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Antioxidant and anti-inflammatory activities of methanol extract and its fractions from the brown seaweed *Spatoglossum schroederi*

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Abstract In the present study, the brown seaweed *Spatoglossum schroederi* was submitted to extraction procedures in order to obtain methanol (MET) extract and its hexane (HEX) and chloroform (CLR) fractions. All samples were evaluated for total phenolic and flavonoid contents as well as antioxidant and anti-inflammatory properties. The HEX fraction was the richest in total phenolic content, while CLR in total flavonoid content. MET, HEX, and CLR exhibited potent dose-dependent antioxidant activity by the total antioxidant capacity (TOAC) and reducing power methods and linear regression evidenced that the antioxidant activity of *S. schroederi* is due mainly to the flavonoid content. Following, MET extract and its fractions were evaluated for anti-inflammatory activity. Algae samples inhibited significantly the paw edema induced by carrageenan or dextran, myeloperoxidase activity, neutrophil migration induced by carrageenan, IL-1β concentration, and increase on IL-10 level in the peritoneal fluid of animals. Our results suggest that antioxidative and anti-inflammatory present in the brown seaweed *S. schroederi* may be related to the presence of flavonoids.

Keywords Flavonoid content · TOAC · Paw edema · Peritonitis

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Introduction

Reactive oxygen species (ROS) and reactive nitrogen species may play important cell roles, such as mediators of signaling processes and immune responses (Dröge 2002). However, when uncontrolled levels of free radicals are produced in cells, it results in oxidative stress which may provoke molecular damages (Aher et al. 2011). Several diseases are associated with oxidative stress, such as hypertension, cancer, and inflammatory processes (Ratnam et al. 2006; Minelli et al. 2009). The use of antioxidant compounds, such as polyphenols, carotenoids, and vitamins, may reduce the risk of diseases and increase life-span (Goldberg & Katz 2007; Dutot et al. 2012). Moreover, many studies have suggested an inverse correlation between flavonoid consumption and inflammatory diseases (Nijveldt et al. 2001).

Inflammation is a complex process that involves cellular and humoral events and tissue recovery (Aller et al. 2006). The process is displayed by inflammatory stimuli that induce cytokine production followed by vasodilatation, leukocyte recruitment, and free radicals production (Klebanoff 2005). High levels of ROS production may locally damage tissues, prolonging the inflammation (Inoue et al. 2003; Valko et al. 2007; Nguemfo et al. 2009). Taking into consideration the involvement of ROS in inflammatory process, antioxidant compounds could present potential anti-inflammatory effect.

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Recent studies have shown that algae are promising organisms to provide new bioactive molecules, such as antioxidants (Terracciano et al. 2006; Cardozo et al. 2007; Holdt & Kraan 2011). Since seaweeds live in oxygen- and light-rich environments and no damage is observed in their cellular components, they can be good sources of antioxidant agents (Zubia et al. 2007; Pandithurai & Murugesan 2014a; Rengasamy et al. 2014).

The brown seaweed *Spatoglossum schroederi* (C.Agardh) Kützing is mainly found in tropical seas. This alga has been reported to possess antithrombotic effect and activity against HIV (Leite et al. 1998; Rocha et al. 2005; Queiroz et al. 2008). These activities have been attributed to sulphated polysaccharides fraction, and few studies have been performed with organic extracts obtained from the algae. In our study, *S. schroederi* was submitted to extraction and fractionation processes with organic solvents, evaluated for the phenolic and flavonoid contents and then investigated for its antioxidant and anti-inflammatory potential.

Materials and methods

Preparation of methanol extract and its fractions

Spatoglossum schroederi was collected from Pacheco Beach, State of Ceará, Brazil, from December 2011 to January 2012. The seaweed was washed thoroughly with fresh water to remove epiphytes, dried overnight at room temperature, and then milled with liquid nitrogen. Methanol extract and its fractions were obtained according to Cho et al. 2010 with some modifications. The milled seaweed was extracted three times with 100 % methanol (1:20 ratio) under shaking at room temperature for 24 h. Then the extract was centrifuged at 10, 000 rpm for 10 min at 25 °C. The supernatant was collected and concentrated to 100 mL in a rotary evaporator (maximum temperature 40 °C). Forty milliliter was separated and dried to obtain methanol extract (MET), and 60 mL was dried, dissolved in distilled water, and then partitioned sequentially with hexane (HEX), chloroform (CLR), and ethyl acetate. The resulting solvent fractions were evaporated until dry to give the HEX, CLR, ACE, and aqueous fractions. MET extract and each solvent fraction weights were estimated gravimetrically. Only the MET extract and HEX and CLR fractions were used in our study because recoveries of ethyl acetate and aqueous fraction were low. Samples were stored in the dark at –20 °C until use.

Total phenolic content

The total phenolic content was determined according to López et al. (2011). MET, HEX, or CLR (100 μL) was added to tubes containing 8.4 mL, followed by addition of 0.5 mL of Folin–Ciocalteu reagent and 1 mL of 20 % sodium carbonate. Tubes were shaken and let stand for 1 h at 25 °C in dim light. Measurements were performed at 765 nm. A standard curve was prepared with gallic acid, and results were given as milligram of gallic acid equivalents (GAE) per gram of seaweed sample.

