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Analysis of two drying methods on the yield and activity of sulfated polysaccharides extracted from *Halymenia* sp. (Rhodophyceae)

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ABSTRACT. Sulfated polysaccharides (SPs) have attracted growing interest for various biotechnological applications. We evaluated the efficiency of two methods of drying SPs (M I and II) extracted from *Halymenia* sp Rhodophyceae in order to compare the yield, purification and anticoagulant activity. The total SPs (TSPs) were first extracted with papain in 100 mM sodium acetate (pH 5.0) containing cysteine and EDTA (5 mM). The TSPs obtained were dried in an oven (M I) or lyophilized (M II) and then examined by ion exchange chromatography (DEAE-cellulose) using the NaCl gradient technique. The fractions were analyzed by 0.5% agarose gel electrophoresis and the *in vitro* anticoagulant activity was evaluated by the activated partial thromboplastin time test using normal human plasma and compared to heparin (HEP) (193.00 IU mg^{-1}). There was a difference in TSP yield of 19.05% and similar chromatographic SP profiles. Electrophoresis revealed fractions with distinct resolutions. The fractions eluted with 0.75 M of salt (M I and II) were the most active, measuring 27.40 and 72.66 IU mg^{-1}, respectively, when compared to HEP. Therefore, obtaining SP with anticoagulant activity from *Halymenia* sp. is more efficient by freeze-drying.

Keywords: Halymeniales, sulfated macromolecules, drying methods, blood coagulation.

Análise de dois métodos de secagem sobre o rendimento e atividade de polissacarídeos sulfatados extraídos da rodofícea *Halymenia* sp.

RESUMO. Os polissacarídeos sulfatados (PS) têm despertado interesse crescente para diversas aplicações em biotecnologia. Avaliou-se a eficiência de duas metodologias de secagem de PSs (M I e II) extraídos da rodofícea *Halymenia* sp. a fim de se comparar o rendimento, a purificação e a atividade anticoagulante. Inicialmente, os PS totais (PSTs) foram extraídos com papaína em tampão acetato de sódio 100 mM (pH 5,0) contendo cisteína e EDTA (5 mM). Os PSTs foram secados em estufa (M I) ou liofilizados (M II), seguido por cromatografia de troca iônica (DEAE-celulose) utilizando um gradiente de NaCl. As frações foram analisadas por eletroforese em gel de agarose a 0,5% e a atividade anticoagulante "in vitro" avaliada por meio do teste do tempo de tromboplastina parcial ativada usando plasma humano normal e comparadas à heparina (HEP) (193.00 UI mg^{-1}). Verificaram-se diferença no rendimento de PSTs (19,05%) e semelhantes perfis cromatográficos de PS, revelando, por eletroforese, frações de PS distintas em grau de resolução entre os métodos. As frações eluídas com 0,75 M de sal (M I e II) foram as mais ativas, com valores de atividade da ordem de 27,40 e 72,66 UI mg^{-1}, respectivamente, em relação à HEP. Portanto, a secagem por liofilização seria uma forma mais eficaz na obtenção de PSs com atividade anticoagulante de *Halymenia* sp.

Palavras-chave: Halymeniales, macromoléculas sulfatadas, métodos de secagem, coagulação sanguínea.

Introduction

Algae are autotrophic organisms with great ecological value and can be found in a wide range of marine and freshwater environments (FONSECA et al., 2009; AMORIM et al., 2011). They have a large number of natural products, with applications in nutrition, cosmetics, food processing and pharmacology (SMIT, 2004; PIRES et al., 2008; CAMPO et al., 2009).

The biosynthesis of compounds known sulfated polysaccharides (SPs) has attracted great interest in various fields of biotechnology, where they have been studied for potential use as gelling agents, thickeners and stabilizers of aqueous mixtures and emulsions in the food industry (CAMPO et al., 2009), anticoagulants and antithrombotics (ATHUKORALA et al., 2006; FONSECA et al., 2008; MOURÃO, 2004; RODRIGUES et al., 2009) and immunostimulants for

fishes and shrimps (CHOTIGEAT et al., 2004; ARAÚJO et al., 2008). In marine algae these compounds are known as galactans in red algae (Rhodophyta) (POMIN; MOURÃO, 2008), fucans or fucoidans in brown algae (Phaeophyta) (CHOTIGEAT et al., 2004), with arabinogalactans and rhamnoses being the most common in green algae (Chlorophyta) (ZHANG et al., 2008), while in animals they are called glycosaminoglycans (RODRIGUES et al., 2011).

