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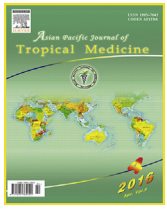

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Bioactive extracts of red seaweeds *Pterocladiella capillacea* and *Osmundaria obtusiloba* (Floridophyceae: Rhodophyta) with antioxidant and bacterial agglutination potential

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ABSTRACT

Objective: To evaluate the antioxidant, antibacterial and bacterial cell agglutination activities of the hexane (Hex) and 70% ethanol (70% EtOH) extracts of two species of red seaweeds *Pterocladiella capillacea* (*P. capillacea*) and *Osmundaria obtusiloba*.

Methods: *In vitro* antioxidant activity was determined by DPPH radical scavenging assay, ferric-reducing antioxidant power assay, ferrous ion chelating assay, β-carotene bleaching assay and total phenolic content quantification. Antimicrobial activity was tested using the method of disc diffusion on Mueller-Hinton medium. The ability of algal extracts to agglutinate bacterial cells was also tested.

Results: The 70% EtOH extract of the two algae showed the highest values of total phenolic content compared to the Hex extract. The results of DPPH for both extracts (Hex, 70% EtOH) of *Osmundaria obtusiloba* (43.46% and 99.47%) were higher than those of *P. capillacea* (33.04% and 40.81%) at a concentration of 1 000 μg/mL. As for the ferrous ion chelating, there was an opposite behavior, extracts of *P. capillacea* had a higher activity. The extracts showed a low ferric-reducing antioxidant power, with optical density ranging from 0.054 to 0.180. Antioxidant activities of all extracts evaluated for β-carotene bleaching were above 40%. There was no antibacterial activity against bacterial strains tested. However, the extracts of both species were able to agglutinate bacterial Gram positive cells of *Staphylococcus aureus* and Gram negative cells of *Escherichia coli*, multidrug-resistant *Salmonella* and *Vibrio harveyi*.

Conclusions: This is the first report of the interaction between these algal extracts, rich in natural compounds with antioxidant potential, and Gram positive and Gram negative bacterial cells.

1. Introduction

Seaweeds are renewable natural resources with great potential for application in the pharmaceutical, food, nutraceutical and cosmetics industries. These plants are rich in bioactive compounds and water-soluble (B-complex and C) and fat-soluble (provitamin A and vitamins D, E and K), dietary fibers, proteins, poly-unsaturated fatty acids and minerals, such as calcium, magnesium, phosphorus, potassium, sodium, iron and iodine [1–5].

Red marine algae are macroscopic, multicellular, benthic organisms, commonly marketed as food in Asian countries in the form of spices, condiments, pasta, and also employed in the phycocolloids industry (agar, carrageenan) as a food additive acting as gelling agents, emulsifiers, in water retention and other physical properties [6].

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In the metabolism of a living organism, reactive oxygen species (ROS) are normally produced and eliminated by enzymatic and non-enzymatic defense systems. However, under stressful conditions, defense systems can fail, and the accumulation of ROS and other free radicals can cause irreversible damage to proteins, amino acids, lipids and DNA. Thus, oxidative stress has been associated with several diseases such as cancer, hypertension, diabetes, atherosclerosis, neurological disorders and inflammatory disorders [7–9].

In addition to the damage to cellular components, ROS can also promote the degradation of oils and fats present in foods, leading to the appearance of odors and rancid flavor, which contributes to decrease the quality and nutritional security, in view of the formation of potentially toxic secondary metabolites [8,10].

Dietary antioxidants are added to food to slow down the oxidative deterioration [8] and widely used in the prevention of chronic diseases [11]. Nevertheless, consumers are demanding new alternative natural antioxidants obtained from plant sources to replace the synthetic antioxidants currently used, such as butylhydroxyanisole (BHA), butylhydroxytoluene and tert butylhydroquinone, which could be toxic and exert carcinogenic effects [12].

During the life cycle of seaweeds, they are exposed to high concentrations of oxygen and light intensity, and this combination favors the formation of free radicals and other oxidizing agents. This suggests that the absence of oxidative damage to structural components of algae and their stability against adverse conditions is related to the presence of antioxidants [6,13,14].

