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Antimicrobial Effect of a Crude Sulfated Polysaccharide from the Red Seaweed *Gracilaria ornata*

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ABSTRACT

The aim of this study was to determine the yield, chemical composition, specific rotation (SR), infrared (IR) spectroscopy and the effect on bacterial growth of a crude sulfated polysaccharide (SP) from the red marine alga G. ornata *(Go). Go-1 (25°C), Go-2 (80°C), and Go-3 (80°C) were sequentially extracted and yielded 9.2%. The contents of sulfate (5.88-10.3%) and proteins (0.1-3.7%) were small. The values of SR were* $[\propto]_D^{20°f}$ *-19.0, -51.0, and -56.5, respectively. IR spectrums showed the presence of galactose-4 sulfate and absence of 3,6-anydrogalactose-2 sulfate, galactose-6 sulfate and galactose-2 sulfate. SR and IR techniques confirmed SPs. Go-3 was tested on the growth of bacteria (*Bacillus subtilis, Staphylococcus aureus, Enterobacter aerogens, Escherichia coli, Pseudomonas aeruginosa, Salmonela choleraesuis *and* Salmonela typhi*), but only* E. coli *was inhibited.*

Key words: Rhodophyta, sulfated macromolecules, chemical analysis, antimicrobial

INTRODUCTION

Red seaweeds are rich sources in sulfated-polysaccharides (SPs) (Ray and Lahaye 1995; Melo et al. 2002; Pereira et al. 2005; S.F-Tischer et al. 2006; Araújo et al. 2008; Rodrigues et al. 2009; Rodrigues et al. 2010; Graça et al. 2011; Rodrigues et al. 2011; Rodrigues et al. 2012). These polyanionic polymers play an important role in ionic, mechanical and osmotic functions, being found at high concentrations in the extracellular matrix of marine algae. Their structures and the sulfate contents markedly vary among species (Pereira et al. 2005; Pomin and Mourão 2008; Zhang et al. 2010; Amorim et al. 2011).

The polysaccharide agar (a SP having galactose as the major monometer) is mainly found in Gelidiaceae, Gracilariaceae, Phyllophoraceae and Ceramiaceae families, which can be extracted by different procedures (Melo et al. 2002; Mazumder

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et al. 2002; Maciel et al. 2008). The genus *Gracilaria* is currently the major source of SPs worldwide, and various studies have been done on their biology, ecology and phycocolloid characterization (Pomin and Mourão 2008).

The *Gracilaria* species are distributed throughout the tropical regions of the world. Algae from this genus are important producer of SPs (Mazumder et al. 2002; Melo et al. 2002; Marinho-Soriano and Bourret 2005; Pomin and Mourão 2008), and can be found in wild and cultured species (Marinho-Soriano and Bourret 2003; Maciel et al. 2008; Bezerra and Marinho-Soriano 2010). These compounds are widely studied as thickening, gelling and stabilizing agents to various biotechnological applications (Melo et al. 2002; Maciel et al. 2008; Pomin and Mourão 2008).

Polysaccharides from *Gracilaria* genus are mainly composed of alternating 3-linked β-D-galactopyranosyl residues (A-units) and 4-linked α-L-galactopyranosyl (or 3,6-anhydrogalactopyranosyl) residues (B-units). This backbone is further modified by different substitutions (Mazumder et al. 2002; Melo et al. 2002; Maciel et al. 2008).

In Brazil, several species of high commercial value have been described, such as *Gracilaria cervicornis*, *Hydropuntia cornea* (Marinho-Soriano et al. 2001), *G. gracilis*, *G. dura*, *G. bursa-pastoris* (Marinho-Soriano 2001), *G. cornea* (Melo et al. 2002), *G. birdiae* (Maciel et al. 2008) and *G. domingensis* (Salles et al. 2010). Some *Gracilaria* species have been reported as rich in SPs possessing antitumor (Fernández et al. 1989), antiviral against herpes simplex virus types 1 and 2 (Mazumder et al. 2002; Duarte et al. 2004), and other with effects to minimize stress in cultured fishes (Araújo et al. 2008). Although SPs modulate a large number of biological activities (Leite et al. 1998; Ghosh et al. 2004; Pereira et al. 2005; Qi et al. 2005; S.F-Tischer et al. 2006; Fonseca et al. 2008; Zhou et al. 2004; Rodrigues et al. 2009; Ananthi et al. 2010; Sinha et al. 2010; Graça et al. 2011; Siqueira et al. 2011), the antimicrobial activity has been rarely reported (Rao and Parekh 1981; Hellio et al. 2001; Chotigeat et al. 2004).

