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Extraction and anticoagulant activity of sulfated polysaccharides from *Caulerpa cupressoides* var. *lycopodium* (Vahl) C. Agardh (Chlorophyceae)

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ABSTRACT. The reportedly low standard quality of heparin (HEP) for use in cardiac surgeries has led to concern in the Brazilian and international markets. Sulfated polysaccharides (SPs) from seaweeds have been regarded as promising substitutes for HEP. The aim of this study was to sequentially extract total SPs (TSPs) from *Caulerpa cupressoides* (Chlorophyceae) with papain in 100 mM sodium acetate buffer (pH 5.0) containing 5 mM cysteine and 5 mM EDTA, followed by fractionation by ion-exchange chromatography (DEAE-cellulose), and then evaluate the anticoagulant potential of SP fractions by activated partial thromboplastin time (APTT) using normal human plasma and compare it to standard HEP (193 IU mg⁻¹). The obtained fractions were chemically characterized by chemical composition and agarose gel electrophoresis. The yield was 4.61%, and three fractions of SP (F I, F II and F III) eluted with 0.50, 0.75 and 1.00 M of NaCl, respectively, were observed on chromatography profiles; however, differences in charge densities patterns and degree of resolution among them were revealed by electrophoresis. SPs were capable of modifying APTT only in fractions eluted with 0.75 M of NaCl, whose activities were 23.37 and 25.76 IU mg⁻¹, respectively, and the charge density was prerequisite to activity. Therefore, *C. cupressoides* is a source of SPs possessing low anticoagulant potential compared to HEP.

Keywords: chlorophyta, sequential extractions, physical-chemical characterization, coagulation test.

RESUMO. Extração e atividade anticoagulante dos polissacarídeos sulfatados da clorofíceia *Caulerpa cupressoides* var. *lycopodium* (Vahl) C. Agardh. O baixo padrão de qualidade outrora declarado da heparina (HEP) para o uso em cirurgias cardíacas tem levado preocupação nos mercados nacional e internacional. Os polissacarídeos sulfatados (PSs) de algas marinhas têm sido considerados como promissores substitutos para HEP. Objetivou-se a extrair sequencialmente PSs totais (PSTs) da clorofíceia *Caulerpa cupressoides* com papaína em tampão acetato de sódio 100 mM (pH 5,0) contendo cisteína 5 mM e EDTA 5 mM, fracionar por cromatografia de troca iônica (DEAE-celulose) e avaliar o potencial anticoagulante das frações de PS por meio do tempo de tromboplastina parcial ativada (TTPA), utilizando plasma humano normal e comparando-se à HEP padrão (193 IU mg⁻¹). As frações obtidas foram caracterizadas quimicamente em composição química e por eletroforese em gel de agarose. O rendimento de PSTs foi 4,61% e os perfis cromatográficos, em DEAE-celulose, indicaram a separação de três frações de PS (F I; F II e F III) eluídas nas concentrações 0,50; 0,75 e 1,00 M de NaCl, respectivamente, revelando, por eletroforese, diferenças em termos de densidade de cargas e grau de resolução. Os PSs foram capazes de modificar o TTPA somente nas frações eluídas com 0,75 M de NaCl, cujas atividades foram 23,37 e 25,76 IU mg⁻¹, respectivamente, quando a densidade de cargas foi pré-requisito para atividade. Portanto, *C. cupressoides* é uma fonte de PSs com baixos potenciais anticoagulantes comparados à HEP.

Palavras-chave: clorofíceia, extrações sequenciais, caracterização físico-química, teste de coagulação.

Introduction

Sedentary lifestyle, poor eating habits and stress are immunosuppressive agents that can potentially lead to thromboembolic disease. Heart attack, stroke, deep-vein thrombosis and pulmonary embolism are the most prevalent

cardiovascular diseases worldwide, usually leading to patient death or else partial or total disability (NADER et al., 2001; RANG et al., 2007).

Heparin glycosaminoglycan (HEP) is a mucilage material obtained commercially from bovine lung and swine mucosa. It is administered therapeutically

as an anticoagulant and antithrombotic agent in the prevention and treatment of patients suffering from thromboembolic disease and for heart surgery involving extracorporeal circulation, such as in hemodialysis (NADER et al., 2001). However, this sulfated polysaccharide (SP) is also known for its undesirable side effects, such as hemorrhage (main adverse effect) and thrombocytopenia (RANG et al., 2007; THOMAS, 1997). Moreover, it has become increasingly difficult to acquire porcine HEP in the international market as a result of the rising demand for low-molecular-weight HEPs (ALBAN, 2005). There have also been reports of a reduction in quality standards for HEP preparations used routinely in heart surgeries under extracorporeal circulation. A significant change in molecular weight resulting from the presence of contaminants and low anticoagulant activity recorded by APTT test for some commercial HEP brands have been recently reported by Melo et al. (2008).

