Hidrobiológica 2025, 35 (1): 49-56

UNIVERSIDAD AUTÓNOMA METROPOLITANA
Casa abierta al tiempo
Unidad Iztapalapa

HIDROBIOLÓGICA
http:/hidrobiologica.izt.uam.mx
ISSN: 2448-7333

OPEN ACCESS
Research Article
April, 2025

Anticoagulant activity of sulfated polysaccharides obtained from the brown seaweed *Stephanocystis dioica*

Actividad anticoagulante de polisacáridos sulfatados obtenidos del alga parda *Stephanocystis dioica*

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Recibido: 20 de agosto de 2024. ***Aceptado:*** 10 de octubre de 2024. ***Publicado:*** abril de 2025.

ABSTRACT

Background: Brown algae are recognized as a source of sulfated polysaccharides of great economic value and importance in biomedical studies due to their diverse biological activities such as anticoagulant, antioxidant, antiviral, among others. **Goals:** In this study, an aqueous extract from the brown seaweed *Stephanocystis dioica* was evaluated to determine its potential anticoagulant activity. **Methods:** An aqueous extraction was carried out at room temperature to obtain sulfated polysaccharides, which were semi-purified by fractional precipitation with ethanol to obtain three fractions. These fractions were employed in prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays to evaluate anticoagulant activity at different concentrations (6.25-100 µg mL^{-1}). The partial chemical composition (fucose, uronic acids, and sulfates) and SO_4/sugar residue ratio were also determined. **Results:** In the PT assay, at a concentration of 100 µg mL^{-1}, fraction F3 exhibited the greatest coagulation time (76 s), which was four times that of the control. In the aPTT assay, the three fractions extended the control time (28.8 s) to more than 300 s at a concentration of 100 µg mL^{-1}. Partial chemical analysis showed that fractions F1, F2, and F3 are sulfated heteropolysaccharides rich in fucose, with lower concentrations of uronic acids and other sugars. In the infrared spectrum, the observed vibrations at 820 cm^{-1} indicate a twist of the sulfate group at the equatorial position of the sugar ring at 2-O and/or 3-O positions. **Conclusions:** The results showed that sulfated polysaccharide from *Stephanocystis dioica* has potential anticoagulant activity.

Keywords: anticoagulant activity, bioactive, brown seaweed, fucoidan, uronic acids.

RESUMEN

Antecedentes: Las algas pardas son reconocidas como fuente de polisacáridos sulfatados de gran valor económico e importancia en estudios biomédicos debido a sus diversas actividades biológicas como anticoagulante, antioxidante, antiviral, entre otras. **Objetivos:** Para este estudio se evaluó un extracto acuoso obtenido del alga parda *Stephanocystis dioica* para determinar su potencial actividad anticoagulante. **Métodos:** Se realizó una extracción acuosa a temperatura ambiente para obtener polisacáridos sulfatados, los cuales se semipurificaron por precipitación fraccionada con etanol, obteniendo tres fracciones. Estas fracciones se emplearon en ensayos de tiempo de protrombina (TP) y tiempo de tromboplastina parcial activada (TTPa) para evaluar la actividad anticoagulante a diferentes concentraciones (6.25-100 µg mL^{-1}). También se determinó la composición química parcial (fucosa, ácidos urónicos y sulfatos) y la relación SO_4/residuos de azúcar. **Resultados:** En el ensayo TP, a una concentración de 100 µg mL^{-1}, la fracción F3 mostró el mayor tiempo de coagulación (76 s), el cual fue cuatro veces superior al tiempo del control. En el ensayo TTPa, las tres fracciones prolongaron el tiempo del control (28.98 s) a más de 300 s a una concentración de 100 µg mL^{-1}. El análisis químico parcial mostró que las fracciones F1, F2 y F3 son heteropolisacáridos sulfatados ricos en fucosa, con menores concentraciones de ácidos urónicos y otros azúcares. En el espectro infrarrojo, las vibraciones observadas a 820 cm^{-1} indican una torsión del grupo sulfato en la posición ecuatorial del anillo de azúcar en las posiciones 2-O y/o 3-O. **Conclusiones:** Los resultados mostraron que el polisacárido sulfatado de *Stephanocystis dioica* tiene una potencial actividad anticoagulante.

Palabras clave: ácidos urónicos, actividad anticoagulante, alga parda, bioactividad, fucoidan.

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To quote as:
García-Zamora, G. M., M. Muñoz-Ochoa, D. L. Arvizu-Higuera, Y. E. Rodríguez-Montesinos & J. M. López-Vivas. 2025. Anticoagulant activity of sulfated polysaccharides obtained from the brown seaweed *Stephanocystis dioica*. *Hidrobiológica* 35 (1): 49-56.

DOI:10.24275/QTZI8162

INTRODUCTION

Brown seaweeds are important sources of sulfated polysaccharides and hold special interest in the search for natural products (Liyanage *et al.,* 2023). These compounds are found mainly in the cell walls or extracellular matrices of brown seaweeds, although they have also been isolated from microorganisms (e.g., cyanobacteria) and marine invertebrates (e.g., jellyfish, sea urchins, and sea cucumbers) (Yang & Zhang, 2009; Senthilkumar *et al.,* 2013). Sulfated polysaccharides are of great economic value and are important in biomedical research due to the variety of biological activities they exhibit, either by themselves or by inducing complex reaction cascades (Inácio *et al*., 2020).

Fucoidan is one of the most studied sulfated polysaccharides due to its anticoagulant, antithrombotic, antiviral, and antiproliferative activities (Inácio *et al*., 2020). As such, a promising opportunity to develop better and safer anticoagulant drugs with fewer and less severe side effects has opened up with fucoidan isolated from brown seaweeds (Pereira *et al.,* 2002; Athukorala *et al.,* 2006; Cumashi *et al., 2*007; Wang *et al.,* 2019). However, the high structural complexity of this sulfated polysaccharide makes it difficult to establish a clear relationship between its structure and bioactivity.