Total flavonoid content

The total flavonoid content was determined according to Cox et al. 2010. Aliquots (250 μL) of MET, HEX, or CLR were added to 1.25 mL of distilled water and 75 μL of 5 % $NaNO_2$ solution. After 6 min, 150 μL of 10 % $AlCl_3.H_2O$ solution was added. Five minutes later, 0.5 mL of 1 M NaOH solution was added and then total volume was filled up to 2.5 mL with distilled water. Absorbance was determined at 510 nm. Quercetin was used to prepare the standard curve and results were given as milligram of Quercetin equivalents (QE) per gram of seaweed sample.

Antioxidant activities

For antioxidant assays samples at 0.5, 1.0, 1.5, and 2.0 mg mL^{-1} of MET extract, HEX or CLR fractions, dissolved in methanol, were prepared. All antioxidant experiments were performed in triplicate.

Total antioxidant capacity The total antioxidant capacity (TAOC) was developed according to Sun et al. (2011) with some modifications. Aliquots (0.15 mL) of MET, HEX, or CLR were mixed with 1.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Tubes were incubated at 95 °C for 90 min in darkness. Following, relational mixtures were cooled to 25 °C, and absorbance was measured at 695 nm. Ascorbic acid was used to generate a standard curve. Total antioxidant capacity was given as milligram of ascorbic acid equivalent antioxidant capacity (AscAEAC) per gram of seaweed sample. BHT 0.1 mg mL^{-1} was used as positive control.

Reducing power Reducing power was evaluated according to Zubia et al. (2007) with some modifications. This assay measured total antioxidant capacity of an extract evaluating the redox potential of its compounds. MET extract or HEX or CLR fractions (0.5 mL each) were mixed with 1.25 mL phosphate buffer (0.2 M, pH 6.6) and 1.25 mL potassium ferricyanide ($K_3Fe(CN)_6$; 1 %) for 20 min, at 50 °C. Following, tubes were cooled and mixed with 1.25 mL trichloroacetic acid (10 %), and 1.25 mL of mixture was transferred to tubes containing 1.25 mL distilled water and 0.250 mL $FeCl_3.6H_2O$ (0.1 %). The mixture was left for 10 min at 25 °C, and absorbances were measured at 700 nm. BHT 0.1 mg mL^{-1} was used as positive control.

DPPH radical scavenging activity 2,2-Diphenylpicrylhydrazyl (DPPH) assay was based on Cho et al. (2010) with modifications. An aliquot of MET, HEX, or CLR (0.75 mL) was mixed with 0.75 mL of 0.1 mM DPPH followed by incubation for 30 min. Absorbance was read at 515 nm. The percentage of scavenged DPPH was calculated using the following equation: DPPH scavenging activity (%)=$([A_c-A_s]/A_c)\times 100$. Where A_c is the control absorbance (0.75 mL of methanol with 0.75 mL of DPPH solution) and A_s is the sample absorbance. BHT 0.1 mg mL^{-1} was used as positive control.

Animal assays

Swiss mice weighing 20–25 g were housed in temperature-controlled rooms and received water and food ad libitum until use. Experiments were according to ethics and biosecurity guidelines and approved by the Animal Research and Care Ethics Committee of the Federal University of Ceará.

Paw edema induced by carrageenan and dextran Mice paw edema was induced by carrageenan (Cg, 500 μg paw^{-1}) or (Dxt, 500 μg paw^{-1}), both of which were prepared in saline. A volume of 0.1 mL was injected via the subplantar route into the right hind paw of the animal. The paw volume was measured immediately before the irritant injection and at selected time intervals thereafter (at 1, 2, 3, and 4 h for Cg and at 30 min as well as 1, 2, and 3 h for Dxt) using a hydroplethysmometer. The *S. schroederi* samples (MET, CLR, and HEX) were dissolved in 0.1 mL 10 % Tween 80 in saline (100 mg kg^{-1}) and injected intraperitoneally (i.p.) 1 h before the injection of carrageenan or dextran. In these experiments, the control group received 10 % Tween 80 in saline (i.p.). Indomethacin (INDO, 10 mg kg^{-1}, i.p.) dissolved in 10 % Tween 80 in saline was injected 30 min before the inflammatory stimulus as a positive control for paw edema inhibition. The results are expressed as the increase in paw volume (mL), which was calculated by subtracting the basal volume.

Determination of myeloperoxidase activity Myeloperoxidase (MPO) activity was measured in the paw of animals injected with carrageenan into the plantar surface and pre-treated with MET extract and CLR and HEX fractions. After 4 h of inflammatory stimulus administration, 50–100 mg of the tissue was harvested by incision of the surface of the right hind paws and MPO activity was determined (Bradley et al. 1982).

Cell migration into peritoneal cavity Carrageenan (500 μg) was injected intraperitoneally in 250 μL of sterile saline. Four hours later, mice were sacrificed by cervical dislocation under anesthesia (Ketamine 80 mg kg^{-1} plus xylazine 10 mg kg^{-1}; intramuscular injection) and the peritoneal cavity was washed with 1.5 mL of heparinized phosphate buffered saline (PBS) to harvest peritoneal fluid contained in cells. Total cell counts and differential cell counts were performed as described previously (Souza & Ferreira 1985). MET, CLR, and HEX (100 mg kg^{-1}), dissolved in 0.1 mL 10 % Tween 80 in saline were separately injected via intraperitoneal route 1 h before injection of carrageenan. Control groups received only sterile saline or indomethacin (INDO: 10 mg kg^{-1}; i.p.). Results are presented as the number of leucocytes or neutrophils per milliliter of peritoneal exudates.