Heparin (HEP), a SP obtained naturally from animals, is a therapeutic agent used worldwide for prevention and treatment of venous thrombosis caused by different etiologies. Nevertheless, the use of HEP can be accompanied by various risk factors, such as hemorrhage and thrombocytopenia, thus potentially complicating its clinical use (MOURÃO; PEREIRA, 1999).

Various SP from seaweed have been isolated and characterized, and showed to have anticoagulant activity. The use of different techniques to obtain these compounds can influence their biological activity. Farias et al. (2000) and Matsubara et al. (2001) isolated, respectively, sulfated galactans from the seaweeds *Botryocladia occidentalis* (Rhodophyceae) and *Codium cylindricum* (Chlorophyceae) with potent anticoagulant activities. The sulfated galactan extracted from the red seaweed *Gelidium crinale* also demonstrated anticoagulant activity (PEREIRA et al., 2005). A potent anticoagulant was isolated from the brown kelp *Ecklonia cava*, from among the seven species studied (ATHUKORALA et al., 2006). The species *Monostroma latissimum* (Chlorophyceae) (ZHANG et al., 2008) and *Halymenia pseudofloresia* (Rhodophyceae) also contain SPs with anticoagulant activity, in the latter case better than that of HEP (RODRIGUES et al., 2009).

The aim of this study was to evaluate the use of two methods of drying the SPs extracted from a species of red seaweed of the *Halymenia* genus, by comparative biochemical analyses. The anticoagulant potential from the isolated SPs were also evaluated, thus contributing to the bioprospection of new natural heparinoids present in these organisms.

Material and methods

Collection of the seaweed and extraction of the total sulfated polysaccharide (TSP)

Specimens of red seaweed (*Halymenia* sp. J. Agardh) were collected from the tidal zone at Flecheiras-Trairí Beach in the State of Ceará, Brazil in July 2008 and taken to the Carbohydrates and Lectins Laboratory (CarboLec) of the Department of Biochemistry and Molecular Biology, Federal University of Ceará. In the laboratory, the algae were cleaned to remove epiphytes and other encrusting organisms, washed with distilled water, dehydrated in a Marconi model MA 035 forced-air drying oven (40°C; 15h), and ground for subsequent extraction of the TSPs. Two methods were used to obtain the TSPs, according to the protocol described by Farias et al. (2000), but differing regarding the drying procedure used.

Method I (M I)

Firstly the ground material (5 g) was hydrated in 250 mL of 100 mM sodium acetate buffer (NaAc) (Vetec Química) (pH 5.0) containing EDTA (QEEL) and cysteine (5 mM) (Sigma Chemical), and digested in a crude papain solution (30 mg mL^{-1}) (Vetec Química) for 6 hours at 60°C in bain-marie (Marconi, model MA 159). Then the material was filtered, centrifuged (2725 × *g*, 4°C, 30 min.) and 48 mL of 10% cetylpyridinium chloride (CCP) (Sigma Chemical) was added to the supernatant for precipitation of the SPs (24h, 25°C). After a new centrifuging, the polysaccharide extract was washed (200 mL, 0.05% CCP), dissolved in 174 mL of 2 M NaCl: commercial-grade-ethanol (100:15, v v^{-1}) and precipitated again by adding 200 mL of commercial ethanol (24h; 4°C). Next the material was washed in 200 mL of 80% commercial ethanol (2 ×), commercial-grade-ethanol (200 mL, 1 ×). Finally, the material was dried in a Marconi model MA 035 forced-air drying oven (3h, 60°C).