The Brazilian coast has an extensive coastline with great biodiversity of marine organisms yet to be explored. Red seaweeds *Pterocladiella capillacea* (*P. capillacea*) and *Osmundaria obtusiloba* (*O. obtusiloba*) are abundant in the coast of the state of Ceará throughout the year. Although there are several studies on biological activity of extracts of seaweeds in recent decades, there is no information on the antioxidant, antimicrobial and bacterial agglutination potential of these species of the coast of the state of Ceará.

Considering the above, the purpose of this study was to evaluate the antioxidant, antimicrobial and bacterial agglutination activity of the hexane (Hex) and 70% ethanol (70% EtOH) extracts of the red seaweeds *P. capillacea* and *O. obtusiloba*.

2. Material and methods

2.1. Collection of seaweeds and species identification

Specimens of the red seaweed *P. capillacea* (S. G. Gmelin) Santelices & Hommersand were collected in March 2008, at Pacheco Beach, municipality of Caucaia, State of Ceará. Individuals of the red seaweed *O. obtusiloba* (C. Agardh) R. E. Norris were collected in September 2010, at Paracuru Beach, São Gonçalo do Amarante, State of Ceará. Samples were taken under low tide conditions, with the permission of the Brazilian Institute of Environment and Renewable Natural Resources (SISBIO 33913-1).

The collected algae were taken to the laboratory. Washed with distilled water to remove impurities and macroscopic epiphytes and then placed on absorbent paper to remove excess water and frozen at −24 °C until analysis.

Species were identified by Professor Kelma Maria dos Santos Pires-Cavalcante of the Department of Fisheries Engineering, Federal University of Ceará. The voucher specimens of *P. capillacea* and *O. obtusiloba* were deposited in the Herbarium Prisco Bezerra, Department of Biology at the same university, under the numbers 447310 and 56432, respectively.

2.2. Preparation of extracts

Fresh algae were dried in a recirculating air oven at 40 °C for 15 h, and then ground. Portions of dehydrated *P. capillacea* (134 g) and *O. obtusiloba* (120 g) were subjected, separately, to exhaustive extraction with cold hexane and then with 70% EtOH. The Hex and 70% EtOH extracts were concentrated in rotary evaporator.

2.3. Quantification of total phenolic content (TPC)

TPC was estimated by the Folin–Ciocalteu method described by Kumar *et al.* [15]. To 200 μL of algal extracts at 1 000 μg/mL were added 1.4 mL distilled water, 100 μL Folin–Ciocalteu reagent and 300 μL sodium carbonate (20%). After 30 min incubation at room temperature in the dark, absorbance was read at 760 nm in a microplate reader (Biochrom Asys UVM 340).

TPC was calculated based on the standard curve of gallic acid at a concentration range of (0.005–0.500) mg/mL, and the results are expressed in milligrams (mg) of gallic acid equivalent/g extract.

2.4. DPPH radical scavenging capacity

DPPH radical scavenging capacity was determined according to Blois [16]. The sample consisted of a mixture of 0.5 mL aliquot of algal extracts at different concentrations (7.81–1 000 μg/mL) and 2.5 mL methanol solution of DPPH at 75 μM. In the blank sample, the methanol solution of DPPH was replaced with methanol, and in the control, were used only 3 mL methanol solution of DPPH at 75 μM. The tubes (sample, blank sample and control) were incubated at room temperature in the dark for 30 min and the absorbance was read at 517 nm in a microplate reader (Biochrom Asys UVM 340). Ascorbic acid was used as positive control at the same concentrations of algal extracts. The DPPH radical scavenging percentage was calculated according to Eq. (1).

$$\text{DPPH radical scavenging capacity } (\%) = \left[1 - \frac{(Abs_{sample} - Abs_{blank})}{Abs_{control}}\right] \times 100\% \quad (1)$$

2.5. Ferrous ion chelating ability (FIC)

The FIC was determined according to the method of Wang *et al.* [17]. The sample consisted of 1 mL of algal extracts at different concentrations (7.81–1 000 μg/L), 1.35 mL distilled water, 50 μL ferrous chloride (2 mM) and 100 μL ferrozine (5 mM). In the blank sample, 100 μL distilled water replaced ferrozine, while in the control, 1 mL water was used instead of the algal extract. Sample, blank sample and control were incubated at room temperature for 10 min, and the absorbance was read at 562 nm in a microplate reader (Biochrom Asys UVM 340). The ethylenediaminetetraacetic acid was used as a positive control at the same concentrations of algal extracts. The percentage of ferrous ion chelating ability was calculated according to the Eq. (2).