From the red seaweed *G. ornata*, Leite et al. (2005) purified and characterized a protein (lectin) that affected the development of cowpea weevil *Callosobruchus maculates* larvae. In the present study, the antimicrobial effect of a crude SP from the native red seaweed *G. ornata* was investigated.

MATERIALS AND METHODS

Marine alga

The red marine alga *G. ornata* Areschoug was collected from the Mucuripe Beach (Fortaleza, Ceará State, Brazil). The material was cleaned of epiphytes, washed with distilled water, and stored at –20°C until use.

Bacteria

Bacillus subtilis (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) (Gram-positive), *Enterobacter aerogens* (ATCC 13048), *Escherichia coli*, *Pseudomonas aeruginosa* (ATCC 25619), *Salmonela choleraesuis* (ATCC 10708) and *Salmonela typhi* (ATCC 65344) (Gram-negative) were used. They were obtained from the Department of Microbiology Laboratory, Federal University of Ceará, Brazil, and kept in AGAR nutritive medium (Difco) at 4°C.

Reagents

Bovine Serum Albumin, Coomassie Brilhant Blue G-250, Agar Sabouraud and Bacto-peptona (Sigma Chemical Co., St. Louis, E.U.A); Agar for cell count (Oxoid LtDa, Hampshire, England); and other reagents were commercially purchased.

SPs extraction

G. ornata was submitted to different extraction conditions for obtaining different crude SPs extracts based on Amorim et al. (2011). Initially, the algal tissue was submitted to mechanical stirring for 24 h at room temperature (25°C) in water at 1.5% (w/v). The residue was removed by centrifugation (5.000 × *g* for 15 min at 4°C). The supernatant was precipitated with absolute EtOH (1:3, v/v), centrifuged, redissolved in distilled water, dialyzed against water, freeze-dried and denominated Go-1. The algal residue was re-extracted but this time at 80°C for 4 h, followed by centrifugation under the same conditions. The hot extraction was repeated once more, using the second extraction residue. The supernatants were precipitated with absolute EtOH (1:3, v/v), and denominated Go-2 and Go-3 for the second and third extractions, respectively.

A mass of 0.2 g of each of the crude Go-1, Go-2 and Go-3 extracts were dissolved in hot distilled water (60°C), and centrifuged. The clear supernatant was then freeze-dried. Extract Go-3 was used in microbiological assays because of its yield and solubility behavior.

Chemical analysis
The crude SPs extracts were estimated for their chemical composition. Total sugars (TSs) content was determined by the phenol-sulfuric acid analysis using galactose as standard (Dubois et al. 1956) using a spectrophotometer (AMERSHAM BIOSCIENCES ULTROSPEC 1100) at 490 nm. After acid hydrolysis of the soluble polysaccharides (1 mL of HCl for 5 h at 100°C), free sulfate (FS) was measured by the BaCl2/gelatin method (Dodgson and Price 1962). Contaminant proteins (CPs) content was measured by Bradford's method (1976), using bovine serum albumin as the standard.

Specific rotation (SR)
The SPs solutions of *G. ornata* crude extracts were prepared at 0.2% in deionized water (25°C). Then, the SR of crude SPs extracts were determined in Perkin Elmer polarimeter (model 341) at 589.3 nm in sodium D line.

Infrared spectroscopy (IR)
The IR spectras of crude SPs extracts were also determined. The Fourier transform IR spectra (FT-IR) were recorded with a SHIMADZU IR spectrophotometer (model 8300) between 400 and 4000 cm^{-1}. The samples were analyzed as KBr pellet.

