# Preliminary data of antioxidant activity of green seaweeds (Ulvophyceae) from the Southwestern Atlantic and Antarctic Maritime islands

# Datos preliminares de la actividad antioxidante de algas verdes (Ulvophyceae) del Antlántico del Sur y de las islas marítimas de Antártida

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## ABSTRACT

**Background.** Seaweeds must survive in highly competitive environments and thus develop defense strategies that may produce highly diversified antioxidant compounds. **Goals**. The main objective of this work was to assess the antioxidant activity of green seaweeds. **Methods**. Six species of ulvophycean chlorophytes were collected during spring/summer, between the Antarctic (*Monostroma hariotii, Protomonostroma rosulatum* and *Ulva hookeriana* – formerly as *U. bulbosa*) and the southwestern Atlantic Ocean (*Gayralia brasiliensis, Protomonostroma undulatum* and *Ulva fasciata*). They were then tested for their antioxidant activities using the 2.2-diphenyl-picrylhydrazyl (DPPH) radical scavenging method and by quantification of their phenolic (expressed as gallic acid equivalent – GAE – and carotenoid contents. **Results**. Among the evaluated species, *P. rosulatum* and *U. hookeriana* showed high antioxidant potential (77.9±2.8 and 53.1±15.0%, respectively) and high phenolic content (176±6.0 and 144.7±8.9 µg GAE $g^{-1}$, respectively). These species were collected on King George Island (South Shetland archipelago, around the Antarctic Peninsula) and their higher antioxidant potential may be associated with adaptation to the high incidence of UV rays in this region during summer. In general, tested seaweeds, mainly the samples collected in the Antarctic and Chilean Patagonia, showed higher values of phenolic (from 58.3±2.0 to 144.7±8.9 µg GAE $g^{-1}$) and carotenoid contents (from 23.4±0.2 to 51.5±0.1 µg β-carotene $g^{-1}$). **Conclusions**. The presence and levels of these compounds suggest that the target seaweeds may have high antioxidant potential. Also the antioxidant activity could be associated with the occurrence area of the species instead of the order or taxonomic group to which they belong.

**Key words:** Green seaweeds, reactive oxygen species, phenolic contents, photoprotectants.

## RESUMEN

**Antecedentes.** Las algas marinas tienen que sobrevivir en entornos altamente competitivos, lo que hace que desarrollan estrategias de defensa que pueden dar lugar a una gran diversidad de compuestos antioxidantes. **Objetivos.** El principal objetivo de este trabajo fue evaluar la actividad antioxidante de algas verdes. **Métodos**. Seis especies de clorófitos (Ulvophyceae) recogidos durante el primavera/verano entre la Antártida (*Monostroma hariotii*, *Protomonostroma rosulatum* y *Ulva hookeriana* – antes como *U. bulbosa*) y el Atlántico Sur (*Gayralia brasiliensis*, *Protomonostroma undulatum* y *Ulva fasciata*) fueron probados por su actividad antioxidante usando el método de secuestro de radicales libres con 2,2-diphenil-picrylhidrazil (DPPH) y pela cuantificación de contenidos fenólicos (expreso como equivalente de ácido gálico (GAE)) y contenidos de carotenoides. **Resultados**. Entre las especies evaluadas, *P. rosulatum* y *U. hookeriana* mostraron alto potencial antioxidante (77,9±2,8 y 53,1±15%, respectivamente) y alto contenido fenólico (176±6,0 y 144,7±8,9 µg GAE $g^{-1}$, respectivamente). Estas especies fueron recolectadas en la Isla Rey Jorge (cerca de la Península Antártida) y su mayor potencial antioxidante puede estar asociado con la adaptación a la alta incidencia de los rayos UV en esta región. En general, las algas marinas probadas, principalmente las muestras recogidas en la Patagonia Antártida y chilena, mostraron valores más altos de fenólico (de 58,3 ± 2,0 a 144,7 ± 8,9 mg GAE $g^{-1}$) y el contenido de carotenoides (de 23,4 ± 0,2 a 51,5 ± 0,1 mg $g^{-1}$ β-caroteno). **Conclusiones**. La presencia y los niveles de estos compuestos sugieren que las algas marinas diana pueden tener un alto potencial antioxidante. También la actividad antioxidante podría estar asociada con el área de ocurrencia de las especies en lugar de la orden o grupo taxonómico a la que pertenecen.

