antigen p53 (PDB ID 6XRE), caspase-3 (PDB ID 1I3O), mucosal address in cell adhesion molecule 1 (PDB ID 1BQS), and nuclear factor NF-kappa-B p105 subunits were the anticancer target proteins (PDB ID 1SVC). The remaining undesirable chains were cut out of the antigen p53 protein from PDB ID 6XRE, where the M chain was selected for cellular cancer. The undesired chains E and F were removed during the protein production wizard module's review phases and only the chains containing caspase-3 were restored. These were also found in the PDB ID 1I3O. The preprocessing stages were accomplished using the Maestro platform's Protein preparation wizard module, which assigned bond orders, added hydrogens, formed zero-order bonds to the metals and di-sulphate bonds, changed seleno-methionines into methionine, and filled in the missing side chains. The OPLS4 force field was employed to optimize the structure utilizing hydrogen bond optimization and energy minimization (Maestro, Schrödinger, LLC, New York, NY, USA). The PubChem databases were used to retrieve the three-dimensional structural data files (3D SDF) for the fucoidan (PubChem ID 129532628). As part of the LigPrep module's preparation, the structures were energy minimized using the OPLS4 force field [46], producing 32 distinct states of stereoisomeric and tautomeric entities (Schrödinger Release 2021-2: LigPrep, LigPrep, Schrödinger, LLC, New York, NY, USA).

2.10.2. Molecular Docking

The Glide XP (extra precision) mode of Maestro's Glide docking module was used to dock the target proteins and ligand molecules. The 2D interaction diagram of a ligand-protein complex molecule was generated using the ligand interaction module after the docked ligand and protein interaction were analyzed by an XP pose viewer to obtain the optimistic pose. The resulting XP posture was further assessed to explore the interaction between the ligand molecules and target proteins during the binding process [47].

2.11. Statistical Investigation

The data were statistically analyzed using Probit analysis, and the results were presented as mean ± SD. The T-test was then used, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Molecular Weight, Solubility, and TGA Analysis of Polysaccharides

Fucoidan was recovered from acetone, while the pigments, lipids, and low molecular polysaccharides were recovered from the *S. ilicifolium* sample using methanol. After separating the sulfated polysaccharides, the sulfate concentration and uronic acid level of the fraction were 18.3% and 11.7%, respectively (Table 1). Figure 1 shows the retention factor (Rf) of *S. ilicifolium* analyzed by HPLC. The Rf values were 2.743, 5.018, 6.921, and 17.989. The HPLC analysis of the monosaccharide composition indicated the presence of sugars, such as fucose (59.1%), galactose (18.3%), xylose (4.1%), and mannose (6.8%) (Table 2). Fucoidan gel permeation chromatography demonstrated the polymer's dependability. Based on the calibration with standard dextrans, the apparent MW of fucoidan was 37 kDa, chondrioitin sulphate was 50 kDa, dextran sulphate was 40 kDa, and heparan sulphate was 17 kDa (Figure 2). The patterns of intra- and intermolecular hydrogen bonding influenced

the solubility of fucoidan, which were typical for polysaccharides and derivatives. The solubility of fucoidan in various solvents and water was up to 500 mg/mL, but it was not soluble in DMSO, ethanol, methanol, acetone, acetonitrile, ethyl acetate, chloroform, or hexane (Supplementary Table S1). The TGA was used to determine the thermal stability of untreated seaweeds (Figure 3). The graphs represent the sample's weight loss after continuous heating till 800 °C. Thermal breakdown began at 41.92 °C and the concludes were obtained at a temperature of 580.47 °C.

Table 1. Chemical analysis of *Sargassum ilicifolium*.

S. No.	*Sargassum ilicifolium*	Sulphated Polysaccharides Composition
1.	Total sugar	63.91%
2.	Total sulphate content	18.3%
3.	Total uronic acid	11.7%

Table 2. Monosaccharide composition of *Sargassum ilicifolium*.

S. No.	Monosaccharide	(%)
1.	Fucose	59.1
2.	Galactose	18.3
3.	Mannose	6.8
4.	Xylose	4.1

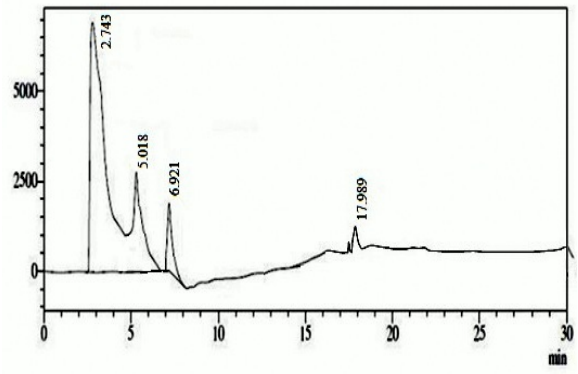

Figure 1. Chromatogram of fucose, galactose, mannose and xylose from hydrolysate fucoidan *Sargassum ilicifollium*.

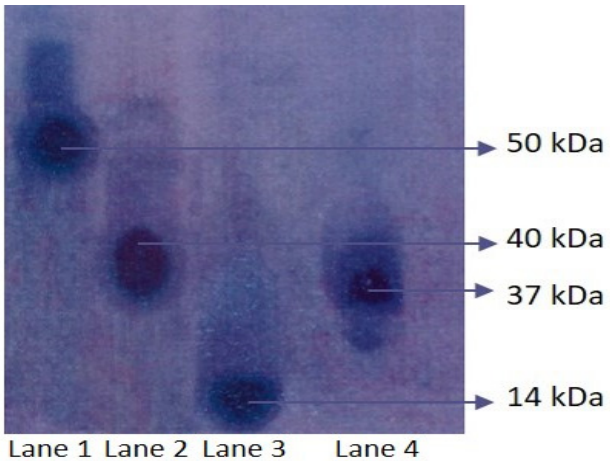

Figure 2. Molecular weight Lane 1—Chondrioitin sulphate; Lane 2—Dextran sulphate; Lane 3—Heparan sulphate; Lane 4—Fucoidan.

