ACTIVITY of SULFATED POLYSACCHARIDES from MICROALGAE *Porphyridium cruentum* over DEGENERATIVE MECHANISMS of the SKIN

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Abstract— **Marine microalgae are an innovative source of bioactive compounds which have achieved great relevance in medical, pharmaceutical and cosmetic fields. Using bubble column photobioreactors we have obtained a constant and controlled culture of the red microalga *Porphyridium cruentum*, known for its high production of sulfated polysaccharides (SPs), which have demonstrated activity on several biological mechanisms. In this paper we have evaluated the inhibitory effect of these compounds on three enzymes which are involved in the degradation of key substrates for the good appearance of skin. The results of the present study indicated that the SPs of *P. cruentum* do not display inhibitory activity on bacterial collagenase neither significant antioxidant activity. However, a high inhibition of hyaluronidase equivalent to an average of 96.6 ± 0.3% was obtained when the SPs were applied in a concentration ranging from 0.25 to 2.5 mg/mL. In addition, an inhibition of 46.0 ± 7.1 % was caused on enzymatic activity of elastase at a concentration of 5 mg/mL. These in vitro studies suggest that the SPs of marine microalga *Porphyridium cruentum* can be used as active ingredients in cosmetic and pharmaceutical formulations in order to maintain good health and appropriate skin appearance.**

Keywords- Biotechnology, microalgae, Porphyridium cruentum, polysaccharides, enzyme inhibition, skin care, anti-aging products.

I. INTRODUCTION

As the largest organ of the human being, the skin acts as a protective barrier by isolating the organism from its surrounding environment and helping to maintain the integrity of all the inner structures, at the same time that can serve as a communication system with the environment [1]. However, beyond its biological function, the skin plays an important role in the health of humans, greatly defining their image, allowing them to be or not in harmony with the beauty standards of today's society. Biochemical changes, which are unleashed by the aging process in addition to external agents, induce such damages on skin as the appearance of wrinkles, spots, loss of elasticity and firmness, inflammation, and thinning of the dermal matrix [2].

The main biochemical imbalance produced is the increased activity of collagenase, elastase, and hyaluronidase enzymes. This fact leads to an accelerated degradation of the three major components of the skin: collagen (protein responsible for giving firmness), elastin (protein that gives elasticity) and hyaluronic acid (glycosaminoglycan, which gives volume due to water retention) [3]. Another significant alteration caused by aging and by certain environmental factors (solar radiation, pollution, climate, e.g.) is the high generation of reactive oxygen species (ROS) such as the hydroxyl radical and the superoxide anion, involved in mutagenesis, carcinogenesis and aging itself [4].

The use of a substance providing protection against oxidative stress and that also inhibits the activity of these enzymes could lead to a better dermal matrix, improving the health and appearance of skin. For this reason, various substances have been studied for several decades in order to know their ability to inhibit collagenase, elastase, and hyaluronidase. Most of those inhibitory substances are synthetically produced or are even obtained from plants or other natural sources [3, 5-7]. When they exhibit a significant inhibitory activity they can be used as active ingredients in cosmetic and pharmaceutical formulations.

Various marine organisms are a source of new and interesting compounds, which offer a broad chemical diversity and valuable bioactive properties of interest for pharmaceutical, cosmetic and food industries [8]. They offer advantages over those obtained by synthetic methods because they have better physicochemical, biochemical, and rheological characteristics, maintaining their stability at different pH and temperature ranges [9]. For all these

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reasons, products from marine sources are achieving great preference in current market.

Among marine organisms, microalgae are considered one of the best sources of important compounds. Their main secondary metabolites include lipids, polysaccharides, pigments and amino acids with several very attractive properties for various industries [10, 11]. Indeed, the cosmetics industry is one of the most interested, as evidenced by the appearance on the market of various cosmetic products derived from these compounds [12].

For several decades, microalgae have been obtained on a large scale thanks to bioprocesses such as screening, prospecting, growing and scaling techniques. This has allowed meeting the high demands for their compounds and strengthening the biotechnology industry [13, 14], without affecting the balance of the marine ecosystem by avoiding the constant removal of organisms from their natural habitat.

