

Partial characterization and biological activity of *Sargassum lapazeanum* (Fucales, Phaeophyceae)

Caracterización parcial y actividad biológica de *Sargassum lapazeanum* (Fucales, Phaeophyceae)

Valeria Alexandra Villegas-Silva, Mauricio Muñoz-Ochoa[✉], Yoloxochitl Elizabeth Rodríguez-Montesinos^{*✉}, Dora Luz Arvizu-Higuera[✉]

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ABSTRACT

Background. The Baja California peninsula contains a wide variety of habitats and climatic conditions and is thus home to an enormous wealth of marine algae. Algae belonging to the *Sargassum* genus are often dominant in communities of the Gulf of California due to their high biomass. **Goals.** This study aimed to evaluate the potential of *Sargassum lapazeanum* as a source of compounds with antioxidant, antibacterial, or anticoagulant activity in addition to its proximal composition and alginate content. **Methods.** An ethanolic extract (EE) was obtained from dried algae to evaluate antibacterial activity by means of the disc agar diffusion method and antioxidant activity by the stable free radical method (DPPH). The crude fucoidan (CF) obtained was fractionated by different chromatographic techniques, and its anticoagulant activity was evaluated by prothrombin time (PT) and activated partial thromboplastin time (APTT) assays. A structural characterization was also conducted. In addition, the proximal composition and alginate content and quality were determined. **Results.** The EE and its different fractions showed selective activity against bacteria of the *Vibrio* genus, such as *V. harveyi* and *V. parahaemolyticus*, in addition to free radical scavenging activity. The lowest half maximal effective concentration ($EC_{50} = 39.96 \mu\text{g mL}^{-1}$) was obtained from fraction F6. Crude fucoidan and its fractions showed anticoagulant activity, with values comparable to those observed for heparin at concentrations of $10 \mu\text{g mL}^{-1}$. The sodium alginate yield was 22.8%, with low viscosity and high gel strength. **Conclusions.** *Sargassum lapazeanum* has high potential to be a source of compounds with antioxidant, antibacterial, and anticoagulant activity.

Keywords: biological activity, brown seaweed, phytochemistry, polysaccharides, Gulf of California.

Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional. Av. Instituto Politécnico Nacional s/n. Col. Playa Palo de Santa Rita, La Paz, Baja California Sur, 23096. México

***Corresponding author:**

Yoloxochitl Elizabeth Rodríguez-Montesinos:
e-mail: yrodriguez@ipn.mx

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RESUMEN

Antecedentes. La península de Baja California cuenta con una gran variedad de hábitats y condiciones climáticas, por lo que alberga una enorme riqueza de algas marinas destacando la presencia de las algas pertenecientes al género *Sargassum* por su alta dominancia en biomasa. **Objetivos.** Evaluar el potencial de *Sargassum lapazeanum* como fuente de compuestos con actividad antioxidante, antibacteriana y anticoagulante, su composición proximal y contenido de alginato. **Métodos.** Se obtuvo un extracto etanólico a partir del alga seca para evaluar su actividad antibacteriana por medio del método de difusión en agar con discos y la actividad antioxidante por el método de radical libre estable (DPPH). El fucoidano crudo obtenido se fraccionó por medio de distintas técnicas cromatográficas, se evaluó la actividad anticoagulante por medio de las pruebas de tiempo de protrombina (TP) y tiempo de tromboplastina parcial activada (TTPA) y se llevó a cabo su caracterización estructural. Además, se determinó la composición proximal del alga, el contenido y calidad del alginato. **Resultados.** El extracto etanólico, así como sus diferentes fracciones presentaron una actividad selectiva contra bacterias del género *Vibrio* como *V. harveyi* y *V. parahaemolyticus*, además presentó actividad secuestrante de radicales libres, obteniendo la menor EC_{50} ($39.96 \mu\text{g mL}^{-1}$) con la fracción F6. El fucoidano crudo y sus fracciones mostraron actividad anticoagulante con valores comparables a los observados por la heparina a concentraciones de $10 \mu\text{g mL}^{-1}$. El rendimiento del alginato de sodio obtenido fue de 22.8%, con una baja viscosidad y alta fuerza de gel. **Conclusiones.** Los ensayos mostraron que *S. lapazeanum* es un alga con un alto potencial como fuente de compuestos con actividad antioxidante, antibacteriana y anticoagulante.

