frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 20 December 2023
DOI 10.3389/fmars.2023.1327408

OPEN ACCESS

EDITED BY
El-Sayed Salama,
Lanzhou University, China

REVIEWED BY
Mohamed Ashour,
National Institute of Oceanography and Fisheries (NIOF), Egypt
Ahmed E. Alprol,
National Institute of Oceanography and Fisheries (NIOF), Egypt

*CORRESPONDENCE
Manel Dhahri
manel.dhahri@gmail.com

RECEIVED 24 October 2023
ACCEPTED 04 December 2023
PUBLISHED 20 December 2023

CITATION
Dhahri M (2023) *Cystoseira myrica*: from beach-cast seaweed to fucoidan with antioxidant and anticoagulant capacity. *Front. Mar. Sci.* 10:1327408. doi: 10.3389/fmars.2023.1327408

Cystoseira myrica: from beach-cast seaweed to fucoidan with antioxidant and anticoagulant capacity

Manel Dhahri*

Biology Department, Faculty of Science, Taibah University, Yanbu, Saudi Arabia

This study highlights the potential of the brown algae *Cystoseira myrica*, collected from raw beach seaweed wastes, as a reliable source of bioactive fucoidan. Fucoidans are natural bioactive sulfated polysaccharides that are gaining popularity worldwide due to their diverse biochemical composition, attractive physical properties, and functional pharmacological activities. The aim of this work is to valorize the beach-Accumulated brown algae *C. myrica* by fucoidan extraction and to investigate its anticoagulant and antioxidant activity. Fucoidan was extracted using several steps of papain digestion followed by cetyltrimethylammonium bromide (CTAB) precipitation and calcium chloride treatment to avoid the coextraction of other polysaccharides. Structural features of the extracted fucoidan were investigated by Fourier transform infrared spectroscopy (FT-IR), solid-state nuclear magnetic resonance (NMR) and high-resolution mass spectroscopy. Agarose gel electrophoresis was used to confirm the purity of the isolated fucoidan from *C. myrica.* Anticoagulant properties were studied *in vitro* by activated partial thromboplastin time (aPTT) and prothrombin time (PT) assays. Furthermore, the antioxidant activity was investigated by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Fe chelating ability. Our results indicate that the approach used was effective in extracting fucoidan with a yield of 3.07%, a high amount of sulfate (27.79%), and fucose was found to be the major monosaccharide component. The extracted fucoidan showed an interesting anticoagulant activity. It prolonged aPTT significantly at a concentration of 10 µg/ml and prolonged PT at high doses. This demonstrated that fucoidan from *C. myrica* may affect intrinsic pathways while having little impact on the extrinsic mechanism of coagulation. However, the extracted fucoidan significantly exhibited an interesting antioxidant capacity as shown by the higher ABTS radical scavenging activity and Ferrous ion-chelating effect. The current findings suggest that fucoidan isolated from *C. myrica* has unique structural, antioxidant and anticoagulant properties and offers innovative therapeutic possibilities.

KEYWORDS

marine waste, brown algae, fucoidan, sulfate, antioxidant, anticoagulant

1 Introduction

Marine biomass is a valuable biotechnological resource that exhibits a wide range of composition and functional capabilities, owing to the presence of diverse bioactive components such as polyphenols, peptides, and polysaccharides (Rudovica et al., 2021). Algae have garnered significant attention in the discipline of biotechnology due to their economic potential, as they are abundantly produced in nature and often discarded as waste material on beaches. The composition of algal biomass makes it a viable option for a growing array of applications, which continues to expand in scope (González Fernández et al., 2023). The presence of algae in coastal environments can be turned from an unpleasant and potentially hazardous phenomenon into a source of pharmaceutical candidates. Seaweeds are becoming more fascinating due to their potential as a renewable reservoir of bioactive chemicals (Ashour et al., 2022). The expanding range of uses for seaweeds in the food, feed (Abdelrhman et al., 2022), cosmetic, and medicine sector highlights the need to investigate their chemical composition (El-Shenody et al., 2019). A notable amount of attention is being paid to pharmaceutical candidates from new natural marine resources that have increased health-promoting qualities (Shinde et al., 2019; Jin et al., 2023). Marine species are rich in sources of beneficial metabolites. Marine polysaccharides, among the marine-based valued bioactive substances, are known as unique and attractive resources for the food and pharmaceutical industries (Mousavian et al., 2022). Great amounts of polysaccharides with different chemical properties and several bioactivities are found in seaweeds (Yu et al., 2021). Among these, fucoidan has shown promise as a therapeutic biomolecule. Fucoidans are sulfated heteropolysaccharides discovered in 1913, found in marine invertebrates and brown seaweed (Jin et al., 2013). They are predominantly made of fucose residues and sulfate groups. Fucoidan has a complex structure and is commonly composed of (1 → 3)- L-fucopyranose monomers that have a sulfate group at C-4. However, further C-2 sulfation and 2-O-a-L-fucopyranosyl branching were reported (Yu et al., 2021; Zayed et al., 2023).They also include minor amounts of protein, galactose, mannose, xylose, glucose, and uronic acids (Mak et al., 2014). They have wide ranging biological and therapeutic activities, including anti-inflammatory, antioxidant, anti-coagulant, anti-viral, anti-tumor, anti-allergic, anti-hepatopathy and immunomodulatory agents. The Food and Drug Administration (FDA) has authorized fucoidan for use in the generally recognized as safe (GRAS) category (Citkowska et al., 2019). Particularly in the recent years, there has been a significant increase in the number of published articles about fucoidan and their therapeutic effects (Wang et al., 2019; Wen et al., 2021). It has been widely reported that the chemical and pharmacological properties of fucoidan vary significantly depending on the algal species, location, harvest season, technique of extraction, and other factors (Lim and Aida, 2017; Qu et al., 2019). Cardiovascular diseases are amongst the most prevalent worldwide and their fatality rate is increasing year after year (Michel et al., 2020). Cardiovascular diseases are treated with anticoagulants, antioxidants, and anti-inflammatories (Manggau et al., 2022). Anticoagulant and antithrombotic compounds work by reducing platelet aggregation and thrombus formation, blocking the coagulation system, and interfering with future plaque extension. Fucoidans have shown promise as a preventive and alternative treatment for thrombus-induced cardiac and cerebrovascular problems. In addition to their potential applications as heparin-like effect compounds, they have also increasingly been studied for their biodegradability and non-toxicity. The body's production of reactive nitrogen species and reactive oxygen species (ROS) encourages oxidative stress, which has several physiological effects, including risk of thrombus formation and platelet hyperactivity. Previous work has revealed that reactive oxygen species can influence both thrombus development and resolution (Gutmann et al., 2020). In addition, it is well established that platelet activation is reduced in the presence of antioxidants such as vitamin C and polyphenols (Olas, 2022), as well as being increased in the presence of ROS donors, showing the significance of ROS-mediated effects on platelet activation (Gutmann et al., 2020).

The unique ecosystems and adaptations of the Red Sea in Saudi Arabia have made it a potentially valuable resource for bioactive chemicals with remarkable biological activity. Marine macroalgae are receiving more recognition in the biotechnology sector due to their economic prospects. This is mostly due to their abundance in the environment and the fact that they are often thrown as waste material on beaches. Additionally, they are rich in distinctive bioactive chemicals. In this study, the brown algae *C. myrica*, a beach-cast macroalgae (Figure 1), was evaluated to isolate fucoidan as an additional, value-added product. To the best of our knowledge, no prior work has extracted fucoidan from the above-mentioned algae and studied its bioactivity. As a result, the objective of this study was to extract fucoidan by combining papain extraction, CTAB and $CaCl_2$ precipitation from the macroalgae biomass (*C. myrica)* collected from the coast of Yanbu, Saudi Arabia

FIGURE 1
Beach-Accumulated brown algae *C. myrica* from the Yanbu coast, Saudi Arabia.

in order to avoid the coextraction of other polysaccharides. The chemical composition and proprieties of fucoidan were assessed by colorimetric assays, Fourier-transform infrared spectroscopy (FTIR), NMR and high-resolution mass spectroscopy. Its antioxidant and anticoagulant capacities were also tested with several *in vitro* techniques.