The marine microalga *Porphyridium cruentum* can synthesize and secrete to the culture medium high amounts of SPs [15] which have shown significant antioxidant and anti-inflammatory activity, interfering with the formation and propagation of free radicals and inhibiting various mechanisms that induce or increase the activity of certain enzymes [4, 16, 17].

We have implemented various cultures of *Porphyridium cruentum* in bubble column photobioreactors, obtaining a constant and optimized production of SPs. Once these SPs were purified, their antioxidant capacity was evaluated using the DPPH, ABTS and FRAP methods. Besides, the SPs inhibitory activity on collagenase, elastase and hyaluronidase was studied through spectrophotometric assays.

The results of this study are in line with the new trends of the worldwide cosmetic industry and can serve as reference for further studies focused on the research, design, and development of new cosmeceutical products with high activity on tissue biochemistry, in order to achieve not only cosmetic but also nutritious, protective and regenerative effects on skin.

II. MATERIALS AND METHODS

A. Reagents

Clostridium histolyticum collagenase (C9891), porcine pancreatic elastase type IV (E0258), elastin-orcein (E1500), and bovine testicular hyaluronidase type VI-S (H3631), were purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA). Sodium chloride, sodium acetate trihydrate, chloramine T trihydrate, and ρ-dimethylamino-benzaldehyde, all of analytical grade, were obtained from Merk & Co. Inc. Collagen was purchased in Bell Chem International S.A and sodium hyaluronate in Vico GR Ltd. All other reagents used in this study were of analytical grade.

B. Equipment

All spectrophotometric measurements were performed with a Powerwave XS2 microplate spectrophotometer (Biotek®).

C. Culture and growth of the strain

The microalga *Porphyridium cruentum* (CIBNOR POC-1) was donated by Dr. Ever Morales from the microalgae strain collection of photosynthetic organism's laboratory at the University of Zulia (Maracaibo, Venezuela). The organism was grown in a bubble column photobioreactor using the modified Hemerick´s medium [18] under conditions of temperature and light intensity of 22 ± 2ºC and 5 ± 0.5 klux, respectively.

D. Extraction and purification of the SPs

After 20 days of culture, biomass was separated from medium by centrifugation at 4000 rpm at 10ºC for 10 min. The supernatant was filtered through a glass fiber membrane (pore size 1.6 µm) to remove any remaining cells from solution and then it was lyophilized. Subsequently a rough amount of 10 g of dry sample was weighed and redissolved in 80 mL of deionized water. This solution was placed inside a regenerated cellulose membrane (MWCO 3500 Da, Fisherbrand®), previously hydrated, and then it was put into a container with deionized water and constantly magnetic stirring. Once the conductivity readings were below 10 µS, salt-free solution was collected and lyophilized. The weight of the extracted SPs was measured on an analytical balance in order to determine its concentration in the culture medium.

E. Quantification of proteins and major monosaccharides

The spectrophotometric tests described by Bradford, 1976 [19] and Lowry et al, 1951 [20] were performed in order to make a quantitative assessment of protein content in the SPs.

For detection and quantification of the carbohydrates of interest (xylose and glucose), a 0.5 mg/mL SPs solution was assessed using the phenol-sulfuric acid method, as recommended by Masuko et al, 2005 [21]. The concentration of each monosaccharide in the sample was determined by interpolating the results in calibration curves. Four replicates of each standard and each sample at different concentrations were used for the test.

F. Enzyme assays

Each test was performed in triplicate using standards reported in the literature, known for their high inhibitory activity, which have been used in this study as positive controls to validate each assay. Negative controls were prepared by adding water instead of inhibitor. The quantification of the inhibitory effect of the SPs, and those of the reference compounds, were calculated using the following equation:

$$(1)\ \%\ \text{Inhibition} = [(An - As)/An] \times 100$$

Where An is the absorbance of negative control and As is the absorbance of the sample measured at the wavelength indicated for each test.