Cytokines IL-1β and IL-10 measurements After the peritonitis assay, samples of peritoneal fluid were collected and the levels of and IL-1β and IL-10 were evaluated using sandwich Enzyme-linked immunosorbent assay (ELISA) according to the supplier's protocol. The results are expressed as picograms (pg mL^{-1}) of each cytokine per peritoneal cavity washed.

Statistical analysis

Antioxidant activity results were given as mean and standard deviation (mean±SD). Statistical analysis was calculated by Tukey's test ($P<0.05$). Linear regression was performed to indicate the relationship between phenolic or flavonoid contents and antioxidant activity results. For anti-inflammatory assays, the statistical analysis was performed through ANOVA followed Bonferroni's post-test ($P<0.05$). Data were analyzed using GraphPad Prism 5 software.

Results

Table 1 shows extraction yield, total phenolic, and total flavonoid contents of methanol extract and its hexane and chloroform fractions obtained from *S. schroederi*. The yield of extractable components was expressed as % (*w*/*w*) of dried seaweed or total methanol extract for MET extract and HEX and CLR fractions. Total phenolic content and total flavonoid content were determined from the calibration curves of gallic acid and quercetin, respectively. The HEX fraction was the richest in total phenolics ($P<0.05$). No statistical differences were observed in total flavonoid content between HEX and CLR fractions ($P>0.05$).

Figure 1 shows TAOC results for *S. schroederi*. This figure shows that the total antioxidant capacity of MET, HEX, and CLR was dependent on the tested concentration. The TOAC increased significantly at 1.5 and 2.0 mg mL^{-1} compared to 0.5 and 1.0 mg mL^{-1} concentrations ($P<0.05$). Moreover, no significant difference was observed in total antioxidant capacity among MET, HEX, or CLR when the same concentration was tested. As expected, the BHT, a synthetic antioxidant used as positive control, had strong antioxidant activity in the TOAC assay.

Table 1 Extraction yield, total phenolic, and total flavonoid contents of methanol extract (MET) and hexane (HEX) and chloroform (CLR) fractions from *Spatoglossum schroederi*

Sample	Yield (%)[a]	Total phenolic content (mg GAE g^{-1})	Total flavonoid content (mg QE g^{-1})
MET	11.0	11.75±0.04a	215.39±10.76d
HEX	36.2	14.10±0.17b	278.79±29.12e
CLR	56.6	6.84±0.10c	279.63±8.91e

Data are mean±SD (n=3). The same letter do not differ significantly from each other by Tukey's test (P<0.05)

[a] Methanol extract yield was calculated as *w*/*w* of dry seaweed, and fractions yields were calculated as *w*/*w* total methanol extract

The reducing power of MET, HEX, and CLR was dose-dependent (Fig. 2). Moreover, except for the HEX fraction at the dose of 1.0 mg mL^{-1}, no significant differences were observed among reducing power of fractions when tested at 0.5, 1.5, and 2.0 mg mL^{-1} (P>0.05). BHT exhibited potent antioxidant activity.

The DPPH assay showed that the capacity of MET, HEX, and CLR to promote reduction of DPPH did not differ among tested concentration (Fig. 3). The MET extract exhibited the best performance, producing a DPPH scavenging activity near to 53 % at 1.5 and 2.0 mg mL^{-1} concentration.

As shown in Fig. 4a, c, a marginal correlation was observed between the antioxidant activities and total phenolic content (R^2<0.75) by TOAC and reducing power methods. However, the correlation coefficient among *S. schroederi* samples and total flavonoid content was higher than 0.90 (R^2>0.90) (Fig. 4b, d). No correlation was observed for total phenolic and flavonoid contents by DPPH radical scavenging activity (R^2<0.50). This indicates that the antioxidant activity of *S. schroederi* is due mainly to high flavonoid content in MET extract and its fractions.

Tables 2 and 3 show the effects of MET, HEX, and CLR on the paw edema induced by carrageenan or dextran, respectively. As expected, treatment with carrageenan (Cg) or dextran (Dxt) induced a significant increase in mice paw volume (P<0.05) and indomethacin reduced the paw edema induced by carrageenan (third hour=100 %; fourth hour=97 %) or dextran (first hour=68 %; second hour=56 %; third hour=89 %). A significant reduction of edema was observed in all groups treated with *S. schroederi* extracts prior to Cg or Dxt treatments, at all periods of evaluation (P<0.05). In both experiments, the HEX fraction exhibited the highest performance at peak of inflammatory responses. In the edema induced by carragennan, the HEX fraction promoted inhibition rates of 94 and 97 % at the third and fourth hours, respectively, when compared to the carrageenan group (Table 2). Moreover, it was observed that pre-treatment of animals with HEX promoted an inhibition rate of 93 % at the first hour, compared to the control group treated with dextran alone (Table 3). Furthermore, MET extract and HEX and CLR fractions were as efficacious as indomethacin (P>0.05), a commercial drug used as an anti-inflammatory agent.