2 Materials and methods

2.1 Fucoidan extraction from the biomass macroalgae

C. myrica algae were collected from coastlines in Yanbu, Saudi Arabia (Figure 1). Seaweed was immediately rinsed in seawater upon collecting and then washed several times with clean water to remove co-collected impurities. The algae were dried at low temperatures before being processed to improve the surface-to-volume ratio. Before fucoidan extraction, algae powder was treated with ethanol twice to remove low molecular weight components, pigments and liposoluble compounds and then filtered, dried, and stored at -80°C. Fucoidan, laminarin and alginate are the three principal polysaccharides found in brown algae (Li et al., 2021). Firstly, sulfated polysaccharides were extracted by enzymatic digestion with papain (100 mg) for 24 hours at 60°C (Dhahri et al., 2020). Then, fucoidan was precipitated with 0.4% (w/v) of the cationic detergent cetyltrimethylammonium bromide (CTAB) (Dhahri et al., 2020). This step is added to avoid the coextraction of the Laminarin, a polysaccharide naturally found in brown algae. Laminarin is a neutral polysaccharide and so will not react with CTAB and remain water soluble (Dobrinčić et al., 2020). Then, to eliminate alginate, polysaccharide solution was precipitated by $CaCl_2$ 2%, and the solution was kept at 4°C overnight to precipitate alginate. The supernatant was recuperated after centrifugation at 14000 rpm for 10 min. Fucoidan was precipitated by ethanol and the precipitate was recuperated by centrifugation at 10000 rpm for 20 min. To eliminate protein, fucoidan was treated by the Sevag method (Li et al., 2019). The extracted fucoidan was dialyzed with a cut-off value of 3500 Da and finally lyophilized to obtain the crude fucoidan F_{Cm}.

2.2 Physicochemical characterization

2.2.1 Chemical composition analysis

The Dische method (Dische and Shettles, 1948) and Carbazole (Cesaretti et al., 2003) method were used to determine the amount of fucose and uronic acid. Fucose and uronic acid content were estimated by using the slopes of standards. The amount of sulfate was determined by sulfate assay kit (MAK132) from sigma Aldrich. The results are represented as weight % of the dry sample. Elemental analysis was used as described by Dhahri et al. to estimate the molar ratio of carbon (C), hydrogen (H), sulfur (S), and nitrogen (N) (Dhahri et al., 2020).

2.2.2 Polysaccharide hydrolysis, derivatization, and high-resolution mass spectroscopy

According to Dhahri et al, F_{Cm} was hydrolyzed with TFA (4 M) and then derivatized using 1-phenyl-3-methyl-5-pyrazolone (PMP) as well as standards (Dhahri et al., 2023). We used an Orbitrap IDX spectrometer which is characterized by a dependable mass accuracy (5 ppm mass error) and high resolution (> 120,000) to evaluate the m/z of the extracted polysaccharides. Samples were analyzed in positive mode electrospray ionization. The derivatized samples were chromatographed using an Acquity UPLC HSS C18 column (2.1x100mm, 1.8µ). For separation, the mobile phase A was water + 0.1% formic acid + 25 mM ammonium formate. The mobile phase B was acetonitrile + 0.1% formic acid. Monosaccharide standards (fucose, galactose, glucose, xylose, arabinose mannose and rhamnose) were used for the detection and quantitation of monosaccharides in extracted fucoidan, F_{Cm}.

2.2.3 Spectroscopic analysis

The structural properties of F_{Cm} were studied by FT-IR analysis and solid-state ^{13}C NMR and compared to fucoidan standard purchased from Molekula. Briefly, samples including F_{Cm} and standard fucoidan were mixed with KBr, pressed, and examined using an FT-IR spectrometer (Nicolet FTIR iS10, Thermo Scientific, USA) with a scanning range of 400 to 4000 cm^{-1}. The solid-state ^{13}C NMR spectrum was analyzed using the WB Bruker 400 AVANCE III spectrometer (Bruker BioSpin, Rheinstetten, Germany) (Alkordi et al., 2015; Chisca et al., 2015; Dhahri et al., 2020).

2.2.4 Agarose gel electrophoresis

The purity of the extracted fucoidan was studied by agarose gel electrophoresis, performed as described by Stelling et al. (Stelling et al., 2019). Agarose gel (0.5%, w/v) in 0.05 M 1,3 diamino propane/acetate buffer (pH 9.0) was used to separate 10 µg of F_{Cm} and fucoidan standard. The separated samples were fixed with 0.1% CTAB before staining with 0.1% toluidine blue in acetic acid: ethanol:water (0.1:5:5, v/v). Finally, acetic acid:ethanol (0.1:1,v/v) was used for destaining.

2.3 Anticoagulant activity

The anticoagulant activity of extracted fucoidan was evaluated by using human plasma (P9523) from sigma. Before testing, the plasma was prepared as described by Escobedo et al. (Escobedo et al., 2006). Briefly, after warming at room temperature, the plasma was reconstituted by adding the appropriate amount of deionized water and the liquid was gently swirled until a suspension is formed. Plasma was used in the following assays to study the anticoagulant activity. F_{Cm} solution at various concentrations was prepared in HEPES buffer (20 mM HEPES, 150 mM NaCl, 0.1% polyethylene glycol Mr 8000, pH 7.4) (Pratt and Monroe, 1992). The clotting agents were injected into the tested samples using a multichannel pipette. The anticoagulant assays were performed on a Synergy 2 Multi-Mode Microplate reader

in kinetic mode and coagulation time was calculated from the absorbance measured at 405 nm (Pratt and Monroe, 1992; Indumathi and Mehta, 2016).

2.3.1 Activated partial thromboplastin time assay

aPTT assays were performed as described by Pratt et al. (Pratt and Monroe, 1992). Briefly, 30 µl of plasma was incubated with 30 µl of various concentrations of F_{Cm} solution and 30 µl of aPTT reagent in microplate wells. Heparin was used as a reference. The plate was incubated for 5 min at 25°C and the absorbance was recorded at 405 nm for 1200 s. Assays were performed in triplicate.

2.3.2 Prothrombin time assay

PT assay was performed by mixing 30 µl of plasma with 30 µl of F_{Cm} solution at various concentrations. After 2 min of incubation, clotting was initiated by adding 30 µl of 5% thromboplastin in 25 mM in 25 mM CaCl2. The absorbance at 405 nm was recorded for 1200 s. The rate of change of absorbance was proportional to clot formation.

2.3.3 Fluorometric assay

Factor thrombin and Xa screening kits were used according to the manufacturer's instructions to detect the direct inhibition of F_{Cm} on thrombin and factor Xa. The factor Xa inhibitor screening kit (MAK239) and thrombin Inhibitor Screening Kit (MAK243) from sigma use the capability of Factor Xa and thrombin, respectively, to cleave a synthetic substrate, thereby releasing a fluorophore, AMC, which can be measured by a fluorescence reader. In the presence of a Factor Xa inhibitor or thrombin specific inhibitors, the amount of the cleavage reaction is decreased or fully stopped. The loss in the fluorescence intensity can be correlated to the amount of inhibitor present in the assay solution.

2.4 Antioxidant activities

2.4.1 ABTS radical scavenging assay

ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting the ABTS solution (7 mM) with potassium persulfate (2.45 mM). After incubation at room temperature in the dark for 12 to 16 hours, the $ABTS^{\bullet+}$ solution was diluted with PBS to get an absorbance at 734 nm of 0.7. Then, 10 µl of F_{Cm} or fucoidan standard purchased from Molekula was mixed with 190 µl ABTS reagent in 96 well plate (Acharya, 2017). After shaking the plate and incubating for 5 minutes in the dark, the absorbance was determined at 734 nm. The following equation was used to calculate the degree of scavenging:

$$ABTS\ scavenging\ effect\ (\%) = \frac{Abs0 - Abs1}{Abs0} \times 100$$

Where: Abs0 is the absorbance of the control (water instead of the sample) and Abs1 is the absorbance of the sample.

2.4.2 Metal chelating assay

Ferrous ion chelating activity was investigated according to the method described by Zhang et al. (Zhang et al., 2019) and compared to fucoidan standard purchased from Molekula. In 96-well microplate, the reaction mixture including 50 µL of samples at various concentrations, 160µL of deionized water and 20µL of FeSO4 solution (0.30 mmol/L) was incubated for 5 min at room temperature. 30µL of ferrozine solution (0.80 mmol/L) was then added to each well. The absorbance was measured at 562 nm, after 15 min of incubation and the capacity of samples to chelate ferrous ion was calculated using the following equation:

$$Chelating\ ability\ (\%) = \frac{Abs0 - Abs1}{Abs0} \times 100$$

Abs0 is the absorbance of the control (water instead of the sample) and Abs1 is the absorbance of the sample.

2.5 Statistical analysis

Analysis was carried out in triplicate and results are presented as mean standard deviation (SD). ANOVA was used to evaluate the data, and differences are considered statistically significant if the value of $p < 0.05$.