SPs are structural components of the cell wall of marine algae, in which they are found in high concentrations (PAINTER, 1983; PEREIRA et al., 2005; RODRIGUES et al., 2009, 2010a). The use of these molecules as alternative sources of new heparinoids is justified by the fact that algae are phylogenetically distant from mammals, significantly reducing contamination by viral particles (LEITE et al., 1998). Some seaweed SPs have already been described as heparinoids. For instance, Farias et al. (2000) and Pereira et al. (2005) isolated sulfated galactans with anticoagulant activity from marine red algae *Botryocladia occidentalis* and *Gelidium crinale*. When these galactans were evaluated in experimental models of thrombosis in rats, different biological effects were found between the two species; *G. crinale* featured procoagulant effects at low doses, and was regarded as a promising therapeutic source for patients with bleeding disorders (hemophilia) (FONSECA et al., 2008). They are thus suggested as biotechnology tools to better understand the physiopathology of thromboembolic diseases.

Marine green algae also contain SPs with anticoagulant activity; however, their biological potential has been little explored. The difficulty in characterizing the chemical structure of these compounds makes it harder to relate structure to biological function (ZHANG et al., 2008). Matsubara et al. (2001), while evaluating the anticoagulant potential of polysaccharides extracted in water medium from marine green alga *Codium*

cylindricum, observed a different anticoagulant mechanism from that reported for HEP. SPs from chlorophycean *Monostroma latissimum*, when fragmented in several molecular weights, produce different effects on blood coagulation *in vitro* (ZHANG et al., 2008).

Genus *Caulerpa* Lamouroux is represented by benthonic marine green algae, macroscopically featuring creeping thallus formed by rhizomes that expand along the substrate, fixed by structures known as rhizoids (JOLY, 1965; BRAYNER et al., 2008). Several species of this genus can be found along the Brazilian coast (JOLY, 1965; BEZERRA-NETO et al., 2008; BRAYNER et al., 2008; VANDERLEI et al., 2010) and some studies report important biological properties of their SPs, such as antiviral, anticoagulant and antitumor activities (GHOSH et al., 2004; JI et al., 2008). Studies have shown that by using sequential extraction it is possible to identify new SPs with anticoagulant activity in marine algae (RODRIGUES et al., 2009, 2010a).

Our group has contributed with studies on the chemical characterization and identification of new SPs with anticoagulant activity present in marine algae native to the coast of the state of Ceará, Brazil (PEREIRA et al., 2005; FONSECA et al., 2008; RODRIGUES et al., 2010b). Among them is chlorophycean *Caulerpa cupressoides* (RODRIGUES et al., 2010c). Thus, continuing with these investigations, the present study had as objective to evaluate, using sequential extraction, the anticoagulant potential of SPs from this species, also contributing to the characterization of these molecules isolated from representatives of the same genus.

Material and methods

Collection and extraction of total sulfated polysaccharides

Specimens of *Caulerpa cupressoides* var. *lycopodium* (Vahl) C. Agardh (recorded under no. 4977 at the Prisco Bezerra herbarium of the Federal University of Ceará) were collected at Pacheco Beach, Caucaia, Ceará State, and taken in plastic bags to the Carbohydrate and Lectins Laboratory (CarboLec) at the Biochemistry and Molecular Biology, Department of the Federal University of Ceará for further studies.

Total SPs (TSPs) extraction was carried out as previously described by Rodrigues et al. (2010c). To that end, algae (5 g) dehydrated at room temperature and macerated with liquid N₂ were hydrated in 250 mL of 100 mM sodium acetate buffer (pH 5.0) (Vetec Química) containing 5 mM EDTA (QEEL) and 5 mM cysteine (Sigma Chemical), and

subjected to digestion with 17 mL of crude papain solution (30 mg mL⁻¹) during 6 hours at 60°C in water bath (MARCONI, model MA 159). Next, the material was filtered, and SPs in the mixture were concentrated by adding 16 mL of cetylpyridinium chloride (CPC) (Sigma Chemical) at 10% (24h, 25°C). Following precipitation, the polysaccharide extract was rinsed (CPC 0.05%; 200 mL), 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). Right after the second precipitation, the obtained material was rinsed with 200 mL of 80% commercial-grade ethanol (2 ×), commercial-grade ethanol (200 mL, 1 ×) and lyophilized in order to obtain TSPs. For optimum TSPs yield, the residues obtained from the extractions were re-digested with papain (RODRIGUES et al., 2009, 2010a).

Ion-exchange chromatography (DEAE-cellulose)

TSPs (33 mg) were first dissolved in 50 mM AcNa buffer (2 mg mL⁻¹) and subjected to ion-exchange chromatography in a DEAE-cellulose column (Sigma Chemical) equilibrated and percolated with 50 mM AcNa buffer until complete removal of non-retained polysaccharides, followed by SP fractioning by elution with the same equilibrium buffer containing NaCl in different concentrations (0.50, 0.75 and 1.00 M) using a fraction collector (FRAC-920) with flow adjusted to 60 mL h⁻¹. The obtained fractions (3 mL min⁻¹) were monitored by metachromatic reaction using 1,9-dimethylmethylene blue (DMB) (Sigma-Aldrich) (FARNDALE et al., 1986). SP fractions were dialyzed against distilled water and freeze-dried for later assays.