The effect of MET extract and HEX and CLR fractions from *S. schroederi* on carrageenan-induced myeloperoxidase activity in mice paws tissue

Carrageenan produced a marked increase in MPO activity (65.00±4.30 U mg^{-1} of tissue) 4 h after inflammatory stimuli injection when compared to the saline group (4.32±1.09 U mg^{-1} of tissue), and this increase was reduced by

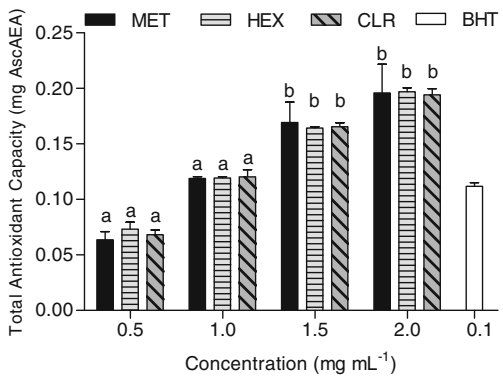


Fig. 1 Total antioxidant capacity (TAOC) of MET extract and HEX and CLR fractions from *S.schroederi*. Each *bar* represents the means±SD (n=3). *MET*: methanol extract; *HEX*: hexane fraction; *CLR*: chloroform fraction. *The same letters* do not differ significantly from each other by Tukey's test (P<0.05)

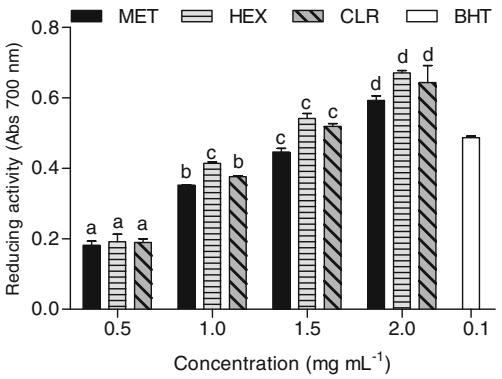


Fig. 2 Reducing power of MET extract and HEX and CLR fractions from *S. schroederi*. Each *bar* represents the means±SD (n=3). *MET*: methanol extract; *HEX*: hexane fraction; *CLR*: chloroform fraction. *The same letters* do not differ significantly from each other by Tukey's test (P<0.05)

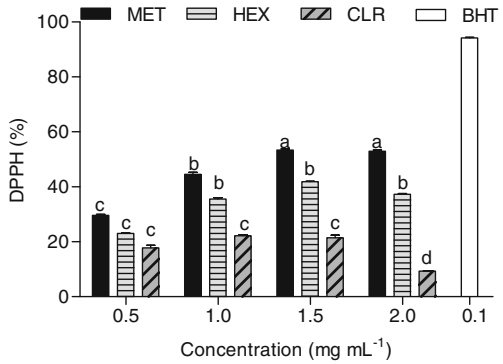


Fig. 3 DPPH radical scavenging activity of MET extract and HEX and CLR fractions from *S. schroederi*. Each *bar* represents the means±SD (n=3). *MET*: methanol extract; *HEX*: hexane fraction; *CLR*: chloroform fraction. *The same letters* do not differ significantly from each other by Tukey's test (P<0.05)

pre-treatment with MET (60.7 %), HEX (66.7 %), or CLR (86.6 %) or indomethacin (26.45±3.78 U mg^{-1} of tissue) (Fig. 5).

Carrageenan also induced an intense increase in the total leukocyte count of 4700±483×10^3 cells mL^{-1} (Fig. 6a) as well as neutrophil count (4129±372.0×10^3 cells mL^{-1}) when compared to the saline group (Fig. 6b). The administration of MET extract and its fractions 1 h before carrageenan injection significantly reduced this peritoneal leukocyte count with inhibition rates of 68.0 % (MET), 71.3 % (HEX), and 54.7 % (CLR). This inhibition was accompanied by intense reduction of neutrophil migration into the peritoneal cavity of mice (MET 71 %, HEX 59.9 %, and CLR 74.3 %). Animals administered only with indomethacin exhibited reduced leukocyte and neutrophil counts as shown in Fig. 6.

The administration of MET, HEX, and CLR decreased the IL-1β concentration (Fig. 7a), while HEX and CLR fractions