3 Results and discussion

3.1 Extraction

Sulfated polysaccharides isolated from brown algae are recognized as fucoidans (Usov et al., 2022). The process of commercial manufacture of fucoidans from brown algal biomass was first proposed in 1952 by Black et al. (Black et al., 1952) and has been adjusted recently, aiming to enhance yields and purity (Jayawardena et al., 2022). *Fucus vesiculosus* and *Ascophyllum nodosum* are the two popular sources of commercial fucoidans, which have similar chemical and biological characteristics (Usov et al., 2022). Numerous studies are currently being conducted to isolate fucoidans with interesting therapeutic effects using various extraction techniques. In this study, fucoidan was extracted from *Cystoseira myrica* by combining papain extraction, CTAB and $CaCl_2$ precipitation. Fucoidan was obtained with a ratio of 3.07% and contains 3.25% of uronic acid and 35.12% of fucose (Table 1). This fucoidan yield was higher than that extracted from brown seaweed (Lakshmanasenthil et al., 2014; Fauziee et al., 2021; Tsou et al., 2022). For instance, fucoidan was extracted from brown seaweed *T. ornata* with a yield of 1.8% (Lakshmanasenthil et al., 2014). Fucoidan obtained by isolation with water and ethanol gradient precipitation from the brown seaweed *Sargassum pallidum* had a ratio of isolated concentrations ranging between 0.31% to 0.96% and had low amounts of sulfate (3.84-6.85%) (Xin et al., 2016). In addition, fucoidan was extracted from *S. polycystum* with a ratio of 4.51 ± 0.24% and contained 38.76 ± 0.26%, 22.35 ±

0.23%, 3.9 ± 1.8%, and 4.7 ± 0.43% of carbohydrate, sulphate, uronic acid and protein, respectively (Palanisamy et al., 2017). Extraction method, seaweed harvesting season, location, and level of maturation may all influence fucoidan yield and chemical composition (Rani et al., 2017; Baba et al., 2018). Recently, Tsou et al, has extracted fucoidan samples at two temperatures (65 and 80°C) from *Sargassum ilicifolium* collected from five different locations in southern Taiwan and confirmed that location and temperature of extraction can significantly influence the yield (1.47%-2,73%) and the chemical composition of fucoidan (Tsou et al., 2022). The amount of sulfate and the position of sulfation have been reported to influence the bioactivity of fucoidan as well as the amount of fucose (Jayawardena et al., 2022). The amount of sulfate in the extracted fucoidan F_{Cm} is comparable to fucoidan obtained from *S. polycystum (*Palanisamy et al., 2017*)* and higher when compared to the brown seaweed *S. pallidum* (Xin et al., 2016). A sulfated polysaccharide extracted from *C. myrica* collected from the Jazan coasts, Saudi Arabia, with an antiviral effect present with an amount of sulfate of 22.3% (Mohamed et al., 2015). As shown in Table 2, the carbon (C%), hydrogen (H%) and sulfur content (S%) were comparable to commercial fucoidan. In contrast, the isolated fucoidan had low nitrogen content (N%), differing from commercial fucoidan that lacks nitrogen, indicative of the presence of some amino-containing compounds (amino sugars or protein) (Saravana et al., 2016). Additionally, the monosaccharide composition of fucoidan isolated from *C. myrica* was determined by high-resolution mass spectrometry (HR-MS) and summarized in Table 1. F_{Cm} contains fucose as the major sugar component with a ratio of 86.48%. In addition, galactose (0.42%), glucose (1.26%), xylose (0.42%), and arabinose (0.42%) were also detected. The result is comparable to the monosaccharide composition of fucoidan from *F. vesiculosus* that shows a high content of fucose of 84.0%, in addition to the presence of galactose (7.3%), xylose (6.0%) and mannose (2.0%) (Lahrsen et al., 2018). Hence, the algal species and extraction method have a strong influence on the chemical composition of fucoidan. For instance, fucoidan isolated from *U. pinnatifida* contains four major monosaccharides including fucose, galactose, xylose, and glucose, which were also found in different proportions in the fucoidan standard from the brown seaweed species U. pinnatifida (Koh et al., 2019). *C. myrica* could be an interesting source of "good quality fucoidan" given that F_{Cm} presents a high level of fucose and sulfate (January et al., 2019).

3.2 Spectroscopic analysis

FTIR and NMR analysis are efficient techniques to elucidate structural properties and have been widely used by researchers to characterize extracted fucoidan from natural sources. The FTIR spectra shown in Figure 2 indicates that the extracted sulfate polysaccharide from *Cystoseira myrica* presents similar structural properties to commercial fucoidan. It is possible to see two distinct absorbance bands at 3423 cm^{-1} and 2940cm^{-1}, which are the most prevalent spectral features related to CH and OH stretching of polysaccharides. The wavelength range between 550-1800 cm^{-1} is the fingerprint region for fucoidan in the FTIR spectra. The peaks at 2929 cm^{-1} and 1627 cm^{-1} relate to CH stretching vibration and to the presence of a carbonyl group from uronic acid (Ganapathy et al., 2019). The bands ranging from 1200 to 970 cm^{-1} suggest the presence of C-O-C glycosidic linkages as well as C-C and C-O-C pyranose rings (Daub et al., 2020). The IR band at 1412 cm^{-1} can be attributed to stretching of the neutral monosaccharide -CH_2 groups and the fucosyl residue -CH_3 groups (Apostolova et al., 2022). The O=S=O stretching vibrations of sulfates, a distinctive characteristic of sulfated polysaccharides like fucoidans, can be attributed to the major peak between 1220 and 1270 cm^{-1} and a smaller peak at 583 cm^{-1} (Fernando et al., 2020) (Daub et al., 2020). The peak around 820 cm^{-1} corresponds to the bending vibration of COS (Fernando et al., 2017).

Solid-state NMR (Figure 3) showed structural similarities between extracted fucoidan and commercial fucoidan. As described in the literature, the spectra displayed strong chemical shift signals at 175.7 for fucoidan and 180.2 for F_{Cm}, that indicate the presence of the carbonyl group. In addition, an intense peak at 101.1 for fucoidan and 100.8 related to the anomeric carbon (C1) (Lim et al., 2016; Ganapathy et al., 2019). The difference between the anomeric carbons (C1) of F_{Cm} and fucoidan standard could be due to the position of sulphate groups, which influence the chemical shift of anomeric carbons, as illustrated by Daniel et al. (Daniel et al., 2001; Lim et al., 2016). The intense peaks at 17.1 ppm (F_{Cm}) and 16.96 ppm (fucoidan standard) were due to methyl groups (C6) of α-L-fucopyranose units (Synytsya et al., 2010; Lim et al., 2016). A comparable chemical shift at 17.92 ppm was obtained with α-fucose monomer. It is well recognized that location, season, and environment can affect the chemical properties of fucoidan (Lim et al., 2016).

3.3 Agarose gel electrophoresis

Agarose gel electrophoresis was used to confirm the purity of the isolated fucoidan from *C. myrica*. An agarose gel of F_{Cm} stained with toluidine blue showed a clear single spot that migrates as far as the fucoidan standard (Figure 3D), validating the purity and the presence of fucoidan in F_{Cm}. The results confirmed the effectiveness of the employed technique by combining enzymatic digestion, CTAB precipitation and calcium chloride treatment for fucoidan

TABLE 1 Chemical composition of fucoidan isolated from *C. Myrica*.

Ratio (%)	Uronic acid (%)	Fucose (%)	Sulfate (%)	Monosaccharide composition (%)				
				Fucose	Galactose	Glucose	Xylose	Arabinose
3.07	3.25	35.12	27.79	86.48	0.42	1.26	0.42	0.42

TABLE 2 Elemental analysis results of extracted and commercial fucoidan.

	F_{Cm}	Fucoidan standard
N (%)	1.682	0
C (%)	26.630	28.117
H (%)	4.893	5.254
S (%)	6.216	6.495
DS [1]	0.525	0.519

[1]DS = 2.25 × % S/% C.

extraction. The small difference in the electrophoretic mobilities was caused by the complex formed between sulfated polysaccharides and diamine, which resulted in CTAB precipitation. These changes resulted from the structure of fucoidan and sulfated patterns (Ganapathy et al., 2019).

3.4 Anticoagulant activities

The anti-coagulant activity of F_{Cm} was studied *in vitro* by aPTT and PT assays that characterized the pathways of the cascade of coagulation. APTT assay evaluated coagulation activity via both the intrinsic and common coagulation pathways, and PT assay mostly evaluated the extrinsic pathway (Yao and Yim, 2021). These pathways converge at the common point where prothrombin is transformed to thrombin, resulting in the production of fibrin threads (Dhahri et al., 2020). In the current study, anticoagulant activity of F_{Cm} was studied by microplate reader which is highly reproducible and allows us the analysis of a large number of samples at the same time (Walkowiak et al., 1997).