Both the chemical composition and biological properties of algae depend on multiple factors, including seasonal variation, geographic location, species, nutrient concentrations in seawater, and other factors related to the processes of obtaining and extracting polysaccharides (Rioux *et al.,* 2007; Pradhan *et al*., 2020). Thus, each new fucoidan isolated from a brown seaweed is a unique compound with specific structural features that confer different physicochemical properties, which are responsible for the diversity of biological activities with potential applications in medicine and pharmacology that have been identified among sulfated polysaccharides (Muñoz-Ochoa *et al*., 2009; Inácio *et al*., 2020).

In Mexico, the studies of *Stephanocystis dioica* (N. L. Gardner) Draisma, Ballesteros, F. Rousseau & T. Thibaut have only focused on its taxonomy, distribution, and ecological roles (Pedroche & Sentíes, 2020). Our interest in searching for novel and alternative bioactive compounds led us to explore the potential of *S. dioica* to serve as a source of sulfated polysaccharides with anticoagulant activity. The results of our study will complement our current understanding of the chemical composition of this alga and its biological properties.

MATERIAL AND METHODS

Sampling. *Stephanocystis dioica* was collected in Popotla, Baja California, Mexico (32° 18' 06" N, 117° 02' 43" W) in summer 2014 from the subtidal zone at 2 m depth (Fig. 1). The samples were washed with tap fresh water to remove epiphytes and sand, after which they were dried in the shade for two days. The dried samples were cut into small pieces and stored in clean containers until use.

Crude fucoidan (CF). One hundred grams of dried algae were macerated with ethanol, which was changed every third day. The extracts were passed through Whatman No. 4 filter paper (25 µ) and the algae were extracted twice again under the same conditions. After filtration, the ethanolic extract (EE) was evaporated under reduced pressure at 40 °C. The dry EE was stored in vials at - 4 °C until use. The residual algal tissue was dried at 45 °C and stored for fucoidan extraction.

Fucoidan extraction was conducted by the method of Muñoz-Ochoa *et al.* (2009). A sample (50 g) of dried residual algal tissue (algae used for EE) was placed in 600 mL of distilled water at room temperature (28 °C) and stirred continuously for 2 h. Then, the algal tissue was removed by simple filtration using a cloth, and the resulting aqueous solution was precipitated with two volumes of ethanol. This process was performed twice more under the same conditions. The obtained crude fucoidan (CF) extract was dried at 45 °C in an oven.

Partial fucoidan purification. The CF extract was dissolved in 100 mL of distilled water, and the insoluble material was removed by centrifugation at 2500 rpm for 10 min (Beckman TJ-6/R, Beckman Coulter, Brea, CA, USA). The clarified solution was precipitated with 10 mL of 10% $CaCl_2$ and centrifuged at 2500 rpm for 10 min. The supernatant was removed and subjected to fractional precipitation with three volumes of ethanol. The precipitate from each volume of ethanol was recovered by centrifugation at 2500 rpm for 10 min. The recovered precipitates were dried in an oven at 45 °C and stored in vials. They were labeled as fractions F1, F2, and F3 (Muñoz-Ochoa *et al*., 2009).

Anticoagulant activity. The anticoagulant activity of the extracts (F1, F2, and F3) was evaluated with prothrombin time (PT) and activated partial thromboplastin time (aPTT) coagulation assays, following the instructions of the manufacturer (Siemens, Munich, Germany). Briefly, 90 µL of citrated human plasma was mixed with 10 µL of extract solution and incubated for 1 min at 37 °C. After incubation, 200 µL of PT reagent (Thromborel S, Siemens) was added to the mixture, and the coagulation time was recorded. For the aPTT assay, 100 µL of aPTT reagent (Actin, Siemens) was added to the mixture followed by 100 µL of 0.025 M $CaCl_2$, and the coagulation time was recorded. Distilled water was used as a negative control. Dilutions of CF and F1, F2, and F3 (100, 50, 25, 12.5, and 6.25 µg mL^{-1}) were prepared to determine the lowest concentration at which anticoagulant activity was still notable (Muñoz-Ochoa *et al*., 2009).

Partial fucoidan characterization. A structural characterization of the fractions obtained from the *S. dioica* extracts was conducted with Fourier transform infrared spectroscopy with a total refraction attenuator (FTIR–ATR) (Spectrum Two, Perkin Elmer, Waltham, MA, USA). Each spectrum was obtained from the sum of 24 replicates at a resolution of 4 cm^{-1} in the spectral range of 4000-500 cm^{-1}.

Determination of fucose. The fucose concentration was determined by the colorimetric cysteine-sulfuric acid method (Dische, 1955) using L-fucose (Sigma) as the standard. Briefly, 1 mL of each CF fraction (100 µg mL^{-1}) was placed in a previously cooled test tube, and 4.5 mL of 85.7% H_2SO_4 solution were added. The tubes were allowed to cool for 1 min in ice-water, and then the water bath temperature was raised to 100 °C for 10 min. After which, the samples were allowed to cool to room temperature, and a total of 100 µL of 3% (w/v) cysteine solution was added. After mixing, the solutions were allowed to stand for 30 min, and absorbance was measured at 396 and 427 nm (OD_{396} and OD_{427}, respectively). The absorbance of fucose (OD_{fuc}) was calculated using the formula:

$$OD_{fuc} = OD_{396} - OD_{427} \quad \text{Eq. (1)}$$

Determination of uronic acids. The relative uronic acid concentration was estimated by the baseline method based on the infrared spectra, considering the ratio of the area between the peaks of 1000 and 1180 cm^{-1}, which is characteristic of sugars, and the area between the peaks