**Palabras clave:** Algas verdes, especies reactivas del oxígeno, contenidos fenólicos, fotoprotectores.

## INTRODUCTION

*Monostroma, Protomonostroma* and *Gayralia* are foliose green seaweeds widely distributed throughout the Southern Hemisphere, with a concentrated biomass between the South Atlantic, Antarctic Peninsula and adjacent archipelagos. These ecosystems show variable and extreme chemical and physical conditions. Benthic organisms, such as seaweeds, can be exposed to high ultraviolet (UV) radiation, herbivory and fouling in higher latitudes, which are factors that may drive the development of defense strategies associated with their secondary metabolism (Verpoorte, 2000; Hartmann, 2007).

The isolation and chemical characterization of secondary metabolites are relevant since some of these compounds demonstrate biological activities against human pathologies (Camarero *et al.*, 1990; Gagioti *et al.*, 1995; Maschek & Baker, 2008). Specifically, antioxidants are the primary natural product known to be inhibitors of reactive oxygen species (ROS) accumulation and can prevent skin oxidative stress, skin cancer and premature skin aging (Guaratini *et al.*, 2007; Guaratini *et al.*, 2012). As such, they have been often applied in the pharmaceutical, nutraceutical and cosmetic industries (Guaratini *et al.*, 2007; Hwang *et al.*, 2010; Guaratini *et al.*, 2012).

Phenolic compounds are the major substances responsible for antioxidant activity in land plants (Hayase & Kato, 1984). In seaweeds, they are primarily involved in UV protection, anti-herbivory defense, pathogen resistance and epiphyte growth defense (Amsler & Fairhead, 2006; Bittencourt-Oliveira *et al.*, 2005).

In addition, carotenoids, a pigment class common in plants and algae, also protect cells against ROS (Hollnagel *et al.*, 1996; Olson, 1999; Naguib, 2000; Gressler *et al.*, 2010, 2011).

Polar ecosystems are the most extreme on the planet, and the organisms inhabiting such environments, including seaweeds, are highly adapted (Dummermuth, 2003). These organisms tolerate low temperatures, long ice-covering periods, low levels of visible irradiance during the winter as well as high quantities and long durations of solar incidence during the summer. Moreover, the stratospheric ozone, which protects organisms from harmful UV radiation, suffers seasonal oscillations over the Antarctic headed by climate global changing and cyclonic winds around this polar area. Therefore, interest in the seaweeds adaptations in polar zones with regard to their production of defense compounds against high levels of UV and fluctuations in ozone levels has been increasing (Dummermuth, 2003).

Recently, Zamora *et al.* (2010) described high levels of antioxidant production enzymes in the shoots of *Deschampsia antarctica* Desvaux, an Antarctic vascular plant, subjected to drought stress. In addition, other studies have found strong antioxidant activity in Antarctic lichen species (Paudel *et al.*, 2008; Luo *et al.*, 2009) and filamentous fungi (Tosi *et al.*, 2010). Apart from those studies, no description of antioxidant activity in Antarctic seaweeds have been reported to date.

In other instance, in the South Atlantic area many studies searching for antioxidant activity in seaweeds have been done in the last 10 years, but only a few of them considered green seaweeds. Cruces *et al.* (2012) reported a positive correlation between the antioxidant activities of two sub-Antarctic brown seaweeds in response to UV radiation. Raymundo *et al.* (2004), using the ferric thiocyanate method to measure the antioxidant activity, found levels of inhibition of lipid peroxidation above 70% in *Ulva intestinalis* Linnaeus and *Chaetomorpha antennina* (Bory de Saint-Vicent) Kützing extracts collected in southern Brazil. Sousa *et al.* (2008) tested the antioxidant potential by assaying for $\alpha$ and $\beta$-carotene and $\alpha$-tocopherol (vitamin E) levels in 32 seaweed species from northeastern Brazil, and higher contents of these substances were obtained mainly in green seaweeds.