As shown in Figure 4, F_{Cm} prolonged clotting time in a concentration dependent manner. Several previous studies have found that fucoidan prolongs APTT. At a concentration of 10 µg/ml, F_{Cm} prolonged aPTT significantly by 1.5-fold. F_{Cm} was more active than fucoidan isolated and purified from *Ceratophyllum Submersum L.* (16.38% of sulfate, 42.11% of fucose) and extended the clotting time at 500 µg/ml by approximately 2-fold compared with our fucoidan that doesn't show any clot formation at the same concentration (Figure 4). The effect of fucoidan extracted from brown seaweed *F. vesiculosus L.* (27.0% of sulfate) of the Barents Sea was slightly higher than the effect of F_{Cm} that causes a prolongation of aPTT by 2.5-fold at 10 µg/ml (Pozharitskaya et al., 2020). F_{Cm} had a potential anticoagulant activity but lower than that of heparin ($p<0.05$). In fact, the same effect of heparin (10µg/ml) was obtained at 500 µg/ml of F_{Cm}. The effect of F_{Cm} on aPTT was more pronounced than in the PT assay as shown in Figure 5. The result of the PT assay was expressed as the ratio of the clotting time in the presence and absence of fucoidan (Figure 5). F_{Cm} doesn't show any effect at low concentrations ranging from 10 to 100 µg/ml. However, F_{Cm} concentrations above 200 µg/ml resulted in an extension of prothrombin time. The greatest prolongation of 2.67-fold was obtained with F_{Cm} at 500 µg/ml. These findings are comparable to fucoidan isolated from *F. vesiculosus L.* and *Tasmanian U. pinnatifida*, that had no effect on PT *in vitro* at low concentrations, but high concentrations above 80 µg/ml and 125 µg/ml, respectively, enhanced PT (Irhimeh et al., 2009; Pozharitskaya et al., 2020). Consequently, fucoidan from *C. myrica* demonstrated strong anti-coagulant effects. At high doses, it prolongs PT and considerably prolongs aPTT in a concentration dependent manner. This demonstrated that F_{Cm} may affect intrinsic pathways while having little impact on the extrinsic mechanism of coagulation.

The inhibition of enzymes and cofactors of the common pathway may also cause an increase of aPTT. The common pathway begins with the activation of factor X (to factor Xa) via either the extrinsic or intrinsic pathways. It is the last step in the coagulation cascade and results in the synthesis of thrombin and fibrin (Palta et al., 2014). For this reason, fluorometric assays using thrombin and factor Xa inhibitor screening kits were employed to check the direct effect of the extracted fucoidan on thrombin or factor Xa. No inhibition was observed when thrombin or factor Xa was incubated with F_{Cm} alone over a range of concentrations (results not shown). The anticoagulant mechanism of fucoidan

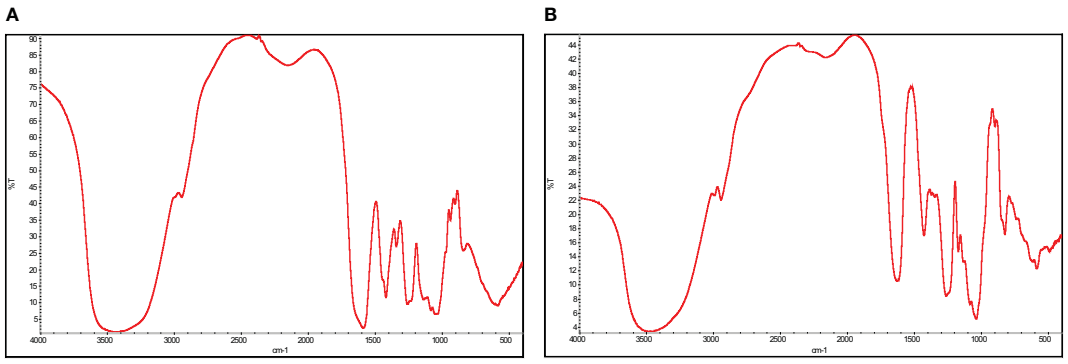

FIGURE 2
Fourier transform infrared analysis of the extracted fucoidan, F_{Cm}, from *Cystoseira myrica* **(A)** compared to standard fucoidan **(B)**.

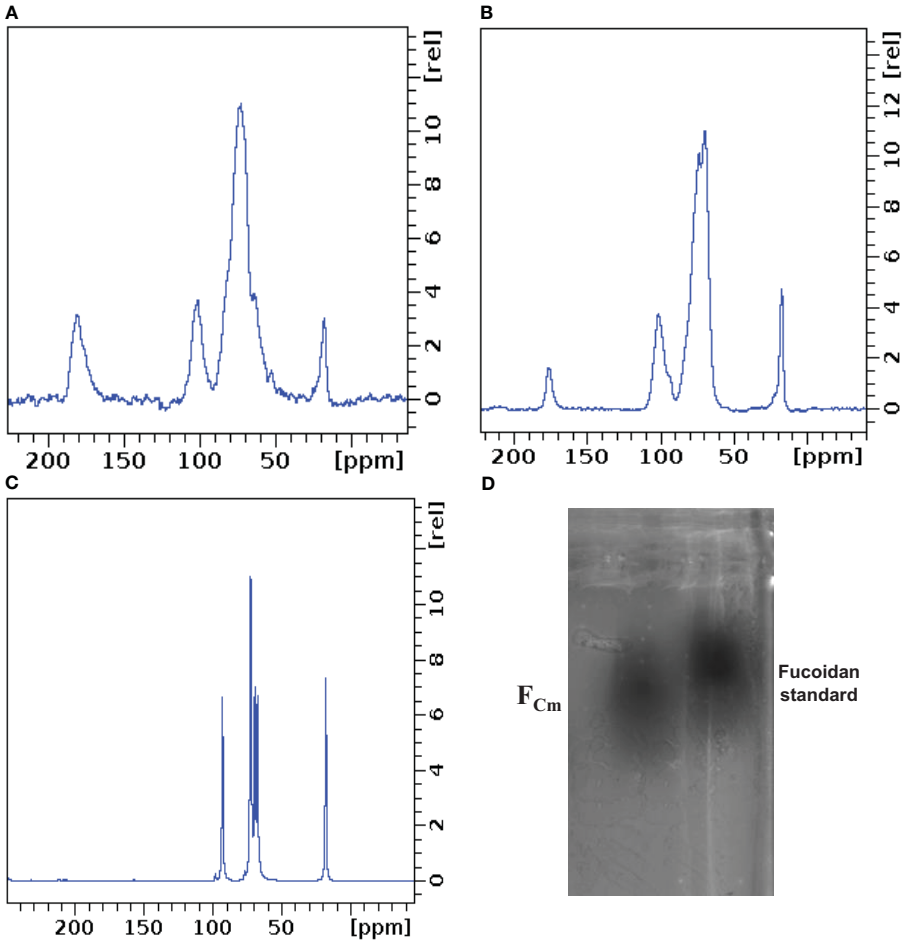

FIGURE 3
Solid state NMR of F_{Cm} **(A)**, fucoidan standard **(B)** and fucose **(C)**. Agarose gel electrophoresis of F_{Cm} and fucoidan **(D)**.