Microbiological assays
Nutritive agar
The nutritive agar was prepared with 18 g agar, 13 g nutrient medium and 1 L distillated water, mixed and submitted to heat, and then distributed in assay tubes containing 5 mL volume each one. After that, the tubes were sterilized (121°C, 15 min) and then the contents were transferred in Petri plates for solidification. PVC films were used as edible and stored in oven (37°C, 24 h). After sterilization, all the plates were maintained at 4°C until use.

Maintenance of cultures
All bacteria in agar nutritive medium (Difco) with sterile mineral oil were maintained at 4°C.

Evaluation of effect of *G. ornata* SPs (Go-3) through disc diffusion
Extract Go-3 was evaluated for its antimicrobial activity by plate diffusion assay in agar nutritive medium according to Cappuccino (1986). The bacterial cultures in exponential growth phase (10^8 cell mL^{-1}) were used for cultivation in agar Muller-Hinton plates. Samples of 30 µL were applied in sterile filter paper discs of 6 mm diameter and then placed under the medium. All the plates were incubated at 35°C for 24 h and monitored by halos formation around the discs. The results was expressed as the halo diameter. The assays were performed in duplicate with three repetition.

Evaluation of effect of *G. ornata* SPs (Go-3) on the development of eubacteria in mineral liquid medium
The effect of Go-3 on the development of eubacteria in mineral liquid medium was evaluated. All the bacterial cultures obtained from the nutritive agar in Petri plates (35°C, 24 h) were transferred to assay tubes containing 9.0 mL of 0.15 M NaCl sterile solution, and then the bacterial cultures were adjusted to a cell density of 10^3 - 10^4 colony forming unit (CFU mL^{-1}) by optical density (OD) measurement in spectrophotometer (PHARMACIA BIOTECH ULTROSPEC 2000) at 630 nm (OD = 0.02-0.04). Afterwards, Go-3 was directly dissolved in mineral medium for obtaining of a final solution of 1 mg mL^{-1} and after a sterilization through 0.22 µm membrane filter (Millex-GP, Millepore) was used in the assay. The test was performed using 3.15 mL of Go-3 collected from each assay tube containing 3.15 mL mineral medium and 0.7 mL bacteria solution to make the final volume as 7.0 mL. All tubes were incubateded at 35°C and monitored for 60 h using spectrophotometer (PHARMACIA BIOTECH ULTROSPEC 2000) at 630 nm.

Evaluation of the effect of *G. ornata* SPs (Go-3) through standard assay using chemical agents in liquid medium
The effect of Go-3 on the development of eubacteria in mineral liquid medium was evaluated. All the bacterial cultures obtained from the nutritive agar in Petri plates (35°C, 24 h) were transferred to assay tubes containing 5.0 mL of nutritive sterile. After a 24 h, the standard assay for chemical agents test was done, which consisted of preparations of consecutive dilutions of Go-3 from initial concentration of 1.0 mg mL^{-1} in 0.15 M NaCl. Then, 0.1 mL of inoculum was added in each tube, followed by incubation at 35°C. Aliquots were collected at 10, 20, 30 and 60 min, and 12 and 24 h for subculture preparations. The same protocol was performed for the cell count. Each culture was diluted until 10-8 cells (in series)

in 0.15 M NaCl sterile. One hundred micro liter of each suspension was used for cultivation in the plates containing PCA medium, using a Drigalski. All the plates were incubated at 35°C for 24 h and the number of CFU mL^{-1} was estimated. The assays were carried out in triplicate, each one with two repetitions. All the experimental procedure was performed under aseptic conditions, using a laminar flow unit. Finally, the colonies from each plate were pinched and subcultivated in nutritive agar medium for bactericidal or bacteriostatic confirmation.

Microscopic analysis of bacteria cultivations in presence of Go-3

The purity of culture was monitored by Gram method as described by Soares et al. (1987). Briefly, fresh culture preparations were examined for possible cell morphological alterations in the presence of Go-3. Laminas were observed in optical microscopy (100 ×).