G. Collagenase assay

The enzyme assay was based on the method reported by Madhan et al, 2007 [22], with some modifications. For the enzymatic hydrolysis an 8 mg/mL solution of collagen was used as substrate. As enzyme, a stock collagenase solution of 3.6 x 103U/mL was prepared in a 0.1 M Tris-HCl buffer (pH 7.4) containing 0.006 mM CaCl2. Inhibitors and enzyme were incubated for 30 minutes at 25°C before adding the substrate. The 1 mL reaction mixture containing 902.83 U of enzyme, 2.4 mg of substrate and 250 µL of the SPs at different final concentrations (2.5, 1.25, 0.63 and 0.25 mg/mL) was incubated for 8 hours at 37°C. Finally, samples were centrifugated at 8500 rpm for 15 minutes at 4ºC and hydroxyproline was detected in 500 µL of supernatant by the method of Kolar, 1990 [23] adapted for a microplate reading. To validate the method we compared the effect of the SPs with the caused by a positive control containing EDTA at a concentration of 0.25 mg/mL. The spectrophotometric measurements were made at a wavelength of 558 nm.

H. Elastase assay

This test was based on the protocol described by Sachar et al, 1955 [24]. The principle of the method is the elastin-orcein hydrolysis, which is initially insoluble in water but when it is digested by elastase, it generates soluble products which can be determined spectrophotometrically at a wavelength of 590 nm. For this assay, an elastase solution of 0.399 U/mL dissolved in Tris-HCl buffer (pH 8.8) and a substrate solution at a final concentration of 4.7 mg/mL were used. The experiment began by adding 500 µL of the SPs to 500 µL of enzyme and then incubating for 30 minutes at 25ºC. Then, the reaction was obtained with the addition of the substrate and then the reaction mixture was incubated at 37ºC for 4 hours. Subsequently, all reaction mixtures were centrifugated for 15 minutes at 8500 rpm and 4ºC, and then the absorbance of the supernatant was read at 590 nm, using a solution containing the same amount of substrate dissolved in the buffer as blank. In order to validate the test, the inhibition caused by the SPs was compared to those caused by EDTA (disodium salt) at 15 mg/mL. At this concentration EDTA exhibits a high inhibition of elastase [25].

I. Hyaluronidase assay

The method described by Aronson and Davidson, 1967 [26] served as reference for carrying out this test. A solution of 147.56 U/mL of bovine hyaluronidase dissolved in 0.1 M acetate buffer (pH 3.9) with 0.1 M NaCl was prepared. A volume of 250 µL of enzyme was preincubated with 100 µL of the inhibitor for 30 minutes at 25ºC. After this time, sodium hyaluronate was added to a final concentration of 0.5 mg/mL and then the reaction mixture was incubated for 6 hours at 37ºC. The reaction was finished by adding 0.4 N NaOH and 0.8 M potassium tetraborate and then was heated in boiling water for 3 minutes. The tubes were left to stay for 5 minutes in an ice bath and under these conditions the color reagent was added. This reagent consisted of a mixture containing 10% of ρ-dimethylamino-benzaldehyde and 12.5% of HCl 10 M dissolved in glacial acetic acid according to the recommendations of Reissig et al, 1955 [27]. A few minutes after adding the color reagent, the reaction mixture turned pink-colored and it was read at a wavelength of 585 nm. Tannic acid was used as positive control at a final concentration of 2.5 mg/mL, since, according to literature, this compound inhibits the enzyme by 75-80% [6].

J. Evaluation of antioxidant activity

Three spectrophotometric methods were applied to assess the reducing ability of the SPs at variable concentrations (100-400 ppm) to act on preformed radicals at different phases. The first method applied was the FRAP assay (Ferric Reducing/Antioxidant Power), carried out according to Benzie and Strain, 1996 [28], using ascorbic acid as a standard to construct the standard curve. The second method was the ABTS radical cation decolorization assay, based on the quantification of bleaching of the radical species by its interaction with hydrogen or electron donors [29]. Finally, a DPPH free radical scavenging activity quantification was carried out by measuring the degree of discoloration of a 2,2-diphenyl-1-picrylhydrazyl solution at the wavelength of 517 nm, using the same amount of DPPH dissolved in 100 µL of deionized water as blank [30].

The results of DPPH and ABTS tests were expressed as TEAC (Trolox equivalent antioxidant capacity), and the FRAP assay results as NAAT (ascorbic acid equivalent antioxidant capacity).

K. Statistical analysis

All tests were performed in triplicate and data are presented as the media ± standard deviation (SD) of values. Statistical significance was determined using the Student´s t- test. P values <0.05 were considered statistically significant with a 95.0% confidence interval. Statistical analysis was performed using the Statgraphics Centurion's software (version 15.01.1903, StatPoint, Herndon, VA, USA).