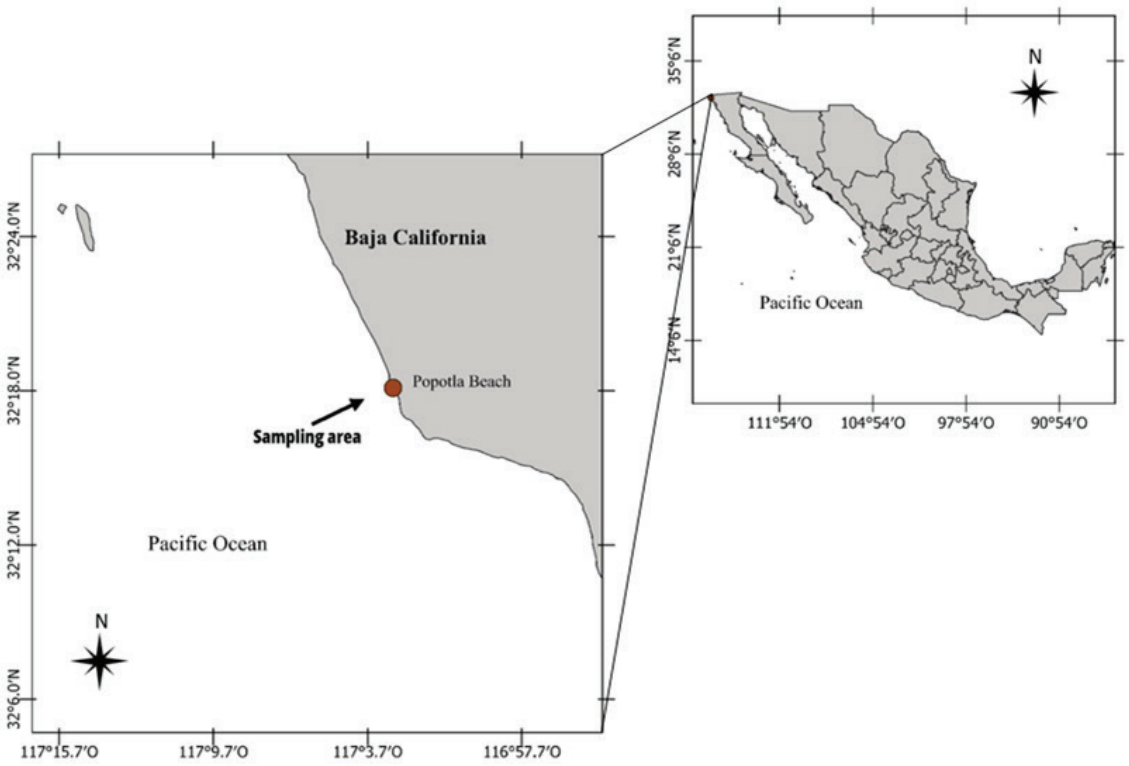

Figure 1. Location of the sampling area of *Stephanocystis dioica* collected in Popotla, Baja California, Mexico.

of 1590 and 1650 cm^{-1}, which is characteristic of uronic acids (Bociek & Welti, 1975).

Determination of sulfates. Sulfate content was estimated by infrared spectroscopy using the baseline method proposed by Lijour *et al.* (1994). The absorption bands around 1230 cm^{-1}, which are characteristic of sulfate, and 1020-1180 cm^{-1}, which are characteristic of the hemi-acetal ring of the carbon skeleton of sugars, were used with the most probable baseline. The SO_4/sugar residue relationship was determined based on the average between the maxima of the two bands. Equation (2) was used to determine the composition of the sulfated polysaccharides:

$$TS = \%Fuc + \%UA + \%Others - \%SO_4 \quad \text{Eq. (2)}$$

where is total sugars, is the percentage of fucose, is the percentage of uronic acids, is the percentage of sulfates, and includes mannose, galactose, acetyl groups, and proteins (Li *et al.,* 2008; Jiao *et al.,* 2011; Wijesinghe & Jeon, 2012).

RESULTS

Extract yields. The yield of the CF extract was 9.78%. Fraction F1 exhibited a higher yield (2.22%) than those of fractions F2 and F3 (1%).

Anticoagulant activity tests. In the PT assay, the coagulation time of fraction F1 was similar to that of the control (17.45 s) at all evaluated concentrations, while those of fractions F2 and F3 were similar to the control time at a concentration of 6.25 µg mL^{-1}, with clot formation times of 18.12 s and 19.10 s, respectively. At a concentration of 100 µg mL^{-1}, fraction F3 exhibited the greatest coagulation time (76.0 s), which was almost four times that of the control, followed by fraction F2, which exhibited a coagulation time (52.85 s) that was three times that of the control. Fractions F2 and F3 exhibited similar coagulation times, which were nearly double that of the control at a concentration of 50 µg mL^{-1} (Table 1).

In the aPTT assays, the high anticoagulant potential of *S. dioica* was evident. At a concentration of 100 µg mL^{-1}, all fractions exhibited coagulation times greater than 300 s, surpassing the coagulation time of the control (28.89 s) by almost ten times. Even at the lowest concentration (6.25 µg mL^{-1}), the three fractions showed strong anticoagulant activity, with a coagulation time for fraction F1 that was almost double that of the control (51.06 s), while fractions F2 and F3 exhibited coagulation times that were four (127.6 s) and three (91.06 s) times greater than that of the control, respectively. The highest anticoagulant activity in the aPTT assay occurred in the F3 fraction at a concentration of 12.5 µg mL^{-1} with a time greater than 300 s (Table 1).