Method II (M II)

Firstly the ground material (5 g) was hydrated in 250 mL of 100 mM sodium acetate buffer (NaAc) (Vetec Química) (pH 5.0) containing EDTA (QEEL) and cysteine (5 mM) (Sigma Chemical), and digested in a crude papain solution (30 mg mL^{-1}) (Vetec Química) for 6 hours at 60°C in bain-marie (Marconi, model MA 159). Then the material was filtered, centrifuged (2725 × *g*, 4°C, 30 min.) and 48 mL of 10% cetylpyridinium chloride (CCP) (Sigma Chemical) was added to the supernatant for precipitation of the SPs (24h, 25°C). After a new centrifuging, the polysaccharide extract was washed (200 mL, 0.05% CCP), dissolved in 174 mL of 2 M NaCl: commercial-grade-ethanol (100:15, v v^{-1}) and precipitated again by adding 200 mL of commercial-grade-ethanol (24h, 4°C). Next the material was washed in 200 mL of 80% commercial ethanol (2 ×), commercial-grade-ethanol (200 mL, 1 ×). Finally, the material was submitted to dialysis (43 × 27 mm cellulose membrane, Sigma-Aldrich) by three

consecutive exchanges with distilled water over a period of 24 hours, followed by freeze-drying in a Labonco FreeZone 4.5 apparatus, to eliminate possible contaminants present in the TSP extract.

Ion exchange chromatography on DEAE-cellulose column

The TSP (15 mg) dissolved in 50 mM NaAc buffer (2 mg mL^{-1}) were subjected to ion exchange chromatography in a DEAE-cellulose column (12 × 1.5 cm) (Sigma Chemical), balanced and percolated with 50 mM NaAc buffer until complete removal of the non-retained polysaccharides, followed by fractionation of the SP by stepwise elution with the same equilibrium buffer containing different concentrations of NaCl (0.50, 0.75, 1.00 and 1.25 M) using a Frac-920 fraction collector (GE Life Sciences) with the flow adjusted to 60 mL h^{-1}. The SP fractions (3.0 mL) were monitored by metachromatic property using 1-9-dimethylmethylene blue (DMB) (FARNDALE et al., 1986) in a spectrophotometer (Amersham Biosciences Ultrospec 1100) adjusted at 525 nm. The metachromatic fractions thus obtained were exhaustively dialyzed (43 × 27 mm cellulose membranes, Sigma-Aldrich) against distilled water through five daily exchanges for three consecutive days to eliminate the NaCl utilized in the SPs fractionation. The sulfate content was determined by numerical integration of the area under the chromatograph obtained in the DEAE-cellulose column, using the Origin 7.0 program, converted into a percentage integrated metachromatic area (RODRIGUES et al., 2011). Then the fractions were concentrated by freeze-drying (Labconco, model FreeZone 4.5) for the subsequent assays.

Agarose gel electrophoresis

To evaluate the possible molecular differences regarding loading densities and resolution pattern, the TSPs and SP fractions (25 μg) were analyzed by 0.5% agarose gel electrophoresis (DIETRICH; DIETRICH, 1976) in 50 mM 1.3 diaminopropane-acetate buffer (pH 9.0) (Aldrich) between the methods, in this way, the SP fractions were applied in the gel and processed at a constant voltage (110 V) for 60 min. Then the samples present in the gel were fixed with a solution of 0.1% *N*-cetyl-*N*-*N*-*N*-trimethylammonium bromide solution (Vetec Química) for 24 hours, after which the gel was stained with 0.1% toluidine blue (Vetec Química). Finally, it was discolored in a solution containing absolute ethanol, distilled water and concentrated acetic acid (4.95:4.95:0.1, v v^{-1} v^{-1}).