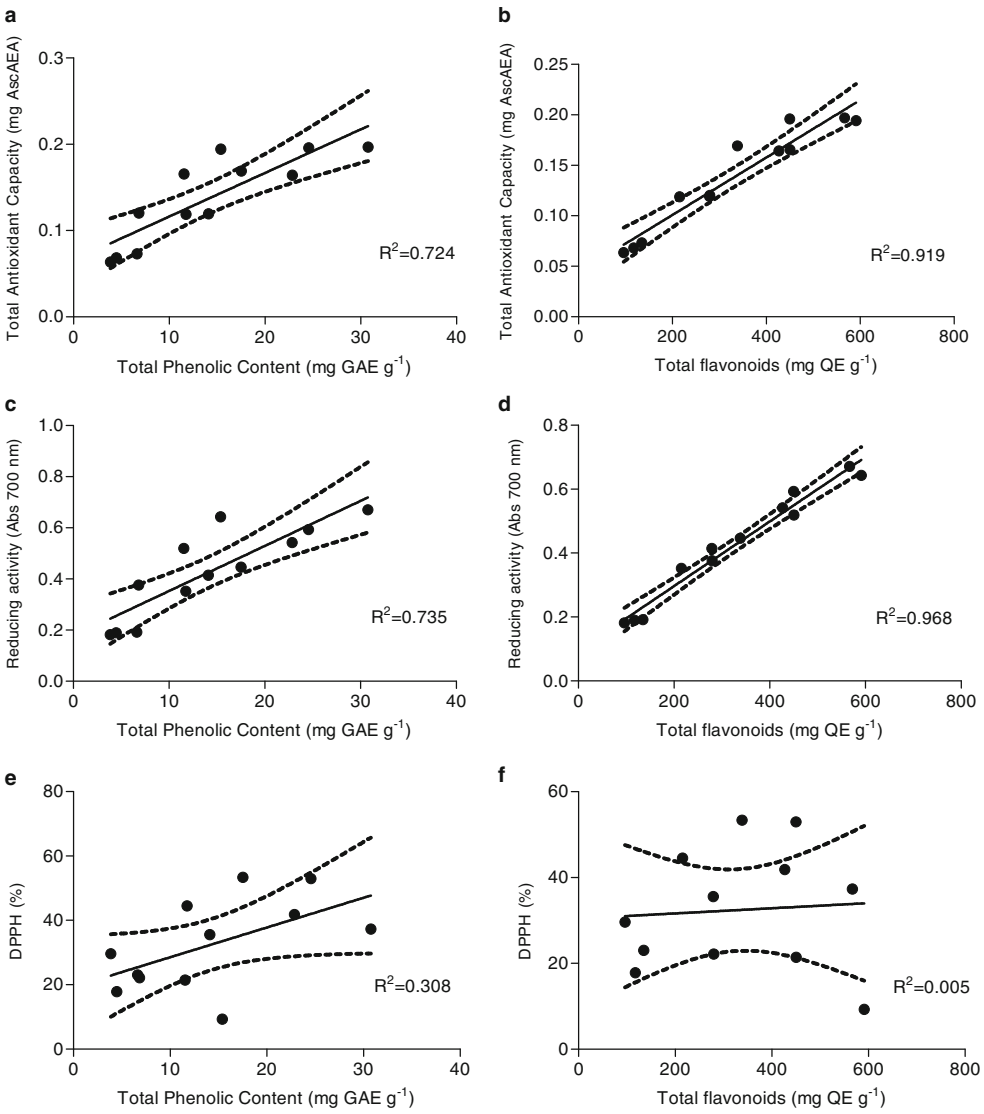


Fig. 4 Antioxidant activities of MET extract and HEX and CLR fractions from *S. schroederi* related to total phenolic (*on the left*) and total flavonoid (*on the right*) contents. Total antioxidant capacity (**a**, **b**), reducing power (**c**, **d**), and DPPH radical scavenging activity (**e**, **f**). Linear regression curves are represented by *solid lines* and 95 % confidence intervals are indicated by *dashed lines*

Table 2 Effect of MET extract and HEX and CLR fractions from *S. schroederi* on paw edema induced by carrageenan

Treatment	Paw edema in mL (inhibition rate in %)			
	1 h	2 h	3 h	4 h
Tween 80	0.027±0.008	0.015±0.009	0.012±0.009	0.000±0.000
Cg	0.057±0.010	0.065±0.009	0.080±0.009	0.095±0.003
MET	0.035±0.016 (39)	0.015±0.010* (77)	0.042±0.017 (47)	0.045±0.009* (53)
HEX	0.007±0.007* (87)	0.005±0.005* (92)	0.005±0.003* (94)	0.002±0.002* (97)
CLR	0.015±0.003 (74)	0.005±0.005* (92)	0.022±0.005* (72)	0.022±0.005* (76)
INDO	0.017±0.006 (70)	0.015±0.006* (77)	0.000±0.000* (100)	0.002±0.002* (97)

Values are given as the mean of five animals as analyzed by ANOVA followed by Bonferroni's post-test. Mice received a dose of 100 mg kg^{-1} ; i.p. of MET, HEX, or CLR 1 h before inflammatory stimuli

*Significant difference compared with Cg group ($P<0.05$)

increased the IL-10 level in the peritoneal fluid of the animals (Fig. 7b).

Discussion

Algae exhibit a diversity of potential activities such as antioxidant and anti-inflammatory activities, and this versatility is attributed to their bioactive compound content (Faulkner 2002; Liu et al. 2011). The presence of proteins, sulfated polysaccharides, carbohydrates, fatty acid, amino acids, sterols, phenols, and flavonoids are examples of identified molecules (Bhakuni & Rawat 2005; Kim & Ta 2011; Jimenez-Escrig et al. 2011; Kim & Li 2011; Gosch et al. 2014; Pandithurai & Murugesan 2014b). In the present work, the brown seaweed *S. schroederi* was evaluated for the presence of active molecules with biological properties.

To determinate the constituents of the *S. schroederi* MET extracts and its fractions, we measured the level of the total phenolics and total flavonoids in the samples. We verified that that the HEX fraction was the richest in total phenolics and the CLR fraction in total flavonoid content. The determination of total flavonoid content in algae is important due to their wide range of biological activities, such as antioxidant and anti-inflammatory activities (Machado et al. 2008). According these findings, we can infer that the samples of *S. schroederi* are rich in compounds with antioxidant and anti-inflammatory effects.