RESULTS AND DISCUSSION

Yield

The total yield of SPs extracted from the red alga *G. ornata* at room temperature (25°C) and sequentially at 80°C (twice) was 9.2% (w/w%). Lower yields were obtained in Go-2 and Go-3 (4.1 and 4.9%, respectively), while the lowest was 0.2% (Go-1) (Fig. 1). This species presented a total SPs yield higher to that from the red seaweeds *Gelidium crinale* (2.6%) (Pereira et al. 2005), *Botryocladia occidentalis* (4%) (Farias et al. 2000) and *G. birdiae* (6.5%) (Maciel et al. 2008), but lower than those of *G. cornea* (21.4%) (Melo et al. 2002), *G. bursa-pastoris* (38.8%), *G. dura* (33.5%), *G. gracilis* (30.0%) (Marinho-Soriano 2001), *Halymenia pseudofloresia* (47.14%) (Rodrigues et al. 2009) and *Halymenia* sp (53.96%) (Rodrigues et al. 2010), respectively. In a recent study, Amorim et al. (2011) performed three differential successive aqueous extractions from *H. floresia* and obtained that at high temperatures (80°C), 34.6% SPs were extracted against 4% at 25°C.

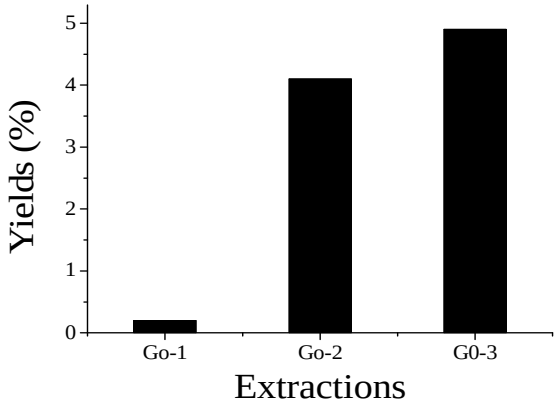

Figure 1 - Yields of crude SPs extracts (Go-1, Go-2 and Go-3) isolated by sequential extractions in water (25°C and 80°C) from the red seaweed *Gracilaria ornata*. Yields are express as percentage weight of alga desihydrated weight.

The lowest yield in Go-1 (25°C) could be due to the presence of precursors or other non-gel promoting structural elements (Murano et al. 1997; S.F-Tischer et al. 2006), whereas in Go-2 and Go-3 (80°C) suggested the presence of floridean starch granules in *G. ornata* (Mazumder et al. 2002; S.F-Tischer et al. 2006). According to Rodrigues et al. (2009, 2010), the employment of successive extractions could be a valuable strategy for the identification of new SPs. However, Chotigeat et al. (2004) reported that accentuated variations in the yield of seaweeds SPs could also occur among different species. SPs yield from *Gracilaria* species has been correlated with the environmental factors (Marinho-Soriano and Bourret 2003). From this point of view, *G. ornata* SPs deserved further studies. These aspects are supported due to the use of this class of macromolecules in several

economical fields (Farias et al. 2000; Mazumder et al. 2002; Duarte et al. 2004; Pereira et al. 2005; S.F-Tischer et al. 2006; Araújo et al. 2008; Siqueira et al. 2011). The high cost of these polymers in the international market encourages to discover new natural sources of phycocolloids for biotechnology (Melo et al. 2002; Maciel et al. 2008; Campo et al. 2009; Silva et al. 2010).

Chemical analysis

The chemical composition varied among the crude SPs extracts obtained (Table 1). Extract Go-1 had the lowest FS content (5.88%) and highest CPs content (3.70%) compared to Go-2 and Go-3 extracts. These results reported were in accordance with others SPs from *Gracilaria* species (Mazumder et al. 2002; Melo et al. 2002; Maciel et al. 2008), but the sulfate content of algae SPs could be variable among the different species (Pereira et al. 2005; S.F-Tischer et al. 2006; Zhang et al. 2010; Amorim et al. 2011). The CPs content of the extracts was very small, suggesting the presence of amino acids (Mazumder et al. 2002; Ghosh et al. 2004; Amorim et al. 2011).

Specific rotation

The SPs solutions of *G. ornata* crude extracts (Go-1, Go-2 and Go-3) prepared at concentration of 0.2% in deionized water (25°C) showed SR raging from –19.0 to –56.5 (Tab. 1), suggesting that the SPs from *G. ornata* belonged to the L-series, similar to reported for other natural SPs found in *Gracilaria* species (Pomin and Mourão 2008) and in the brown seaweed *Padina tetrastomatica* (Karmakar et al. 2009).