$$\text{Ferrous ion chelating ability } (\%) = \frac{\left[\text{Abs}_{\text{control}} - \left(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}\right)\right]}{\text{Abs}_{\text{control}}} \times 100\% \quad (2)$$

2.6. Ferric ion reducing power (FRAP)

FRAP was determined by the method described by Ganesan *et al.* [18]. To 1 mL algal extracts at different concentrations (7.81–1 000 μg/L) were added 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). This mixture was incubated at 50 °C for 20 min, cooled in ice-water and then added of 2.5 mL trichloroacetic acid (10%). After stirring, 2.5 mL were taken and mixed with 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%). After 10 min incubation at room temperature, the absorbance was read at 700 nm in a microplate reader (Biochrom Asys UVM 340). The BHA was used as a positive control at the same concentrations of algal extracts. The increase in absorbance indicated increase of FRAP, that is, the higher the absorbance, the greater the FRAP.

2.7. Degradation of β-carotene (BCB)

Degradation of β-carotene was determined by the method of Chew *et al.* [19], with minor modifications. There were added 2.5 mg β-carotene and 40 mg linoleic acid, both solubilized in chloroform, to 400 mg Tween 40 emulsifier. In a rotary evaporator, the chloroform was evaporated and 100 mL oxygen-saturated ultrapure water (Milli-Q) was added. Then, the mixture was vigorously stirred to form an emulsion; 3 mL of this emulsion was mixed with 1 mL algal extracts at different concentrations (7.81–1 000 μg/mL) and the initial absorbance was read at 470 nm. The tubes were incubated at 50 °C for 3 h and, after that, the absorbance was read again at the same wavelength; the two readings were made in a microplate reader (Biochrom Asys UVM 340). The BHA was used as positive control at the same concentrations of algal extracts. The antioxidant activity was calculated according to Eq. (3).

$$\text{Antioxidant activity } (\%) = \left(\frac{\text{Abs}_{3\text{h}}}{\text{Abs}_{\text{initial}}}\right) \times 100\% \quad (3)$$

2.8. Antibacterial activity

The antibacterial activity of algal extracts was determined by antibiogram test using the well diffusion method, following recommendations of the Clinical and Laboratory Standard Institute [20]. In the tests, there were used standard strains of *Escherichia coli* (*E. coli*) ATCC 25922, *Staphylococcus aureus* (*S. aureus*) ATCC 25923 e *Pseudomonas aeruginosa* ATCC 27853. The inocula were prepared respectively in test tubes containing 0.85% NaCl saline solution and turbidity adjusted according to 0.5 McFarland scale in a spectrophotometer. Inocula were uniformly distributed on the surface of Petri dishes containing Muller-Hinton agar (Difco) with sterile swabs, and the wells were drilled after absorption of the inoculum in the culture medium. The algal extracts were grouped according to the solvent used for preparation and the diluents was used as a negative control. Each well received 50 μL algal extract at 1 000 μg/mL and, then, the plates were incubated at 35 °C for 24 h. The procedure was performed in duplicate.

2.9. Bacterial agglutination assay

The ability of algal extracts to agglutinate bacterial cells was tested according to the method described by Imamichi and Yokoyama [21], using as indicator strains *E. coli* (ATCC 25922), multidrug-resistant *E. coli* (Lamap collection) *S. aureus* (ATCC 25923), multidrug-resistant *Salmonella* Infante (Lamap collection) and *Vibrio harveyi* (*V. harveyi*) (Lamap collection). Bacterial cultures were grown at 35 °C for 24 h and collected by centrifugation at 1 00 ×*g* for 10 min. Cells were washed three times with saline phosphate buffer pH 7.2 and fixed in the same buffer added with 4% formaldehyde and maintained at 4 °C for 16 h. Once fixed, the bacteria were washed again with Tris–HCl buffer pH 7.5 and diluted to obtain at 600 nm an optical density corresponding to 0.5 McFarland scale. The bacterial inoculum was mixed with an equal volume (50 μL) of algal extract (1 000 μg/mL) on a slide and kept at room temperature for 2 h. The negative control was done using the bacterial suspension in distilled water. Agglutination was analyzed by optical microscopy (Olympus CBB).