Structural characterization of fucoidan. The infrared spectrum of the CF fractions ranged from 4000 to 500 cm^{-1} (Fig. 2). In the diagnostic region, extensive signals around 3300 cm^{-1} due to the vibration of hydroxyl group bonds (OH) were detected, while the signals around 2900 cm^{-1} were due to the stretching of methyl bonds (CH). When analyzing the fingerprint region for each spectrum, the characteristic bands reported for fucoidans were evident. The signals between 1630 and 1415

cm^{-1} were attributed to the vibrations of the bonds of the carboxyl acid group belonging to the uronates, with higher intensity vibrations observed in the F1 fraction.

The observed difference in the intensity of the signals around 1220-1230 cm^{-1} for the three CF fractions indicates a concentration of sulfate ester groups (S=O), with a low sulfate concentration for fraction F1 and a higher sulfate concentration for fraction F2, which was corroborated by the vibrations at 570 cm^{-1}. The signals observed around 820 cm^{-1} indicate sulfate ester group torsion at the equatorial position of the sugar ring at the 2-O and/or 3-O positions, with higher intensity bands for fraction F2.

The spectrum also showed bands at 1024-1022 cm^{-1} corresponding to the vibrations of the hemiacetal bonds of the sugar ring (C-O-C), with bands of greater intensity for fraction F2 as well as other vibrations within this peak that indicated the presence of more than one type of sugar. The small shoulder at 963 cm^{-1} observed with fractions F2 and F3 agrees with the signals reported for fucoidans given the vibration of fucose methyl group residues (CH_3) (Muñoz-Ochoa *et al*., 2009; Ptak *et al*., 2021; Augustyniak *et al*., 2024).

Partial chemical characterization. The chemical analysis of the three fractions indicated that fraction F1 is partially composed of sulfates (10%) and total sugars (90%), of which 15% was fucose and 60% uronic acids. Fraction F1 showed the lowest SO_4/sugar residue ratio of 0.11. On the other hand, fraction F2 was partially composed of sulfates (38%) and total sugars (62%), of which 20% was fucose and 13% uronic acid. In contrast to fraction F1, fraction F2 showed the highest SO_4/sugar residue ratio (0.61). Finally, fraction F3 was partially composed of sulfates (27%) and total sugars (72%), of which 18% was fucose and 8% uronic acids. Fraction F3 showed a SO_4/sugar residue ratio of 0.38 (Table 2).

DISCUSSION

Currently, many products contain various functional compounds obtained from natural marine sources. Indeed, brown seaweeds have been widely used to produce industrially useful ingredients. Numerous studies have focused on sulfated fucoidans and their bioactive properties, with anticoagulant activity receiving notable attention (Wijesinghe & Jeon, 2012). It is now understood that the degree of anticoagulant activity depends in large part on the degree of sulfation, structure, and molecular weight of the fucoidan (Zvyagintseva *et al.,* 2003; Jiang *et al.,* 2010; Wijesinghe & Jeon, 2012; Wang *et al*., 2019).

In the present study, the dry base yield of the CF obtained from *S. dioica* was 9.78%. Lower yields have been reported for *Laminaria japonica* Areschoug (1.9%), *Saccharina longicrusis* (Bachelot Pylaie) Kuntze (1.3%), and *Ascophyllum nodosum* (Linnaeus) Le Jolis (2.6%) (Rioux *et al.,* 2007; Zhang *et al.,* 2008). It is important to emphasize that fucoidan content varies among brown seaweeds due to the developmental differences among species and the influence of other factors, including local environmental conditions, seasonal variations, and the extraction method (Rioux *et al.,* 2007; Inácio *et al*., 2020).

In this study, all fucoidans were able to prolong blood coagulation time. The results of the PT assay suggest that fucoidans obtained from *S. dioica* inhibited the extrinsic pathway, as all fractions exhibited clotting times longer than that of the control. Indeed, the coagulation time of fraction F3 was four times that of the control at a concentration of 100 µg mL^{-1}. A more evident inhibitory effect was observed in the aPTT assay, which inhibited the intrinsic pathway. All fractions doubled the coagulation time established by the control (28.9 s), even at the minimum concentration.

Similar results to those of the aPTT assay in this study have been reported for *Sargassum* species, with times greater than 300 s for *S. vulgare* C. Agardh and *S. horneri* (Turner) C. Agardh and a time of 200 s for *S. siliquastrum* (Mertens ex Turner) C. Agardh (De Lara-Isassi & Álvarez-Hernández, 1999; Athukorala *et al.,* 2007). Other sulfated heteropolysaccharides with similar anticoagulant activity have been isolated from brown seaweeds, including *Dictyota dichotoma* (Hudson) J. V. Lamouroux, *Padina pavonia* (Linnaeus) J. V. Lamouroux*, P. tetrastromatica* Hauck (Abel-Fattah *et al.,* 1974; Dobashi *et al.,* 1989), *Ecklonia cava* Kjellman, *A. nodosum*, and *Undaria pinnatifida* (Harvey) Suringar (Athukorala *et al.,* 2006). Thus, the fucoidans obtained from brown seaweeds are active modulators of coagulation and constitute potential therapeutic compounds and viable alternatives to heparin (Mourão, 2004; Cumashi *et al.,* 2007).

The specific anticoagulant effects of fucoidans are determined by their composition and chemical structure, especially the positions of sulfate groups and sulfated fucose chains (Jiang *et al.,* 2010; Wijesinghe & Jeon, 2012; Dore *et al.,* 2013). Among fucoidans, the bonds, branching, and positions of the monosaccharides and the arrangement and content of the sulfate groups notably differ, which seriously impedes clear relationships from being established between chemical structures and biological activity (Cumashi *et al.,* 2007; Holtkamp *et al.,* 2009; Jiao *et al.,*

Table 1. Coagulation times obtained in the prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays of the three polysaccharide fractions (F1, F2, and F3; Mean ± SD, n = 3).