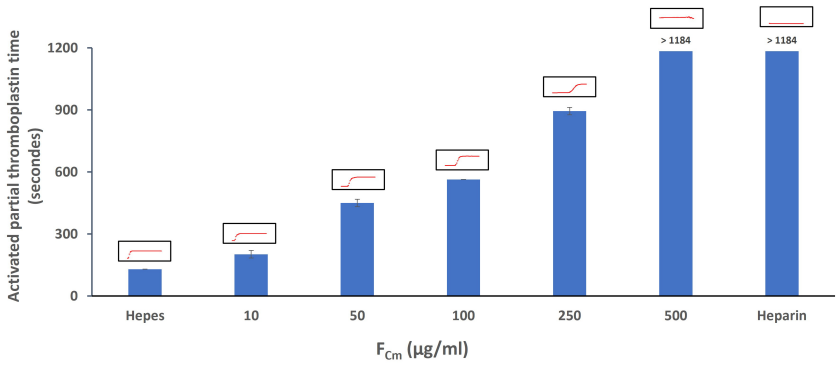

FIGURE 4
Activated partial thromboplastin time in the absence or presence of extracted fucoidan FCm (10 - 500 µg/mL) and standard heparin (10µg/ml). The red curves correspond to the actual instrument output of the reading on a microplate well, indicating increasing absorbance at 405 nm as a function of time.

has been previously studied. Fucoidan, like heparin, involves antithrombin or heparin cofactor II for thrombin and factor Xa inhibition (Lapikova et al., 2008). The antithrombotic effect *in vivo* has also been confirmed in several studies. Ustyuzhanina et al. studied the antithrombotic activities of three fucoidans from brown seaweeds that have different structural properties including sulfate content, sulfation pattern and monosaccharide composition (Ustyuzhanina et al., 2013). Molecular weight, glycoside

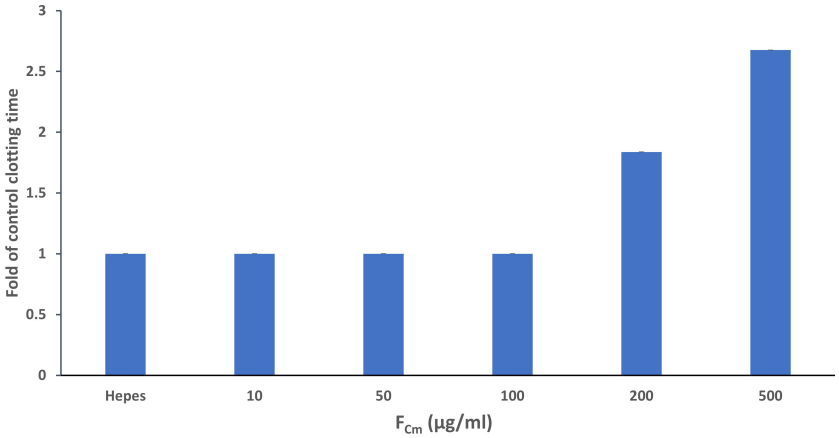

FIGURE 5
Prothrombin time in the absence or presence of extracted fucoidan F_{Cm} (10 −500 µg/mL). Data shown are the mean and standard deviation.

branching, sulfate/total sugar ratio, sulfate content and position have a significant impact on the anticoagulant activity of fucoidan (Wang et al., 2019). For instance, Jin et al. compared the anticoagulant activity of eleven fucoidans by studying the correlation between structure and anticoagulant activity. They also showed that the anticoagulant action of fucoidans was influenced by both the molar ratio of fucose to galactose and the average molecular weight (Zayed et al., 2023).

3.5 Antioxidant activities

The antioxidant activity of extracted fucoidan was studied by ABTS assay and Fe chelating test and compared to commercial fucoidan as shown in Figure 6.

3.5.1 ABTS assay

The ABTS scavenging assay depends on the decolorization of $ABTS^{\bullet+}$ when it reacts with a hydrogen-donating antioxidant (Huang et al., 2018). F_{Cm} showed ABTS radical scavenging activity dose-dependently (Figure 6A). It was also found that the antioxidant activity of F_{Cm} was 93.6% at concentrations of 5000 µg/ml and the IC50 was 1148 ± 0.02 µg/mL. The ABTS radical scavenging effect of F_{Cm} is similar to the antioxidant effect of fucoidan extracted from *Kjellmaniella crassifolia* (Tang et al., 2022). In contrast, commercial fucoidan shows a very low scavenging effect of 5.98 ± 0.6% at 5000 µg/ml concentration, with a comparable amount of sulfate (Table 1). Barahona et al. studied the kinetic profiles of the antioxidant capacity of seaweed polysaccharides with $ABTS^{+}$ radical cations and confirmed that sulfate content and antioxidant activity were not correlated, whereas monosulfation position in the monosaccharide residues and antioxidant capacity were found to be positively correlated (Barahona et al., 2011). In addition, the link between antioxidant activity and chemical structure was studied for five fucoidans from different brown seaweeds, showing that although a sulfate group is necessary for antioxidant activity, fucoidans with high sulfate group content did not necessarily exhibit higher antioxidant activity (Ajisaka et al.,

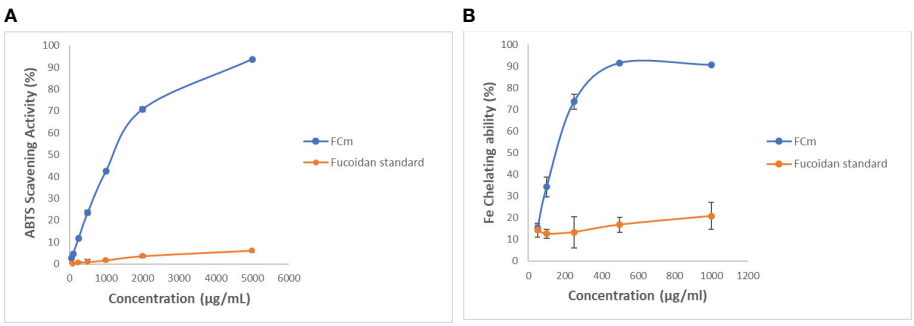

FIGURE 6
ABTS radical scavenging activity **(A)** and ferrous ion-chelating effect **(B)** of extracted fucoidan obtained from *C. myrica* and standard fucoidan at different concentrations. Data shown are the mean and standard deviation.

2016). The molecular mass of polysaccharides and their bioactivities are highly connected, for example Lahrse et al. have proved that pharmacological activities of depolymerized fractions obtained by hydrothermal and H_2O_2 treatment of fucoidan from seaweed *F. vesiculosus* decreased with decreasing molecular weight (Lahrsen et al., 2018).

A low scavenging activity of 11% and 4.1% was observed for the crude fucoidan (1000 µg/ml) obtained from *Fucus evanescens* by hot acidic extraction and with aqueous calcium chloride solution, respectively (Imbs et al., 2015). Moreover, sulphated polysaccharides extracted from the seaweed *F. vesiculosus* by two different techniques (autohydrolysis and microwave-assisted extraction) showed different ABTS scavenging capacity, with a maximum of inhibition of 49.1 ± 1.1% at 1000 µg/mL (Rodriguez-Jasso et al., 2014). Fucoidan has been shown to have a variety of ABTS radical scavenging effects (Ashayerizadeh et al., 2020) that can be influenced by extraction techniques, solvents, seaweed origin, and physicochemical structures. The difference of the antioxidant activity between commercial fucoidan and extracted fucoidan could be due to coextracted secondary metabolites such as terpenoids and polyphenols (Schneider et al., 2015; Lahrsen et al., 2018), for instance. Shneider et al. have compared the antioxidant effects of four different batches of crude commercial fucoidan by DPPH test and found that they exhibited a significant batch-to-batch DPPH scavenging effect variance that could correlate with the phenolic content (Schneider et al., 2015).

3.5.2 Fe chelating ability

Ferrous ions were revealed to stimulate lipid peroxidation and have been recognized as an effective pro-oxidant in food systems (Huang et al., 2016). The ferrous ion chelating activity of F_{Cm} and commercial fucoidan at different concentrations is shown in Figure 6B. The extracted fucoidan F_{Cm} exhibited a dose-dependent ferrous ion-chelating effect. FCm was proved to have a maximal ferrous ion-chelating activity at 500 µg/ml of 91.23%. The IC50 of F_{Cm} for chelating 50% of ferrous ion was 166.9 ± 0.87 µg/ml revealing a greater metal ion-binding ability. Our results were higher than that obtained by Tsou et al. from *S. ilicifolium* extracted at two temperatures (65 and 80°C) with antioxidant effects of 93% and 92% at concentrations of 1000 µg/mL (Tsou et al., 2022). Furthermore, Ashayerizadeh et al. have recovered fucoidan from *Sargassum tenerrimum* by two water-extraction methods and their antioxidant capacity to chelate ferrous ions were 78.3% for DFM1 and 89.4% for DFM2 at 10 mg/mL concentration (Ashayerizadeh et al., 2020). The standard fucoidan (Figure 6B) revealed a weak ferrous ion-chelating effect of 20.83% at 1000 µg/ml which was comparable to the antioxidant activity of the crude fucoidan isolated from *L. japonica* that present a chelating effect of 24.95% at 1180 µg/ml (Wang et al., 2008). Recently, Kong et al. have isolated a fucoidan from *Saccharina japonica* that has the capacity of reducing cellular ROS and cell death in Vero cells stimulated by 2,2-azobis(2-amidinopropane) hydrochloride (AAPH) (Kong et al., 2022). It has previously been demonstrated that the sulfate/fucose ratio has an obvious correlation with the chelating ability (Wang et al., 2008). Also, it appears that various fucoidan processing methods and pre-treatments of seaweeds have an impact on their ion-chelating activity (Ashayerizadeh et al., 2020). According to recent research, several brown algal polysaccharides found in cell walls are related in a complicated way with polyphenols (Usov et al., 2022) that are also related to the antioxidant activity (Tsou et al., 2022). The demonstrated antioxidant activity of F_{Cm} boosts their significance as a potential new source of natural additives (Dore et al., 2013).