Agarose gel electrophoresis

Due to the lack of standardization for algae, the study used animal glycosaminoglycans – chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS) and heparin (HEP) – to characterize SPs obtained from algae with regard to standards and charge density. Thus, TSPs and SP fractions (25 µg) were analyzed by 0.5% agarose gel electrophoresis (Bioagency) in 50 mM 1,3-diaminopropane acetate buffer (Aldrich) (pH 9.0). SPs were applied on the gel and run under constant voltage (110 V) during 60 min. After the procedure, the SPs present in the gel were fixed 0.1% *N*-cetyl-*N,N,N*-trimethylammonium bromide solution (Vetec Química) for 24 hours. The gel was next stained with 0.1% toluidine blue and dyed with a solution containing absolute ethanol, distilled water and

concentrated acetic acid (4.95:4.95:0.1, v v⁻¹ v⁻¹) as described by Dietrich and Dietrich (1976).

Chemical analyses

Total carbohydrates (TCs) content in the fractions was determined by the phenol-sulfuric acid method (DUBOIS et al., 1956) on a microplate format (MASUKO et al., 2005) using D-galactose to obtain the standard curve in an Elisa reader (Amersham Biosciences, model Biotrak II). Contaminating proteins (CPs) content was estimated using the method described by Bradford (1976), using bovine serum albumin as the standard.

Anticoagulant assay

For the test, normal citrated human blood was obtained from different donors at the Hematology and Hemotherapy Center of Ceará (HEMOCE) and Activated Partial Thromboplastin Time (APTT) was measured according to manufacturer specifications. First, the blood was centrifuged (73.75 × *g*, 15 min.) to obtain platelet-poor plasma. To perform the test, 50 µL of human plasma were incubated at 37°C for 3 min. with 10 µL of SP solution and 50 µL of APTT reagent (CLOT, Bios Diagnóstica). After incubation, 50 µL of 25 mM calcium chloride (CLOT, Bios Diagnóstica) were added to the mixture to trigger the coagulation cascade. Trials were performed in triplicate; coagulation time was recorded automatically with a coagulometer (DRAKE, QUICK-TIMER model) and anticoagulant activity was expressed in international units (IU) per mg of polysaccharide, using HEP with 193 IU mg⁻¹ as reference.

Results and discussion

Yield

The methodology of using enzymatic digestion of proteins by proteolytic enzymes (papain) (6h, 60°C) to extract TSPs resulted in three consecutive extractions for *C. cupressoides*. The largest quantity of these compounds was obtained in the first extraction (3.15%), whereas during the second and third extractions yields decreased sharply to 1.11% and 0.35%, respectively, totaling 4.61% of TSPs, from dehydrated (25°) and macerated algae.

In studies with marine algae *B. occidentalis* and *G. crinale* (Rhodophyceae), Farias et al. (2000) and Pereira et al. (2005) obtained, respectively, 4.00 and 2.60% yields of TSPs, using digestion papain (24h, 60°C). Extracting alginate (non-sulfated polysaccharide) from phaeophycean *Sargassum vulgare*, Torres et al. (2007) obtained a better yield (16.90%), when submitted to acid medium (60°C). To perform

three consecutive TSPs extractions in the presence of papain for 24h at 60°C, Rodrigues et al. (2009, 2010a) obtained 47.14 and 53.96% of TSPs for Rhodophyceae *Halymenia pseudofloresia* and *Halymenia* sp., respectively, after oven-drying (24h, 60°C).

Therefore, the variation in yield of these compounds among different species also involves the use of various techniques. In the present study, three consecutive TSP extractions resulted in a marked reduction in yield from *C. cupressoides*. This suggests an extraction of TSPs from different layers of algal tissue, thus favoring the identification of new bioactive compounds (RODRIGUES et al., 2009, 2010a), which can also be applied in other economic sectors, such as in agricultural technology (ARAÚJO et al., 2008).

Ion-exchange chromatography (DEAE-cellulose)

Given the low yield obtained in the third extraction (0.35%), only the first two TSPs extracts were used. Thus, the chromatography profiles obtained in ion-exchange column (DEAE-cellulose) showed three separate SP fractions (F I, F II and F III) eluted at concentrations of 0.50, 0.75 and 1.00 M of NaCl in both extractions, respectively (Figure 1). The fraction with highest yield was obtained in F II, eluted with 0.75 M of NaCl, in comparison to the other fractions obtained in both extractions (Table 1).