Extract concentration	F1		F2		F3	
(µg mL^{-1})	PT (s)	aPTT (s)	PT (s)	aPTT (s)	PT (s)	aPTT (s)
100	19.70±0.72	>300	52.85±3.69	>300	76.00±4.24	>300
50	18.70±0.61	234.5±10.6	32.42±2.70	>300	33.94±0.53	>300
25	17.11±0.91	ND	25.66±1.13	ND	23.93±1.55	>300
12.5	16.15±0.44	59.79±2.10	20.37±0.22	243.8±1.59	20.83±0.54	>300
6.25	15.53±0.07	51.06±1.40	18.12±0.26	127.6±2.94	19.10±0.86	91.06±3.73
Control	17.45±2.07	28.89±0.97	17.45±2.07	28.89±0.97	17.45±2.07	28.89±0.97

ND = Not determined

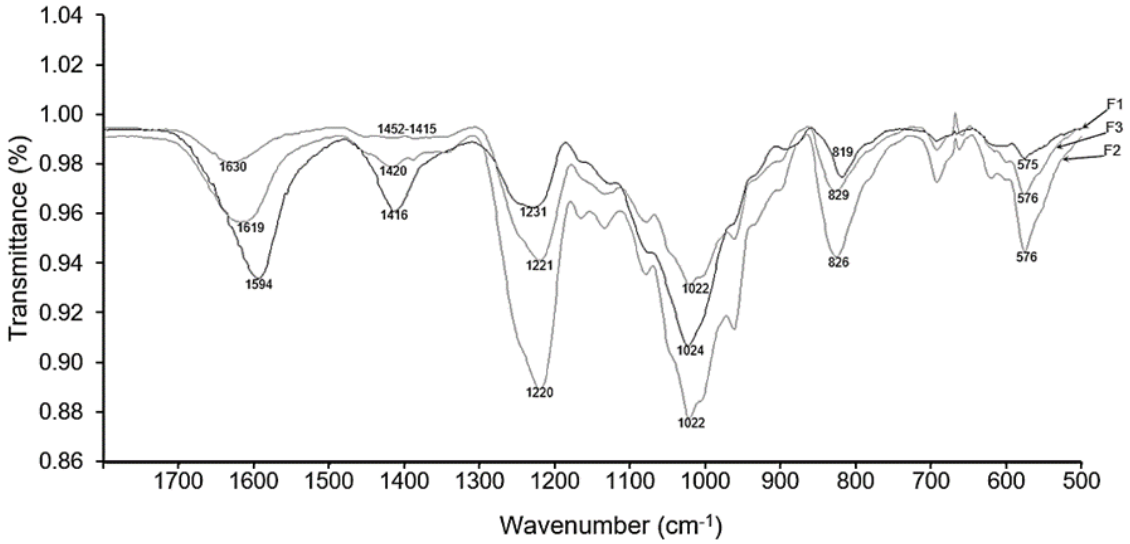

Figure 2. Comparison of the spectra obtained from the crude fucoidan fractions (F1, F2, and F3) of *Stephanocystis dioica*

2011). The anticoagulant capacity of *S. dioica* observed in this study can be attributed to the ability of heterofucans to inhibit the catalytic activity of thrombin by acting directly on the enzyme or by activating inhibitors in the plasma.

On the other hand, we observed that prior to the formation of the clot, F2 and F3 promoted the formation of platelet aggregates. In a previous study, Manne *et al.* (2013) reported that fucoidans obtained from *Fucus vesiculosus* Linnaeus promoted platelet aggregation, highlighting its potential alternative use as a CLEC-2 agonist in treating hemophilia, given that CLEC-2 is a C-type lectin-like type II transmembrane receptor. Zhu *et al.* (2010) evaluated the effect of high and low molecular weight fucoidans from *Laminaria japonica* on platelet aggregation. These authors reported a pro-aggregation effect of high molecular weight fucoidans and an inhibitory effect of low molecular weight fucoidans, indicating that *L. japonica* is a promising candidate to develop treatments for thrombosis-related cardiovascular diseases. Due to the platelet aggregation effect we detected with the F2 and F3 fractions, the majority of the fucoidans produced by *S. dioica* are likely of high molecular weight.

In the present study, the infrared spectra of the three CF fractions were similar to those that have been previously published for sulfated heterofucans obtained from brown seaweeds (Chevolot *et al.,* 1999; Silva *et al.,* 2005). In addition, the absorption patterns of the three CF fractions were similar, which suggests that all fractions contained similar functional groups, although they exhibited differences in composition that were evident in the different intensities of the absorption peaks (Fig. 2). In addition, the sulfate groups of the three fractions were in the equatorial position (C-O-S) according to the moderate band detected around 820 cm^{-1}. This has been generally attributed to sulfates in fucose residues at the O-2 and/or O-3 positions, which is a common feature reported for anticoagulant fucoidans (Nishino *et al.,* 1989; Patankar *et al.,* 1993; Dore *et al.,* 2013). In addition, the fucoidans presented glycosidic bonds of the L-fucose type with α- (1 → 3) and α- (1 → 4) bonds responsible for anticoagulant activity, as has been reported for a heterofucans obtained from *A. nodosum* (Chevolot *et al.,* 1999).