Palabras clave: actividad biológica, algas pardas, fitoquímico, polisacáridos, Golfo de California.

INTRODUCTION

Sargassum C. Agardh is a brown seaweed (Phaeophyceae) genus harboring at least 615 taxonomically accepted species, making it the most abundant genus within the Fucales order (Guiry & Guiry, 2022). *Sargassum* species are distributed throughout the tropical and subtropical zones of both hemispheres and often form dense underwater forests. These forests constitute key habitats that supply shelter to many species, such as fishes, invertebrates, and other algae. In the Gulf of California, *Sargassum* species are often dominant within communities due to their biomass (Suárez-Castillo *et al.*, 2013). *Sargassum* species are of interest to study due to their capacity to synthesize secondary metabolites, including terpenoids, florotannins, polyphenols, volatile hydrocarbons, sulfated polysaccharides, and products of mixed biogenetic origin. As such, *Sargassum* species have unique chemical compositions, making them important sources of phycocolloids and compounds that may be used in fertilizers, fodder, food supplements, and compounds of pharmaceutical interest (Mascheck & Baker, 2008; Echavarría *et al.*, 2009).

There are five *Sargassum* species that form underwater forests in the Gulf of California: *S. johnstonii* Setchell & N. L. Gardner; *S. herporhizum*, Setchell & N. L. Gardner; *S. sinicola* Setchell & N. L. Gardner; *S. horridum* Setchell & N. L. Gardner; and *S. lapazeanum* Setchell & N. L. Gardner (Suárez-Castillo *et al.*, 2013).

A few studies have been conducted with *S. lapazeanum* that have spanned a variety of topics including biogeography (Phillips, 1995), population dynamics (Rivera & Scrosati, 2006), taxonomy (Andrade-Sorcia *et al.*, 2008; Norris, 2010), and biotechnology (Patrón-Prado *et al.*, 2011). However, despite technological advances, no information is yet available for the biological activity of *S. lapazeanum*. Therefore, the aim of this study was to evaluate the potential of *S. lapazeanum* as a source of biologically active compounds, such as those with antimicrobial, antioxidant, and anticoagulant activity. In addition, this study aimed to conduct a partial characterization of fucoidan active fractions and their structural nature.

MATERIALS AND METHODS

Sampling. *Sargassum lapazeanum* was collected by hand in the intertidal zone of the Tarabillas site (24°27'55.1" N, 110°41'20.1" W), which is located in La Paz, Baja California Sur, Mexico. Seaweed samples were collected at 1 m depth and transported to the laboratory in buckets at room temperature. In the laboratory, the seaweeds were thoroughly washed with tap water to remove epiphytes and sand and then sun-dried. After which, the seaweed samples were ground into particles (1 mm) and stored in plastic bags at room temperature until analysis.

Proximal analysis. A proximal analysis of the algal tissue was conducted at Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in La Paz, Mexico, following the methods of the Association of Official Analytical Chemists (AOAC 2005). All analyses were conducted in triplicate. Moisture content was determined as the difference in sample weights before and after treatment at 110 °C for 4 h (AOAC 930.15). Ash content was determined as the difference in dry weight before and after calcination at 600 °C for 2 h (AOAC 942.05). Crude protein content was determined using a FP-528 Nitrogen/Protein Determinator (LECO, Saint Joseph, MI, USA) after determining the total nitrogen content and multiplying by a factor of 6.25 (AOAC 990.03). Crude lipid content was

determined by direct extraction with diethyl ether using a Foss Tecator Soxtec Avanti 2050 Extraction System (FOSS Analytical, Höganäs, Sweden; AOAC 920.39). Crude fiber content was determined by the successive hydrolysis method (AOAC 978.10).

Ethanolic extract (EE). Three hundred grams of algae were extracted with ethanol (96%) at room temperature (25 °C) for one week, changing the solvent every other day. All obtained filtrates were combined into a final solution, which was concentrated to dryness with a RE500 rotary evaporator (Yamato, Orangeburg, NY, USA) at 40 °C. The obtained ethanolic extract (EE) was stored at -18 °C.