III. RESULTS AND DISCUSSION

A. Extraction and dialysis of SPs

A total volume of 1.2 L of culture medium was filtered and freeze-dried, obtaining 20.5 g of extract before dialysis. At the end of the whole procedure, a SPs extract of 431.9 mg was obtained. Based on these data it was estimated that the approximate concentration of the SPs in the culture medium was 0.036% (w/v).

B. Quantification of proteins and major monosaccharides

No readings were obtained in both Bradford as in the Lowry test, thus demonstrating the absence of proteins in the SPs extract, so it was unnecessary to carry out a method for protein extraction.

According to Geresh et al, 2002 [31] the SPs of *Porphyridium cruentum* are comprised mainly of 10 different carbohydrates of which xylose, galactose and glucose are the most important. With the spectrophotometric assay, we confirmed the presence of xylose and glucose at a concentration of 0.5 ± 0.03 mg/mL and 0.4 ± 0.01 mg/mL, respectively.

C. Collagenase assay

The method was based on the hydrolysis of collagen by collagenase to release hydroxyproline, which was oxidized to pyrrole-2-carboxylic acid and subsequently complexed with ρ-dimethylaminobenzaldehyde, thus causing the formation of a colored complex that was read at a wavelength of 558 nm [32]. To study the inhibitory effect on the collagenase activity, we compared the absorbance of negative control with the absorbance of the samples. An increase on enzyme activity in the presence of the SPs was observed in a dose-dependent manner as shown in Figure 1.

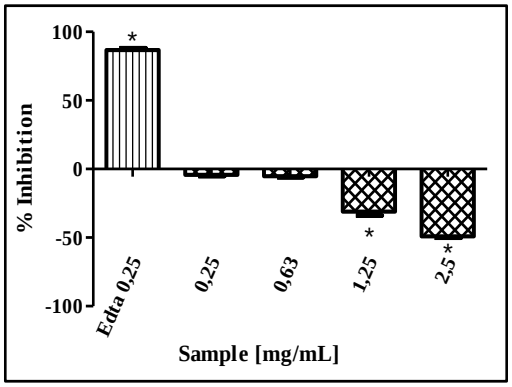

Figure 1. Representation of the SPs effect on collagenase activity. The degradation of the substrate increased in direct proportion to the concentration of the sample. Data are expressed as % inhibition, and each column represents the mean ± SD for the standard and for each concentration of the SPs. The symbol (*) indicates statistical difference compared with the control group, $p<0.05$.

A final concentration of 2.5 mg/mL produced a 49.1 ± 1.9% induction in the activity of the enzyme. As the SPs concentration decreased in the reaction mixture, there was a reduction of this induction, with 31.1 ± 4.9% for the concentration of 1.25 mg/mL, 5.2 ± 1.5% for 0.63 mg/mL and 4.3 ± 1.8% for the concentration of 0.25 mg/mL.

EDTA was also evaluated under the same conditions in which the SPs were tested. In other studies, EDTA has shown a significant inhibitory effect on collagenase through its ability to chelate the calcium ions necessary for the activation of the enzyme [5]. When EDTA was tested to a final concentration of 0.25 mg/mL, it triggered an inhibition of 86.7 ± 2.6%, and at concentrations of 0.63 mg/mL and 1.25 mg/mL caused total inhibition of the enzyme (100 ± 1.2%). In experiments conducted with an excess of calcium, EDTA had little or no inhibitory activity, this may due to a reactivation of the enzyme caused by the presence of non-chelated calcium, as reported by other authors [5].

As happened with EDTA, the SPs were also expected to inhibit the enzyme by kidnapping the calcium necessary for activation of the enzyme, thanks to the chelating properties of these sulphated compounds [33]. However, instead of an inhibition, or inactivity, the SPs provoked an enzyme induction as proved by the manifestation of further degradation of the substrate with respect to the negative controls. Although this result called our attention, it could be explained according to other reports. Actually, in other previous studies some compounds were evaluated as inhibitors of *Clostridium histolyticum* collagenase, resulting in an inductive effect caused by one of them [34]. This effect occurred with alendronate sodium, a molecule that acted as enzymatic inductor in a dose-dependent manner, even though it proved to be one of those which interacted more strongly with *Clostridium histolyticum* collagenase thanks to the high binding energies involved. This kind of inductive effect happened because that union was not made randomly but mainly with the Arg929 amino-acid residue of the enzyme. This fact reminds us that the inhibitory effect depends not only on the strength or magnitude of binding energies between the enzyme and the ligand, but also on the kind of interaction and the groups involved.