4 Conclusions

This study proposes an effective approach to extract fucoidan, an active polysaccharide that have therapeutic applications, from the beach-accumulated brown C. myrica algae. In the current study, the chemical properties and the purity of the isolated fucoidan were determined and confirmed using several techniques. F_{Cm} has dual anticoagulant and antioxidant properties, which may be influenced by the combination of various factors, including monosaccharide composition, the degree of sulfates, uronic acid content and others. Results indicate that fucoidan had prominent anticoagulant activity *in vitro* that may affect intrinsic pathways while having little impact on the extrinsic mechanism of coagulation since it significantly prolongs aPTT in a concentration dependent manner and it prolongs PT at high doses. However, F_{Cm} significantly exhibited an interesting antioxidant capacity as shown by the higher ABTS radical scavenging activity and Ferrous ion-chelating effect. According to the findings, *C. myrica* algae can be used as a competitive feedstock to make high-value sulfated polysaccharides like fucoidan that present beneficial effects, suggesting possible future innovative therapeutic possibilities for numerous diseases. This study provides a mechanism for scaling up marine macroalgae biomass conversion into value-added goods and particularly, the presence of the brown C. myrica algae accumulated in coastal environment can be turned from an unpleasant phenomenon into a source of pharmaceutical candidates.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

MD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing, Funding acquisition, Resources, Validation, Visualization.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia through the project number 445-9-730.

Acknowledgments

The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number 445-9-730.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abdelrhman, A. M., Ashour, M., Al-Zahaby, M. A., Sharawy, Z. Z., Nazmi, H., Zaki, M. A., et al. (2022). Effect of polysaccharides derived from brown macroalgae Sargassum dentifolium on growth performance, serum biochemical, digestive histology and enzyme activity of hybrid red tilapia. *Aquac. Rep.* 25, 101212. doi: 10.1016/j.aqrep.2022.101212

Acharya, K. (2017). Simplified methods for microtiter based analysis of in *vitro* antioxidant activity. *Asian J. Pharm. (AJP)* 11 (02).

Ajisaka, K., Yokoyama, T., and Matsu, K. (2016). Structural characteristics and antioxidant activities of fucoidans from five brown seaweeds. *J. Appl. Glycosci. (1999)* 63 (2), 31–37. doi: 10.5458/jag.jag.JAG-2015_024

Alkordi, M. H., Haikal, R. R., Hassan, Y. S., Emwas, A.-H., and Belmabkhout, Y. (2015). Poly-functional porous-organic polymers to access functionality–CO 2 sorption energetic relationships. *J. Mater. Chem. A* 3 (45), 22584–22590. doi: 10.1039/C5TA05297A

Apostolova, E., Lukova, P., Baldzhieva, A., Delattre, C., Molinie, R., Petit, E., et al. (2022). Structural characterization and *in vivo* anti-inflammatory activity of fucoidan from Cystoseira crinita (Desf.) Borry. *Mar. Drugs* 20 (11), 714. doi: 10.3390/md20110714

Ashayerizadeh, O., Dastar, B., and Pourashouri, P. (2020). Study of antioxidant and antibacterial activities of depolymerized fucoidans extracted from Sargassum tenerrimum. *Int. J. Biol. Macromol.* 151, 1259–1266. doi: 10.1016/j.ijbiomac.2019.10.172

Ashour, M., Al-Souti, A. S., Hassan, S. M., Ammar, G. A., Goda, A. M.-S., El-Shenody, R., et al. (2022). Commercial seaweed liquid extract as strawberry biostimulants and bioethanol production. *Life* 13 (1), 85. doi: 10.3390/life13010085

Baba, B. M., Mustapha, W. A. W., and Joe, L. S. (2018). Effect of extraction methods on the yield, fucose content and purity of fucoidan from Sargassum sp. obtained from Pulau Langkawi, Malaysia. *Malaysian J. Anal. Sci.* 22 (1), 87–94. doi: 10.17576/mjas-2018-2201-11

Barahona, T., Chandía, N. P., Encinas, M. V., Matsuhiro, B., and Zúñiga, E. A. (2011). Antioxidant capacity of sulfated polysaccharides from seaweeds. A kinetic approach. *Food Hydrocoll.* 25, 3, 529–535. doi: 10.1016/j.foodhyd.2010.08.004

Black, W., Dewar, E., and Woodward, F. (1952). Manufacture of algal chemicals. IV —laboratory-scale isolation of fucoidin from brown marine algae. *J. Sci. Food Agric.* 3, 3, 122–129. doi: 10.1002/jsfa.2740030305

Cesaretti, M., Luppi, E., Maccari, F., and Volpi, N. (2003). A 96-well assay for uronic acid carbazole reaction. *Carbohydr. Polymers* 54 (1), 59–61. doi: 10.1016/S0144-8617(03)00144-9

Chisca, S., Duong, P., Emwas, A.-H., Sougrat, R., and Nunes, S. P. (2015). Crosslinked copolyazoles with a zwitterionic structure for organic solvent resistant membranes. *Polymer Chem.* 6 (4), 543–554. doi: 10.1039/C4PY01293C

Citkowska, A., Szekalska, M., and Winnicka, K. (2019). Possibilities of fucoidan utilization in the development of pharmaceutical dosage forms. *Mar. Drugs* 17 (8). doi: 10.3390/md17080458

Daniel, R., Berteau, O., Chevolot, L., Varenne, A., Gareil, P., and Goasdoue, N. (2001). Regioselective desulfation of sulfated l-fucopyranoside by a new sulfoesterase from the marine mollusk Pecten maximus. Application to the structural study of algal fucoidan (Ascophyllum nodosum). *Eur. J. Biochem.* 268 (21), 5617–5626. doi: 10.1046/j.1432-1033.2001.02497.x

Daub, C. D., Mabate, B., Malgas, S., and Pletschke, B. I. (2020). Fucoidan from Ecklonia maxima is a powerful inhibitor of the diabetes-related enzyme, α-glucosidase. *Int. J. Biol. Macromol.* 151, 412–420. doi: 10.1016/j.ijbiomac.2020.02.161

Dhahri, M., Sioud, S., Alsuhaymi, S., Almulhim, F., Haneef, A., Saoudi, A., et al. (2023). Extraction, characterization, and antioxidant activity of polysaccharides from Ajwa seed and flesh. *Separations* 10 (2), 103. doi: 10.3390/separations10020103

Dhahri, M., Sioud, S., Dridi, R., Hassine, M., Boughattas, N. A., Almulhim, F., et al. (2020). Extraction, characterization, and anticoagulant activity of a sulfated polysaccharide from Bursatella leachii viscera. *ACS Omega* 5 (24), 14786–14795. doi: 10.1021/acsomega.0c01724

Dische, Z., and Shettles, L. B. (1948). A specific color reaction of methylpentoses and a spectrophotometric micromethod for their determination. *J. Biol. Chem.* 175 (2), 595–603. doi: 10.1016/S0021-9258(18)57178-7

Dobrinčić, A., Balbino, S., Zorić, Z., Pedisić, S., Bursać Kovačević, D., Elez Garofulić, I., et al. (2020). Advanced technologies for the extraction of marine brown algal polysaccharides. *Mar. Drugs* 18 (3), 168. doi: 10.3390/md18030168

Dore, C. M., das, C.F.A.M.G., Will, L. S., Costa, T. G., Sabry, D. A., de Souza Rego, L. A., et al. (2013). A sulfated polysaccharide, fucans, isolated from brown algae Sargassum vulgare with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. *Carbohydr. Polym.* 91 (1), 467–475. doi: 10.1016/j.carbpol.2012.07.075

El-Shenody, R. A., Ashour, M., and Ghobara, M. M. E. (2019). Evaluating the chemical composition and antioxidant activity of three Egyptian seaweeds: Dictyota dichotoma, Turbinaria decurrens, and Laurencia obtusa. *Braz. J. Food Technol.* 22. doi: 10.1590/1981-6723.20318

Escobedo, J. O., Wang, W., and Strongin, R. M. (2006). Use of a commercially available reagent for the selective detection of homocysteine in plasma. *Nat. Protoc.* 1 (6), 2759–2762. doi: 10.1038/nprot.2006.399