Ethanolic extract (EE) fractionation. Fifteen grams of EE was fractionated in a normal phase silica gel chromatography column using a mixture of the following solvents: CH₂Cl₂:100, CH₂Cl₂:EtOH 90:10, CH₂Cl₂:EtOH 25:75, CH₂Cl₂:EtOH 50:50, EtOH 100, EtOH:H₂O 50:50 y H₂O 100%. Seven fractions (F1–F7) were obtained. Subsequently, fraction F2 was selected for a second fractionation because it showed the highest antibacterial activity. This fraction was separated using the same chromatographic system with a mixture of the following solvents: CH₂Cl₂:100, CH₂Cl₂:MeOH 95:5, CH₂Cl₂:MeOH 90:10, CH₂Cl₂:MeOH 80:20, CH₂Cl₂:EtOH 70:30, CH₂Cl₂:MeOH 50:50, EtOH 100, and H₂O 100%. Fourteen subfractions (FF1–FF14) were obtained as a result.

Crude fucoidan content and purification. Crude fucoidan (CF) was obtained following the method of Muñoz-Ochoa *et al.* (2009) with modifications. One hundred grams of algae were soaked in 600 mL of distilled water. The mixture was then placed in a water bath (Precision Scientific, Chicago, IL, USA) at 55 °C with continuous stirring for 2 h. The mixture was filtered, and the extract liquor was centrifuged at 3000 rpm for 30 min (Beckman centrifuge TJ-6, Palo Alto, CA, USA). The clarified solution was decanted and precipitated with 40 mL of 10% calcium chloride to remove any soluble residual alginate, which was separated by centrifugation a 3000 rpm for 30 min. The clarified solution was precipitated with three volumes of ethanol. Then, the precipitate was separated by centrifugation for 15 min at 3000 rpm and dried at 50 °C.

The CF was dissolved in 150 mL of distilled water and partially purified by fractional precipitation with three volumes of ethanol, and the precipitate was recovered after each volume was added. The three precipitates (CFF1, CFF2, and CFF3) were obtained and dried at 55 °C for 24 h and stored at -18°C until analysis.

Antibacterial activity. This was evaluated in duplicate by the disc agar diffusion method of Kirby-Bauer (Bauer *et al.*, 1966). For this study, *Staphylococcus aureus* Rosenbach 1884 (BAA-42); *Escherichia coli* Escherich1885 (BAA-196); *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872; *Vibrio harveyi* Filippo Pacini 1854 (14126), and *Vibrio parahaemolyticus* Filippo Pacini 1854, were obtained from the American Type Culture Collection (ATCC) and maintained in Mueller-Hinton media for marine bacteria (Mueller-Hinton 2.5% NaCl). Paper discs (6.5 mm diameter) impregnated with 2 mg of each sample (EE, F1–F7) were placed on plates of Mueller-Hinton agar that had been previously inoculated with a suspension of each test microorganism containing 1×10⁸ cells mL⁻¹. Inhibition zones were measured after 24 h of incubation at 37 °C. Ampicillin and erythromycin were used as positive controls, and solvents were used as negative controls.

Antioxidant activity. Free radical scavenging activity was evaluated using the bioautographic and colorimetric methods. For the former, the antioxidant constituents were analyzed using thin layer chromatogra-

phy (TLC) following the methodology of Wang *et al.* (2012). Each sample (1 mg mL⁻¹) was dissolved in the appropriate solvent, and 10 µL of each solution was loaded onto TLC plates. The plates were developed in a CH₂Cl₂:MeOH (9:1) system, sprayed with 0.2% DPPH solution in methanol, and left in the dark for 30 min at room temperature. After this time, antioxidant activity was detected by a change in color from purple to yellow, reflecting discoloration.

The colorimetric assay was conducted in duplicate following the method of Molyneux (2004). A stock solution of each sample [40 mg in 5 ml of EtOH:H₂O (9:1)] was tested at different concentrations (400, 200, 100, 50, and 25 µg mL⁻¹), and dose-effect curves were prepared. Each concentration (1 mL) was mixed with 4 mL of 0.02% DPPH solution in methanol and placed in the dark for 30 min at room temperature. The absorbance at 517 nm was measured in a spectrophotometer (Milton Roy, Spectronic 20D, Rochester, NY, USA). A blank was also prepared to obtain the color correction factor.