It has been suggested that in addition to the position of the sulfate group and the length of the sulfated fucose chain, the difference in fucose, uronic acid, and sulfate content among fucoidan fractions may be related to the observed difference in bioactivity (Shanmugam & Mody, 2000; Mourão, 2004; Cumashi *et al., 2*007; Li *et al.,* 2008). This agrees with what has been reported in previous studies, namely that compounds containing higher amounts of uronic acids and neutral sugars (other than fucose) and small amounts of sulfates show little or no inhibitory effect on the thrombin-fibrinogen reaction (Shanmugam & Mody, 2000; Trejo, 2004; Awad *et al.,* 2009). In this study, the characteristics of the F1 fraction included bands of greater intensity for sugars and uronates (1590 and 1650 cm^{-1}) and a composition high in uronic acids (60%) and low in sulfates (10%) (Table 2).

On the other hand, fucoidans with higher fucose and sulfate content and lower uronic acid and neutral sugar content were also obtained with fractions F2 and F3. For these fractions, absorption bands of higher intensity were observed for the sulfate group (1230 cm^{-1}). In addition, both fractions exhibited high sulfated fucose compositions (Fig. 2), namely 38% sulfates and 20% fucose for fraction F2 and 27% sulfates and 18% fucose for fraction F3 (Table 2).

Ciancia *et al*., 2010 cited that the SO_4/sugar residue ratio must approach or be greater than unity for potent anticoagulant activity to develop in fucoidans obtained from brown seaweeds. Thus, fucoidans with SO_4/sugar residue ratios < 0.3 should show low or no bioactivity; however, it is not yet fully understood if the degree of sulfation in fucoidans increases or decreases anticoagulant activity. In agreement with these authors, the SO_4/sugar residue ratio of fraction F1 (0.11) reflects the low bioactivity observed in the PT and aPTT tests. In contrast, fractions F2 and F3 exhibited higher SO_4/sugar residue ratios of 0.61 and 0.38, respectively, which were consistent with higher anticoagulant activity when compared to that of fraction F1 (Table 2). Similar results have been

Table 2. Partial chemical composition expressed as a percentage of the polysaccharide fractions (F1, F2, and F3) obtained from *Stephanocystis dioica*, and the SO_4/sugar residue ratio. (*Mean ± SD, n = 3).

Fraction	Total sugars	Fucose*	Uronic acids	Sulfates	Other components**	SO_4/sugar residues
F1	90.09	14.39 ± 0.00	60.36	9.91	15.34	0.11
F2	62.11	20.19 ± 0.02	13.04	37.88	28.88	0.61
F3	72.46	17.76 ± 0.02	7.97	27.53	46.73	0.38

**Other components: acetyl groups and proteins

reported for sulfated fucoidans with chemical characteristics and similar anticoagulant activity isolated from *Eisenia bicyclis* (Kjellman) Setchell (sulfated fucans), *U. pinnatifida* (fucogalactan sulfate), and *Ecklonia kurome* Okamura (sulfated galactofucan; Dobashi *et al.,* 1989).

It is important to mention that the bioactivity reported in this study does not exclude the possibility of *S. dioica* exhibiting variable anticoagulant activity. Bioactivity is influenced by specific biosynthetic factors that are directly related to the physiological requirements of the algae. This leads to the formation of generally heterogeneous and branched algal polysaccharides with different structures and bioactivity (Matsubara, 2004). Furthermore, we must consider that different coagulation pathways interact with different factors, thus the heterofucans extracted from *S. dioica* may or may not be active depending on the coagulation pathway being evaluated (Shanmugam & Mody, 2000; Dore *et al.,* 2013).

This study is the first to provide a partial characterization of fucoidan isolated from *S. dioica* and its anticoagulant activity. The results contribute to our fundamental understanding of sulfated polysaccharides from this brown alga and highlight the great pharmacological potential of fucoidans as anticoagulant agents with potential for use in the treatment of thrombotic diseases.

In conclusion, the sulfated polysaccharides produced by *Stephanocystis dioica* are heterofucans composed mainly of fucose, uronic acids, and sulfate. A direct relationship was observed between the sulfate content and anticoagulant activity observed. Platelet pro-aggregation was also observed, suggesting that the fucoidans present in *S. dioica* are of high molecular weight. It is necessary to deepen the study of sulfated polysaccharides to develop alternative compounds to treat cardiovascular diseases like thrombosis, platelet aggregation disorders, and other maladies that involve regulating blood coagulation mechanisms.

ACKNOWLEDGMENTS

The authors thank to Instituto Politécnico Nacional for financially supporting this research. Also, thanks to Comisión de Operación y Fomento de Actividades Académicas (COFAA) and Estímulo al Desempeño de los Investigadores (EDI), and Sistema Nacional de Investigadoras e Investigadores (SNII).

REFERENCES

Abel-Fattah, A. F., M. M. D. Hussein & H. M. Salem.1974. Some structural features of sargassan, a sulphated heteropolysaccharide from *Sargassum linifolium*. *Carbohydrate Research* 33: 19-24. DOI: 10.1016/S0008-6215(00)82936-5

Athukorala, Y., W. K. Jung, T. Vasanthan & Y. J. Jeon. 2006. An anticoagulative polysaccharide from an enzymatic hydrolysate of *Ecklonia cava*. *Carbohydrate Research* 66: 184-191. DOI: 10.1016/j.carbpol.2006.03.002

Athukorala, Y., K. W. Lee, S. K. Kim & Y. J. Jeon. 2007. Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. *Bioresource Technology* 98: 1711-1716. DOI: 10.1016/j.biortech.2006.07.034

Augustyniak, A., J. Przyborska & H. McMahon. 2024. Differential effects of Fucus vesiculosus fucoidan on fibroblast and macrophage cell lines inflammatory activation. *Results in Chemistry* 7: 101443. DOI: 10.1016/j.rechem.2024.101443

Awad, N. E., H. M. Motawe, M. A. Selim & A. A. Matloub. 2009. Antitumourigenic polysaccharides isolated from the brown algae, *Padina pavonia* (L.) Gaill and *Hydroclathrus clathratus* (C. Agardh) Howe. *Medicinal and Aromatic Plant Science and Biotechnology* 3: 6-11.