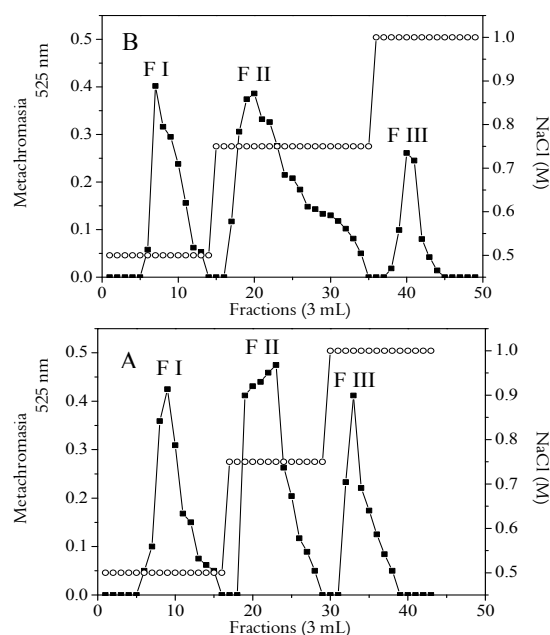


Figure 1. Ion-exchange chromatography of TSPs obtained in the first (A) and second (B) extractions from *Caulerpa cupressoides*. The column was equilibrated and eluted with 50 mM sodium acetate buffer (pH 5.0) containing 5 mM cysteine and 5 mM EDTA. The SPs adsorbed in gel were eluted by adding NaCl (0.50, 0.75 and 1.00 M). (■—■) metachromasia; (○—○) NaCl concentration (M).

In the present studies we observed that the methodology resulted in similar chromatographic profiles for all TSP extractions from chlorophycean *C. cupressoides*, whereas marked differences during fractioning in DEAE-cellulose of sulfated galactans from red algae *H. pseudofloresia* and *Halymenia* sp. were reported by Rodrigues et al. (2009, 2010a).

Therefore, the studies demonstrate that the use of this technique makes it possible to identify SPs with different characteristics among macroalgae species. The efficiency in separating SPs demonstrated in Figure 1 can be advantageous, considering the complex and heterogeneous nature of these compounds, which encourages studies on structural characterizations and its relationships with biological activities (FARIAS et al., 2000; PEREIRA et al., 2005; RODRIGUES et al., 2009, 2010a).

Chemical analyses

The chemical composition of freeze-dried fractions obtained from DEAE-cellulose also revealed differences among the extractions (Table 1). Overall, the TCs content of the fractions was similar in the first extraction (F I and F II), while lower levels (F I and F III) were found in the second extraction. No soluble proteins were detected, denoting the efficacy of the method in the enzymatic digestion of proteins by proteolytic enzymes for the extraction of TSPs present in *C. cupressoides*, as also previously described to other studies (RODRIGUES et al., 2010b, 2010c).

Table 1. Yield and chemical composition of SP fractions obtained by ion-exchange chromatography (DEAE-cellulose) from chlorophycean *Caulerpa cupressoides*.

Extractions	Fractions	Yield (%)	Chemical composition (%)	
			*TCs	*CPs
1 st	F I	15.38	25.50	-
	F II	21.29	26.40	-
	F III	3.12	9.65	-
2 nd	F I	7.57	11.20	-
	F II	33.03	35.70	-
	F III	4.24	17.70	-

*Average of three determinations; -: not detected.

Such disparities in the chemical composition of SP fractions from *C. cupressoides* are the result of complex and heterogeneous characteristics of these macromolecules, when compared to other studies (FARIAS et al., 2000; PEREIRA et al., 2005; ZHANG et al., 2008; AZEVEDO et al., 2009; RODRIGUES et al., 2010b).

Agarose gel electrophoresis

Agarose gel electrophoresis revealed differences between the resolution degree of fractions and TSPs, as well as in the charge densities of SP

fractions obtained from the different extractions (Figure 2). The fractions eluted in the first extraction (F I, F II and F III) (Figure 2A) showed differences in charge density among them on gel, in which fraction F II, eluted with 0.75 M of NaCl, showed a higher resolution degree and similar mobility to glycosaminoglycan heparan sulfate (HS) (Figure 2A). Conversely, fractions F I and F III practically were not observed, suggesting a smaller presence of sulfate groupings. During the second extraction, practically the same electrophoretic profile was observed (Figure 2B). However, small differences were observed in negative charge densities between fractions F II and F III (eluted with 0.75 and 1.00 M of NaCl, respectively), when subjected to potential difference. When compared among them, (Figure 2C), fraction F II (Cc1 – first extraction and Cc2 – second extraction, respectively) also showed the same mobility pattern among extractions and unfractionated HEP, although with different resolutions on gel.