Considering that meteorological and oceanographic traits along the Antarctic region influence air and seawater mass circulation throughout the Southwestern Atlantic and due to the scarcity of studies evaluating chlorophyte bioactive compounds from the Antarctic Peninsula and Southwestern Atlantic Ocean, this contribution aims to analyze and compare the antioxidant activity of three South American ulvophycean species (*Gayralia brasiliensis* Pellizzari, M.C. Oliveira *et* N. S. Yokoya, *Protomonostroma undulatum* (Wittrock) K. L. Vinogradova and *Ulva fasciata* Delile) and three other species from King George island around the Antarctic Peninsula (*Protomonostroma rosulatum* Vinogradova, *Monostroma hariotii* Gain and *Ulva hookeriana* (Suhr) Hariot). This study is the first report, although preliminary, of antioxidant activities in seaweeds collected in the Maritime Antarctic islands and surroundings.

## MATERIALS AND METHODS

Seaweed material (approx. 500 g wet weight) was handly collected during spring/summer seasons in the intertidal zones of the Antarctic Peninsula surroundings and Southwestern Atlantic Ocean locations showed in Figure 1, Tables 1 and 2. Samples were washed with local seawater to remove epiphytes, associated fauna and sediments. In the laboratory, the seaweeds were washed with distilled water before freezing. The analyzed species, sites, sampling dates and voucher numbers of the species deposited in the "Maria Eneyda P. K. Fidalgo" Herbarium of Instituto de Botânica de São Paulo (SP) are listed in Table 1.

Aiming to obtain crude chlorophyte extracts, the algae were defrosted and dried in a laminar flow hood (Splabor model SP 808/6-HLV) at room temperature (approx.. 25ºC) for approximately 48 h. Dried algal samples were triturated into a powder using liquid nitrogen. The material was weighed, and the volume of solvents to be added was calculated for each sample. For each 10g of algae sample was added 50 mL of the particular solvents. The first extraction was conducted with methanol P.A. for 48h before subsequently filtration with cotton. The filtered extract was poured into a 500 mL beaker to evaporate in a laminar flow hood. The second extraction, conducted with dichloromethane P.A. (50 mL of solvent to each 10 g of algae), was performed with the algae fraction retained after the filtration. After 48 h, the extract was filtered, added to the same beaker used before and evaporated completely in a laminar flow hood.

The antioxidant properties assay on seaweed samples using DPPH (2.2-diphenyl-picrylhydrazyl) radical scavenging was described by Kim *et al.* (2002). Briefly, a 2.9 mL aliquot of a methanolic solution of the DPPH radical (Sigma, 0.1 mM in 80% methanol) was added to 0.1 mL of each sample (in 80% methanol). All algal extracts were solubilized with 80% methanol and evaluated at three different dilutions (1.0, 0.5 and 0.16 mg $mL^{-1}$). A blank sample was prepared with 80% methanol as control. The absorbance decrease at 517 nm relative to the negative control was measured with a UV-visible spectrophotometer with a microplate reader after 30 minutes of sample incubation. The percentage of absorbance decrease, indicating antioxidant activity (AA), was calcu-