2.10. Statistical analysis

Results of CFT of extracts of the seaweed species *P. capillacea* and *O. obtusiloba* were subjected to one-way analysis of variance followed by Tukey's test ($P < 0.05$).

To investigate the correlation between CFT and antioxidant activities of algal extracts, we used the Pearson correlation coefficient, where the CFT was considered the independent variable (x) and each *in vitro* antioxidant methodology, the dependent variable (y).

3. Results

3.1. TPC

Quantitation of TPC in the Hex and 70% EtOH extracts in the two species of red seaweeds *P. capillacea* and *O. obtusiloba* was based on the standard curve of gallic acid. The correlation (r = 0.9992, $P < 0.05$) between the absorbance (760 nm) and concentrations, varying in the range of (0.005–0.500) mg/mL, enabled its quantification (y = 0.0755 + 4.896x, n = 8). TPC values of 70% EtOH extracts of *P. capillacea* and *O. obtusiloba* were 1.48 and 2.55 higher than the Hex extracts, respectively. Extracts of *O. obtusiloba* showed CFT values significantly higher than *P. capillacea* (Figure 1).

3.2. DPPH radical scavenging capacity

The DPPH radical scavenging capacity by Hex and 70% EtOH extracts of the algae *P. capillacea* and *O. obtusiloba* was evaluated at different concentrations of the extracts and the results are illustrated in Figure 2. The 70% EtOH extract of *O. obtusiloba* (99.47% ± 0.22%) exhibited a strong DPPH activity, followed by the Hex extract of *O. obtusiloba* (43.46% ± 0.19%), 70% EtOH of *P. capillacea* (40.81% ± 0.60%), Hex of *P. capillacea* (33.04% ± 2.08%) at a concentration of 1 000 μg/mL. Aside from the 70% EtOH extracts of *O. obtusiloba* at concentrations of 500 μg/mL and 1 000 μg/mL, no other showed activity similar to ascorbic acid.

3.3. FIC

The results of FIC indicated that the 70% EtOH (54.70% ± 0.60%) and Hex (52.27% ± 1.01%) extracts of *P. capillacea* exhibited the best activities, followed by Hex (33.00% ± 0.24%) and 70% EtOH (27.70% ± 1.30%) extracts of *O. obtusiloba* at a concentration of 1 000 μg/mL. None of these extracts showed activity similar to ethylenediaminetetraacetic acid (Figure 3).

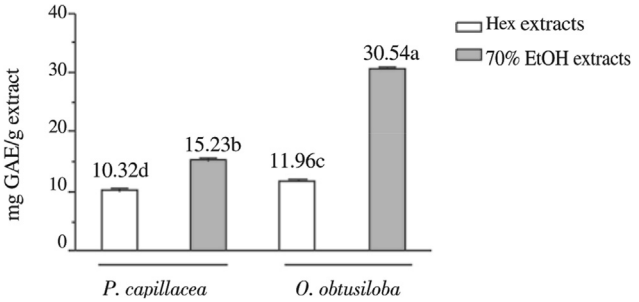

Figure 1. Total phenolic content of the Hex and 70% EtOH extracts of *P. capillacea* and *O. obtusiloba*.
Different letters indicate statistically significant differences (Tukey's test, $P < 0.05$). GAE: gallic acid equivalent.

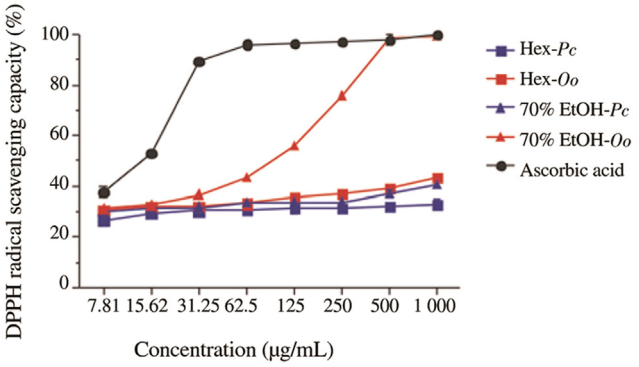

Figure 2. DPPH radical scavenging capacity of the Hex and 70% EtOH extracts of *P. capillacea* (*Pc*) and *O. obtusiloba* (*Oo*).