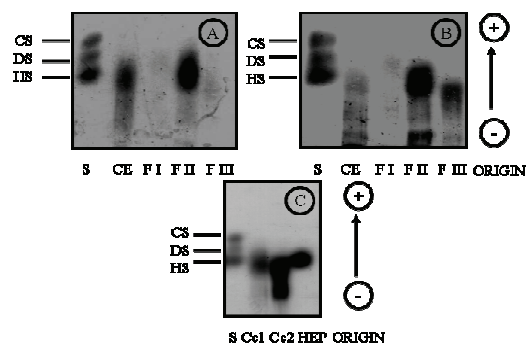


Figure 2. Resolution of SP fractions (1st – A and 2nd – B extractions) of marine green algae *Caulerpa cupressoides* by agarose gel electrophoresis and compared to unfractionated HEP (C). TSPs (CE), fractions (F I (0.50 M); F II (0.75 M) and F III (1.00 M)) and standards (S) of chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS) and HEP in gel were stained with 0.1% toluidine blue.

The higher resolution degree observed for fraction F II of the first TSPs extraction (Cc1) compared to that of the second extraction (Cc2) (Figure 2C) suggests the occurrence of SPs with different negative charge patterns in different coenocyte portions of *C. cupressoides*. This suggests that the procedure of separating these SPs by ion-exchange chromatography (DEAE-cellulose) combined with the electrophoresis technique proved to be a very efficient tool for partial characterization of these polymers, given that marine green algae have more complex components in their chemical structure (D-galactose, D-xylose, D-glucuronic acid, L-arabinose and L-rhamnose),

thus making it more difficult to elucidate the native chemical structure of these compounds (PAINTER, 1983; ZHANG et al., 2008).

Rodrigues et al. (2009, 2010a), using the TSPs re-extractions technique for Rhodophyceae, also observed marked differences in DEAE-cellulose fractioning and the electrophoresis procedure. The authors concluded that more homogenous SPs in negative charges occurred in the third extraction compared to the first and second extractions in species from genus *Halymenia*. This constitutes an important tool for future characterization of those macromolecules.

Considering our study, it is known that the cell wall of algae generally consists of two components: fibrillary (wall skeleton) and amorphous (mucilage matrix) (PAINTER, 1983). Extracellular-matrix polysaccharides are linked to mechanical, osmotic and ionic functions, favoring the survival of these organisms in marine environments (KLOAREG; QUATRANO, 1998). However, the absence of defined cells or cell organization that could allow a microscopic differentiation among the different species of the genus *Caulerpa* (structure known as coenocyte) (JOLY, 1965; BRAYNER et al., 2008) suggest that perhaps the detection of these macromolecules may in the future be regarded as an additional biotechnology tool for strategies aiming to identify species without microscopic identification. Alternatively, the application of the technique of enzymatic digestion of proteins by proteolytic enzymes (papain) in the consecutive extraction of TSPs from marine algae of genus *Caulerpa* followed by analysis of these compounds by ion-exchange chromatography on DEAE-cellulose column and agarose gel electrophoresis may be regarded as the first record using a marine green alga. Thus, further studies are recommended, including a larger number of species from this genus, in order to strengthen these observations, as well as list these compounds with regard to their possible use as taxonomic, phylogenetic and biogeographic molecular markers (TEIXEIRA, 2002; RODRIGUES et al., 2009).

Anticoagulant assay

The anticoagulant assay, performed according to the APTT test, revealed SP fractions capable of altering normal coagulation time (Table 2). The minimum SP concentration to prolong APTT was 0.10 mg mL⁻¹ in fractions F II for both extractions; their activities were 23.37 and 25.76 IU mg⁻¹, respectively. No prolongations were detected at a high SP concentration (1.00 mg mL⁻¹) in the SP fractions separation in 0.50 and 1.00 M elutions of

NaCl. Anticoagulant activity was also dose-dependent, requiring a high concentration greater than 0.75 mg mL⁻¹ of SP (F II – first extraction) to effectively prolong APTT, whereas for the fraction obtained at the same concentration (0.75 M of NaCl) in the second extraction from *C. cupressoides*, activity was significantly prolonged above 0.25 mg mL⁻¹ of PS (F II – 2nd). Thus, the anticoagulant activity of SPs isolated from that species was lower than that of unfractionated HEP, and the APTT test demonstrated that these SPs inhibited the intrinsic and/or common coagulation cascade, whereas prothrombin time (data not shown) was not altered, denoting that these compounds do not inhibit coagulation through the extrinsic pathway (ZHANG et al., 2008; AZEVEDO et al., 2009; RODRIGUES et al., 2009, 2010a, b).

Table 2. Anticoagulant activity of SP fractions obtained by ion-exchange chromatography (DEAE-cellulose) from chlorophycean *Caulerpa cupressoides* in relation to HEP.