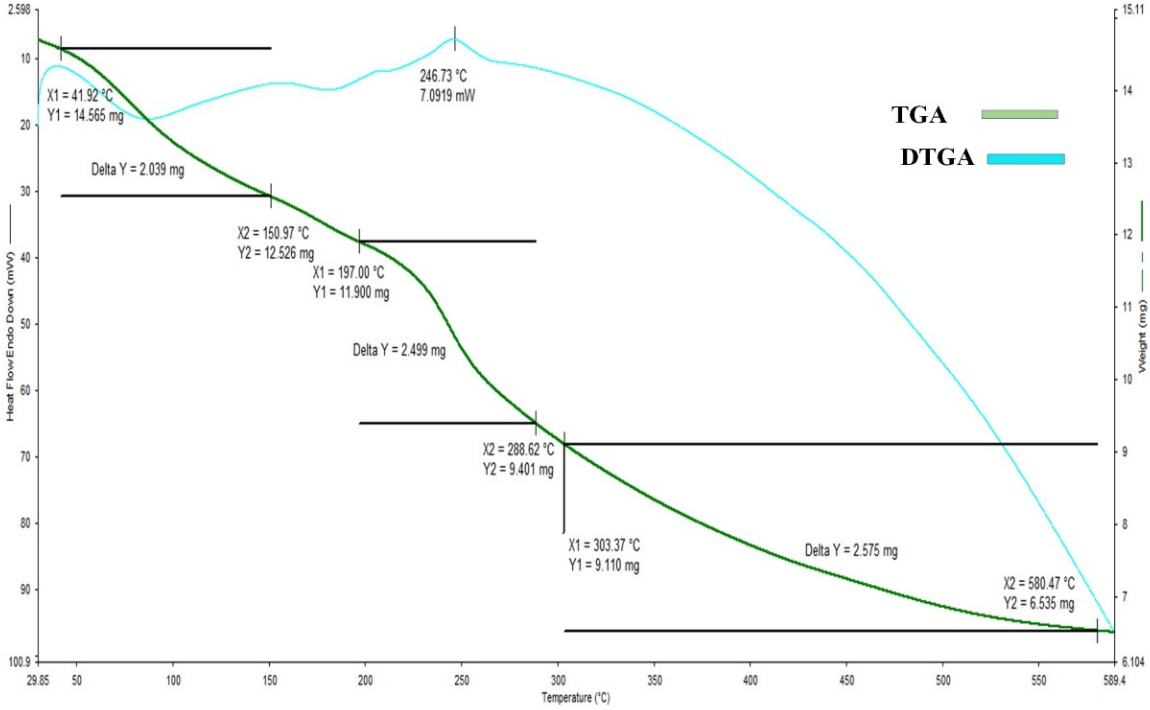

Figure 3. Thernogravimetric and different thermogravimetric analysis of fucoidan from the brown seaweed *Sargassum ilicifollium*.

3.2. Characterization of Fucoidan from S. ilicifolium

The FT-IR analysis of fucoidan extracted from *S. ilicifolium* was shown in Figure 4. The carbohydrate O–H stretching tremor was assigned to the bands at 3292.42 and 3258.45 cm^{-1} (Table 3, Figure 4). The stretching and bending vibrations of the alkyl groups (–CH_2−, CH_3) were assigned to the absorption band at 2930.59 cm^{-1}, indicating the existence of C=O stretching vibrations of O-acetyl groups. CH_2 was assigned to the band at 1412.35 cm^{-1} (galactose, xylose). The presence of sulphate groups is indicated by the fucoidan characteristic absorption band at 1246.34 cm^{-1} (S=O Stretching, Fucose, O-acetyl group). The absorbance at the wavelength of 1029.0 cm^{-1} indicates C-O-SO3 vibration. The C-O-S bending vibration of the sulphate substitute at the axial C_2 and C4 positions were assigned to the bands at 882.96 and 816.06 cm^{-1}, respectively. Figure 5 depicts that the ^1H NMR range of fucoidan isolated from *S. ilicifolium*. The signal at 5.00 ppm represents the components of fucoidan L fucopyranosyl units, the 4.74 ppm signal represents the 3-linked α-L-fucose, and the 4.56 ppm signal at β-D-Galactose (G) represents the 3,6-α-L-anhydrogalactose residues. The 3.98 ppm represents the 3-linked ß-D-Galactose. The shape, diameter, and length of numerous polysaccharides have been characterized using AFM. The topography images of the fucoidan surface revealed the sample, soft template heights, and the bright spot. The AFM microscopy results exposed the peaks and troughs all over the surface. Figure 6 depicts various distorted forms with huge diameters and lengths. The size of the AFM was 390 nm. The shape and size of fucoidan were characterized by SEM examination, revealing the thread-like exterior morphology (Figure 7).

Table 3. The FT-IR analysis of *S. ilicifolium*.

S. No.	Peak Area	Functional Groups
1.	2930.59, 2361.89^{-1} cm	Stretching vibrations and bending vibrations of alkyl groups (–CH_2– and –CH_3)
2.	1245.34^{-1} cm	S=O Stretching vibration
3.	1029.00^{-1} cm	C-O-SO_3 vibration
4.	882.96, 816.06^{-1} cm	C-O-S bending vibration of the sulphate substitute at the axial C_2, C_4 position.

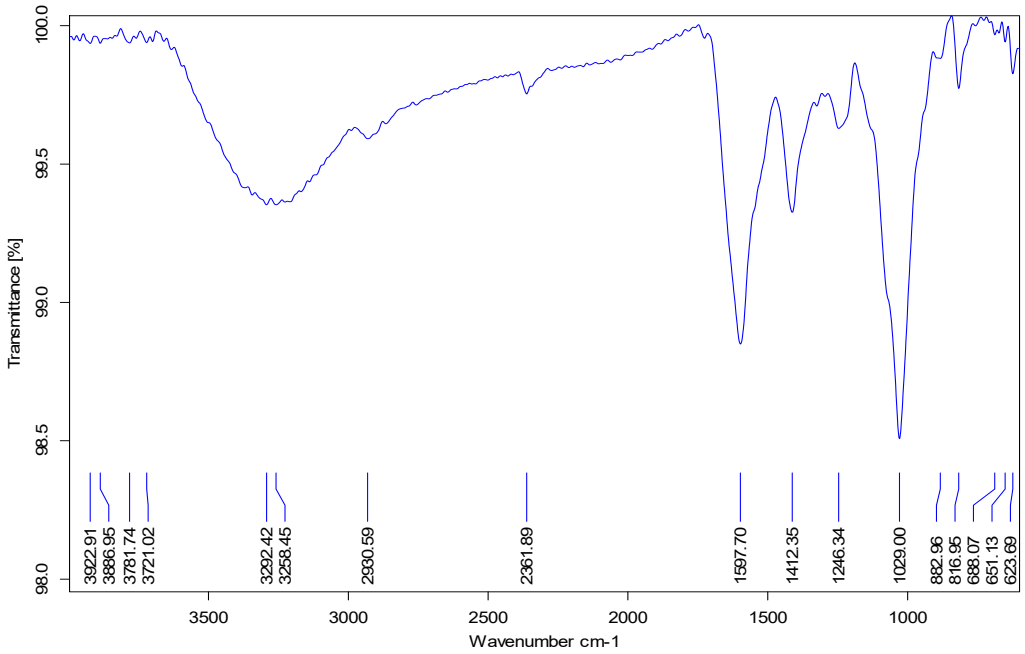

Figure 4. FT-IR spectra of fucoidan fraction isolated from the brown seaweed *Sargassum ilicifollium*.