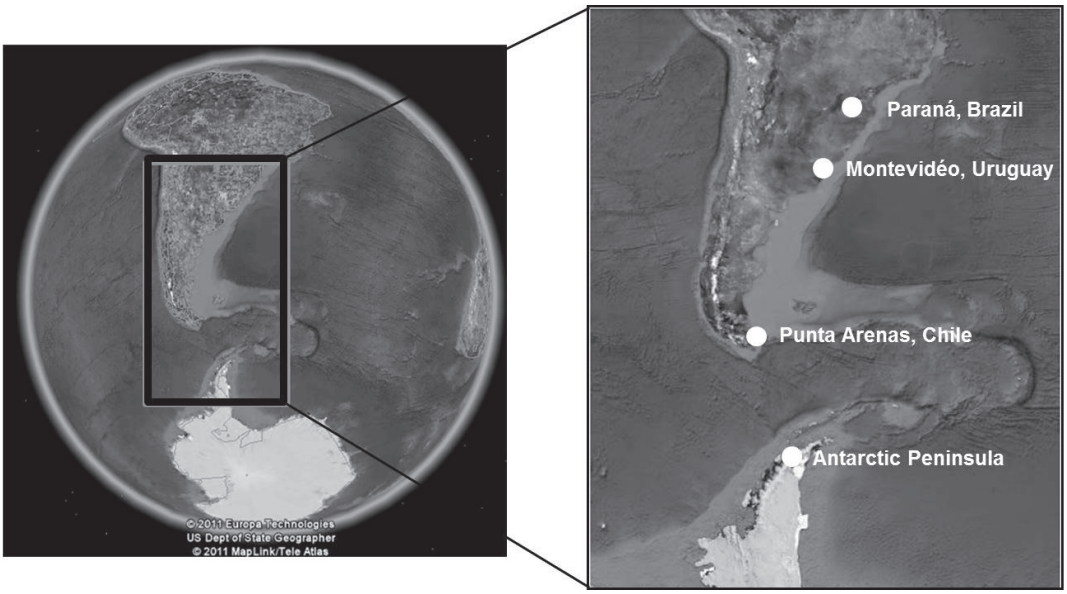


Figure 1. Map showing the sampling locations at Southwestern Atlantic Ocean. In Paraná, Brazil, seaweeds were collected at the inner sector of Antonina Bay and outer sector of Guaratuba Bay. In Montevideo, Uruguay, samplings were made at the Rio de La Plata outfall. In Punta Arenas, Chile, seaweeds were sampled at Fuerte Bulnes beach. In Antarctic Peninsula, samplings took place at Punta Plaza and Botany Point beaches.

lated using the equation: $\%AA = \{(Abs_{control} - Abs_{sample})/Abs_{control}\} \times 100$. All tests were performed in triplicate.

Phenolic contents were determined using the method described by Rhandir *et al.* (2002) with modifications. A 40 µL aliquot of each algae extract was added to a reaction mixture containing 3.16 mL of distilled water, 200 µL of Folin-Ciocalteu reagent and 600 µL of $Na_2CO_3$ (20% in water) and then thoroughly mixed. After 2 hours incubation in a dark chamber, the absorbance was measured at 750 nm using a UV spectrophotometer. The total phenolic content was analyzed using a gallic acid standard curve. The results from triplicate tests were expressed as µg of gallic acid equivalent (GAE) per gram of dry biomass.

Carotenoid quantification was carried out according to Aman *et al.* (2005) with modifications. 3 mg of each algae crude extract were solubilized in 3 mL of hexane P.A. and the absorbance was read at 450 nm using a UV spectrophotometer. The results from triplicate tests and the mean absorbance obtained were used in a regression equation calculated from the β-carotene standard curve ($y = 0.0856x + 0.0074$, where x is the average and y is the concentration). The results were expressed as µg of β-carotene per gram of dry mass.

To compare the AA of the distinct extracts of marine chlorophytes, a one-way analysis of variance (ANOVA) was performed. Variance homogeneity was tested by a Cochran test, and the normal distribution of the data was observed by a histogram. Tukey's post-hoc test was used when the data showed significant differences ($p < 0.05$). When the ANOVA assumptions were not attempted, a non-parametric Kruskal-Wallis test was used. A linear regression was made between the phenolic contents and the radical scavenging assay. All analyses were performed in Statistica Software version 07 (StatSoft, 2004) and the graphic was made using Microsoft Excel® 2010.

Table 1. Sampled chlorophyte species, sampling sites, coordinates, sampling dates and voucher numbers.