Extraction	Fraction	NaCl (M)	APTT test *					IU mg ⁻¹ ***
			1.00 **	0.75 **	0.50 **	0.25 **	0.10 **	
			mg mL ⁻¹	mg mL ⁻¹	mg mL ⁻¹	mg mL ⁻¹	mg mL ⁻¹	
1 ^a	F I 0.50 M	-	-	-	-	-	-	-
	F II 0.75 M	217.12 s	101.68 s	83.37 s	61.50 s	45.32 s	23.37	-
	F III 1.00 M	-	-	-	-	-	-	-
2 ^a	F I 0.50 M	-	-	-	-	-	-	-
	F II 0.75 M	240.00 s	240.00 s	161.85 s	71.35 s	46.50 s	25.76	-
	F III 1.00 M	-	-	-	-	-	-	-

*APTT in seconds; **SP concentration to prolong APTT; ***Activity expressed in international units (IU) per mg of SP; - No activity; HEP (193.00 IU mg⁻¹; 0.01 mg mL⁻¹; APTT: 40.15 s); Plasma: 33.0 s.

Side effects from HEP use have led to a search for alternative sources of new anticoagulant compounds (SPs). However, there is little scientific information on the biologic functions of the chemical structures of these compounds (FARIAS et al., 2000; GHOSH et al., 2004; MOURÃO, 2004; PEREIRA et al., 2005; BEZERRA-NETO et al., 2008; FONSECA et al., 2008; ZHANG et al., 2008; AZEVEDO et al., 2009; RODRIGUES et al., 2010b).

Studies indicate some action mechanisms of the polymers in blood coagulation. According to Farias et al. (2000), sulfated D-galactan isolated from *B. occidentalis* (Rhodophyceae) has anticoagulant activity by inhibiting thrombin via antithrombin and heparin cofactor II (endogenous blood coagulation regulators) from the presence of two sulfate esters in a single galactose residue. Matsubara et al. (2001) reported that the polysaccharide isolated from marine green alga *C. cylindricum* has a direct inhibitory mechanism on thrombin, regardless of antithrombin III and heparin cofactor II. More recently, Fonseca et al. (2008), while testing two

sulfated galactans isolated from Rhodophyceae *B. occidentalis* and *Gelidium crinale*, observed that they dissociate *in vivo* systemic events in rats. Their actions involve naturally existing differences among the ratios and/or distributions of sulfated radical in the chemical structure. This results in different mechanisms of interaction with proteases, inhibitors and activators of the coagulation system, and expressing anti- and pro-coagulant activities, as well as anti- and pro-thrombotic actions.

The anticoagulant activity of *C. cupressoides* SPs was detected only in F II (Table 2), in both extractions, which reinforces the resolutions of these compounds when observed by electrophoresis (Figure 2) – activities were 23.37 and 25.76 IU mg⁻¹ for the first and second extractions, respectively. Thus, the activity of these polysaccharides seems to be dependent on negative charge density, given that the other SP fractions from the same species were generally unable to alter normal APTT. On the other hand, the *in vitro* effects of these compounds recorded in coagulation did not occur merely as a function of charge densities (MOURÃO, 2004); further studies are necessary to elucidate their structural requirements of anticoagulation action of SPs from *C. cupressoides* in coagulation (MATSUBARA et al., 2001; PEREIRA et al., 2005; FONSECA et al., 2008; ZHANG et al., 2008), as well as relate them to other biologic activities of interest in biomedicine (GHOSH et al., 2004; MOURÃO, 2004; JI et al., 2008).

Evaluating the *in vitro* anticoagulant activity of SP fractions from red alga *H. pseudofloresia* using citrated rabbit plasma, Rodrigues et al. (2009) observed marked changes in APTT. The fractions obtained in the first (464.20, 211.60, 103.50 and 101.70 IU mg⁻¹) were more active compared to those from the third extraction (137.10, 96.50 and 89.20 IU mg⁻¹). Its actions were considered superior to the existing HEP standard (100 IU mg⁻¹) and to SPs from the same-genus species *Halymenia* sp (RODRIGUES et al., 2010a).

As such, the use of these polysaccharides in several biotechnology processes justifies further studies by our research group, particularly with thrombosis models using animals. Those studies would aim to investigate the effect of these molecules in order to distinguish systemic events and delineate their use in medical practice, such as in the case of patients undergoing heparin therapy and/or thromboembolic disorders (FONSECA et al., 2008). Studies on these compounds are also recommended to characterize other species of the same genus.

Conclusion

Applying the methodology – which uses enzymatic digestion of proteins (papain) in the next extraction, followed by the chromatography procedure in ion-exchange column (DEAE-cellulose), combined with the electrophoresis technique – it is possible to partially characterize sulfated polysaccharides from marine green alga *Caulerpa cupressoides*. These polysaccharides, however, show low anticoagulant potential *in vitro* compared to heparin.