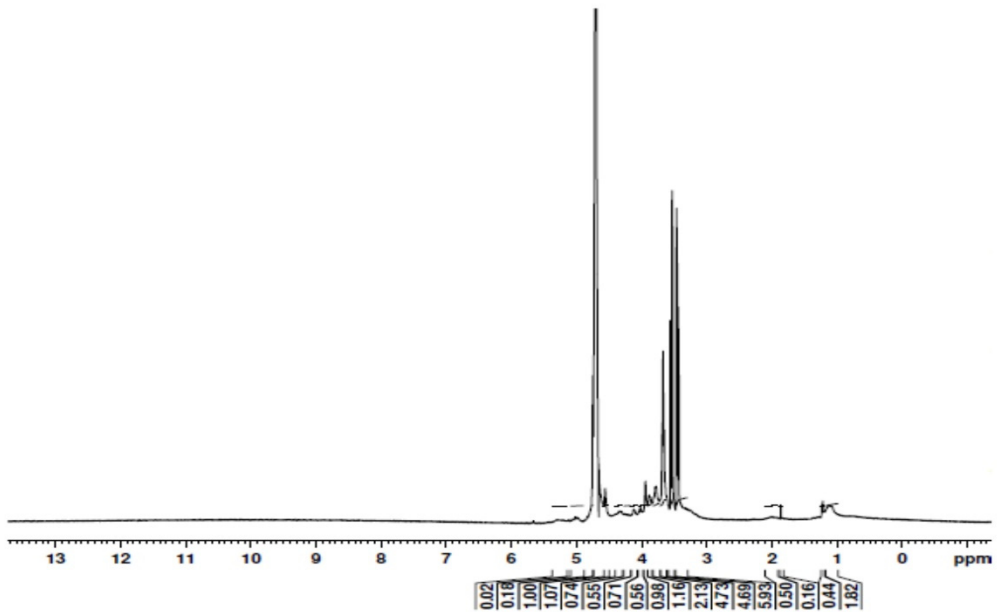

Figure 5. H ^{1}NMR of fucoidan from the brown seaweed *Sargassum ilicifollium*.

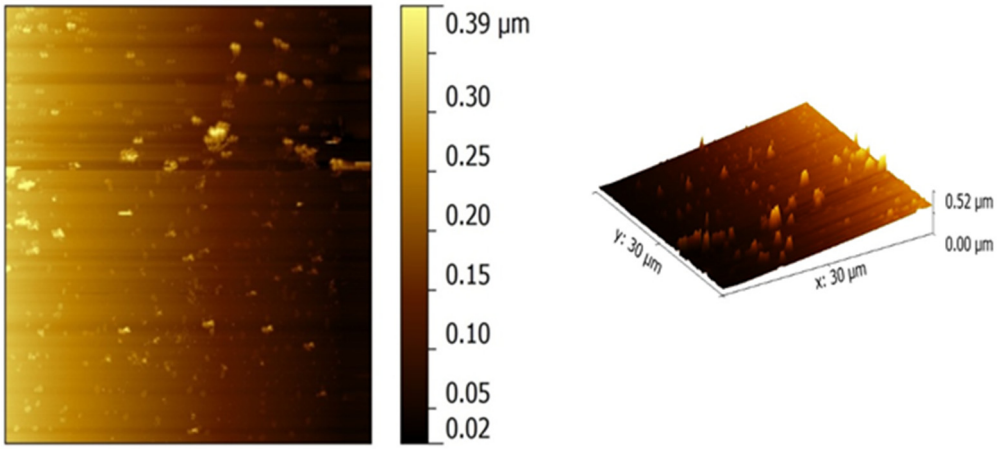

Figure 6. AFM structure of Fucoidan from *Sargassum ilicifolium*.

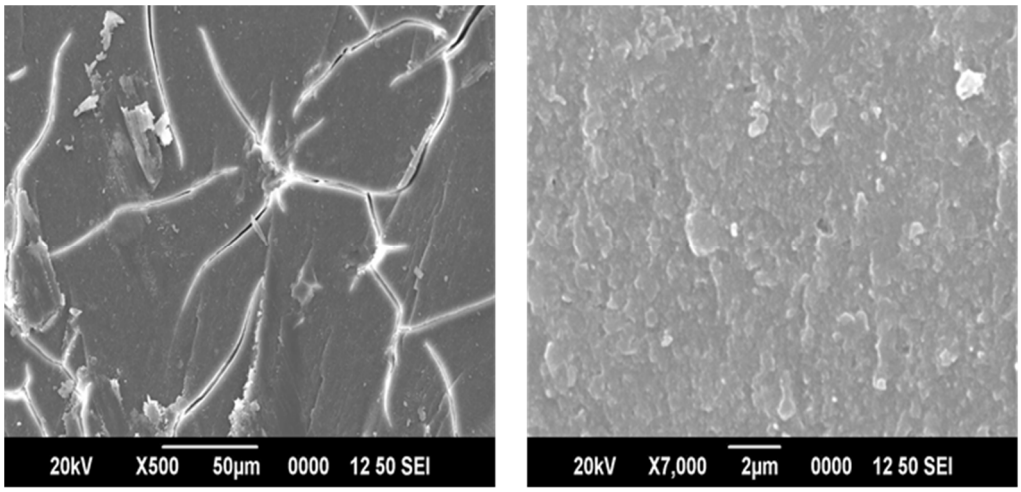

Figure 7. SEM analysis of Fucoidan from *Sargassum ilicifolium*.