In order to evaluate the antioxidant properties of the methanol extract and its fractions, the total antioxidant capacity (TOAC), the reducing power, and the DPPH radical scavenging activity were measured. These methods are simple and widely used for the fast screening of sample antioxidant properties, and they provide quite reliable preliminary information on the presence of anti-oxidatively active constituents in the extracts (Krishnaiah et al. 2011). Our data showed that all samples had antioxidant activity. The TAOC reducing power and DPPH assay were dependent on the concentration used in each assay. The TAOC reflects the non-enzymatic antioxidant defense system capacity, that may be evaluated by phosphomolybdenum method, where molybdenum VI (Mo^{6+}) is reduced to form a green complex of phosphate/Mo^{5+} in acidic pH (Sun et al. 2011). According to our results,

Table 3 Effect of MET extract and HEX and CLR fractions from *S. schroederi* on paw edema induced by dextran

Treatment	Paw edema in mL (inhibition rate in %)			
	0.5 h	1 h	2 h	3 h
Tween 80	0.002±0.002	0.012±0.006	0.008±0.004	0.000±0.000
Dxt	0.096±0.010	0.070±0.009	0.022±0.005	0.022±0.002
MET	0.052±0.008* (45)	0.017±0.006* (75)	0.012±0.005 (44)	0.002±0.002* (89)
HEX	0.022±0.008* (77)	0.005±0.002* (93)	0.000±0.000* (100)	0.012±0.005 (44)
CLR	0.020±0.004* (79)	0.020±0.008* (71)	0.012±0.007 (44)	0.010±0.004 (56)
INDO	0.055±0.005* (43)	0.022±0.005* (68)	0.010±0.004* (56)	0.002±0.002* (89)

Values are given as the mean of five animals as analyzed by ANOVA followed by Bonferroni's post-test. Mice received a dose of 100 mg kg^{-1} ; i.p. of MET, HEX, or CLR 1 h before inflammatory stimuli

*Significant difference compared with Dxt group ($P<0.05$)

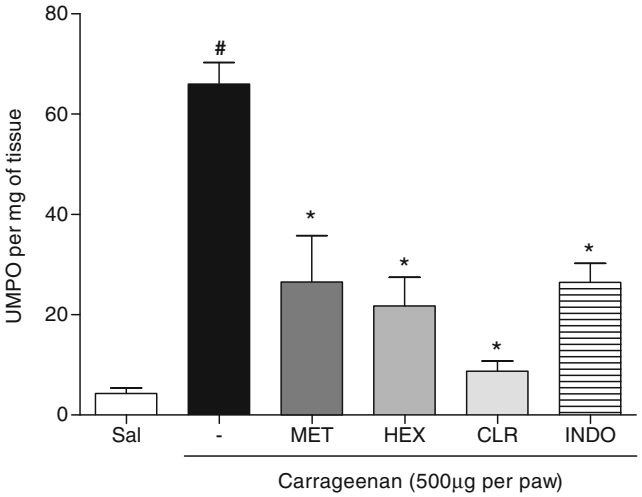


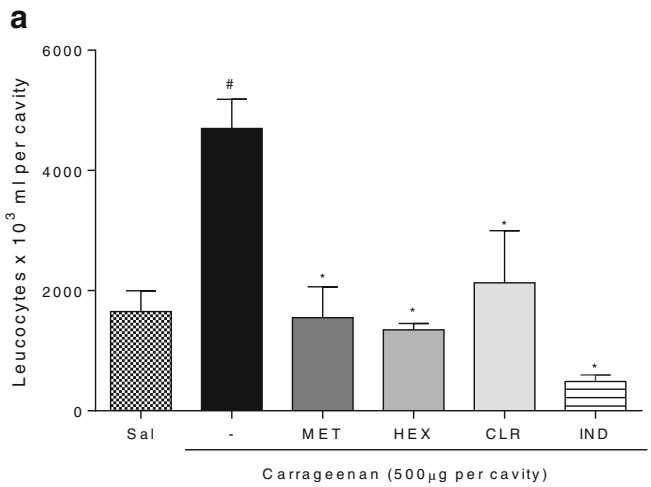
Fig. 5 The inhibitory effect of MET extract and HEX and CLR fractions from *S. schroederi* on carrageenan-induced myeloperoxidase activity in mice paws tissue. Animals received *S. schroederi* samples (100 mg kg^{-1}; i.p.) 1 h before the carrageenan administration (500 µg/paw), and 4 h later the myeloperoxidase activity was evaluated. The values are given as the mean±S.E.M. (n=5). Indomethacin (INDO: 10 mg kg^{-1}, i.p.) was used as a positive control. The *number sign* and *asterisk* indicate significant statistical difference (P<0.05) compared to saline and carrageenan, respectively (ANOVA followed by Bonferroni's post-test)

we infer that the total antioxidant capacity of MET, HEX, and CLR were dependent on the tested concentration.

The reducing power is a method widely used to evaluate the capacity of antioxidant compound for donating electrons. The electrons may react with intermediate oxidized compounds obtained from lipid peroxidation by scavenging free radicals (Aher et al. 2011). Our results show that the *S. schroederi* MET extract and its fractions have important antioxidant properties and that this effect probably is mediated by the flavonoid content.