| Species | Site | Latitude | Longitude | Date | Herbarium Number |
|---|---|---|---|---|---|
| *Protomonostroma rosulatum* | Punta Plaza, King George Island, Maritime Antarctic | 62°05'25"S | 58°24'56"W | Dec 2010 Jan 2011 | SP 427956 |
| *Protomonostroma undulatum* | Fuerte Bulnes, Punta Arenas, Chile | 53°37'44.22"S | 70°55'17.92"W | Mar 2012 | SP 427957 |
| *Ulva hookeriana* | Punta Plaza, King George Island, Maritime Antarctic | 62°05'25"S | 58°24'56"W | Dec 2010 Jan 2011 | SP 427959 |
| *Ulva fasciata* | Matinhos, Paraná, South Brazil | 25º49'8"S | 48º32'29"W | Sep 2012 | SP 427958 |
| *Gayralia brasiliensis* | Guaratuba Bay, Paraná, South Brazil | 25°51'10.92"S | 48°34'13.17"W | Sep 2012 | SP 427960 |
| *Monostroma hariotii* | Botany Point, King George Island, Maritime Antarctic | 62°04'4.57"S | 58°18'35.13"W | Dec 2011 Jan 2012 | SP 427954 |

SP = Initials of Maria Eneyda P. K. Fidalgo Herbarium, of the Instituto de Botânica de São Paulo.

## RESULTS

The AA average values from chlorophyte extracts, obtained by the DPPH method at three extract concentrations, are shown in Table 3. The DPPH radical reduction percentages ranged from 3.5±2.1% in *Ulva fasciata* to 77.9±2.8% in *Protomonostroma rosulatum*. The extract dilution of 1 mg $mL^{-1}$ showed the most satisfactory results. Two species collected in the Antarctic Peninsula, *P. rosulatum* and *Ulva hookeriana* showed the highest apparent AA potential (77.9±2.8% and 53.1±15%, respectively) and the differences were statistically significant from the other analyzed species ($p<0.05$). The species *Protomonostroma undulatum, Gayralia brasiliensis, Monostroma hariotii* and *U. fasciata* did not differ (Tukey´s post-hoc, $p>0.05$) and showed lower antioxidant potential (Table 3).

Table 3 shows the average values of total phenolic contents expressed as the gallic acid equivalent (GAE) detected in the tested species. The lowest value was obtained by *Ulva fasciata* (58.3±2 µg GAE $g^{-1}$) collected in southern Brazil, and the highest value was identified in *P. rosulatum* (176±6 µg GAE $g^{-1}$) sampled in Antarctic. In this analysis, interspecific variance comparisons showed significant differences (Kruskal-Wallis, $p<0.05$). Figure 2 shows a linear regression between the phenolic contents and the DPPH radical scavenging percentages. In general, the radical reduction rates followed the phenolic contents. Values are presented in Table 3.

The average values found for carotenoid contents in the analyzed chlorophytes are presented in Table 3. The values ranged from 23.4±0.2 µg β-carotene $g^{-1}$ in *Ulva fasciata* to 51.5±0.1 µg β-carotene $g^{-1}$ in *Ulva hookeriana*. The comparison of the variances was significantly different (Kruskal-Wallis, $p<0.05$) only between those two species and no significant differences was detected for the other species analyzed.

## DISCUSSION

Among the Ulvophyceaean species collected between the Antarctic Peninsula and the southern Atlantic, *Protomonostroma rosulatum* and *Ulva hookeriana,* from King George Island, Antarctic, showed higher antioxidant potential. This result suggests that the AA levels could be associated with the occurrence area of the analyzed species and not necessarily with the order or taxonomic group to which the species belongs. Futhermore, they indicate that antioxidant potential is maybe an adaptation strategy for increased tolerance to heightened UV irradiation in the algae's habitat.

UV data around the world is controversial due to differences on units of measurements and sort of equipment used to such purpose, besides, long term measurements and data in broad areas are presented as averages. However, following data retrieved from the *Brazilian Weather Forecast and Climate Studies Center* from the *National Institute of Space Research* (CPTEC/INPE - http://www.cptec.inpe.br) we can observe data of IUV max for Paraná sampling station between 5 and 9, considering also a crucial factor when discussing antioxidant activity and its relation with UV rays, the photoperiod in the area and for spring season, 12:12h (light/dark). Southern Chilean coast during summer register IUV data between 10 and 13, presenting a photoperiod of 15:9h. Following data retrieved from the *National Institute of Water and Atmospheric Research* of New Zealand (https://www.niwa.co.nz/sites/niwa.co.nz/, citing Liley & Mckenzie, 2008), during summer NZ, including subantarctic islands and islands around Peninsula, experiences IUV higher than 14, and a photoperiod of 20:4h. Considering these pool of data we justify in part our inferences above.