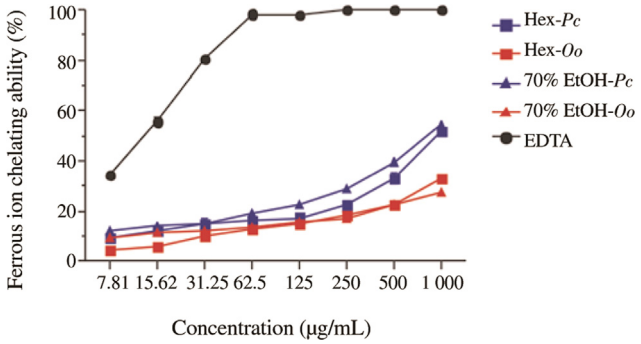

Figure 3. Ferrous ion chelating ability of the Hex and 70% EtOH extracts of *P. capillacea* (*Pc*) and *O. obtusiloba* (*Oo*).

3.4. FRAP

Increase in absorbance was found as the concentration of the extract increased from 7.81 to 1 000 μg/mL. Variations in optical density followed the decreasing order, as shown in Figure 4: BHA (0.158–2.375), 70% EtOH of *O. obtusiloba* (0.078–0.180), Hex of *P. capillacea* (0.109–0.167), 70% EtOH of *P. capillacea* (0.083–0.136) and Hex of *O. obtusiloba* (0.054–0.101).

3.5. BCB

BCB in the Hex and 70% EtOH extracts of the species *P. capillacea* and *O. obtusiloba* was examined at different concentrations, and the results are depicted in Figure 5. The results indicated that 70% EtOH extract of *O. obtusiloba*

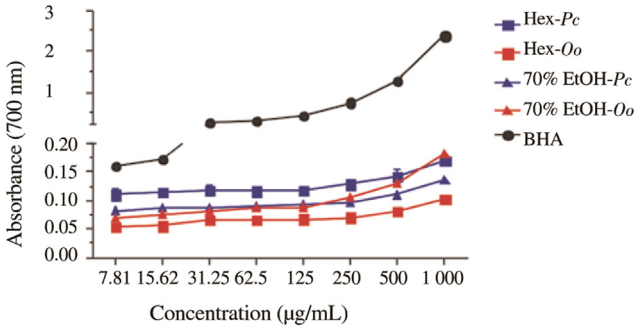

Figure 4. Ferric ion reducing power of the Hex and 70% EtOH extracts of *P. capillacea* (*Pc*) and *O. obtusiloba* (*Oo*).

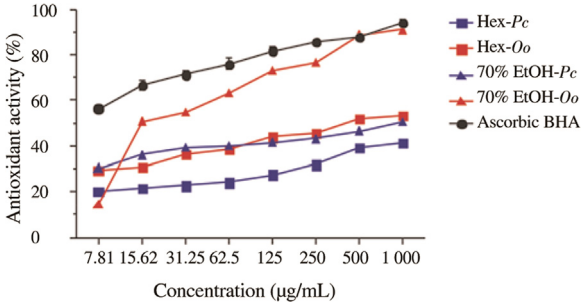

Figure 5. Degradation of β-carotene of the Hex and 70% EtOH extracts of *P. capillacea* (*Pc*) and *O. obtusiloba* (*Oo*).

(91.02% ± 0.15%) showed a strong BCB activity followed by the Hex extract of the same species (52.81% ± 0.49%), 70% EtOH of *P. capillacea* (50.61% ± 0.13%) and Hex of *P. capillacea* (41.33% ± 0.32%) at a concentration of 1 000 μg/mL. Except for the 70% EtOH extract of *O. obtusiloba* at concentrations of 500 and 1 000 μg/mL, no other showed activity similar to that of BHA.

3.6. Antibacterial activity

The Hex and 70% EtOH extracts of the marine algae *P. capillacea* and *O. obtusiloba* at a concentration of 1 000 μg/mL showed no antibacterial activity against standard strains of *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853.

3.7. Bacterial agglutination assay

The Hex and 70% EtOH extracts of the red seaweeds *P. capillacea* and *O. obtusiloba* were able to agglutinate bacterial Gram positive cells of *S. aureus* and Gram negative cells of *E. coli*, multiresistant *Salmonella* and *V. harveyi*, except the multiresistant strains of *E. coli* (Figure 6).