Wilson et al, 2003 [35] have identified the amino acid residues important for enzymatic activity of *Clostridium histolyticum* collagenase. Among all of them, it has shown that interactions between a ligand and the Arg929 amino acid residue of this enzyme result in an inductive effect of the enzyme activity [34, 35]. Since the SPs of *Porphyridium cruentum* are polyanionic due to the presence of glucuronic acid and half-ester sulfate groups [31], electrostatic interaction between these groups and the Arg929 amino acid residue may explain the results obtained in this test.

According to Wilson et al, 2003 [35], Thr957, Tyr970, Leu992, Tyr994 and Tyr996 amino acid residues are essential for enzymatic activity of *Clostridium histolyticum* collagenase. In fact, if the residue Tyr994 is substituted, the enzymatic activity would be reduced up to five times, preventing a total binding of the enzyme with collagen [34]. In this manner, to achieve an inhibition of this enzyme, a stronger interaction with these specific residues may be reached by fractionation or modification of the SPs. However, tests must be made with animal or human collagenase before considering any alternative that involves a physicochemical alteration of the sample. This is because collagenase from mammals contains other amino acid residues [36] which may interact differently with the sample, perhaps leading to an inhibition of the enzyme.

D. Elastase assay

Concentrations of 5.0, 3.33, 2.5, 1.25 and 0.25 mg/mL of the SPs were tested to determine the effect on this enzyme, obtaining in all cases an inhibition that increased in direct proportion to the concentration of sample used. The greatest effect was observed when testing the concentration of 5 mg/mL causing an inhibition of 46.0 ±

7.1%. It was followed by an inhibition of 29.9 ± 3.8% for the concentration of 3.33 mg/mL and 29.3 ± 3.5% for 2.5 mg/mL. The other concentrations (1.25 and 0.25 mg/mL) caused an inhibition of 17.6 ± 2.0% and 1.9 ± 0.3%, respectively, as can be seen in Figure 2.

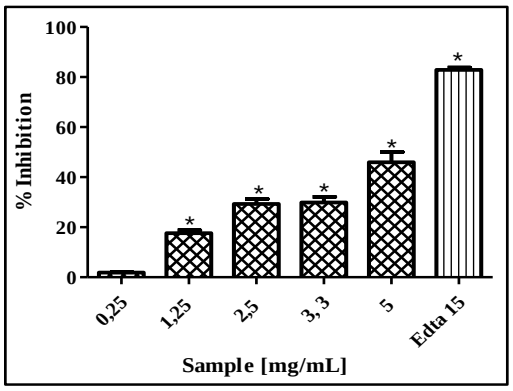

Figure 2. Representation of the SPs inhibitory effect on elastase activity. It is noted that the SPs cause inhibition directly proportional to its concentration, with an IC50 = 5.41 mg/mL. Data are expressed as % inhibition and each column represents the mean ± SD for the standard and for each concentration of the SPs. The symbol (*) indicates statistical difference compared with the control group, $p<0.05$.

From these results, it was determined that at a concentration of 5.41 mg/mL the SPs cause a 50% inhibition of elastase.

As in the previous test, we used EDTA as the standard inhibitor, this time to a final concentration of 15 mg/mL, causing an inhibition of 82.8 ± 1.6%. When it was tested at a final concentration of 5 mg/mL there was an inhibition of 8.84 ± 1.8%. At a concentration of 3.33 mg/mL an inhibition of 5.5 ± 1.3% was caused. As the enzyme used in this essay is not a metalloprotease, the resulting inhibition may be due to a reason other than the EDTA chelating property. We noted that adding EDTA at concentrations as high as 15 mg/mL caused the pH of the enzyme solution to decrease from pH 8.8 to pH 6. We assume that this effect, coupled with the modification of the ionic strength of reaction mixture, results in the inhibition of the enzyme by altering the ideal conditions required for its activity [37]. It has also been reported that high salt concentrations significantly suppress the elastolytic activity of elastase [25].