Infrared spectroscopy

The IR spectrums of crude SPs extracts are shown in Fig. 2. This technique is considered as an useful information for partial characterization of seaweeds SPs (Melo et al. 2002; Maciel et al. 2008; Campo et al. 2009; Silva et al. 2010), such as in the determination of sulfate and 3,6-anhydrogalactose contents in SPs (Mazumder et al. 2002). The original bands at 845-850 cm^{-1} (C-O-S, secondary axial sulfate) were extended and showed the presence of galactose-4-sulfate in the IR spectra of all the crude SPs extracts. Furthermore, the intensity of this signal was corroborated by the FS content (Table 1). This meant that the sulfate occurred at the position C-4 of D-galactose. The IR spectrums of SPs also showed absorption bands at 1250 cm^{-1}, denoting the presence of sulfate ester (Mazumder et al. 2002; Melo et al. 2002; Ghosh et al. 2004; Chattopadhyay et al. 2007a; Karmakar et al. 2009; Silva et al. 2010).

Band absorbance provides information on the occurrence in the crude extracts of 3,6 anhydrogalactose at 930 cm^{-1} (Melo et al. 2002; Silva el al. 2010) and characteristics of agarocolloids at 1375, 1153, 1070, 890 and 761 cm^{-1} based on Melo et al. (2002) and Maciel et al. (2008). 1030 cm^{-1} corresponded to the glycosidic linkage stretch vibration of C-O-H (Ananthi et al. 2010). 3400-3423 (OH stretching) and 2922-2926 (CH stretching) cm^{-1} were also detected (data not shown). However, there were no absorption bands or shoulders detected at 820 and 805 cm^{-1}, showing that 2-sulfate galactose, galactose-6-sulfate and sulfate on C-2 of 3,6-anhydrogalactose were not present. Pomin and Mourão (2008) reported that the chemical structure of SPs from red seaweeds could occur as agaran (L-series), carrageenan (D-series) or both (hybrids D-/L-series).

Therefore, the little differences found in this experiment could be determined by the technique employment. The SPs yield and chemical and IR analyses varied among the differential extractions, with low values recorded at 25°C (Go-1) (Maciel et al. 2008) when compared to those found at 80°C (Go-2 and Go-3) (Figs. 1 and 2, Table 1) (Amorim et al. 2011), suggesting the occurrence, at least, of two "populations" of cell-wall SPs (Ray and Lahaye 1995; Rodrigues et al. 2009; Graça et al. 2011; Siqueira et al. 2011), and that high temperature was an important parameter for *G. ornata* SPs extraction (Amorim et al. 2011).

Table 1 - Chemical composition of crude SPs extracts from red seaweed *Gracilaria ornata*

SPs	°C	* $[\propto]_D^{20°}$	Chemical composition (%)		
			TSs [a]	FS [b]	CPs [c]
Go-1	25	-19.00	33.14	5.88	3.70
Go-2	80	-51.00	57.71	10.30	1.30
Go-3	80	-56.50	62.20	10.30	0.10

a – Dosage by Dubois et al.' method using D-galactose as standard; b – Dosage by Dodgson and Price' method using $NaSO_3$ as standard; c – Dosage by Bradford' method using bovine serum albumin; * – Specific rotation.

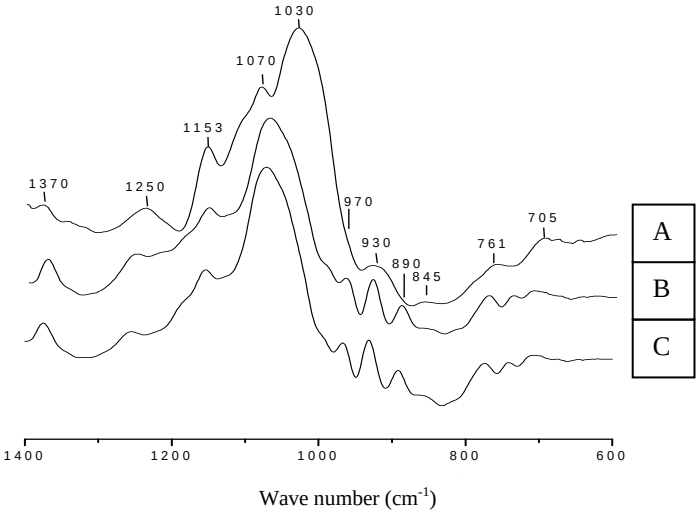

Figure 2 – FT-IR spectrums of Go-1 (A), Go-2 (B) and Go-3 (C) crude SPs extracts from red seaweed *Gracilaria ornata*.