Flavonoids may exert antioxidant effects due to their ability to act as free radical scavengers, hydrogen donating compounds, singlet oxygen quenchers, and metal ion chelators (Pietta 2000; Butkovic et al. 2004; Amić et al. 2007; Boots et al. 2008), and the antioxidant and anti-inflammatory effects of flavonoids may contribute to modulation of the inflammatory process. It is well known that the excess of reactive oxygen species (ROS) is closely associated to the inflammation pathway, leading to secretion of a variety of pro-inflammatory cytokines and chemokines, vasoconstriction contributing to vascular injury in many inflammatory diseases (Bartsch & Nair 2006; Libby 2006; Speranza et al. 2010). The production of ROS can cause oxidative damage by attacking biomolecules such as proteins, lipids, lipoproteins, and DNA (Gurpreet et al. 2006). This scenario favors the expansion and perpetuation of the inflammatory response. Since our results indicated that *S. schroederi* MET extracts and its fractions exhibited antioxidant activity, we evaluated the anti-inflammatory activity of *S. schroederi* samples using classical models of acute inflammation.

We performed the carrageenan and dextran induced paw edema test as the first step to evaluate the action of the *S. schroederi* MET extracts and its fractions on the inflammatory models. We showed that pre-treatment with *S. schroederi* extracts reduced the paw edema induced by carrageenan or dextran at all periods of evaluation. The paw edema promoted by carrageenan induces a biphasic edema. The first phase is characterized by an edema of little intensity and dispersive cellular infiltrate with a predominance of neutrophils that are capable of amplifying the inflammatory response via production of reactive oxygen species and release of inflammatory mediators. The second phase develops after 24 h, displaying a more pronounced edema with a maximum effect between 48



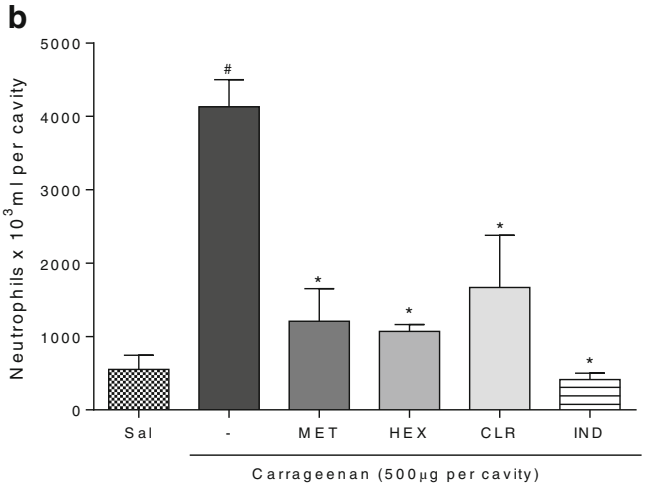


Fig. 6 The inhibitory effect of MET extract and HEX and CLR fractions from *S. schroederi* on cell migration induced by carrageenan in peritonitis model. Mice received samples of *S. schroederi* fraction (100 mg kg^{-1}) 1 hour before an i.p. injection of carrageenan, and the total leucocytes (**a**) and neutrophil migration (**b**) were counted in the peritoneal fluid 4 h later. The values are given as the mean±S.E.M. (n=5). Indomethacin (INDO: 10 mg kg^{-1}) was used as a positive control for the anti-inflammatory activity. The *number sign* and asterisk indicate statistically significant difference (P<0.05) compared to saline and carrageenan, respectively (ANOVA followed by Bonferroni's post-test)