Free radical scavenging activity (SA) was calculated as the percentage of DPPH reduction using the formula:

$$SA (\%) = \frac{A_0 - A_s}{A_0} \times 100 \quad (\text{Eq. 1}),$$

where A₀ is the blank absorbance and A_s is the sample absorbance. With the data, a dose-effect curve was constructed by graphing the reduction percentage of DPPH regarding the concentration of the extract used. Further, the half effective concentration (EC₅₀) values were calculated with a linear regression of the dose-effect curve. Ascorbic acid was used as a positive control.

Fucoidan anticoagulant activity. This was evaluated in duplicate according to instructions of the manufacturer (SIEMENS®), by the increase in prothrombin time (PT) and activated partial thromboplastin time (APTT). The PT and APTT were measured using human plasma treated with sodium citrate and a stock solution of 10 mg mL⁻¹ of CF. The PT assay was carried out by mixing 90 µL of human plasma with 10 µL of stock solution followed by incubation at 37 °C for 1 min. After incubation, 200 µL of PT reagent (preincubated at the same temperature for 10 min) were added to the mixture. The APTT assay was carried out by mixing 90 µL of human plasma with 10 µL of stock solution and 100 µL of APTT reagent followed by incubation at 37 °C for 3 min. After incubation, 100 µL of 0.025 M CaCl₂ (preincubated at the same temperature) were added to the mixture. The time for clot formation for PT and APTT was determined by visual inspection and recorded in seconds. Distilled water was used as a negative control while sodium heparin was used as a positive control in both assays.

Fucoidan Fourier-transform infrared (FTIR) analysis. The CF as well as its fractions (CFF1, CFF2, and CFF3) were structurally characterized

with an IR spectrophotometer (Perkin Elmer, TWO, Waltham, MA, USA) equipped with an attenuator of total reflectance (ATR). Each spectrum was obtained by the addition of 14 scans (resolution of 4 cm⁻¹) in the spectral range of 500–4000 cm⁻¹. In addition, the sulfate content was calculated by the baseline method based on the sulphate/total sugars (SO₄/CHOH) ratio determined from the area under the curve of the bands at 1250 and 1040 cm⁻¹ (Lijour *et al.*, 1994).

Alginate extraction. Sodium alginate was obtained in triplicate following the method of Rodríguez-Montesinos *et al.* (2008). Twenty grams of the algal residual obtained in the fucoidan process were hydrated overnight in 180 mL of 0.1% formaldehyde solution. The solution was drained, and the algal tissue was placed in 400 mL of distilled water. The pH was adjusted to 4 with 1N HCl and constant stirring for 15 min. The solution was drained, and the algal tissue was placed in 400 mL of distilled water. The pH was adjusted to 10 with 10% Na₂CO₃ in a water bath (80 °C) with constant stirring for 2 h. The resulting mixture was vacuum filtered using diatomaceous earth as a filter aid. The obtained solution was precipitated with one volume of ethanol (96%). The precipitate was then filtered, dried for 24 h at 55 °C, and stored at 4 °C. The alginate yield was computed as the percentage of algal dry weight.

Chemical properties of alginate. The viscosity of sodium alginate was measured in a 1% (w/v) alginate solution at 22 °C with a digital viscometer (Brookfield, DV-I, Middleboro, MA, USA) using the appropriate spindle. Alginate gel strength was measured using the 1% alginate solution. A dialysis membrane (2.9 cm diameter) was filled with the alginate solution and immersed overnight in 10% CaCl₂. The gels were cut into three cylinders (2.9 cm diameter × 3 cm length), and gel strength was measured with a texture analyzer (Stable Micro Systems, TA.XT Plus, Godalming, Surrey, UK) (Borras-Chavez *et al.*, 2016).

RESULTS

Proximal analysis. *Sargassum lapazeanum* is mainly composed of a high content of inorganic material and carbohydrates, corresponding to high values of nitrogen-free extract (NFE) and ash (Table 1). The lowest value recorded was that of the ether extract content.