As reported above the inhibitory effect of the SPs on elastase activity was proved. This enzyme is recognized as the only serine protease capable of degrading elastin and triggering various inflammatory processes [38]. Its activity and concentration in the body are increased due to the aging process and other extrinsic factors. It has even been shown that UVA rays cause a release of this enzyme from polymorphonuclear leukocytes, attacking and damaging several connective tissue proteins, leading to inflammation and weakening of the dermal matrix [3]. Based on these facts, the inhibition of elastase by the SPs of *Porphyridium cruentum* may help to fight against the effects of aging and solar radiation. In addition, since this enzyme hydrolyzes virtually all proteins, including those that are structural and supporting for the connective tissue [39], its inhibition would not only help maintain the integrity of elastin but also of collagen and other critical proteins of the skin such as proteoglycans and keratin. Many studies have suggested that SPs could inhibit this protease through electrostatic interactions [40-43]. Redini et al, 1988 [44] indicate that the inhibition of human leukocyte elastase (HLE) by heparin (sulfated glycosaminoglycan) is probably due to the interaction between negative charges of sulfate groups and positively charged guanidinium groups of arginine residues located on HLE surface [45, 46]. These studies also found that the level of inhibition may differ according to molecular weight and degree of sulfation of SPs [45]. These facts serve as support to explain the results obtained in our experiments since the porcine pancreatic elastase (PPE) used by us has a close similarity to the HLE, due to the presence of similar structures in the active sites [47, 48] and the homologue organization of the N-terminal amino acids sequence [49].

E. Hyaluronidase assay

To study the inhibitory effect of the SPs on hyaluronidase, the substrate degradation was quantified by converting the resulting fragment (N-acetyl glucosamine) to a colored complex using ρ-dimethylaminobenzaldehyde. The absorbance of this complex was detected spectrophotometrically at a wavelength of 585 nm. Based on these data we calculated the percentage of inhibition vs the concentration used, as shown in Figure 3.

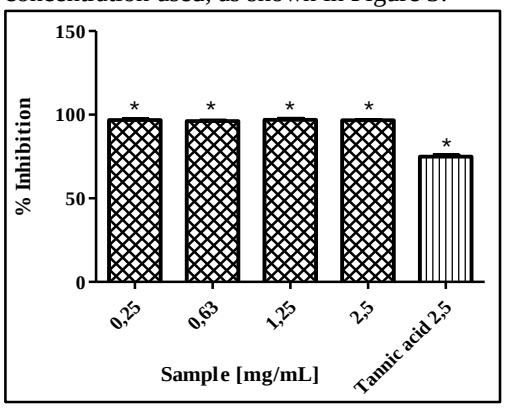

Figure 3. Test results of the hyaluronidase enzyme inhibition. Almost total inhibition of the enzyme can be observed at all concentrations of the SPs used, with statistical significance $p<0.05$ for all concentrations of the SPs and tannic acid. Data are expressed as % of inhibition and each column represents the mean ± SD for the standard and for each concentration of the SPs. The symbol (*) indicates statistical difference compared with the control group, $p<0.05$.

In the graphic above we see that the SPs exhibited an excellent inhibitory effect with an average inhibition of 96.6 ± 0.3% when it was used in a concentration ranging from 0.25 to 2.5 mg/mL. Furthermore, tannic acid tested at a concentration of 2.5 mg/mL produced an inhibition of 75.0 ± 1.3%, much lower than that caused by our SPs evaluated at the same concentration.