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References

- ALBAN, S. The 'precautionary principle' as a guide for future drug development. **European Journal of Clinical Investigation**, v. 35, n. 1, p. 33-44, 2005.
- ARAÚJO, G. S.; FARIAS, W. R. L.; RODRIGUES, J. A. G.; TORRES, V. M.; PONTES, G. C. Administração oral dos polissacarídeos sulfatados da rodofícea *Gracilaria caudata* na sobrevivência de pós-larvas de tilápia. **Revista Ciência Agronômica**, v. 39, n. 4, p. 548-554, 2008.
- AZEVEDO, T. C. G.; BEZERRA, M. E. B.; SANTOS, M. G. L.; SOUZA, L. A.; MARQUES, C. T.; BENEVIDES, N. M. B.; LEITE, E. L. Heparinoids algal and their anticoagulant hemorrhagic activities and platelet aggregation. **Biomedicine & Pharmacotherapy**, v. 63, n. 7, p. 477-483, 2009.
- BEZERRA-NETO, J. T. B.; RODRIGUES, J. A. G.; PONTES, G. C.; FARIAS, W. R. L. Polissacarídeos sulfatados da alga *Caulerpa sertularioides* (GMEL.) HOWE: análise de metodologias de precipitação. **Revista Brasileira de Engenharia de Pesca**, v. 3, n. 2, p. 33-39, 2008.
- BRADFORD, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1-2, p. 248-254, 1976.
- BRAYNER, S.; PEREIRA, S. M. B.; BANDEIRA-PEDROSA, M. E. Taxonomia e distribuição do gênero *Caulerpa* Lamouroux (Bryopsidales–Chlorophyta) na costa de Pernambuco e Arquipélago de Fernando de Noronha, Brasil. **Acta Botânica Brasilica**, v. 22, n. 4, p. 914-928, 2008.
- DIETRICH, C. P.; DIETRICH, S. M. C. Electrophoretic behaviour of acidic mucopolysaccharides in diamine buffers. **Analytical Biochemistry**, v. 70, n. 2, p. 645-647, 1976.
- DUBOIS, M.; GILLES, K. A.; HAMILTON, J. K.; REBERS, P. A.; SMITH, F. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, v. 28, n. 3, p. 350-356, 1956.
- FARIAS, W. R. L.; VALENTE, A. P.; PEREIRA, M. S.; MOURÃO, P. A. S. Structure and anticoagulant activity of sulfated galactans. Isolation of a unique sulfated galactan from the red alga *Botryocladia occidentalis* and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. **Journal of Biological Chemistry**, v. 275, n. 38, p. 29299-29307, 2000.
- FARNDALE, R. W.; BUTTLE, D. J.; BARRET, A. J. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. **Biochimistry et Biophysica Acta**, v. 883, n. 2, p. 173-177, 1986.
- FONSECA, R. J. C.; OLIVEIRA, S. N. M. C. G.; MELO, F. R.; PEREIRA, M. G.; BENEVIDES, N. M. B.; MOURÃO, P. A. S. Slight differences in sulfatation of algal galactans account for differences in their anticoagulant and venous antithrombotic activities. **Thrombosis and Haemostasis**, v. 99, n. 3, p. 539-545, 2008.
- GHOSH, P.; ADHIKARI, U.; GHOSAL, P. K.; PUJOL, C. A.; CARLUCCI, M. J.; DAMONTE, E. B.; RAY, B. In vitro anti-herpetic activity of sulfated polysaccharide fractions from *Caulerpa racemosa*. **Phytochemistry**, v. 65, n. 23, p. 3151-3157, 2004.
- JI, H.; SHAO, H.; ZHANG, C.; HONG, P.; XIONG, H. Separation of the polysaccharides in *Caulerpa racemosa* and their chemical composition and antitumor activity. **Journal of Applied Polymer Science**, v. 110, n. 3, p. 1435-1440, 2008.
- JOLY, A. B. Flora marinha do litoral norte do Estado de São Paulo e regiões circunvizinhas. **Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Série Botânica**, v. 21, n. 294, p. 5-393, 1965.
- KLOAREG, B.; QUATRANO, R. S. Structure of the cell wall of marine algae and ecophysiological functions of matrix polysaccharides. **Oceanography Marine and Biology an Annual Review**, v. 26, n. 1, p. 259-315, 1998.
- LEITE, E. L.; MEDEIROS, M. G. L.; ROCHA, H. A. O.; FARIAS, G. G. M.; SILVA, L. F.; CHAVANTE, S. F.; ABREU, L. D.; DIETRICH, C. P.; NADER, H. B. Structure and pharmacological activities of a sulfated xylofucoglucuronan from the alga *Spatoglossum schröderi*. **Plant Science**, v. 132, n. 2, p. 215-228, 1998.
- MASUKO, T.; MINAMI, A.; IWASAKI, N.; MAJIMA, T.; NISHIMURA, S. I.; LEE, Y. C. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. **Analytical Biochemistry**, v. 339, n. 1, p. 69-72, 2005.
- MATSUBARA, K.; MATSUBARA, Y.; BASIC, A.; LIAO, M. L.; HORI, K.; MIYAZAWA, K. Anticoagulant properties of a sulfated galactan preparation from a marine