Bociek, S. M. & D. Welti. 1975. The quantitative analysis of uronic acid polymers by infrared spectroscopy. *Carbohydrate Research* 42: 217-226. DOI: 10.1016/S0008-6215(00)84263-9

Chevolot, L., A. Foucault, F. Chaubet, N. Kervarec, C. Sinquin, A. M. Fisher & C. Boisson-Vidal. 1999. Further data on the structure of brown seaweed fucans: relationships with anticoagulant activity. *Carbohydrate Research* 319: 154-165. DOI: 10.1016/s0008-6215(99)00127-5

Ciancia, M., I. Quintana & A. S. Cerezo. 2010. Overview of anticoagulant activity of sulfated polysaccharides from seaweeds in relation to their structures, focusing on those of green seaweeds. *Current Medicinal Chemistry* 17: 2503-2529. DOI: 10.2174/092986710791556069

Cumashi, A., N. A. Ushakova, M. E. Preobrazhenskaya, A. D'Incecco, A. Piccoli, L. Totani, N. Tinari, G. E. Morozevich, A. E. Berman, M. I. Bilan, A. I. Usov, N. E. Ustyuzhanina, A. A. Grachev, C. J. Sanderson, M. Kelly, G. A. Rabinovich, S. Iacobelli & N. E. Nifantiev. 2007. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology* 17: 541-552. DOI: 10.1093/glycob/cwm014

De Lara-Isassi, G. & S. Álvarez-Hernández. 1999. Evaluación de la actividad anticoagulante de algas marinas presentes en las costas del Golfo de México y Caribe mexicano. *Revista de la Sociedad Mexicana de Historia Natural* 49: 75-82. Available online at: http://hdl.handle.net/11154/143155

Dische, Z. 1955. New color reaction for determination of sugars in polysaccharides. *Methods of Biochemical Analysis* 2: 313-358. DOI: 10.1002/9780470110188.ch11

Dobashi, K., T. Nishino, M. Fujihara & T. Nagumo. 1989. Isolation and preliminary characterization of fucose-containing sulfated polysaccharides with blood-anticoagulant activity from the brown seaweed *Hizikia fusiforme*. *Carbohydrate Research* 194: 315-320. DOI: 10.1016/0008-6215(89)85032-3

Dore, C. M. P. G., M. G. C. Faustino-Alves, L. S. E. Porfírio-Will, T. G. Costa, D. A. Sabry, L. A. R. de Souza-Rêgo, C. M. Accardo, H. A. O. Rocha, L. G. A. Filgueira & E. L. Leite. 2013. A sulfated polysaccharide, fucans, isolated from brown algae *Sargassum vulgare* with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. *Carbohydrate Polymers* 91: 467-475. DOI: 10.1016/j.carbpol.2012.07.075

Holtkamp, A. D., S. Kelly, R. Ulber & S. Lang. 2009. Fucoidan and fucoidanases–focus on techniques for molecular structure elucidation and modification of marine polysaccharides. *Applied Microbiology and Biotechnology* 82: 1-11. DOI: 10.1007/s00253-008-1790-x

Inácio, A. R., A. C. Carvalho, C. Oliveira, L. Reys, S. S. Silva, N. M. Neves, A. Martins, R. L. Reis & T. H. Silva. 2020. Sulfated Seaweed Polysaccharides. *In*: Oliveira, J. M., H. Radhouani & R. L. Reis (Eds.). *Polysaccharides of Microbial Origin. Biomedical Applications*. Springer, pp. 307-340. DOI: 10.1007/978-3-030-42215-8

Jiang, Z., T. Okimura, T. Yokose, Y. Yamasaki, K. Yamaguchi & T. Oda. 2010. Effect of sulfated fucan, ascophyllan, from the brown alga *Ascophyllum nodosum* on various cell lines: a comparative study on ascophyllan and fucoidan. *Journal of Bioscience and Bioengineering* 110: 113-117. DOI: 10.1016/j.jbiosc.2010.01.007

Jiao, G., G. Yu, J. Zhang & H. E. Stephen. 2011. Chemical structures bioactivities of sulfated polysaccharides from marine algae. *Marine Drugs* 9: 196-223. DOI: 10.3390/md9020196

Li, B., F. Lu, X. Wei & R. Zhao. 2008. Fucoidan: Structure and bioactivity. *Molecules* 13: 1671-1695. DOI: 10.3390/molecules13081671

Lijour, Y., E. Gentric, E. Deslandes & J. Guezennec. 1994. Estimation of the sulfate content of hydrothermal vent bacterial polysaccharides by Fourier transform infrared spectroscopy. *Analytical Biochemistry* 220: 244-248. DOI: 10.1006/abio.1994.1334

Liyanage, N. M., D. P. Nagahawatta, T. U. Jayawardena, K. K. A. Sanjeewa, H. H. A. C. K. Jayawrdhana, J. I. Kim & Y. J. Jeon. 2023. Sulfated Polysaccharides from Seaweeds: A Promising Strategy for Combatting Viral Diseases-A Review. *Marine Drugs* 21(9): 461. DOI: 10.3390/md21090461

Manne, B. K., T. M. Getz, C. E. Hughes, O. Alshehri, C. Dangelmaier, U. P. Naik, S. P. Watson & S. P. Kunapuli. 2013. Fucoidan is a novel platelet agonist for the C-type lectin-like receptor 2 (CLEC-2). *Journal of Biological Chemistry* 288(11): 7717-7726. DOI: 10.1074/jbc.M112.424473