Based on previous results obtained from *Gingko biloba* L., an extract used as indicator of antioxidant potential with 80% of AA (Martins *et al*. 2012), our report infers that *P. rosulatum* showed high antioxidant potential. Dummermuth (2003) quantified AA in extracts of Arctic seaweeds, and the chlorophytes showed higher AA compared to red and brown algae. This author also found that *Monostroma grevillei* var. *arcticum* (Wittrock) Rosenvinge, had higher antioxidant potential compared with other Arctic green seaweeds (Dummermuth, 2003).

In our study, *P. undulatum,* collected in the Chilean Patagonia, and *Monostroma hariotii*, sampled in the Antarctic Peninsula, showed high rates of phenolic contents but showed non-satisfactory antioxidant

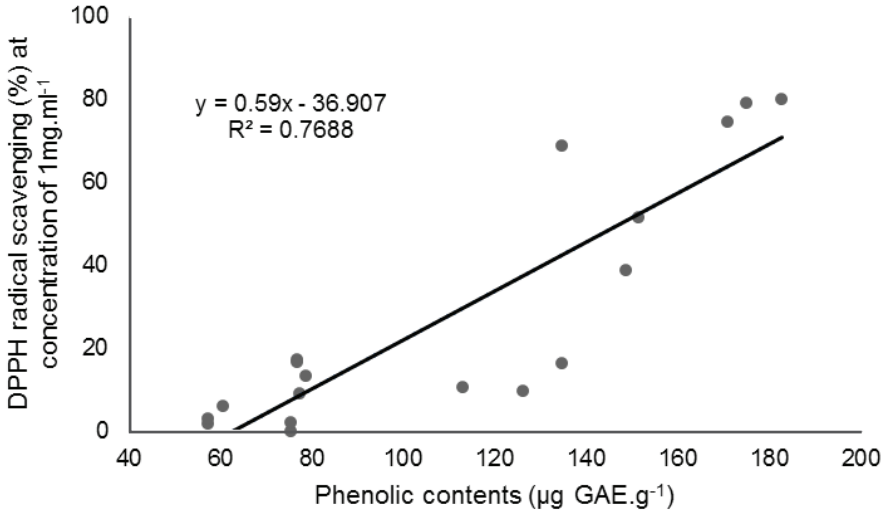


Figure 2. Linear regression between total phenolic contents of the crude extracts of chlorophytes from the Antarctic Peninsula and South Atlantic expressed as the average and standard deviation (n=3) of the gallic acid equivalent (µg GAE $g^{-1}$) and the percentages of DPPH radical scavenging at a concentration of 1 mg $mL^{-1}$. $R^2$= 0.7688; $p < 0.01$.

Table 2. Chlorophyte species sampled, sites, equivalent occurrence zone, average temperature and salinity data and sampling season.

| Species | Site | Occurrence zone | Temperature (°C) | Salinity (ups) | Season | Maximum UV values* |
|---|---|---|---|---|---|---|
| *M. hariotii, Protomonostroma* sp. and *U. hookeriana* | Maritime Antarctic | Polar | 1±2 | 37±1,5 | Summer | >14 |
| *G. oxysperma* | Uruguay | Subtropical | 21±2 | 16±3 | Spring | 7 - 10 |
| *G. brasiliensis* | Brazil | Subtropical | 20±4 | 20±4 | Spring | 5 - 9 |
| *P. undulatum* | Chile | Temperate | 6±4 | 28±5 | Spring | 10 - 13 |

*Data from: www.cptec.inpe.br; www.niwa.co.nz/sites/niwa.co.nz

efficacy using methanolic extracts (<60% of reduction). This result could imply differences in the chemical composition among phenolic compounds of the tested algae, and also differences in the solubility of these compounds in the applied solvent, or the influence of associated antioxidant compounds, such as carotenoids, on the AA efficiency present in these algal extracts.