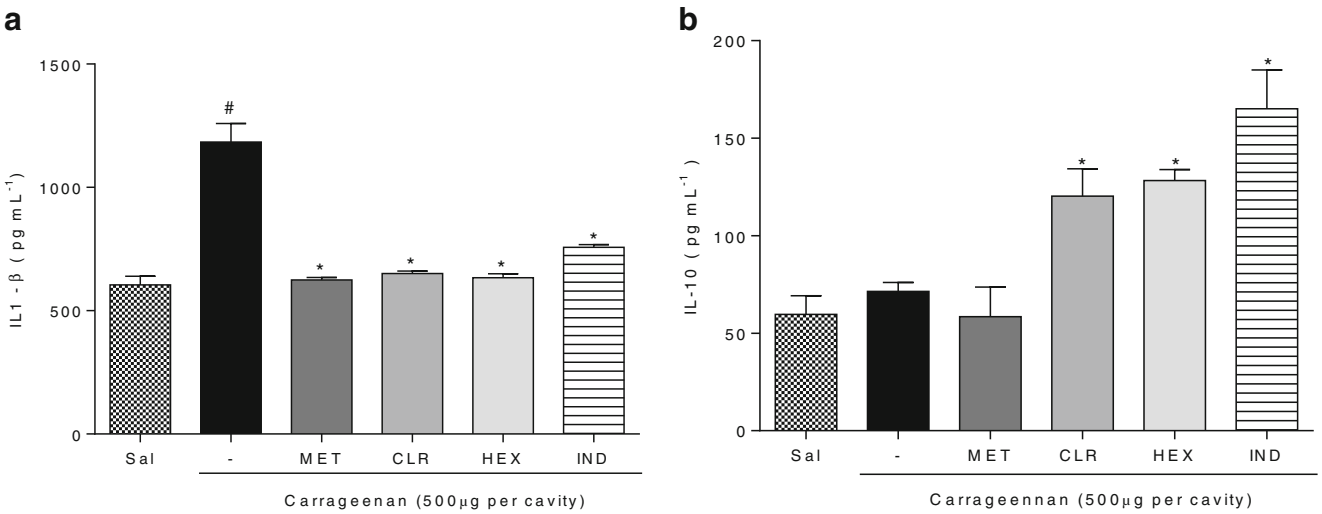


Fig. 7 The effect of MET extract and HEX and CLR fractions from *S. schroederi* on carrageenan-induced cytokine production in peritonitis. Mice received *S. schroederi* fractions (100 mg kg^{-1}) 1 h before an i.p. injection of carrageenan and 4 h later, the levels of IL-1ß (**a**) and IL-10 (**b**) were measured in the peritoneal fluid. The values are given as the mean± S.E.M. (n=5). Indomethacin (INDO: 10 mg kg^{-1}) was used as a positive control for the anti-inflammatory activity. The *number sign* and *asterisk* indicate statistically significant difference (P<0.05) compared to saline and carrageenan, respectively (ANOVA followed by Bonferroni's post-test)

and 72 h with an intense accumulation of macrophages, eosinophils, and lymphocytes (Henriques et al. 1987). On the other hand, dextran promotes inflammation by increasing vascular permeability as a result of mast cell degranulation and the subsequent release of histamine and serotonin (Rowley et al. 2003). Moreover, dextran promotes osmotic edema mainly characterized by an increase in vascular permeability and low levels of protein and neutrophils (Calixto et al. 2004). Our results indicate that anti-edematogenic effects promoted by MET, HEX, and CLR may be related to the inflammatory events involving neutrophil migration, as well as inhibition of the release or activity of inflammatory mediators.

The inflammatory response induced by carrageenan in paw edema involves intense neutrophil infiltration (Carvalho et al. 1996; Hajare et al. 2001). This event can be measured using the neutrophil-specific enzyme myeloperoxidase (MPO), which is an indicator of neutrophil accumulation (Ajuebor et al. 2000). MPO can be released on the outside of the cell, inducing damage to adjacent tissue and thus contributing to the pathogenesis of inflammation (Klebanoff 1999). Our data suggest that the pre-treatment with MET extract and HEX or CLR fractions can reduce the MPO concentration in the paw tissue indicating that part of the anti-inflammatory action of the *S. schroederi* extracts may involve inhibition of neutrophil infiltration.

To confirm that the anti-inflammatory effect promoted by *S. schroederi* extracts is involved in diminishing neutrophil migration, it was tested in the MET extract and its fractions in a carrageenan-induced peritonitis model. This experimental model provides a pharmacological tool to examine acute peritoneal inflammation, which allows quantification of resident macrophage activation and cell migration (Montanher et al. 2007). Our results demonstrated that the administration of MET extract and its fractions significantly reduced peritoneal leukocyte and neutrophil counts in the peritoneal cavity of mice. Leukocytes play an important role in acute inflammatory processes, and tissue damage is a deleterious consequence of intense neutrophil migration (Smiderle et al. 2008). Thus, we can infer that the tested substances can promote the diminution of the inflammatory process modulating the neutrophil invasion into the site of inflammation and thus, reducing tissue damage.

Previous studies have shown that carrageenan injection into the peritoneal cavity induces intense neutrophil migration dependent on the release of pro-inflammatory cytokines, such as TNFα and IL-1β. These cytokines play an important role in maintenance of the inflammatory process (Chaves et al. 2013). On the other hand, in an attempt to control the rise of the process, the organism produces and releases IL-10. Interleukin-10 is considered the most important anti-inflammatory cytokine. It is secreted by a variety of cells including macrophages, dendritic cells, granulocytes, and epithelial cells and downregulates the production of pro-inflammatory cytokines, such as IL-1β (Niiro et al. 1995; Marks et al. 2010). Our data demonstrated that pre-treatment with MET extract and HEX or CLR fractions markedly decreased the IL-1β concentration, while the HEX and CLR fractions increased the IL-10 level in the peritoneal fluid of the animals. Therefore, we can conclude that these extracts blocked the carrageenan-induced inflammatory process in the peritoneal cavity by inhibiting the release of pro-inflammatory cytokines and stimulating the release of IL-10.

In summary, we found that the MET extract of the brown seaweed *S. schroederi* and its fractions (HEX and CLR) are sources of phenolics and flavonoids and the antioxidant activity of these extracts is due mainly to the presence of flavonoids. The extracts also demonstrated an anti-inflammatory effect by reducing vascular and cellular events, decreasing release or production of pro-inflammatory the cytokine IL-1β and increasing the anti-inflammatory cytokine IL-10. These events seem to be related to the anti-oxidative effect of flavonoids present in the extracts. The results suggest that the products extracted from this alga can have a significant importance in experimental trials in an attempt to discover new compounds with anti-inflammatory and antioxidant effects. However, additional experiments concerning isolation of the molecules involved in the observed events remains necessary to clarify this question.

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