4. Discussion

Quantitation of TPC in extracts of the seaweeds *P. capillacea* and *O. obtusiloba*, measured by the spectrophotometric method of Folin–Ciocalteu, is based on the redox reaction of phenolic compounds present in algal extracts with metal ions. In this method, in alkaline medium, phenols reduce phosphomolybdate phosphotungstate, yellow in color, to molybdenum, of blue color [22,23].

The 70% EtOH was the most effective solvent for extracting phenolic compounds from red seaweeds. This because polar solvents efficiently extract a series of polar compounds, such as polyphenols bound to sugars or proteins, phlorotannins, saponins, glycosides and organic acids [24,25]. Moreover, hexane will extract metabolites of low polarity like hydrocarbons, fatty acids, acetogenins, terpenes halogenated or not, carotenoids and tocopherols [26,27].

Variations in the CFT of marine macroalgae may be influenced by extrinsic factors, including herbivory pressure, irradiance, depth, salinity and nutrients; by intrinsic factors, such as, seaweed morphology, age and reproductive stage, but also by the type of solvent used in the extraction of phenolic compounds [19,28,29].

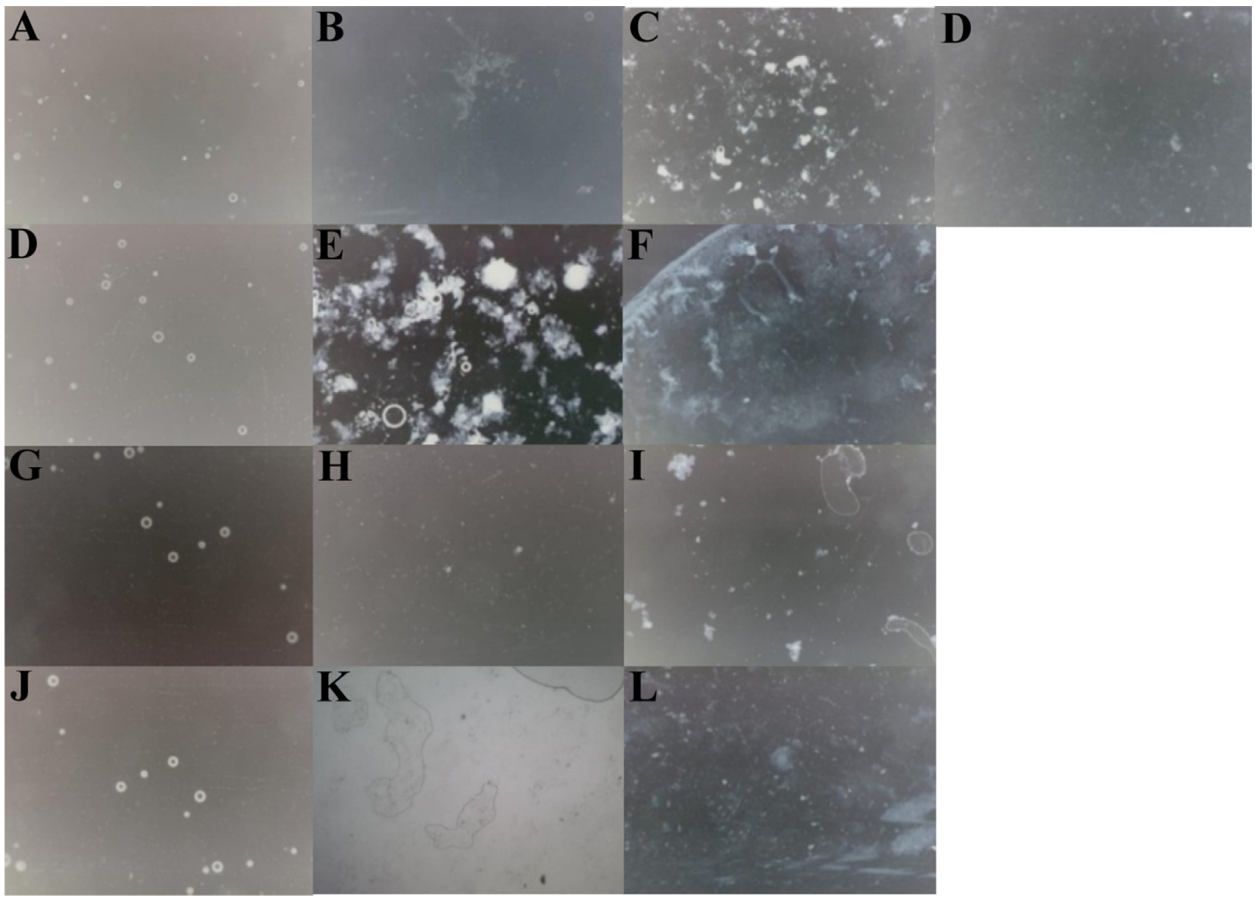

Figure 6. Bacterial agglutination prometed by the Hex and 70% EtOH extracts of *P. capillacea* (*Pc*) and *O. obtusiloba* (*Oo*).
(A) *E. coli* negative control; (B) *E. coli* incubated with Hex-*Pc*; (C) *E. coli* incubated with Hex-*Oo*; (D) *E. coli* incubated with 70% EtOH-*Oo*; (E) *S. aureus* negative control; (F) *S. aureus* incubated with Hex-*Oo*; (G) *S. aureus* incubated with 70% EtOH-*Oo*; (H) multidrug-resistant *Salmonella* negative control; (I) multidrug-resistant *Salmonella* incubated with Hex-*Pc*; (J) multidrug-resistant *Salmonella* incubated with Hex-*Oo*; (K) *V. harveyi* negative control; (L) *V. harveyi* incubated with Hex-*Pc*; (M) *V. harveyi* incubated with 70% EtOH-*Oo*.

The DPPH radical scavenging method, among colorimetric antioxidant assays, has long been used in the search for new natural antioxidant compounds because of radical stability, reproducibility, simplicity, speed and ease of use [7,8,30,31].

The DPPH activity of (50%) dichloromethane: methanol extracts of *P. capillacea* e *O. obtusiloba*, collected in the State of Santa Catarina, at 1 000 μg/mL, were 57.51% and 30.59% [32]. In this study, considering the same concentration, the activities of the extracts (70% EtOH and Hex) of *P. capillacea* were lower, but those of *O. obtusiloba*, showed activities up to three times higher.