3.3. Antioxidant Activity

The antioxidant activity of seaweed extracts in *S. ilicifolium* was demonstrated by the DPPH activity (Figure 8A). In this activity, fucoidan showed the highest inhibition range at 66.09 ± 0.25 µg/mL in 160 µL/mL concentration. The hydroxyl scavenging activity of fucoidan from *S. ilicifollium* extract increased from lower to higher concentrations of 50 to 250 µg/mL (59.04 ± 0.25 µg/mL), and the IC_{50} values of fucoidan was 209.99 (Figure 8B). The calculated superoxide radical scavenging test results and % inhibition at different doses (50 to 250 µg/mL) are depicted in Figure 8C. The maximum concentration was achieved at 250 µg/mL of 68.5 ± 0.64, and the IC_{50} value of fucoidan was 195.43 µg/mL. The ABTS assay and reducing power showed the strong inhibitory activity in 125 and 120 µg/mL (69.78 ± 0.42 and 0.781 ± 0.028), respectively, and the IC_{50} value was 94.25, 44.00 (Figure 8D,E). The APTT and PT models revealed significant variations between fucoidans derived from seaweeds (Table 4). The APTT and PT tests confirm that fucoidan from *S. ilicifolium* poses anticoagulant properties. The APTT test results (98.76 IU at 25 g/mL) were higher than the PT test results (52.93 IU at 25 g/mL), indicating a potential inhibitory activity.

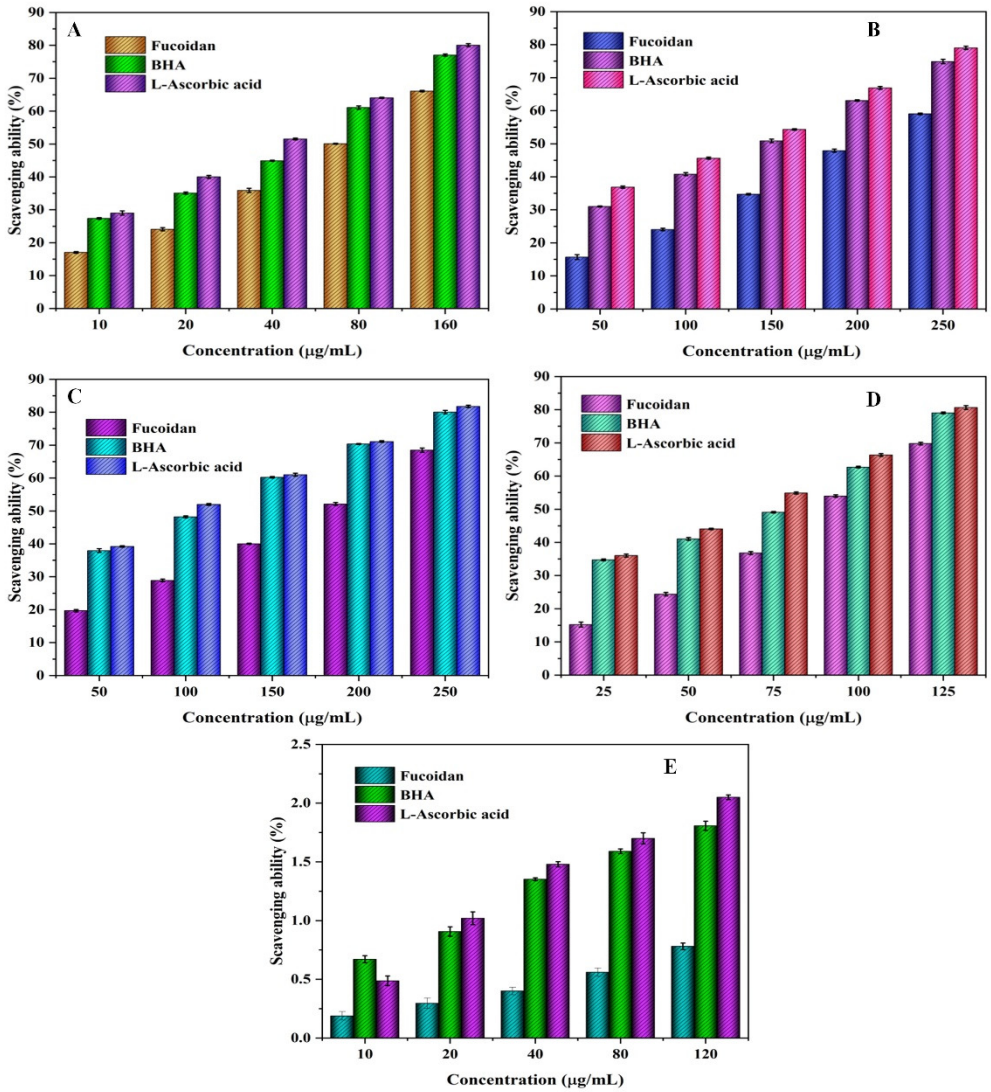

Figure 8. Antioxidant activity of fucoidan *S. ilicifolium*: (**A**) DPPH scavenging assay, (**B**) hydroxyl scavenging activity, (**C**) superoxide radical scavenging activity, (**D**) ABTS assay and (**E**) reducing power.

Table 4. Anticoagulant activity of fucoidan from *Sargassum ilicifolium*.

Extraction/Control	APTT (25 μg/mL)	PT (25 μg/mL)
Fucoidan	98.76	52.93
Heparin	175.50	126.80

3.4. Anticancer Activity

The MTT, Ao/EtBr, MMP, and ROS assays were used to assess the anticancer efficacy of sulfated polysaccharides against the HepG2 cell lines. The cytotoxicity test revealed the fundamental measure of cell metabolism and life. The anticancer efficacy of fucoidan at different concentrations treated with HepG2 cells. The anticancer activity increased with increased concentration of fucoidan (Supplementary Table S2). The HepG2 inhibitory concentration IC_{50} value was 71.33 μg (Figures 9 and 10). Fluorescence staining revealed more apoptotic cells with strong fluorescence, perhaps from the fucoidan-treated cells. Microscopic examination revealed that the integrated HepG2 cell was stained with green fluorescence, indicating the viable cells in untreated controls. The untreated cells' nuclei were spherical and uniform, and their morphology was normal. The fucoidan-treated cells

exhibited cell shrinkage, nuclear condensation, and fragmentation. Flow cytometry was used to investigate the effect of fucoidan on MMP and ROs in the human liver cancer cell line HepG2.