In vitro anticoagulant assay

Evaluation of the anticoagulant activity was done by the activated partial thromboplastin time (APTT) test, according to the manufacturer's specifications. Initially, citrated normal human blood (different donors) was centrifuged (73.75 × *g*; 15 min.) to obtain platelet-poor plasma. To conduct the test, 50 μL of human plasma was incubated at 37°C for 3 min. with 10 μL of the SP solution and 50 μL of the reagent APTT (CLOT, Bios Diagnóstica). After incubation, 50 μL of 25 mM calcium chloride (CLOT, Bios Diagnóstica) was added to the mixture to activate the coagulation cascade. The assays were run in duplicate and the clotting time was registered automatically in a coagulometer (Drake Quick-Timer) and the anticoagulant activity was expressed in international units (IU) as mg of polysaccharide using the unfractionated HEP (193 IU mg^{-1}) from the National Institute for Biological Standards and Control, Potters Bar, England. A polysaccharide concentration range of 0.01 to 0.10 mg mL^{-1} was used to plot the standard HEP curve.

Results and discussion

Yield

Table 1 shows some of the TSPs yields extracted with the two methods and red seaweed species of the *Halymenia* genus tested here, along with results obtained by some other authors. The use of proteolytic enzymes (papain) to digest proteins, followed by the TSPs drying procedures resulted in different yields between the two methods (M I and II). M I outperformed M II in terms of TSP yield (46.00 to 26.95%). Thus, the difference in yield from the dehydrated algal material between the two methods was 19.05%.

Table 1. TSPs yields among red seaweed species of the *Halymenia* genus.

Species	Location	Yield (%)	Reference
Halymenia sp.	Brazil	46.00 (M I) 26.95 (M II)	This study
H. floresia	Brazil	38.60	Amorim et al. (2011)
H. durvillei	Madagascar	18.00 or 15.00	Fenoradosoa et al. (2009)
H. pseudofloresia	Brazil	47.14 (M I)	Rodrigues et al. (2009)

As can be seen, the yield of these compounds can vary according to the species, extraction method and origin of the particular alga. These factors could also be cause variations in the chemical composition of the SPs, thus influencing the various biotechnology applications (CAMPO et al., 2009).

In this study, the difference in yield between the methods was influenced by the technique used (Table 1). The proteolytic extraction of TSP from the red marine algae *B. occidentalis* (4.00%) by Farias et al. (2000) and *G. crinale* (2.60%) by Pereira et al. (2005) resulted in lower yields than from *Halymenia* sp. in this study. Papain digestion to obtain the sulfated galactan (agar) from the red seaweed *Gracilaria cornea* (21.40%) (MELO et al., 2002) had lower TSP yield when compared to M II. The use of M I resulted in three consecutive enzymatic extractions of TSPs from the red seaweed *H. pseudofloresia*, with a total yield of 47.14% (RODRIGUES et al., 2009) and a similar yield from a single extraction here from *Halymenia* sp. (Table 1). The consecutive extraction of TSPs suggests the possibility of identifying new biomolecules for different uses in biotechnology (CAMPO et al., 2009; RODRIGUES et al., 2009; AMORIM et al., 2011).

Purification by ion-exchange chromatography (DEAE-cellulose)

The chromatographic profiles obtained on DEAE-cellulose were similar and indicated the separation of four different SP fractions (F I, F II, F III and F IV), eluted at the respective NaCl concentrations of 0.50, 0.75, 1.00 and 1.25 M, using both methods (Figure 1). The highest yield of SP fractions of all the others observed in the taxon was in F II, eluted with 0.75 M of NaCl (Table 2).

The employment of chromatographic procedures for the separation of SP fractions using DEAE-cellulose column has proved very efficient for marine algae (PEREIRA et al., 2005; ATHUKORALA et al., 2006), revealing characteristics of species and/or remarkable differences between them by obtaining different profiles (RODRIGUES et al., 2009).

Fraction F II, eluted in 0.75 M of salt, obtained the highest yield in both methods. The respective integrated metachromatic areas (IMA) were 47.34% (M I) and 28.84% (M II), as shown in Table 2. However, similar detection of these compounds was observed in M II, when concentrations of 0.5 (F I), 1.0 (F III) and 1.25 M (F IV) of NaCl resulted in IMA values of 27.09, 28.22 and 15.87%, respectively (Figure 1B, Table 2), while comparatively lower yields were obtained by M I, thus indicating that M I is less efficient in obtaining polysaccharide fractions from *Halymenia* sp. The ion-exchange resin DEAE-cellulose was very efficient in separating these molecules obtained from this species.