Antimicrobial activity. The agar disc diffusion method was used to screen for antimicrobial activity. The EE and F1–F7 fractions were assayed against *S. aureus*, *B. subtilis*, *E. coli*, *V. parahaemolyticus*, and *V. harveyi*. None were active against *S. aureus*, *B. subtilis*, or *E. coli*, and only EE and F2 inhibited *V. parahaemolyticus* and *V. harveyi* (Table 2). The F2 fraction was fractionated again, and 14 fractions were obtained. Due to the small quantities obtained for some fractions, only some of these could be tested against *V. parahaemolyticus* and *V. harveyi*. The results indicate that the F2F6, F2F8, F2F9, and F2F10 fractions were active for both bacteria, while the F2F12 fraction was only active against *V. harveyi* (Table 3).

Table 1. Composition of *Sargassum lapazeanum* from Tarabillas, Baja California Sur, Mexico. Mean ± SD (n = 3).

Moisture (%)	Protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	NFE (%)	Gross energy (kJ g ⁻¹)
8.51 ± 0.03	8.54 ± 0.07	0.28 ± 0.03	6.06 ± 0.15	30.52 ± 0.1	85.12	10.55 ± 0.02

NFE = nitrogen-free extract

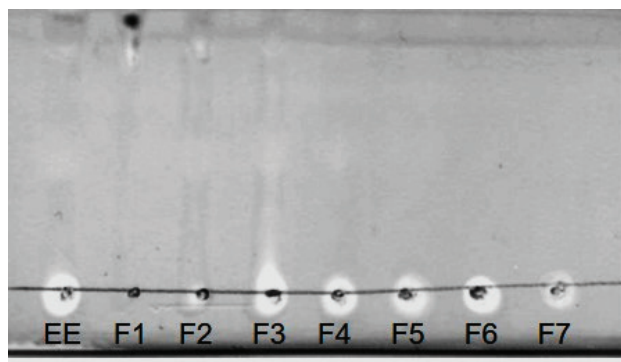


Figure 1. Antioxidant activity of the fractions obtained from the ethanolic extract (EE) of *Sargassum lapazeanum* using the bioautographic method and revealed with a 0.4% DPPH solution. Discolored areas mean free radical scavenging activity.

Antioxidant activity. A TLC bioautography was performed to screen for the antioxidant activity of the EE and its fractions. All samples showing discolored bands were considered antioxidants (Fig. 1). For this reason, all samples were analyzed by the colorimetric method to determine the EC_{50} . The results indicate that F6 presented the lowest EC_{50} of $39.96 \mu\text{g mL}^{-1}$, while the highest EC_{50} corresponded to fraction F1 ($394.21 \mu\text{g mL}^{-1}$) (Fig. 2). On the other hand, ascorbic acid presented an EC_{50} of $1.5 \mu\text{g mL}^{-1}$, which was 26-fold higher than that of F6.

Anticoagulant activity. The CF yield was 3% based on the dry weight of the seaweed. The anticoagulant activity of CF and the CFF1, CFF2, and CFF3 fractions was evaluated, and the results were compared with those of heparin (control) (Table 4). For the APTT assay, all fractions analyzed had clotting times greater than 300 s that were longer than the control (31s). For the TP assay, the CFF2 fraction showed the greatest activity with a coagulation time of 41.9 s; however, all analyzed samples had higher PT values than the control (14 s). Based on the above, the dose-effect curves of the extracts (CF, CFF1, CFF2, and CFF3) were constructed (Fig. 3), and it was observed that most extracts showed coagulation times that were double that of the control (32.8 s) even at minimum concentrations.

Fucoidan FTIR. FTIR-ATR spectroscopy was performed to identify the major characteristic peaks (Fig. 4). The CF, CFF1, CFF2, and CFF3 spectra showed different absorption bands that were characteristic of heterofucan. The bands around 1375 and $960\text{--}970 \text{ cm}^{-1}$ corresponded to the methyl group of fucose, whereas bands between 1417 and 1616 cm^{-1} corresponded to an amide bond and uronic acid residues, respectively. An absorption band around $1210\text{--}1250 \text{ cm}^{-1}$ corresponding to S-O stretching, a band at 830 cm^{-1} corresponding to C-O-S stretching, and an additional band at 570 cm^{-1} confirm a notable number of sulfate groups. Furthermore, the sulfate content in CF, CFF1, CFF2, and CFF3 was 27.85%, 8.10%, 47.48%, and 12.98%, respectively.