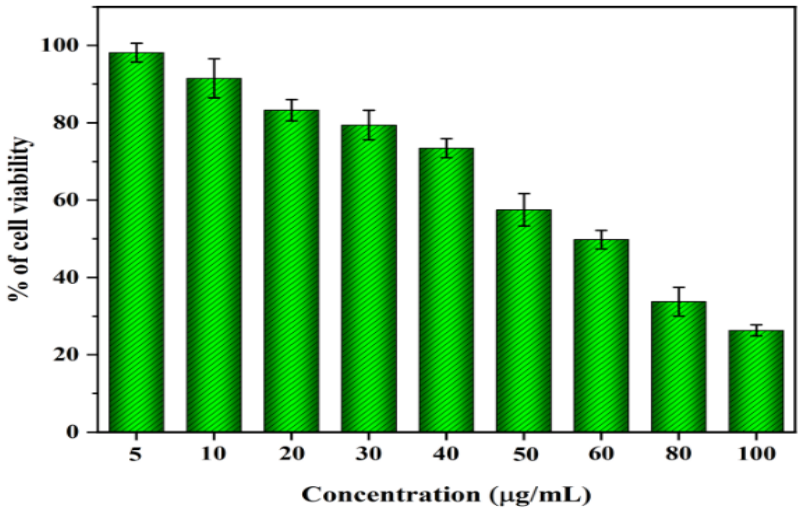

Figure 9. Cytotoxicity of fucoidan from *S. illicifolium* on HepG2 cells.

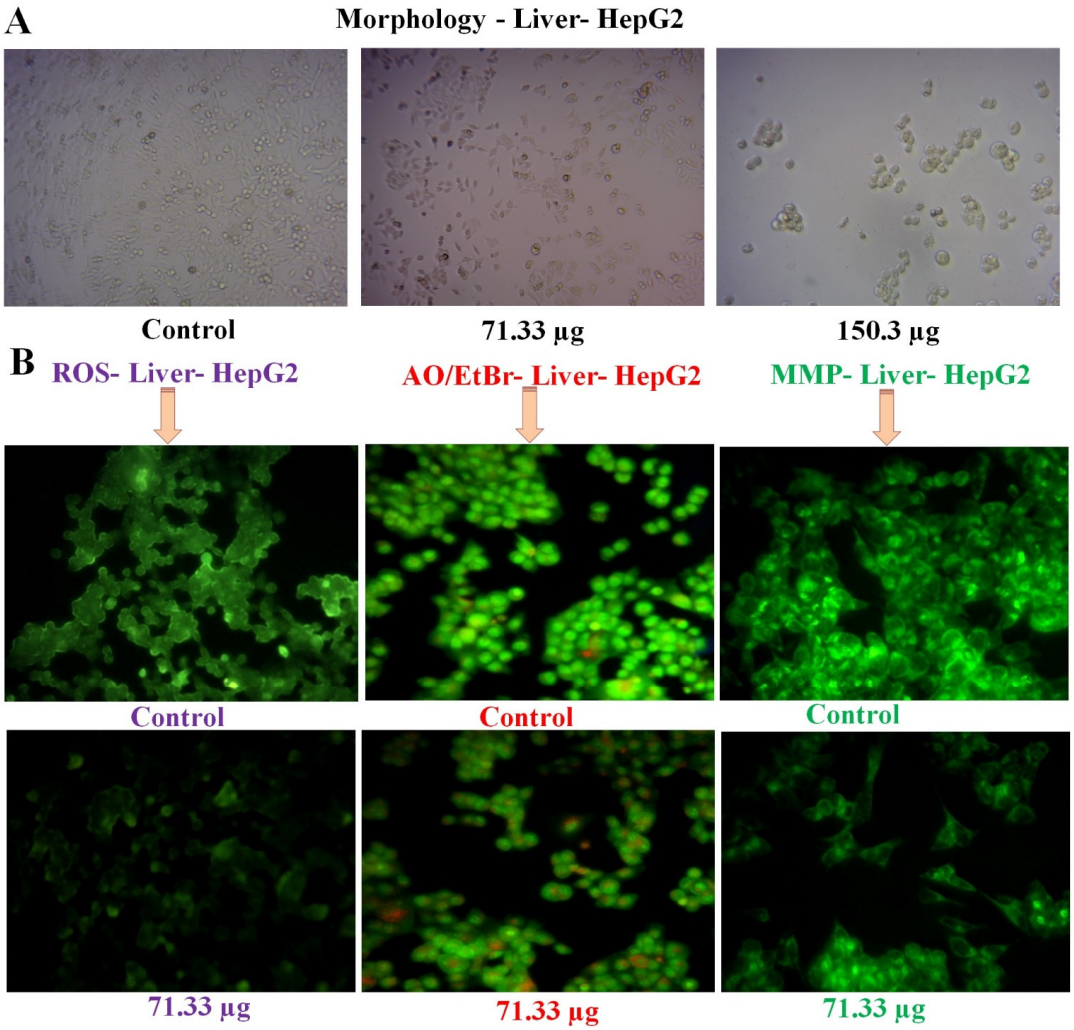

Figure 10. (**A**) Morphology–Liver–Hep G2 fucoidan from *S. ilicifolium* and (**B**) ROS, Ao/EtBr –Liver –Hep G2 fucoidan from *S. ilicifolium*.

3.5. Molecular Docking

Fucoidan was used in the molecular docking experiments against the target proteins selected based on their functional characteristics. The antioxidant and anticancer multitarget proteins were used in the docking experiment as a GLIDE (ligand docking) module, and these targets were normally prioritized according to the docking score computation in the form of G-Score. The lowest energy posture, or "binding confirmation," indicates the efficacy of the docking method. The G-score and GLIDE energy of multitargeted proteins were noted. According to the docking complex, a hydrogen bond contact typically occurs at a distance of roughly 3 Å. The antioxidant target proteins were lipoxygenase (PDB ID 1N8Q), cytochrome p450 (PDB ID 1OG5), NADPH oxidase (PDB ID 2CDU), xanthine dehydrogenase/oxidase (PDB ID 3NRZ), and NADPH oxidase (PDB ID 1DNU). Among them, three target proteins were docked with NADPH oxidase, Cytochrome p450, and xanthine dehydrogenase/oxidase with the docking scores of −7.169, −4.455, and −4.899 Kcal/mol, respectively. The amino acid interaction is summarized in Supplementary Table S3, and their interaction with different protein chains is mentioned in the bracket of three-letter amino acid. Their target protein interaction is depicted in Figure 11. Similarly, caspase-3, nuclear factor NF-kappa-B p105 subunits, cellular tumor antigen p53, and mucosal address in cell adhesion molecule 1 were docked with fucoidan for anticancer studies, and the docking score results were as follows: −5.062, −3.219, −6.205, and −4.222 Kcal/mol, respectively. The interaction among these components is depicted in Figure 12.