These data were not in accordance with other studied *Gracilaria* species SPs. Mazumder et al. (2002) isolated and investigated chemically several SPs present in *G. corticata*. The authors observed that the SPs extracted with water presented the sulfated groups at positions C-4 of the 1,3-linked D-galactose units and C-6 of the 1,4-linked L-galactose residues. In contrast, when the SPs was extracted by alkaline treatment, there was absence of the band at 850 cm^{-1} (sulfate-4-galactose). The obtained SPs alkaline presented a signal more intense at 930 cm^{-1} (3,6-anhydrogalactose). In fact, it has been suggested that 6-sulfate-α-L-galactose may be chemically converted to 3,6-anhydrogalactose after alkaline treatment.

In another study, the FT-IR spectra of crude SP and its obtained fractions from papain digestion from *G. cornea* was reported by Melo et al. (2002). The results revealed the possible presence of sulfate-4-galactose and a shoulder close to 820 cm^{-1} suggestive of sulfate-6-galactose. In contrast, 2-sulfate galactose and sulfate on C-2 of 3,6,-anhydrogalactose were not identified. The results showed that the ratio between galactose and anhydrogalactose was quite far from the ideal agarose ratio, and this chemical aspect was attributed to the absence of gelation in aqueous solutions. According to Percival and McDowell (1967), possibly the use of crude enzyme preparations could modify the structure of the residual polysaccharide.

Diverse others marine organisms are rich sources in SPs possessing highly complex and heterogeneous structures. Pereira et al. (2005) reported the occurrence of -4-α-Gal*p*-(1-3)-β-Gal*p*1, but with a variable sulfation pattern, having 2,3-di-sulfated and 2-sulfated, for the red alga *G. crinale*. The presence of 2-sulfated, 3-linked α-L-galactan, was also characteristics of some marine invertebrates (Pereira et al. 2002). More recently, Aquino et al. (2005) identified sulfated galactans from the marine angiosperms (*Ruppia maritima*, *Halodule wrightii*, and *Halophila decipiens*). The authors concluded that those from the sea grass *R. maritima* were constituted by a regular tetrasaccharide repeating unit that had an intermediate structure when compared to those presents in marine invertebrates and red marine algae.

Sinha et al. (2010) extracted the polysacharides from *Sargassum tenerrimum* (Phaeophyta). The FT-IR spectrum of a fraction also contained a band at 1420 (COO^-) cm^{-1} characteristic of alginate, being the first report for the presence of guluronate in Sargassaceae.

The absence of 2-sulfate galactose, sulfate-6-galactose and sulfate on C-2 of 3,6-anhydrogalactose in the IR spectra (Fig. 2) in the present study suggested the hypothesis of relating

the structural studies of SPs as supplement in morphology, anatomy and life history studies, as auxiliary tools in the elucidation of taxonomic position of these organisms (Usov 1998). It has been reported that different extraction methods could influence the extraction of these polymers (Mazumder et al. 2002; Melo et al. 2002), as observed in this study (Fig. 1). In this context, a more detailed investigation is needed.

The absence of an absorption band in the IR spectra for galactose-6-sulfate was also noted (Fig. 2). The presence of this signal in the algae IR spectra indicated that the sulfate was linked at position C-6 of galactose (Mazumder et al. 2002). In general, the *Caulerpa* SPs occurred with this structural feature (Ghosh et al. 2004; Chattopadhyay et al. 2007b).