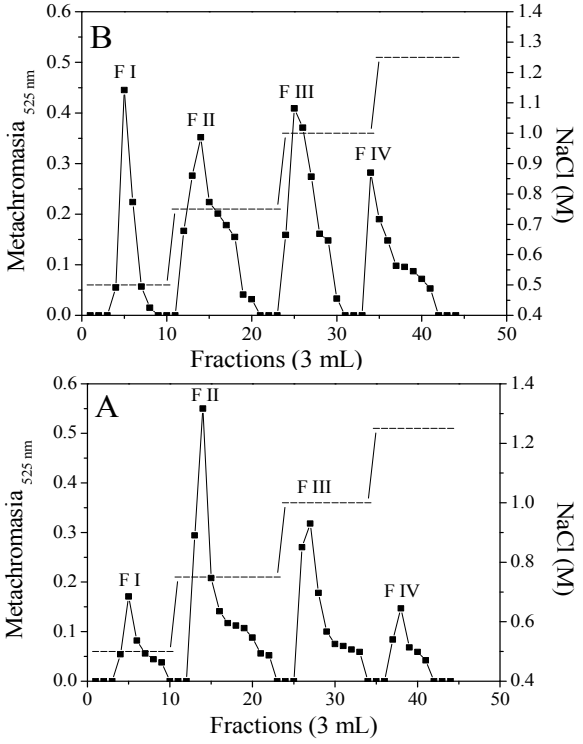

Figure 1. Chromatograms of the TSPs obtained by methods I (A) and II (B) from *Halymenia* sp. on DEAE-cellulose column. The column was equilibrated and washed with 50 mM NaAc buffer (pH 5.0). The SPs adsorbed in the gel were eluted with the 50 mM NaAc buffer (pH 5.0) containing NaCl at different concentrations (0.50, 0.75, 1.00 and 1.25 M). The SPs were monitored by metachromatic property with 1-9-dimethylmethlene blue at 525 nm. (■—■) metachromasia (SPs), (—) NaCl gradient.

Table 2. Integrated metachromatic area (IMA) and yield (%) of the SP fractions obtained by ion-exchange chromatography (DEAE-cellulose) from the red alga *Halymenia* sp.

Method	Fraction	NaCl (M)	IMA (%)	%
I	F I	0.50	12.21	13.33
	F II	0.75	47.34	31.68
	F III	1.00	29.09	21.66
	F IV	1.25	11.36	13.33
II	F I	0.50	27.09	16.00
	F II	0.75	28.84	42.66
	F III	1.00	28.22	20.00
	F IV	1.25	15.85	16.67

Agarose gel electrophoresis

The agarose gel electrophoresis procedure revealed differences in the purification of the TSPs and their SP fractions, as well as in the standard pattern of the SP fractions obtained by both methods (Figure 2). M I indicated SP fractions with negative standard pattern that were highly polydisperse when mutually compared and when compared to the TSP (Figure 2A), while more homogeneous fractions were observed with M II. This indicates a greater degree of purification of these macromolecules (Figure 1B). However,

fractions F I and F IV, eluted with 0.50 and 1.25 M of NaCl, respectively, practically did not appear in the gel, suggesting a lower presence of sulfate radicals (RODRIGUES et al., 2011).

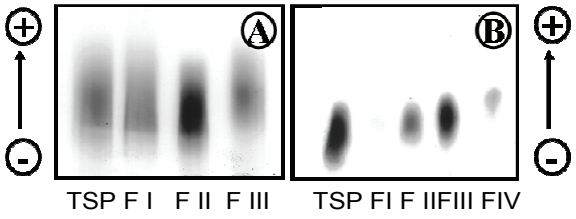

Figure 2. Revelation of the SPs obtained by methods I (A) and II (B) by 0.5% agarose gel electrophoresis. The crude extracts (TSPs) and SP fractions (F I, F II, F III and F IV) present on gel were stained with 0.1% toluidine blue.