Alginate. The sodium alginate yield obtained from the residual algal used for the fucoidan extraction was 22.88% (SD ± 0.91). The viscosity of the alginate obtained was $50.83 \text{ mPa}\cdot\text{s}$ (SD ± 1.04), and its gel strength was $1807.42 \text{ g cm}^{-2}$ (SD ± 10.60).

DISCUSSION

The main constituents of *S. lapazeanum* were NFE (85.1%) and ash (30.5%). Usually, NFE is mainly composed of soluble carbohydrates, such as starches and sugars, although it also includes considerable amounts of alginic acid and sulfated polysaccharides (Carrillo-Domínguez *et al.*, 2002). The ash percentage in this study is comparable to those of other studies on seaweeds, which have indicated that the high organic matter content detected is due to these organisms being able to store mineral elements that are abundant in the marine environment (Carrillo-Domínguez *et al.*, 2002; Casas-Valdez *et al.*, 2006).

The protein content reported in the literature for brown macroalgae varies from 3–15% (dry weight) and depends on multiple factors, including the species and season (Burtin, 2003). Studies with *Sargassum* species have shown that the quality of these proteins is adequate, as essential amino acids are present and digestibility is higher than 70% (Casas-Valdez *et al.*, 2006).

The amount of crude fiber (6.06%) found in this study was low, although comparable to those of some foods intended for human consumption, such as oats (7%) and legumes like beans and chickpeas (5%) (Carrillo-Domínguez *et al.*, 2002). In addition, the amount of gross energy (10.55 kJ g^{-1}) was similar to that found by Di Filippo-Herrera *et al.* (2018) in *S. horridum* ($10.26\text{--}11.55 \text{ kJ g}^{-1}$).

Table 2. Antimicrobial activity (zone of inhibition in mm) of the ethanolic extract (EE) and fractions (2 mg/disc) of *S. lapazeanum*. Mean \pm SD (n = 2).

Extract	Microorganism				
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Vibrio parahaemolyticus</i>	<i>Vibrio harveyi</i>
EE	--	--	--	8.5 ± 0.70	10.0 ± 0
EEF1	--	--	--	--	--
EEF2	--	--	--	8.0 ± 0	8.5 ± 0.70
EEF3	--	--	--	--	--
EEF4	--	--	--	--	--
EEF5	--	--	--	--	--
EEF6	--	--	--	--	8.75 ± 0.35
EEF7	--	--	--	--	9.0 ± 0
Eritromicina	25	13	R	13	13
Ampicilina	10	R	R	7	7

-- Without activity

R = Resistant

Table 3. Antimicrobial activity (zone of inhibition in mm) of the EEF2 fractions (2 mg/disc). Mean \pm SD (n = 2).

Fraction	<i>Vibrio parahaemolyticus</i>	<i>Vibrio harveyi</i>
F2F5	--	--
F2F6	7.75 \pm 0.35	10.75 \pm 1.06
F2F8	8.5 \pm 0.71	11.75 \pm 1.06
F2F9	8.0 \pm 0.71	11.75 \pm 0.35
F2F10	7.75 \pm 0.35	11.50 \pm 0.71
F2F11	--	--
F2F12	--	10.0 \pm 0
-- Without activity		

Most studies conducted with crude marine algae extracts mention high activity against gram-positive bacteria, with *S. aureus* being considered one of the species most susceptible to algal extracts (Rios *et al.*, 2009). However, the ethanolic extract and fractions obtained by solid-liquid fractionation were not active against the gram-positive bacteria used in the assay. Species of the *Sargassum* genus, such as *S. oligocystum* Montagne (Baleta *et al.*, 2011) and *S. latifolium* (Turner) C. Agardh (Dashtiannasab *et al.*, 2012), have shown high potential against bacteria of the *Vibrio* genus. However, these differences in activity can be explained by several factors, such as the species studied, seasonal variation, geographic variation, reproductive state of the algae (De Lara-Issasi & Álvarez-Hernández, 1999), and the type of solvent used for the extraction (Tüney *et al.*, 2006). In addition, some studies have shown that *V. harveyi* is usually sensitive to antibacterial activity tests, presenting more defined inhibition halos than those observed for *V. parahaemolyticus* (Baleta *et al.*, 2011; Dashtiannasab *et al.*, 2012).