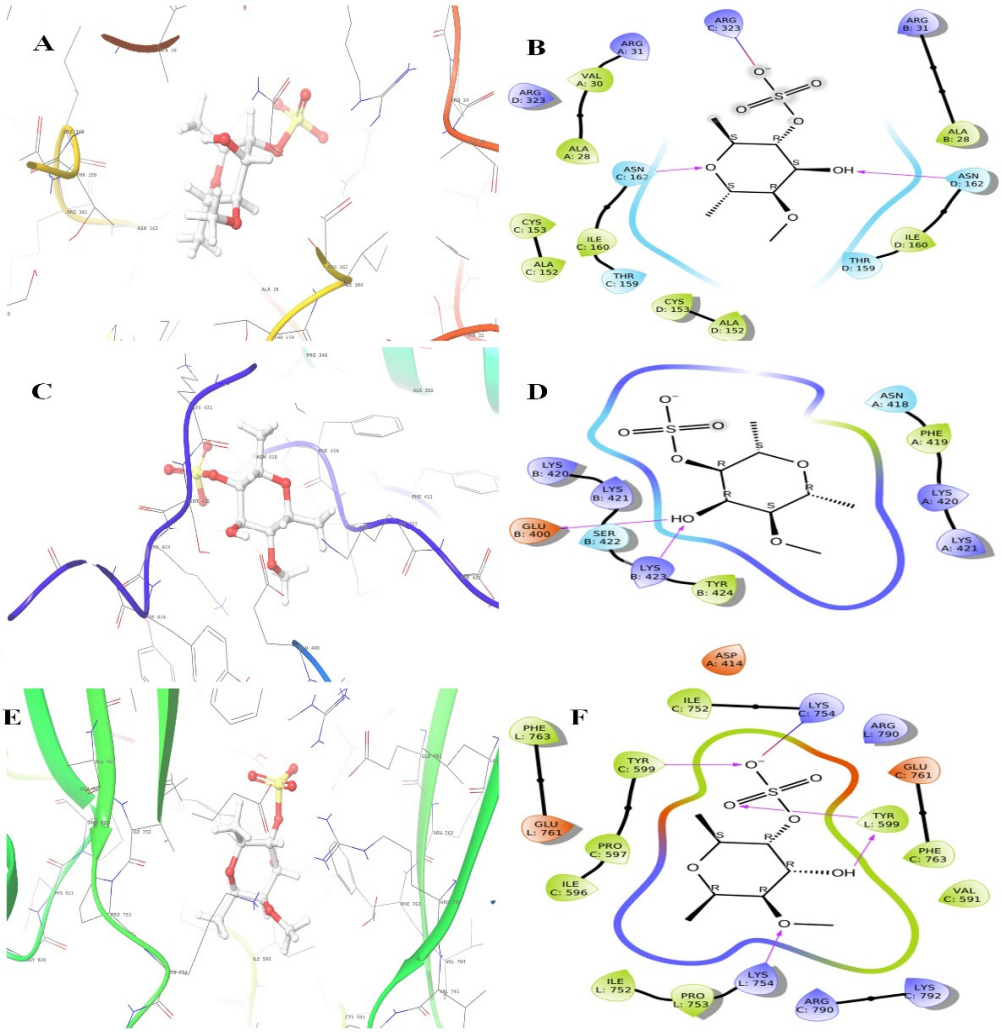

Figure 11. The three-dimensional and two-dimensional structure of docked compound (fucoidan) with antioxidant target proteins were depicted. (**A**,**B**) represents the NADPH oxidase, (**C**,**D**) for

cytochrome p450 and (**E**,**F**) for xanthine dehydrogenase/oxidase. The interaction was observed as hydroxyl and epoxy group of fucoidan.