Matsubara, K. 2004. Recent advances in marine algal anticoagulants. *Current Medicinal Chemistry – Cardiovascular & Hematology Agents* 2: 13-19. DOI: 10.2174/1568016043477314

Mourão, P. A. S. 2004. Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. *Current Pharmaceutical Desing* 10: 967-981. DOI: 10.2174/1381612043452730

Muñoz-Ochoa, M., J. I. Murillo-Álvarez, Y. E. Rodríguez-Montesinos, G. Hernández-Carmona, D. L. Arvizu-Higuera, J. Peralta-Cruz & J. Lizardi-Mendoza. 2009. Anticoagulant screening of marine algae from México, and partial characterization of the active sulphated polysaccharide from *Eisenia arborea*. *CICIMAR Oceánides* 24: 41-51. DOI: 10.37543/oceanides.v24i1.52

Nishino, T., G. Yokoyama, K. Dobashi, M. Fujihara & T. Nagumo. 1989. Isolation, purification, and characterization of fucose containing sulfated polysaccharides from the brown seaweed *Ecklonia kurome* and their blood anticoagulant activities. *Carbohydrate Research* 186: 119-129. DOI: 10.1016/0008-6215(89)84010-8

Patankar, M., S. Oehninger, T. Barnett, R. L. Williams & G. F. Clark. 1993. A revised structure for fucoidan may explain some of its biological activities. *Journal of Biological Chemistry* 268: 21770-21776. DOI: 10.1016/S0021-9258(20)80609-7

Pradhan, B., S. Patra, R. Nayak, C. Behera, S. R. Dash, S. Nayak, B. B. Sahu, S. K. Bhutia & M. Jena. 2020. Multifunctional role of fucoidan, sulfated polysaccharides in human health and disease: A journey under the sea in pursuit of potent therapeutic agents. *International Journal of Biological Macromolecules* 164: 4263-4278. DOI: 10.1016/j.ijbiomac.2020.09.019

Ptak, S. H., L. Sanchez, X. Fretté & D. Kurouski. 2021. Complementarity of Raman and Infrared spectroscopy for rapid characterization of fucoidan extracts. *Plant Methods* 17: 130. DOI: 10.1186/s13007-021-00830-6

Pedroche, F. F. & A. Sentíes. 2020. Diversidad de macroalgas marinas en México. Una actualización florística y nomenclatural. *Cymbella* 6: 4-55. Available online at: https://cymbella.fciencias.unam.mx/articulos/V6/01/Diversidad_de_macroalgas_marinas_en_Mexico.html

Pereira, M. S., F. R. Melo & P. A. S. Mourão. 2002. Is there correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans? *Glycobiology* 12: 573-580. DOI: 10.1093/glycob/cwf077

Rioux, L. E., S. L. Turgeon & M. Beaulieu. 2007. Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers* 69: 530-537. DOI: 10.1016/j.carbpol.2007.01.009

Senthilkumar, K., P. Manivasagan, J. Venkatesan & S. K. Kim. 2013. Brown seaweed fucoidan: biological activity and apoptosis, growth signaling mechanism in cancer. *International Journal of Biological Macromolecules* 60: 366-374. DOI: 10.1016/j.ijbiomac.2013.06.030

Shanmugam, M. & K. H. Mody. 2000. Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. *Current Science* 79: 1672-1683. Available online at: https://www.jstor.org/stable/24104127

Silva, T. M. A., L. G. Alves, K. C. S. de Queiroz, M. G. L. Santos, C. T. Marques, S. F. Chavante, H. A. O. Rocha & E. L. Leite. 2005. Partial cha-

racterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. *The Brazilian Journal of Medical and Biological Research* 38: 523-533. DOI: 10.1590/S0100-879X2005000400005

Trejo, C. I. 2004. Anticoagulantes: Farmacología, mecanismos de acción y usos clínicos. *Cuadernos de Cirugía* 18: 83-90. Available online at: http://revistas.uach.cl/index.php/cuadcir/article/view/2322

Wang, Y., M. Xing, Q. Cao, A. Ji, H. Liang & S. Song. 2019. Biological activities of fucoidan and the factors mediating its therapeutic effects: A review of recent studies. *Marine Drugs* 7(3): 183. DOI: 10.3390/md17030183

Wijesinghe, W. A. J. P. & Y. J. Jeon. 2012. Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review. *Carbohydrate Polymers* 88: 13-20. DOI: 10.1016/j.carbpol.2011.12.029

Yang, L. & L. M. Zhang. 2009. Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate Polymers* 76: 349-361. DOI: 10.1016/j.carbpol.2008.12.015

Zhang, H. J., W. J. Mao, F. Fang, H. Y. Li, H. H. Sun, Y. Chen & X. H. Qi. 2008. Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostroma latissimum*. *Carbohydrate Polymers* 71: 428-434. DOI: 10.1016/j.carbpol.2007.06.012

Zhu, Z., Q. Zhang, L. Chen, S. Ren, P. Xu, Y. Tang & D. Luo. 2010. Higher specificity of the activity of low molecular weight fucoidan for thrombin-induced platelet aggregation. *Thrombosis Research* 125(5): 419-26. DOI: 10.1016/j.thromres.2010.02.011

Zvyagintseva, T. N., N. M. Shevchenko, A. O. Chizhov, T. N. Krupnova, E. V. Sundukova & V. V. Isakov. 2003. Water-soluble polysaccharides of some far-eastern brown seaweeds. Distribution, structure, and their dependence on the developmental conditions. *Journal of Experimental Marine Biology and Ecology* 294: 1-13. DOI: 10.1016/S0022-0981(03)00244-2