Fauziee, N. A. M., Chang, L. S., Mustapha, W. A. W., Nor, A. R. M., and Lim, S. J. (2021). Functional polysaccharides of fucoidan, laminaran and alginate from Malaysian brown seaweeds (Sargassum polycystum, Turbinaria ornata and Padina boryana). *Int. J. Biol. Macromol.* 167, 1135–1145. doi: 10.1016/j.ijbiomac.2020.11.067

Fernando, I. P. S., Dias, M., Madusanka, D. M. D., Han, E. J., Kim, M. J., Jeon, Y. J., et al. (2020). Human keratinocyte UVB-protective effects of a low molecular weight fucoidan from Sargassum horneri purified by step gradient ethanol precipitation. *Antioxid. (Basel)* 9 (4), 340. doi: 10.3390/antiox9040340

Fernando, I. P. S., Sanjeewa, K. K. A., Samarakoon, K. W., Lee, W. W., Kim, H. S., Kang, N., et al. (2017). A fucoidan fraction purified from Chnoospora minima; a potential inhibitor of LPS-induced inflammatory responses. *Int. J. Biol. Macromol.* 104 (Pt A), 1185–1193. doi: 10.1016/j.ijbiomac.2017.07.031

Ganapathy, S., Lingappa, S., Naidu, K., Selvaraj, U., Ramachandiran, S., Ponnusamy, S., et al. (2019). Isolation and bioactive potential of fucoidan from marine macroalgae Turbinaria conoides. *ChemistrySelect* 4, 48, 14114–14119. doi: 10.1002/slct.201903548

González Fernández, L. A., Castillo Ramos, V., Sánchez Polo, M., and Medellín Castillo, N. A. (2023). Fundamentals in applications of algae biomass: A review. *J. Environ. Manage.* 338, 117830. doi: 10.1016/j.jenvman.2023.117830

Gutmann, C., Siow, R., Gwozdz, A. M., Saha, P., and Smith, A. (2020). Reactive oxygen species in venous thrombosis. *Int. J. Mol. Sci.* 21 (6), 1918. doi: 10.3390/ijms21061918

Huang, C.-Y., Kuo, C.-H., and Lee, C.-H. (2018). Antibacterial and antioxidant capacities and attenuation of lipid accumulation in 3T3-L1 adipocytes by low-molecular-weight fucoidans prepared from compressional-puffing-pretreated Sargassum crassifolium. *Mar. Drugs* 16, 1, 24. doi: 10.3390/md16010024

Huang, C.-Y., Wu, S.-J., Yang, W.-N., Kuan, A.-W., and Chen, C.-Y. (2016). Antioxidant activities of crude extracts of fucoidan extracted from Sargassum glaucescens by a compressional-puffing-hydrothermal extraction process. *Food Chem.* 197, 1121–1129. doi: 10.1016/j.foodchem.2015.11.100

Imbs, T. I., Skriptsova, A. V., and Zvyagintseva, T. N. (2015). Antioxidant activity of fucose-containing sulfated polysaccharides obtained from Fucus evanescens by

different extraction methods. *J. Appl. Phycol.* 27, 1, 545–553. doi: 10.1007/s10811-014-0293-7

Indumathi, P., and Mehta, A. (2016). A novel anticoagulant peptide from the Nori hydrolysate. *J. Funct. Foods* 20, 606–617. doi: 10.1016/j.jff.2015.11.016

Irhimeh, M. R., Fitton, J. H., and Lowenthal, R. M. (2009). Pilot clinical study to evaluate the anticoagulant activity of fucoidan. *Blood Coagulation Fibrinol.* 20 (7), 607–610. doi: 10.1097/MBC.0b013e32833135fe

January, G., Naidoo, R., Kirby-McCullough, B., and Bauer, R. (2019). Assessing methodologies for fucoidan extraction from South African brown algae. *Algal Res.* 40, 101517. doi: 10.1016/j.algal.2019.101517

Jayawardena, T. U., Nagahawatta, D., Fernando, I., Kim, Y.-T., Kim, J.-S., Kim, W.-S., et al. (2022). A review on fucoidan structure, extraction techniques, and its role as an immunomodulatory agent. *Mar. Drugs* 20, 12, 755. doi: 10.3390/md20120755

Jin, W., Zhang, Q., Wang, J., and Zhang, W. (2013). A comparative study of the anticoagulant activities of eleven fucoidans. *Carbohydr. Polymers* 91 (1), 1–6. doi: 10.1016/j.carbpol.2012.07.067

Jin, Y., Yu, Q., Li, S., Chen, T., and Liu, D. (2023). Application of seaweed polysaccharide in bone tissue regeneration. *Front. Mar. Sci.* 10. doi: 10.3389/fmars.2023.1202422

Koh, H. S. A., Lu, J., and Zhou, W. (2019). Structure characterization and antioxidant activity of fucoidan isolated from Undaria pinnatifida grown in New Zealand. *Carbohydr. Polym.* 212, 178–185. doi: 10.1016/j.carbpol.2019.02.040

Kong, Y., Wang, L., Jeon, Y.-J., Xu, J., Gao, X., and Fu, X. (2022). *In vitro* and in *vivo* antioxidant activities of a fucose-rich fucoidan isolated from Saccharina japonica. *J. Appl. Phycol.* 35, 353–364. doi: 10.1007/s10811-022-02869-x

Lahrsen, E., Schoenfeld, A. K., and Alban, S. (2018). Size-dependent pharmacological activities of differently degraded fucoidan fractions from Fucus vesiculosus. *Carbohydr. Polym.* 189, 162–168. doi: 10.1016/j.carbpol.2018.02.035

Lakshmanasenthil, S., Vinothkumar, T., Geetharamani, D., Marudhupandi, T., Suja, G., Sindhu, N. J. B., et al. (2014). Fucoidan—a novel α-amylase inhibitor from Turbinaria ornata with relevance to NIDDM therapy. *Biocatal. Agric. Biotechnol.* 3, 3, 66–70. doi: 10.1016/j.bcab.2014.02.003

Lapikova, E., Drozd, N., Tolstenkov, A., Makarov, V., Zvyagintseva, T., Shevchenko, N., et al. (2008). Inhibition of thrombin and factor Xa by Fucus evanescens fucoidan and its modified analogs. *Bull. Exp. Biol. Med.* 146, 328–333. doi: 10.1007/s10517-008-0267-3

Li, S., Wang, A., Liu, L., Tian, G., and Xu, F. (2019). Effect of deproteinization methods on the antioxidant activity of polysaccharides extracted from Lentinus edodes stipe. *J. Food Measurement Characterization* 13, 1382–1389. doi: 10.1007/s11694-019-00054-2

Li, Y., Zheng, Y., Zhang, Y., Yang, Y., Wang, P., Imre, B., et al. (2021). Brown algae carbohydrates: Structures, pharmaceutical properties, and research challenges. *Mar. Drugs* 19 (11), 620. doi: 10.3390/md19110620

Lim, S. J., and Aida, W. M. W. (2017). "Extraction of sulfated polysaccharides (Fucoidan) from brown seaweed," in *Seaweed polysaccharides* (Elsevier), 27–46.

Lim, S. J., Aida, W. M. W., Maskat, M. Y., Latip, J., Badri, K. H., Hassan, O., et al. (2016). Characterisation of fucoidan extracted from Malaysian Sargassum binderi. *Food Chem.* 209, 267–273. doi: 10.1016/j.foodchem.2016.04.058

Mak, W., Wang, S. K., Liu, T., Hamid, N., Li, Y., Lu, J., et al. (2014). Anti-proliferation potential and content of fucoidan extracted from sporophyll of New Zealand Undaria pinnatifida. *Front. Nutr.* 1. doi: 10.3389/fnut.2014.00009

Manggau, M., Kasim, S., Fitri, N., Aulia, N., Agustiani, A., Raihan, M., et al. (2022). "Antioxidant, anti-inflammatory and anticoagulant activities of sulfate polysaccharide isolate from brown alga Sargassum policystum," in *3rd International Conference on Integrated Coastal Management & Marine Biotechnology. IOP Conference Series: Earth and Environmental Science* (IOP Publishing), 012029.

Michel, J., Abd Rani, N. Z., and Husain, K. (2020). A review on the potential use of medicinal plants from Asteraceae and Lamiaceae plant family in cardiovascular diseases. *Front. Pharmacol.* 11, 852. doi: 10.3389/fphar.2020.00852

Mohamed, S. F., Slamy, T. M., and Shaeiri, S. Y. (2015). Characterization of sulphated polysaccharide with antiviral activity from marine brown alga Cystoseira myrica collected from Jazan coasts, KSA. *Int. J. PharmTech Res.* 8, 198–203.