Microbiological assays

In recent years, the risks of the ingestion of contaminated food by human have been significantly increased. Among the major agents are bacteria (Gram-positive and -negative bacteria). Bacteria cause huge damage in several economic fields, including food industry, fish and shrimp farms, etc (Chotigeat et al. 2004; Carvalho et al. 2009). Although the antibiotics are widely used for bacterial control, their indiscriminate administration is considered a problem of public health (Harakeh et al. 2006; Carvalho et al. 2009).

The hypothesis of a possible biological effect of Go-3, which presented the highest yield (Fig. 1) and low CPs content (Table 1), from *G. ornata*, was similar to that reported by Graça et al. (2011), studying the effects of a crude SP extract (named Hf2s) from red seaweed *Halymenia floresia* on gastrointestinal smooth muscle contractility (*in vitro* and *in vivo*). These authors, based on a previous study performed by Amorim et al. (2011), noticed that this crude polysaccharide contained a highest yield and sulfate content, and low CPs content. In addition, low CPs content in *G. ornata* (Go-3) could be important in the solubility behavior of this crude polysaccharide (Ray and Lahaye 1995).

Based on these considerations, the effects of a crude SPs extract (Go-3) on the growth of seven bacteria (*Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive), *Enterobacter aerogens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonela choleraesuis* and *Salmonela typhi* (Gram-negative) was studied.

In this study, no antimicrobial effect was obtained by Go-3 through plate diffusion method, suggesting that the high viscosity of the *G. ornata* SPs in the cultivate medium interfered (Cappuccino et al. 1986). Also, no growth of bacteria was observed in the presence of Go-3 in the mineral medium. This indicated that all the tested bacteria were not capable of utilizing the *G. ornata* SPs as carbon source.

In order to still verify some bactericidal action of Go-3, the method using chemical agents in liquid medium was tested. However, as in the plate diffusion and mineral medium methods, Go-3 had also no effect. Surprisingly, when Go-3 was tested in another inhibition assay, *G. ornata* SPs presented inhibitory effect against *E. coli* bacteria (Gran-negative) ($p<0.05$) (Fig 3). No morphological alteration in the cultured cell in the presence of Go-3 was also observed.

There are a few reports about the antimicrobial effects of seaweeds SPs. According to some literature data, the extracts from the marine algae *Padina gymnospora*, *Dictyota dichotoma* (Rao and Parekh 1981), *Dunaliella bardawill*, *Isochrysis galbana* (Fabregas et al. 1999), *Sargassum muticum* (Hellio et al. 2001) and *S. polycystum* (Chotigeat et al. 2004) possessed antimicrobial effects.

Chotigeat et al. (2004) evaluated *S. polycystum* against three bacteria (*E. coli*, *Staphylococcus aureus* and *Vibrio harveyi*) and found that the *S. polycystum* extract had ability to inhibit the growth of all these bacteria, which caused diseases in shrimp *Litopenaeus vannamei*.

The present study demonstrated that Go-3 only inhibited the growth of *E. coli* (Fig. 3). It showed that Go-3 presented narrow spectrum of action against all the tested bacteria. Generally it is emphasized that structural and conformation aspects of the seaweed SPs, as well as charge density, molecular weight and/or distribution, sulfate content also play an important role for their biological actions (Nishino et al. 1991; Farias et al. 2000; Duarte et al. 2004; Ghosh et al. 2004; Mourão 2004; Zhou et al., 2004; Pereira et al. 2005; Fonseca et al. 2008; Zhang et al. 2008; Rodrigues et al. 2010; Silva et al. 2010; Rodrigues et al. 2012).