- green alga, *Codium cylindricum*. **International Journal of Biological Macromolecules**, v. 28, n. 5, p. 395-399, 2001.
- MELO, E. I.; PEREIRA, M. S.; CUNHA, R. S.; SÁ, M. P. L.; MOURÃO, P. A. S. Controle da qualidade das preparações de heparina disponíveis no Brasil: implicações na cirurgia cardiovascular. **Revista Brasileira de Cirurgia Cardiovascular**, v. 23, n. 2, p. 169-174, 2008.
- MOURÃO, A. P. S. Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. **Current Pharmaceutical Design**, v. 10, n. 9, p. 967-981, 2004.
- NADER, H. B.; PINHAL, M. A. S.; BAÚ, E. C.; CASTRO, R. A. B.; MEDEIROS, G. F.; CHAVANTE, S. F.; LEITE, E. L.; TRINDADE, E. S.; SHINJO, S. K.; ROCHA, H. A. O.; TERSARIOL, I. L. S.; MENDES, A.; DIETRICH, C. P. Development of new heparin-like compounds and other antithrombotic drugs and their interaction with vascular endothelial cells. **Brazilian Journal of Medical and Biological Research**, v. 34, n. 6, p. 699-709, 2001.
- PAINTER, T. J. Algal polysaccharides. In: ASPINALL, G. O. (Ed.). **The polysaccharides**. New York: Academic Press, 1983. p. 195-285.
- PEREIRA, M. G.; BENEVIDES, N. M. B.; MELO, M. R. S.; VALENTE, A. P.; MELO, F. R.; MOURÃO, P. A. S. Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? **Carbohydrate Research**, v. 340, n. 12, p. 2015-2023, 2005.
- RANG, H. P.; DALE, M. M.; RITTER, J. M.; FLOWER, R. J. **Farmacologia**. 6. ed. Rio de Janeiro: Elsevier, 2007.
- RODRIGUES, J. A. G.; TORRES, V. M.; ALENCAR, D. B.; SAMPAIO, A. H.; FARIAS, W. R. L. Extração e atividade anticoagulante dos polissacarídeos sulfatados da alga marinha vermelha *Halymenia pseudofloresia*. **Revista Ciência Agronômica**, v. 40, n. 2, p. 224-231, 2009.
- RODRIGUES, J. A. G.; TORRES, V. M.; ALENCAR, D. B.; SAMPAIO, A. H.; FARIAS, W. R. L. Heparinoides naturais isolados de algas marinhas vermelhas (*Halymenia* sp.) arribadas na costa cearense. **Acta Scientiarum. Biological Sciences**, v. 32, n. 3, p. 235-242, 2010a.
- RODRIGUES, J. A. G.; ARAÚJO, I. W. F.; PAULA, G. A.; BESSA, E. F.; LIMA, T. B.; BENEVIDES, N. M. B. Isolamento, fracionamento e atividade anticoagulante de *iota*-carragenanas da *Solieria filiformis*. **Ciência Rural**, v. 40, n. 11, p. 2310-2316, 2010b.
- RODRIGUES, J. A. G.; VANDERLEI, E. S. O.; QUINDERÉ, A. L. G.; FONTES, B. P.; BENEVIDES, N. M. B. Polissacarídeos sulfatados isolados das clorofíceas *Caulerpa racemosa* e *Caulerpa cupressoides* – extração, fracionamento e atividade anticoagulante. **Acta Scientiarum. Biological Sciences**, v. 32, n. 2, p. 113-120, 2010c.
- TEIXEIRA, V. L. Produtos naturais marinhos. In: PEREIRA, R. C.; SOARES-GOMES, A. (Ed.). **Biologia marinha**. Rio de Janeiro: Interciência, 2002. p. 249-279.
- THOMAS, D. P. Does low molecular weight heparin cause less bleeding? **Thrombosis and Haemostasis**, v. 78, n. 6, p. 1422-1425, 1997.
- TORRES, M. R.; SOUSA, A. P. A.; FILHO, E. A. T. S.; MELO, D. F.; FEITOSA, J. P. A.; PAULA, R. C. M.; LIMA, M. G. S. Extraction and physicochemical characterization of *Sargassum vulgare* alginate from Brazil. **Carbohydrate Research**, v. 342, n. 14, p. 2067-2074, 2007.
- VANDERLEI, E. S. O.; PATOILLO, K. K. N. R.; LIMA, N. A.; LIMA, A. P. S.; RODRIGUES, J. A. G.; SILVA, L. M. C. M.; LIMA, M. E. P.; LIMA, V.; BENEVIDES, N. M. B. Antinociceptive and anti-inflammatory activities of lectin from the marine green alga *Caulerpa cupressoides*. **International Immunopharmacology**, v. 10, n. 9, p. 1113-1118, 2010.
- ZHANG, H. J.; MAO, W. J.; FANG, F.; LI, H. Y.; SUN, H. H.; CHEN, Y.; QI, X. H. Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostroma latissimum*. **Carbohydrate Polymers**, v. 71, n. 3, p. 428-434, 2008.

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