Antibacterial activity of *S. lapazeanum* was present in both polar and non-polar fractions. However, the F2 (non-polar) fraction was selected for fractionation, as it showed the highest activity against *V. parahaemolyticus* and *V. harveyi*. After fractionation, the antibacterial activity in some subfractions increased, reflected in more defined inhibition halos. Fractions F2F8 and F2F9 were the most active against *V. harveyi* (11.75 mm), while fraction F2F8 was most active against *V. parahaemolyticus* (8.5 mm). This shows that the fractionation process was effective in such a way that activity increased. The selective activity against bacteria of the *Vibrio* genus observed in this study suggests that *S. lapazeanum* may be considered as an alternative species to obtain natural antibacterial compounds with applications in the treatment of vibriosis in aquaculture settings (Rubio-Limonta & Silveira-Coffigny, 2012). In a qualitative comparison, it was observed that the fractions of the extracts presented a higher antimicrobial activity than the ampicillin control and lower than the erythromycin control.

The results of the free radical scavenging activity assays showed that *S. lapazeanum* is a potential source of compounds with antioxidant activity, although the activity of the fraction (CFF2) with the highest activity ($EC_{50} = 39.96 \mu\text{g mL}^{-1}$) was 6-fold lower than that found by Zubia *et al.* (2007) who obtained EC_{50} values of 6.64 and 7.14 $\mu\text{g mL}^{-1}$ for *S. ramifolium* Kützinger and *S. pteropleuron* Grunow, respectively.

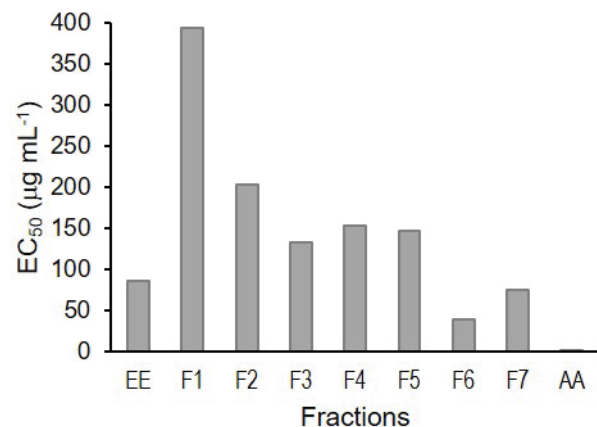


Figure 2. Values obtained for the half maximal effective concentration (EC_{50}) of the fractions of *Sargassum lapazeanum* obtained by polarity gradient. EE = Ethanolic Extract. AA = Ascorbic acid.

Generally, photoautotrophic organisms are exposed to high oxygen and radical stresses and have adapted to these conditions by developing various yet efficient protective systems against reactive oxygen species and free radicals. Some of these systems consist of the production of powerful antioxidants, such as polyphenols, carbohydrates, nitrogenous compounds, phytosterols, carotenoids, chlorophyll derivatives, and other photoprotective pigments (Zubia *et al.*, 2007).

The IR spectra of the fucoidan fractions presented similar absorption patterns, suggesting that all fractions contain the same functional groups; however, differences in composition were present, as evidence by the differences among the intensities of the absorption peaks. The band at 830 cm^{-1} was assigned to the vibration of the C–O–S group,

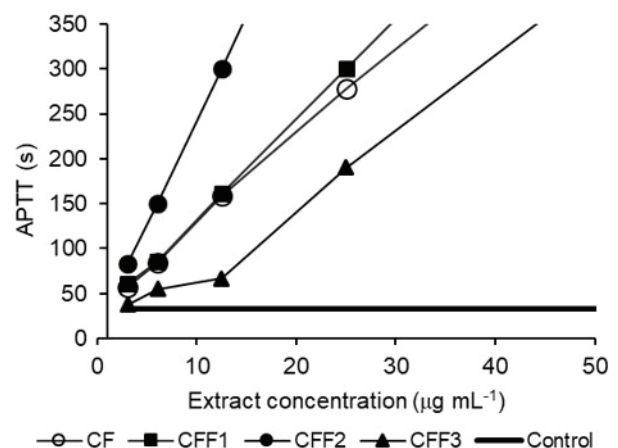


Figure 3. Dose-response curves of anticoagulant activity from the APTT assay employing different fucoidan concentrations from *Sargassum lapazeanum* and fractions obtained by precipitation.