The results show that the SPs inhibit the degradation of hyaluronic acid by an effective and broad inhibition of the hyaluronidase activity. This may be very useful since it has

been demonstrated that several hyaluronidases are related to many pathological disorders such as cancer, osteoarthritis, inflammatory processes and skin diseases [50, 51]. The results obtained in this study may be due to the electrostatic interaction between positive charges of hyaluronidase amino acids and the negatively charged sulfate groups and uronic acids present in the SPs, which results in the formation of a complex that would prevent the enzyme from bonding with the substrate [52]. However, several authors have argued that the biological activities of SPs are likely to be due not only to a property, but to a number of important factors such as molecular weight, sulfation degree, distribution and structure of components, and charge density [31, 53, 54]. It should also be noted that the SPs from *Porphyridium cruentum*, unlike hyaluronic acid and chondroitin sulfates, do not contain the 1,4-linkages between 2-acetamido-2-deoxy-β-D-glucose and D-glucose which are randomly hydrolyzed by hyaluronidase [9]. In this manner, the SPs exhibit their own resistance to degradation caused by endogenous hyaluronidase. This natural resistance may allow the SPs to remain longer in the body, resulting in a prolonged desired effect, than if sodium hyaluronate (also known as hyaluronic acid) is used. This advantage, coupled with the SPs high capacity to retain moisture and act as bio-lubricants [9], would be useful in cosmetic procedures such as lips and wrinkles filling, or in therapeutic treatments such as viscosupplementation employed to reduce symptoms of patients with osteoarthritis.

F. Evaluation of antioxidant activity

Table 1 shows the results of the SPs antioxidant capacity as assessed by DPPH, ABTS and FRAP methods at a concentration of 100 ppm. Higher concentrations were tested without obtaining greater differences in the results.

TABLE 1. Results of the in vitro antioxidant capacity. The data below corresponds to the average of a triplicate ± SD ($p<0.05$) for each method.

SAMPLE	ANTIOXIDANT CAPACITY		
	DPPH - TEAC Trolox μM/g of Sample	**ABTS -TEAC Trolox μM/g of sample**	**FRAP-NAAT Ascorbic acid mg/g of sample**
SPs (100 ppm)	5.88 ± 0.96	40.46 ± 2.97	1.81 ± 0.24

According to these results, the ability of the SPs to remove both DPPH and ABTS•+ radicals and to reduce Fe3+ is very low. This low antioxidant capacity can be explained according to the findings of Sun et al, 2009 [55], taking into account that the SPs are a mixture of polymer chains of various molecular weights. They found a high antioxidant activity when evaluating SPs fractions of low molecular weight, but no significant activity was found by testing non-fractionated SPs, which have high molecular weights [56]. Sun et al, 2009 [55] also claim, as mentioned earlier, that this antioxidant activity can result from a combination of factors, among which stands the content of sulfate groups. It's also important to note that in the literature the need for multiple methods when evaluating the antioxidant capacity of a sample is widely referenced, because antioxidant compounds may act by different mechanisms, depending on the reaction system or on the radical source [57].

IV. CONCLUSIONS

SPs are very promising for incorporation into cosmetic and pharmaceutical formulations since they not only exhibit excellent bioactive capacity but have also proven to be safe and acceptable for use in humans, as verified by various clinical studies [58-60].

Several authors have been interested in evaluating the bioactive potential of the SPs of *Porphyridium cruentum* on different biological processes. In fact, they have shown a very good antiviral activity against herpes simplex virus (HSV 1, 2) and varicella zoster [61], anti-inflammatory [62], antitumor, and immunomodulatory activity [63], among others [64]. A high antioxidant capacity has also been shown when the SPs are evaluated with methods other than those used in this research [4].

According to our in vitro study it is clear that the SPs of *Porphyridium cruentum* inhibit the activity of elastase and hyaluronidase enzymes, showing maximum values of inhibition of 46.0 ± 7.1% and 96.9 ± 1.2% respectively. Inhibition of elastase by SPs not only may allow greater permanence of elastin but also of collagen and many other essential proteins for the skin. Besides, the skin inflammation and irritation which result from the action of chemicals, excessive exposure to sunlight and other biochemical and environmental factors, may be also counteracted by the SPs. On the other hand, the high inhibition caused by the SPs on hyaluronidase activity may contribute to prolong the integrity of hyaluronic acid and the skin´s natural moisture. Moreover, the SPs natural resistance to the action of hyaluronidase is a great advantage for their use as filler substance in various beauty treatments and as a biolubricant for viscosupplementation therapies. All this makes the SPs of *Porphyridium cruentum* good candidates for use in cosmetic or pharmaceutical formulations, as anti-aging, protective, moisturizing, emollient, lubricant and anti-inflammatory agent for all skin types, both to preserve the beauty and vitality of the healthy and young skins, as to treat those who are affected by some kind of disorder.

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