The antioxidant properties of natural products are related to the presence of phenolic compounds that naturally occur in terrestrial and aquatic plants (Hayase & Kato, 1984). In the present study, the higher percentage of AA was obtained in the extract of *P. rosulatum*, whose biomass had a higher content of phenolic compounds, corroborating the previous study. *U. hookeriana,* also collected in King George Island, showed high phenolic contents and was the species showing second highest AA among the ones we sampled in this study.

Trigui *et al*. (2012) studied seasonal variations of AA levels in *Ulva rigida* C. Agardh and concluded that variations in activity correspond to the variations observed in the total phenolic contents. The authors also suggest that these differences could be assigned to seasonal changes in abiotic factors, such as climatic changes, temperature, salinity, nutrients, pollution, epiphytism and different reproductive or metabolic stages of the plant.

Carotenoids are also known to function as antioxidants (Naguib, 2000). Sousa *et al*. (2008) found $\alpha$- and $\beta$-carotene in all tested green algae. Green and yellow vegetables have been reported to have high carotenoid contents (Bendich & Olson, 1989; Grune *et al*., 2010), *e.g.,* carrots (33 µg $g^{-1}$) and pumpkins (53 µg $g^{-1}$) (Rodrigues-Amaya *et al*., 2008). Literature data report that *Enteromorpha* (Ulvales) can present higher ç-carotene contents than some vegetables, showing concentrations nearing 250 µg $g^{-1}$ (Ito & Hori, 1989). These data support our high concentrations detected in *Ulva hookeriana* (previously reported as *Enteromorpha bulbosa* (Suhr) Montagne) sampled in the surrounding islands of Antarctic Peninsula.

Finally, the higher AA observed in *P. rosulatum* and *U. hookeriana* seems to be related to the phenolic and carotenoid contents. Both phenolics and carotenoids are directly related to protection against UV rays in vegetables and likely in seaweeds. One hypothesis for the higher antioxidant potential found in this two species from the Antarctic Peninsula is that these populations are exposed to higher and continuous UV radiation mainly during summer and thus may be naturally selected for high antioxidant potential.

Therefore, these preliminary results suggest that Antarctic Ulvophyceans may have a wide range of chemical constituents from secondary metabolism, mainly phenolic compounds and carotenoids, that can present AA and that probably these contents are higher when compared to the same orders or family taxa collected in temperate or subtropical zones due to adaptive routes. This contribution is the first comparative report of AA in Ulvophyceae species from Brazil, Chile and maritime Antarctic. The high AA of *P. rosulatum,* endemic to King George Island, Antarctic Peninsula, corroborates the relevance of elucidating bioactive compounds from high latitudes as possible new sources of natural products with potential uses in cosmetic, nutraceutical and pharmacological industries for their anti-oxidative stress effects.

Table 3. Marine chlorophyte species, their distribution and extracts analysis results.

| Species | Sites | DPPH radical scavenging (%) | | | Phenolic contents (µg GAE $g^{-1}$) | Carotenoid contents (µg $\beta$-carotene $g^{-1}$) |
|---|---|---|---|---|---|---|
| | | 1 mg $ml^{-1}$ | 0.5 mg $ml^{-1}$ | 0.16 mg $ml^{-1}$ | | |
| *P. rosulatum* | Antarctic | 77.9±2.8 | 40±6 | 14±1 | 176±6 | 29.6±0.04 |
| *P. undulatum* | Chile | 12.1±3.5 | 10.7±2.9 | - | 124.5±10.8 | 25.2±0.6 |
| *U. hookeriana* | Antarctic | 53.1±15 | 38.9±3.4 | 8.4±2 | 144.7±8.9 | 51.5±0.1 |
| *U. fasciata* | Brazil | 3.5±2.1 | - | - | 58.3±2 | 23.4±0.2 |
| *G. brasiliensis* | Brazil | 13.1±4.1 | 15.8±5.2 | - | 77.5±1.0 | 32.2±0.08 |
| *M. hariotii* | Antarctic | 5.1±10.2 | - | - | 75.7±0.8 | 36.3±0.2 |

Results of DPPH radical scavenging percentages in different concentrations of chlorophyte extracts, phenolic and carotenoid contents to evaluate antioxidant activity. Values are expressed as the mean ± standard deviation.

- Undetectable values.

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