Rodrigues et al. (2009) performed three consecutive papain digestion extractions of TSPs from the red seaweed *H. pseudofloresia* and observed pronounced differences in the degree of resolution of these compounds by agarose gel electrophoresis. The authors reported molecules with very polydispersed standard pattern when compared to those obtained in the other extractions from the species, suggesting the technique is an important tool in the process of purification and identification of new biological agents. In this study, the use of the dialysis procedure followed by freeze drying (M II) to obtain TSP from the red seaweed *Halymenia* sp. also proved to be a simple and promising strategy in the resolution of these compounds (Figure 2B), thus reducing the need for purification steps. The complex and heterogeneous nature of these compounds hinders the elucidation of their chemical structures (FENORADOSOA et al., 2009; MELO et al., 2002) and relations with the biological activities of interest in clinical medicine (AMORIM et al., 2011; FONSECA et al., 2008; MOURÃO, 2004; MOURÃO; PEREIRA, 1999; PEREIRA et al., 2005; ZHANG et al., 2008).

Anticoagulant activity

The APTT test is one of the most frequently used to measure sulfated polysaccharides with anticoagulant activity. It precisely indicates the anticoagulant activity of the compound isolated (MOURÃO; PEREIRA, 1999). The species tested here presented SP fractions able to modify the normal APTT by both methods (Table 3). The minimum SP concentrations able to prolong the APTTs were 0.10 and 0.05 mg mL^{-1} for M I and M II, whose respective activity levels corresponded to 27.40 and 72.66 IU mg^{-1} in fraction F II. The lowest activity levels were obtained in the fractions eluted with 0.50, 1.00 and 1.25 M of NaCl in M II (4.67, 41.30 and 39.12 IU mg^{-1}, respectively), while no activity was detected for the SP fractions separated in the elutions with 0.50 and 1.25 M of salt (M I), at a high SP concentration (1.00 mg mL^{-1}). Therefore, the SP fractions isolated from *Halymenia* sp. had inferior anticoagulant activities than the unfractionated HEP. This fact suggests that *Halymenia* sp. polysaccharides naturally are inhibited in intrinsic pathway on cascade of coagulation (ATHUKORALA et al., 2006; RODRIGUES et al., 2009; AMORIM et al., 2011).

Table 3. Anticoagulant activity (APTT) of the SP fractions obtained by ion-exchange chromatography (DEAE-cellulose) of the red seaweed *Halymenia* sp. in relation to HEP.

Method	Fractions	NaCl (M)	Activity		
			⋆mg mL^{-1}	⋆⋆APTT (s)	⋆⋆⋆IU mg^{-1}
I	F I	0.50	#	#	#
	F II	0.75	0.10	57.10	27.40
	F III	1.00	0.10	41.20	19.80
	F IV	1.25	#	#	#
II	F I	0.50	0.50	38.30	4.67
	F II	0.75	0.05	52.30	72.66
	F III	1.00	0.05	34.10	41.30
	F IV	1.25	0.05	32.40	39.12
HEP	-	-	0.01	42.15	193.00
Plasma	-	-	-	30.00	-

⋆Minimum SP concentration to prolong the APTT; ⋆⋆APTT in seconds; ⋆⋆⋆Activity expressed in international units (IU) per mg of SP; #No activity at a high SP concentration (1.00 mg mL^{-1}).

Various SPs anticoagulants have been isolated from different tissues (MOURÃO; PEREIRA, 1999; RODRIGUES et al., 2009, 2011). The side effects of using HEP have prompted the search for alternative sources of natural anticoagulants to prevent and treat diseases related to thrombosis. Farias et al. (2000) reported strong anticoagulant activity of a sulfated D-galactan extracted from the red seaweed *B. occidentalis*. The activity was measured by the inhibition of thrombin via antithrombin and heparin cofactor II (plasma regulators of blood clotting), by the presence of these two sulfate esters in a single galactose residue. Matsubara et al. (2001) reported that the polysaccharide isolated from the green seaweed *C. cylindricum* has a direct inhibiting mechanism on thrombin independent of antithrombin III and heparin cofactor II.