For the Hex extract, there was a negative correlation between the CFT and DPPH radical scavenging capacity ($r = -0.7154$; $P = 0.0200$), *i.e*, the phenolic compounds were not the major responsible for DPPH activity, but apolar compounds like glycolipids, phospholipids, steroids, terpenes, fatty acids, carotenoids and tocopherols, which are present in these extracts [3–5,32,33].

Unlike the Hex extract, 70% EtOH extract demonstrated a strong correlation between the CFT and the DPPH radical scavenging capacity ($r = 0.8817$, $P = 0.0007$). Red seaweeds, especially the Order Ceramiales, which includes *O. obtusiloba*, are the main producers of secondary metabolites; the main characteristic of these algae is their great ability to synthesize phenolic compounds and derivatives, as sulfated or non-sulfated bromophenols, which have a relevant role in protecting tissues exposed to oxidative stress, against different diseases including cancer, diabetes, cardiovascular and neurodegenerative diseases [34–36].

Souza *et al.* [14] also reported a correlation between the CFT and the DPPH scavenging ($r = 0.9590$) for methanol and ethanol extracts of two red seaweed species, *Gracilaria birdiae* and *Gracilaria cornea* of the coast of the State of Ceará.

The ability to chelate metals is very important, since the transition metals catalyze the peroxidation of lipids forming undesirable compounds that attack several types of molecules [15]. The binding of antioxidant compounds with metal ions can be assessed by the ferrous ion chelating assay. An extract with high binding ability with metal ions can prevent or inhibit various reactions capable of producing reactive hydroxyl radicals [19].

Hex and 70% EtOH extracts of the algae studied presented moderate ($r = 0.5976$; $P = 0.0680$) and good ($r = 0.7146$; $P = 0.0202$) correlation between the TPC and FIC, respectively. According to Wang *et al.* [17], phenolic compounds are not strong metal chelating agents. Other compounds, such as dietary fibers (agar, carrageenan, and alginate), present in algal extracts are also known for their ability to chelate metal and there is evidence of their inhibitory effects on the absorption of ferrous ion [37].