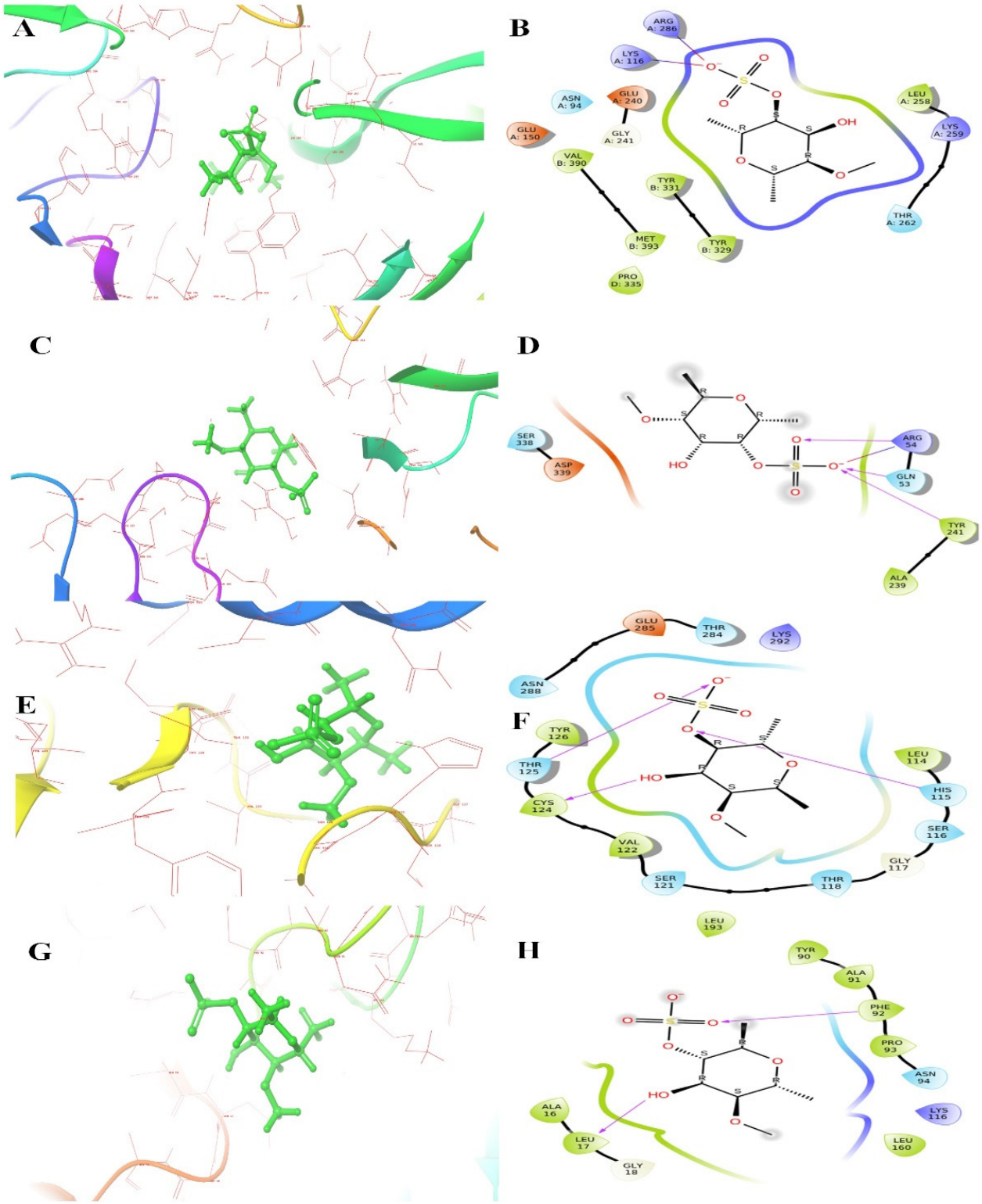

Figure 12. The three-dimensional and two-dimensional structures of docked compound (fucoidan) with antioxidant target proteins were represented as caspase-3 (**A**,**B**), nuclear factor NF-kappa-B p105 subunits (**C**,**D**), cellular tumor antigen p53 (**E**,**F**), and mucosal address in cell adhesion molecule 1 (**G**,**H**). The interaction was observed as hydroxyl and epoxy group of fucoidan.

4. Discussion

The abundance of polysaccharides in the naturally occurring sea algae is directly associated with the presence of several kinds of biomolecules. Furthermore, the polysaccharides in the marine animals vary greatly from those in the terrestrial habitats. In addition to fucoidan, marine brown algae have been shown to include sulfated polysaccharides, such as alginate, which are not observed in other macroalgal species. The sulphate concentration of fucoidan was 8.07 ± 0.13%, which was much lower than the value reported for sulfated polysaccharides isolated from abalone [13]. The confirmation experiment results are closely connected to *Sargassum* sp. having a sulphate concentration of 47.6 ± 0.59% *w/w* by utilizing distilled water at 65 °C [11,48]. Sulfate (7.56%) and uronic acid (0.32%) were found in *S. fluitans* [49]. Carbohydrates, galactose, uronic acid, and sulphate groups were slightly changed depending on the extraction technique, season, and local environmental variations. However, the chemical composition of some species, such as *S. polycycystum*, *S. graminifolium*, and *S. wightii* are different [50–52].

Brown algal sulfated polysaccharide monosaccharide composition varies based on species, the extraction process, location, and climatic conditions [53–55]. The chemical composition of fucoidan changed according to the extraction procedure, analytical methodology, season, and location. *Sargassum tenerium* had 16.33% uronic acid, 39.04% fucose, 7.62% mannose, 31.50% galactose, and 56.89% carbohydrate. The presence of fucose (59.3 ± 0.43%) showed the carbohydrate content of isolated fucoidan [4]. A similar study was reported in *Turbinaria decurrens,* and the fucose content (59.3%) is present [55]. The xylose (3.01%) is present in *M. oxyspermum* [56]. The molecular weights of fucoidan might vary depending on geographical location, harvesting season, and species. The physical and chemical characteristics of these fucoidans varied depending on the species and population age [57]. The molecular weight varied in *U. pinnatifida* (51.7 kDa), *S. latissimi* (395.4 kDa), and *Cladosiphon* sp. (1927.2 kDa) [38]. Fucoidan isolated from *U. pinnatifida* had an MW of around 378 kDa. The solubility of seaweed was more or less prevalent in *S. wightii* and *S. plagiophyllum* [33,51]. Fucoidan derived from *S. fusiforme* had an MW of 12.4 kDa and a sulphate content of 7.5%, which did not inhibit angiogenesis in the HMEC-1 cells [8]. As for bioactivity, the molecular weight serves two functions. In general, high-molecular-weight fucoidans are more effective than low-molecular-weight fucoidans. However, low-molecular-weight fucoidans have superior pharmacokinetic properties [58]. The solubility of fucoidan from *S. plagiophyllum* and *Fucus vesiculosus* was the highest in water and DMSO [37,59]. All seaweeds exhibited the highest weight loss between 170 and 280 °C, which was related to the high carbohydrate contents of three species, such as *Sargassum thunbergii, Mastocarpus stellatus, Ulva* sp. [60]. In the last stage (550–800 °C), the weight of the seaweeds barely changed, and the remaining weight represented the ash content, which might contain the minerals of polysaccharides, such as sulfates, phosphates, and carbonates [61].

The FT-IR spectrum analysis of marine algae *Chondrus crispus* and lambda-carrageenan showed two weak bands at 815 and 830 cm^{-1}, which was consistent with the previous results [62]. The FT-IR analyses of marine brown algae *P. pavonica* and *S. vulgare* revealed a wide band (about 1195–1237 cm^{-1} for *Padina* and 1210–1280 cm^{-1} for *Sargassum*) ascribed to (S=O), a distinctive part in fucoidan [63]. The NMR study showed the chemical shift of fucoidan at an anomeric signal of 5.00–5.5 ppm, revealing that L pyrenose was concentrated in fucoidan [64]. The signals at 4.681 corresponded to 3-linked α-L-fucose, which was the main constituent of fucoidan [65]. The signal at 4.505 ppm (f β-d-Galactose (G) was linked to 3,6-α-l-anhydrogalactose in the sulfated polysaccharide from *M. oxyspermum* [65]. The fucoidan from *S. siliquosum* possesses (1→3)-linked or (1→4)-linked L-fucose residues as the backbone structure [66]. A similar study on *Ecklonia maxima* shows that the signal at 1.10 ppm is linked to the C6 methyl protons of L-fucose. The peaks in the range of 3.4–4.0 ppm denote the sugar residues [67]. The AFM is a single molecular spectroscopic technology to detect the conformational changes in morphological structures of polysaccharides and molecular assembling. AFM provides visualization of different types of sulfated

polysaccharides [68]. In a similar study, AFM was employed to analyze *S. plagiophyllum* [33]. The AFM image shows a three-dimensional view of the spherical shape with its polydispersity nature [69]. The SEM image of F2-fucoidan shows a worm-like structure in *S. plagiophylum* [33]. The SEM image of crude seaweed polysaccharide shows the irregular shape of *Sargassum longifolium*, which is highly undesirable for biomedical applications [70].