Recently, Fonseca et al. (2008) carried out a study comparing two sulfated galactans isolated from the red seaweeds *B. occidentalis* and *G. crinale*, involving the relations between their differences in the proportions and/or distribution of the sulfate radicals in the chain with the distinct interaction mechanisms of these galactans among proteases, inhibitors and activators of the clotting system, to elucidate their relations on anti- and pro-coagulation

activities, besides the pro- and antithrombotic action of these compounds.

The results of this study suggest that the differences in the anticoagulant activities of the fractions between the extraction methods strengthen the resolutions of these compounds when observed by electrophoresis (Figure 2). The greatest activities (Table 3) were attained by the fractions that showed the highest definitions in the gel and charge densities (Figure 2), mainly fraction F II (0.75 M of salt), whose activities were 27.40 and 72.66 IU mg^{-1} in both methods. On the other hand, the coagulant action of the SPs does not occur only as a function of the charge densities (Figure 2), but also according to the monosaccharide composition, sulfatation, and particularly the occurrence of disulfated units (MOURÃO, 2004). Therefore, complementary studies to clarify the anticoagulant action mechanisms of the SPs from *Halymenia* sp. suggest subsequent studies for chemical characterization, using the obtaining M II.

Rodrigues et al. (2009) reported accentuated anticoagulant activity of SP fractions isolated from the red seaweed *H. pseudofloresia*, utilizing citrated rabbit plasma, using M I of this study. The authors observed that the APTT changed between the different TSP extractions carried out for this species, and that the activities obtained in the first extraction (464.20, 211.60, 103.50 and 101.70 IU mg^{-1}) were greater than those of the third (137.10, 96.50 and 89.20 IU mg^{-1}), respectively, and also were superior to those of the HEP utilized (100.00 IU mg^{-1}). The changes in the APTT among the TSP extractions were not correlated with the metachromatic properties of the SP fractions obtained. Instead, the activities were dose-dependent. This suggested formulating other models of activities of interest to biomedicine, to explore the biological potentials of these fractions.

Mourão and Pereira (1999) reported that it is not enough to compare a required dose to an antithrombotic action. Other aspects must also be considered, such as the persistence of the effect, levels of plasma circulation, correlation between the anticoagulant and antithrombotic action, and availability and absorption when the drug is administered by different routes.

The use of enzymes is suggested by Athukorala et al. (2006) to be more efficient in the extraction of the bioactive compounds contained in algae for carrying out biological assays, because it enables dissolving the polysaccharides naturally allocated in membranes and other constituent algal tissues. Besides this, enzymes have the property of digesting the bonds of materials of the cell wall of algae, although the hydrolysis rate depends on the type of bond and length of the polysaccharide chain (HEO et al., 2005).

In the present study, the use of M II enhanced the anticoagulant activity (Table 3). According to Pitombo (2005), freeze drying is considered the most efficient drying mechanism to obtain biotechnology products because it eliminates not only the shrinkage and migration of the dissolved constituents of the material because of the freezing, but it also preserves the volatile constituents, biological activity and molecular structure of the compounds. These are extremely important for the use of these compounds in drugs and other substances of medical interest (antibiotics, anticoagulants, antivirals, enzymes, hormones and blood fractions). Therefore, this method can become an important tool to obtain new macromolecules with biological activities present in algae of the *Halymenia* genus, in view of its various uses of interest in biotechnology. Nevertheless, further research is necessary to explore other potential biological applications of this species.

Conclusion

The isolation of sulfated polysaccharides by proteolytic digestion (papain) followed by the use of two drying methods and ion-exchange chromatography (DEAE-cellulose) of the red seaweed *Halymenia* sp. resulted in fractions with anticoagulant activity inferior to that of heparin. We believe that the use of lyophilization as the drying method to obtain Halymeniaceae polysaccharides can contribute to a better understanding of the action mechanisms, by establishing relationships between the structures of these compounds and their biological functions, as well as reduce the purification steps. We also suggest further technical studies with other species of the genus.

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