In the FRAP assay, antioxidant activity is determined based on the ability of antioxidant compounds, present in algal extracts, to reduce ferric iron (III) to ferrous iron (II) in a colorimetric redox reaction that simply involves the transfer of electrons [19]. Reducing agents present in solution promote the reduction of the Fe^{3+}/ferrocyanide complex to the ferrous form (Fe^{2+}), which can be measured at the absorbance of 700 nm [28]. The greater the absorbance of the mixture, the greater the antioxidant activity of iron reduction [38].

Extracts of *P. capillacea* and *O. obtusiloba* at a concentration of 1 000 μg/mL have presented low values, FRAP values were greater than those of 70% EtOH extracts of the Rhodophyta *Amansia multifida* and *Meristiella echinocarpa* [39]. Farvin and Jacobsen [40], in turn, observed high optical densities, from 0.2 to 0.4, in the ethanolic extract of the Rhodophyta *Palmaria palmata*, *Porphyra purpurea*, *Chondrus crispus* and *Mastocarpus stellatus*.

In Hex extracts of the two species of algae it was registered a low correlation between TPC and FRAP ($r = 0.3185$, $P = 0.3697$), suggesting that phenolic compounds cannot be the main ferric ion reducing agent. Conversely, the 70% EtOH extracts of both algal species showed a very high correlation ($r = 0.9421$; $P < 0.0001$), as previously reported in the literature [9,41].

Oxidation of unsaturated fatty acids present in biological membranes causes the formation of lipid free radicals and destruction of the lipid membrane. Antioxidants can stop the chain reaction by donating hydrogen atoms [42]. The BCB method is simple, sensitive and enables to evaluate the ability of a substance to neutralize the free radicals generated during the oxidation of linoleic acid, thereby preventing the degradation of β-carotene. Once the methodology does not employ high temperatures, it is possible to determine heat sensitive substances [19,43].

Antioxidant activities measured by BCB in the 50% dichloromethane: methanol extract at 1 000 μg/mL, of *P. capillacea* and *O. obtusiloba* collected in the State of Santa Catarina, were around 43% [32]. These results are lower than those for the activities of the extracts (70% EtOH and Hex) of *P. capillacea* and *O. obtusiloba* detected herein.

High correlations between CFT and BCB were verified for the Hex ($r = 0.9364$; $P < 0.0001$) and 70% EtOH ($r = 0.9988$; $P < 0.0001$) extracts of the red algae analyzed. This result was also reported by Souza *et al.* [14], but O' Sullivan *et al.* [8] failed to establish a correlation between these two variables.

Concerning antibacterial activity, El Kassis and Attia [44] found no antibacterial activity of the aqueous extract of *P. capillacea* collected in Abur Thursday, Egypt, compared to strains of *S. aureus*, *P. aeruginosa*, *E. coli* and *Bacillus subtilis*. Abou Zeid *et al.* [45] observed that the aqueous extract of the seaweed *P. capillacea* collected in the same region showed antibacterial activity against *Bacillus cereus*, *S. aureus*, *Streptococcus pyogen* and *Pseudomonas flouresens.*

Compounds isolated from other species of the genus *Osmundaria* exhibit antibacterial activity, as is the case of halogenated lactones, halogenated volatile metabolites and lanosol ethyl ether, isolated from *Osmundaria fimbriata*, *Osmundaria volubilis* and *Osmundaria serrata*, respectively [36].

This is the first report on the ability of secondary metabolites of the red seaweeds *P. capillacea* and *O. obtusiloba* to agglutinate Gram positive and Gram negative bacteria. Among the possible mechanisms of action of these bioactive molecules are damage to the plasma membrane, the inhibition of enzymes and microbial aggregation. It has been suggested that terpenes can promote disruption of the cytoplasmic membrane of the microorganisms, as well as tannins can interact with polysaccharides, inactivating enzymes [46–48].

In conclusion, the evaluation of antioxidant activity using five different methodologies evidenced that extracts of the red seaweeds *P. capillacea* and *O. obtusiloba* have antioxidant potential, except for the FRAP. Although the algal extracts have not presented antimicrobial activity, the possible interaction of these extracts with bacterial Gram positive cells of *S. aureus* and Gram negative cells of *E. coli*, multidrug-resistant *Salmonella* and *V. harveyi* was detected and reported for the first time in this work.

Conflict of interest statement

We declare that we have no conflict of interest.

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