Mousavian, Z., Safavi, M., Azizmohseni, F., Hadizadeh, M., and Mirdamadi, S. (2022). Characterization, antioxidant and anticoagulant properties of exopolysaccharide from marine microalgae. *AMB Express* 12 (1), 27. doi: 10.1186/s13568-022-01365-2

Olas, B. (2022). The antioxidant, anti-platelet and anti-coagulant properties of phenolic compounds, associated with modulation of hemostasis and cardiovascular disease, and their possible effect on COVID-19. *Nutrients* 14 (7), 1390. doi: 10.3390/nu14071390

Palanisamy, S., Vinosha, M., Marudhupandi, T., Rajasekar, P., and Prabhu, N. M. (2017). Isolation of fucoidan from Sargassum polycystum brown algae: Structural characterization, in *vitro* antioxidant and anticancer activity. *Int. J. Biol. Macromol.* 102, 405–412. doi: 10.1016/j.ijbiomac.2017.03.182

Palta, S., Saroa, R., and Palta, A. (2014). Overview of the coagulation system. *Indian J. Anaesthesia* 58 (5), 515. doi: 10.4103/0019-5049.144643

Pozharitskaya, O. N., Obluchinskaya, E. D., and Shikov, A. N. (2020). Mechanisms of bioactivities of fucoidan from the brown seaweed Fucus vesiculosus L. of the Barents Sea. *Mar. Drugs* 18 (5), 275. doi: 10.3390/md18050275

Pratt, C., and Monroe, D. (1992). Microplate coagulation assays. *Biotechniques* 13 (3), 430–433.

Qu, Y., Cao, Z., Wang, W., Wang, N., Li, X., and Pan, J. (2019). Monthly variations of fucoidan content and its composition in the farmed brown alga Saccharina sculpera (Laminariales, Phaeophyceae). *J. Appl. Phycol.* 31 (4), 2623–2628. doi: 10.1007/s10811-019-1753-x

Rani, V., Shakila, R., Jawahar, P., and Srinivasan, A. (2017). Influence of species, geographic location, seasonal variation and extraction method on the fucoidan yield of the brown seaweeds of Gulf of Mannar, India. *Indian J. Pharm. Sci.* 79, 65–71. doi: 10.4172/pharmaceutical-sciences.1000202

Rodriguez-Jasso, R. M., Mussatto, S. I., Pastrana, L., Aguilar, C. N., and Teixeira, J.A.J.C.P. (2014). Chemical composition and antioxidant activity of sulphated polysaccharides extracted from Fucus vesiculosus using different hydrothermal processes. *Chem. Papers* 68, 2, 203–209. doi: 10.2478/s11696-013-0430-9

Rudovica, V., Rotter, A., Gaudêncio, S. P., Novoveská, L., Akgül, F., Akslen-Hoel, L. K., et al. (2021). Valorization of marine waste: use of industrial by-products and beach wrack towards the production of high added-value products. *Front. Mar. Sci.* 8, 723333. doi: 10.3389/fmars.2021.723333

Saravana, P. S., Cho, Y. J., Park, Y. B., Woo, H. C., and Chun, B. S. (2016). Structural, antioxidant, and emulsifying activities of fucoidan from Saccharina japonica using pressurized liquid extraction. *Carbohydr. Polym.* 153, 518–525. doi: 10.1016/j.carbpol.2016.08.014

Schneider, T., Ehrig, K., Liewert, I., and Alban, S. J. G. (2015). Interference with the CXCL12/CXCR4 axis as potential antitumor strategy: Superiority of a sulfated galactofucan from the brown alga Saccharina latissima and fucoidan over heparins. *Glycobiology* 25, 8, 812–824. doi: 10.1093/glycob/cwv022

Shinde, P., Banerjee, P., and Mandhare, A. (2019). Marine natural products as source of new drugs: A patent review, (2015–2018). *Expert Opin. Ther. Patents* 29 (4), 283–309. doi: 10.1080/13543776.2019.1598972

Stelling, M. P., de Bento, A. A., Caloba, P., Vilanova, E., and Pavão, M. S. G. (2019). "Methods for isolation and characterization of sulfated glycosaminoglycans from marine invertebrates," in *The Extracellular Matrix: Methods and Protocols*. Eds. D. Vigetti and A. D. Theocharis (New York, NY: Springer New York), 55–70.

Synytsya, A., Kim, W.-J., Kim, S.-M., Pohl, R., Synytsya, A., Kvasnička, F., et al. (2010). Structure and antitumour activity of fucoidan isolated from sporophyll of Korean brown seaweed Undaria pinnatifida. *Carbohydr. Polymers* 81 (1), 41–48. doi: 10.1016/j.carbpol.2010.01.052

Tang, S., Ma, Y., Dong, X., Zhou, H., He, Y., Ren, D., et al. (2022). Enzyme-assisted extraction of fucoidan from Kjellmaniella crassifolia based on kinetic study of enzymatic hydrolysis of algal cellulose. *Algal Res.* 66, 102795. doi: 10.1016/j.algal.2022.102795

Tsou, M. H., Lee, C. C., Wu, Z. Y., Lee, Z. H., and Lin, H. M. (2022). Bioactivity of crude fucoidan extracted from Sargassum ilicifolium (Turner) C. Agardh. *Sci. Rep.* 12 (1), 15916. doi: 10.1038/s41598-022-19370-7

Usov, A. I., Bilan, M. I., Ustyuzhanina, N. E., and Nifantiev, N.E.J.M.D. (2022). Fucoidans of Brown Algae: Comparison of Sulfated Polysaccharides from Fucus vesiculosus and Ascophyllum nodosum. *Mar. Drugs* 20, 10, 638. doi: 10.3390/md20100638

Ustyuzhanina, N. E., Ushakova, N. A., Zyuzina, K. A., Bilan, M. I., Elizarova, A. L., Somonova, O. V., et al. (2013). Influence of fucoidans on hemostatic system. *Mar. Drugs* 11 (7), 2444–2458. doi: 10.3390/md11072444

Walkowiak, B., Kęsy, A., and Michalec, L. (1997). Microplate reader-a convenient tool in studies of blood coagulation. *Thromb. Res.* 87 (1), 95–103. doi: 10.1016/S0049-3848(97)00108-4

Wang, J., Zhang, Q., Zhang, Z., and Li, Z. (2008). Antioxidant activity of sulfated polysaccharide fractions extracted from Laminaria japonica. *Int. J. Biol. Macromol.* 42 (2), 127–132. doi: 10.1016/j.ijbiomac.2007.10.003

Wang, Y., Xing, M., Cao, Q., Ji, A., Liang, H., and Song, S. (2019). Biological activities of fucoidan and the factors mediating its therapeutic effects: A review of recent studies. *Mar. Drugs* 17 (3), 183. doi: 10.3390/md17030183

Wen, Y., Gao, L., Zhou, H., Ai, C., Huang, X., Wang, M., et al. (2021). Opportunities and challenges of algal fucoidan for diabetes management. *Trends Food Sci. Technol.* 111, 628–641. doi: 10.1016/j.tifs.2021.03.028

Xin, L., Bin, L., Xiao-Lei, W., Zhen-Liang, S., and Chang-Yun, W. (2016). Extraction, fractionation, and chemical characterisation of fucoidans from the brown seaweed Sargassum pallidum. *Czech J. Food Sci.* 34, 5, 406–413. doi: 10.17221/322/2015-CJFS

Yao, Y., and Yim, E. K. (2021). Fucoidan for cardiovascular application and the factors mediating its activities. *Carbohydr. Polymers* 270, 118347. doi: 10.1016/j.carbpol.2021.118347

Yu, J., Li, Q., Wu, J., Yang, X., Yang, S., Zhu, W., et al. (2021). Fucoidan extracted from sporophyll of Undaria pinnatifida grown in Weihai, China–chemical composition and comparison of antioxidant activity of different molecular weight fractions. *Front. Nutr.* 8, 636930. doi: 10.3389/fnut.2021.636930

Zayed, A., Finkelmeier, D., Hahn, T., Rebers, L., Shanmugam, A., Burger-Kentischer, A., et al. (2023). Characterization and cytotoxic activity of microwave-assisted extracted crude fucoidans from different brown seaweeds. *Mar. Drugs* 21 (1), 48. doi: 10.3390/md21010048

Zhang, H., Cai, X. T., Tian, Q. H., Xiao, L. X., Zeng, Z., Cai, X. T., et al. (2019). Microwave-assisted degradation of polysaccharide from Polygonatum sibiricum and antioxidant activity. *J. Food Sci.* 84 (4), 754–761. doi: 10.1111/1750-3841.14449