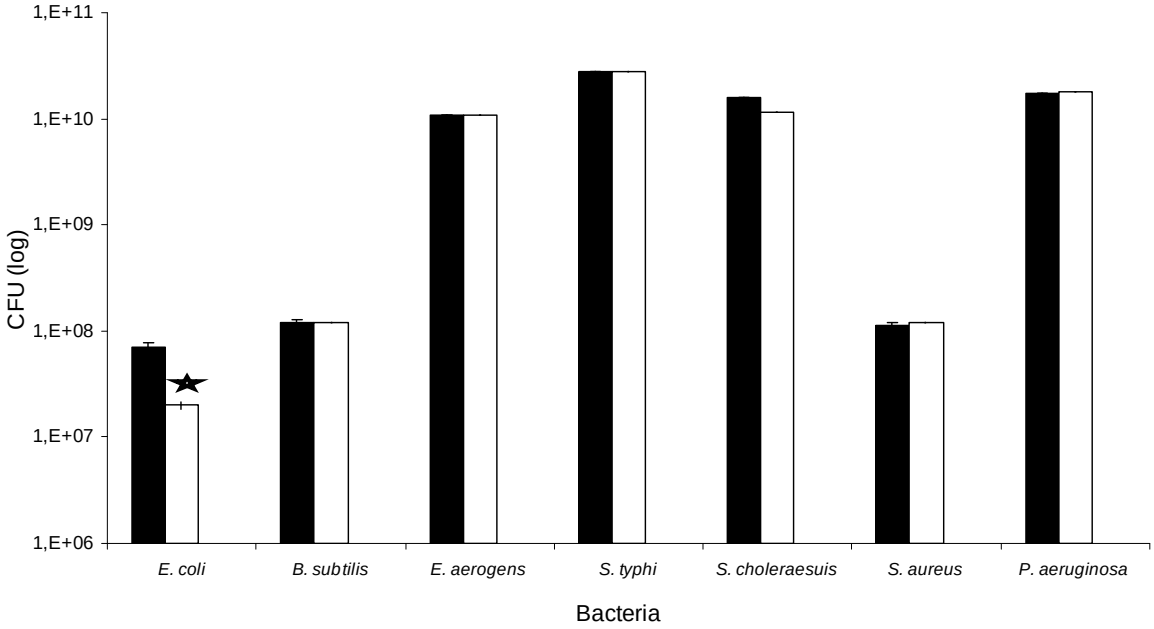

Figure 3 – Effect of the crude SPs extract (Go-3) from the red alga *Gracilaria ornata* on the growth Gran-positive and -negative bacteria. Control group ■ ; Go-3 □ . *P<0.05 statistically significant.

SPs occur in several marine organisms and comprise a group of structurally heterogeneous macromolecules with a diversity of biological activities. SPs from different algal species have demonstrated that each polysaccharide possesses a particular biological action, and as a consequence of the presence of sulfate radicals, these polymers deserve to be evaluated using different biological assays (Mourão and Pereira 1999).

The occurrence of -4-α-Gal*p*-(1-3)-β-Gal*p*1, but with a variable sulfation pattern, having 2,3-di-sulfated and 2-sulfated, has been attributed as a structural requirement for the anticoagulant action of galactans from red algae (Pereira et al. 2005), and as a structure–anticoagulant relationship that also indicate 2-sulfated, 3-linked α-L-galactan, being a potent thrombin inhibitor mediated by antithrombin or heparin cofactor II, for marine invertebrates (Pereira et al. 2002).

More recently, Fonseca et al. (2008) compared a SP isolated from *B. occidentalis* having anticoagulant activity (90 IU mg^{-1}) with that of *G. crinale* (65 IU mg^{-1}) (Pereira et al. 2005) (Rhodophyta) on specific coagulation assays and experimental models of thrombosis. The results indicated that slight differences in the proportion and/or distribution of sulfated residues in these polysaccharides chain was critical for interaction between proteases, inhibitors and activators of the coagulation system, resulting therefore in a distinct pattern in anti- and procoagulant activities and in the antithrombotic action.

It is believed that the absence of antimicrobial activity of seaweeds SPs was related to charge the repulsion between the sulfated groups and cell-wall of bacteria. The inhibition of growth of the bacteria by the addition of *G. ornata* SPs (Go-3) suggested the hypothesis of the presence of glycoprotein-receptors in the cell-surface capable of recognizing and binding to the charged compounds presents in the cell-surface of bacteria (Rostand and Esko 1997). Another hypothesis is the production of enzyme by the bacteria (heparin lyases, chondroitin lyases and chondroitinases) capable of removing SPs on the host cell-surface (Rostand and Esko 1997; Huang et al. 1999).

Therefore, current data indicated that Go-3 from the red alga *G. ornata* exerted selectivity on the growth of *E. coli*. The action mechanism of *G. ornata* SPs on the antimicrobial effect (*E. coli*), including its structural requirements and chemical modifications, should be further investigated.

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