indicating the presence of a sulfate group in the axial position of C4 with minor substitutions in carbons C2 and C3 of fucose given the less intense absorption peak at 805 cm^{-1} (Li *et al.*, 2008). Furthermore, the absorption band at 570 cm^{-1} confirmed the presence of a notable number of sulfate groups (Synytsya *et al.*, 2010). As such, the fucoidan obtained from *S. lapazeanum* is described as a heterofucan due to the presence of uronic acids ($1600\text{--}1650\text{ cm}^{-1}$) and neutral sugars ($1180\text{--}1000\text{ cm}^{-1}$) in the IR bands.

The results obtained from the PT and APTT assays demonstrated the high anticoagulant potential of *S. lapazeanum*, as all of the extracts prolonged the clot formation time, extending it to double that of the control, even at minimum concentrations. The fucoidan fractions obtained in the present study showed activities similar to that of heparin, according to the study by Ronghua *et al.* (2003) who reported a coagulation time for heparin of 125 s at concentrations of $10\text{ }\mu\text{g mL}^{-1}$. Based on this,

Table 4. Fucoidan anticoagulant activity obtained by fractional precipitation. Mean \pm SD (n = 3).

Sample	PT (s)	APTT (s)
CF	30.25 ± 0.79	> 300
CFF1	24.50 ± 1.30	> 300
CFF2	41.93 ± 2.49	> 300
CFF3	23.08 ± 1.90	> 300
Control	14.00	33.54

PT = pro-thrombin time
APTT = activated partial thromboplastin time

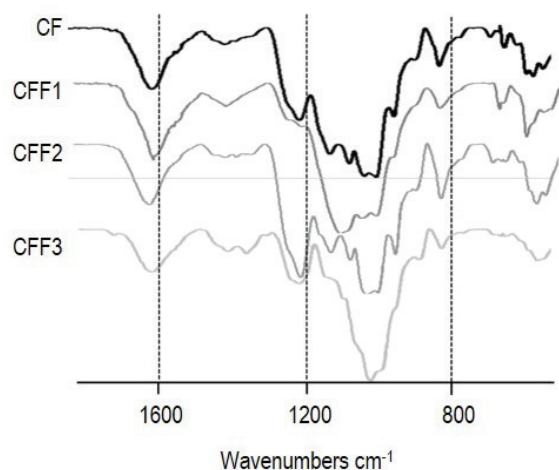


Figure 4. FTIR-ATR spectra of fucoidan fractions from *Sargassum lapazeanum* at $500\text{--}4000\text{ cm}^{-1}$.

it can be said that CFF2 was the most active fraction with the greatest potential, as it doubled this time at the same concentration. This agrees with the potential observed in *S. vulgare* C. Agardh, which was reported to be 10-fold more active than heparin (Guerra-Dore *et al.*, 2013). Heparin is a biomolecule that contains highly sulfated glycosaminoglycan and is the most widely used anticoagulant on the market for the prevention and treatment of cardiovascular diseases. However, the prolonged use of heparin compounds can have adverse effects, such as thrombocytopenia, osteoporosis, and bleeding (Gómez-Ordóñez, 2012).

Regarding the degree of sulfation, the sulfate content in CFF2 (47.48%) was higher compared to those of the other fractions in this study and also presented the most activity. This agrees with the results of previous studies that have indicated that an increase in sulfate content is one of the most important factors that determines anticoagulant activity, which increases according to the degree of fucan sulfation (Muñoz-Ochoa *et al.*, 2009). However, further studies are needed to determine its potential application in humans.

Lastly, the alginate yield (22.8%) obtained from *S. lapazeanum* is high when compared with that reported for *S. cymosum* C. Agardh (15.9%), *S. sinicola* (13-15%), and *S. vulgare* (16.9%; Camacho & Hernández-Carmona, 2012; Torres *et al.*, 2007; Yabur *et al.*, 2007). Due to their thickening, gelling, stabilizing, and film-forming properties, alginates are widely used in food, textile, and pharmaceutical industries (Hernández-Carmona *et al.*, 2012). The results of the present study showed that *Sargassum lapazeanum* is an alga with a high potential as a source of compounds with antioxidant, antibacterial, and anticoagulant activity.

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