The antioxidant properties of isolated fucoidans were investigated by their physical differences related to molecular weights. The extraction analysis revealed the chemical composition of sulfated polysaccharides in *Fucus evanescens* and the amount of polyphenols in different extraction methods [71]. Fucoidan isolated from marine brown algae had the highest DPPH scavenging, reducing ability, and overall antioxidant activity in 1000 µg/mL of *Sargassum polycystum* (61.2, 67.56, and 65.3%) [49]. In a previous study, the IC_{50} of the DPPH scavenging activity of fucoidan extracted from *L. japonica* was found to be 3.7 mg/mL [72]. The DPPH scavenging activity was associated with a hydrogen atom or electron transfer reaction from the sulfate group to the free radical. Higher sulfate content corresponded to a higher antioxidant activity [56]. The sulfate group, activating the hydrogen atom of the anomeric carbon to adsorb the antioxidants exhibited the scavenging ability of *Sargassum thunbergii* [73]. The sulfated polysaccharides derived from *Dictyota menstrualis* and *Dyctiota mertensii* showed the maximum hydroxyl radical scavenging activity inhibition at 8.7 and 7.5%, while the superoxide radical scavengers from *Dictyota cervicornis* and *Dictyota deliculata* showed the highest scavenging activity at 29 and 32%, respectively [40]. *L. nigrescens* and *L. trabeculata* showed the highest scavenging activity in the superoxide and hydroxyl group experiments [74]. The polyphenols found in the seaweed extracts exhibit antioxidant activity. The free radical scavenging properties of sulfated polysaccharides were isolated from *S. myriocystum* [75]. The ABTS scavenging ability of *Monostroma oxyspermum* exhibited higher scavenging activity (25.37–76.81%) at various concentrations than the standard BHT and l-ascorbic acid [57]. Previous study results demonstrated that the inhibitory impact of sulfated polysaccharide on the formation of these radicals was higher in *Laminaria japonica* [72]. The reducing power is generally associated with the presence of reductones, which has been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom. The difference in the antioxidant activity of seaweed polysaccharides might be attributed to the influence of various factors, such as the structure, molecular weight (MW), and chemical composition [30]. The antioxidant activity having higher polyphenol contents in the soluble fraction has a tendency to precipitate. The highly effective polyphenol and flavonoid compounds with their hydroxyl group are present because of the potential scavenging free radicals' activity [76]. The compounds with anticoagulant properties are evaluated using different assays, such as APPT and PT [77]. A previous report showed that the effect of fucoidan on APTT was more active than on PT in *Fucus vesiculosus* [26] or the dry *Fucus vesiculosus* (DFE-1 and DFE-2) extract, which are ointments used in blood clotting measures for cutaneous application [78]. The fucoidans from brown algae (11 sps) with anticoagulant activity exhibited the highest inhibitory impact on thrombin and factor Xa in the presence or absence of thrombin inhibitor and antithrombin III [79]. Many sulfated galactans resemble agar and are a part of the agar group. These are agarose derivatives with higher sulphate groups and fewer 3, 6-anhydro-L-galactose residues. [80]. The purified fucoidan of *T. decurrens* showed a dose-dependent anticoagulation of 387.8 ± 10.3 s [81]. Heparin, a fucoidan could inhibit thrombin formation, but its anticoagulant efficacy is not the same as that of the latter. The anticoagulant and antithrombotic effects of fucoidan have been mediated by catalyzing thrombin inhibition mainly through heparin cofactor II inhibition [82].

The suppressed gene promotes cancer by enhancing unregulated cell growth and partition while breaching the cellular process, cell cycle detain, and cell decease. The molecular weight, monosaccharide content, polymeric backbone structure, and branching degree (protein, sulfate, uronic acid, and solubility) of polysaccharides play a vital role in the development of anticancer activity [24]. Similarly, the recent in vitro studies demonstrated that fucoidan from *T. conoides* showed a dose-dependent anti-proliferation in the HepG2

cancer cells [83]. The in vitro cytotoxicity of fucoidan-derived seaweed from *Sargassum polycystum* showed an IC_{50} of 50 µg/mL, inhibiting the MCF-7 cell line (90.40 ± 0.25%) [49]. Similarly, polysaccharides from the seaweed *C. caledoniae.* Kylin exerted higher anti-angiogenic effect on HeLa cell exposure by lowering VEGF appearance and emission, preventing cancer cells in the vascular tubule organization [84]. The fucoidan from various brown algae, including *L. saccharina*, *L. digitata*, *F. serratus*, *F.distichus*, and *F. vesiculosus* demonstrated an antitumor property that reduced the bond of MDA-MB 231 cancer cells to platelets by approximately 80% under lab conditions [85]. Additionally, fucoidans might induce apoptosis in the MCF-7 cells. In a previous study, the isolated fucoidan from marine macroalgae stimulated chromatin condensation under in vitro conditions of fragmented DNA. Furthermore, the isolated fucoidan might trigger the death of the MCF-7 cells through a caspase-8-dependent route. In vitro experiments showed that fucoidan isolated from the sea algae *Fucus evanescens* with a concentration of 100–1000 µg/mL showed the strongest virucidal and defensive impact on cells infected with tick-borne encephalitis [86].

5. Conclusions

The present study investigated the extraction, purification, and antioxidant activity of sulfated polysaccharides from *Sargassum ilicifolium*. Fucose, galactose, xylose, and uronic acids were detected in the sulfated polysaccharide monosaccharide composition. The DPPH, ABTS, and reducing activity tests revealed the radical scavenging activity of the sulfated polysaccharide samples, indicating that they might function as potent antioxidants. The molecular docking experiment was carried out for anticancer and antioxidant target proteins. The protein NADPH oxidase showed the highest content among the antioxidant targets with the lowest binding score (−7.169 Kcal/mol). Similarly, the cellular tumor antigen p53 protein showed the highest content among the anticancer targets with the lowest binding score (−6.205 Kcal/mol). The highest inhibition of anticancer activity against in vitro HepG2 cells and IC_{50} value was 71.33 µg/mL. These findings report the *S. ilicifolium* biological roles of polysaccharides and develop potential anticancer drugs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app122413010/s1, Table S1: Physico-chemical Property Solubility of fucoidan from *S. illicifollium* in different solvents. Table S2: Anticancer activity of Fucoidan from *Sargassum ilicifolium*. Table S3: The molecular docking experiment was carried out for the compound fucoidan against the target protein of antioxidant and anticancer studies. Their molecular interaction, bond length with glide scores were listed.

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