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Original Article

Brown seaweed *Padina gymnospora* is a prominent natural wound-care product

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ABSTRACT

Seaweeds are related to anti-inflammatory, anti-bacterial and anti-noceptive effects. This work aimed to verify the potential of seaweed *Padina gymnospora* (Kützing) Sonder 1871 to improve wound healing *in vitro*. *P. gymnospora* was collected at a bethonic area in Espirito Santo. Methanolic extract of *P. gymnospora* was obtained by percolation. To determine cytotoxicity, colorimetric MTT tests were performed against normal fibroblasts (L929), macrophages (RAW 264.7) and human ovarian carcinoma (OVCAR-3) cell lines using concentration range of 12–110 $\mu g\,ml^{-1}$. To evaluate *in vitro* wound healing, monolayer of fibroblasts L929 was seeded and artificial wounded. Cell proliferation was blocked by 5 $\mu g\,ml^{-1}$ Mytomycin C. Nitric oxide inhibition was quantified with Raw 264.7 by Griess reaction. Minimal inhibitory concentration (MIC) against *Staphylococcus aureus* was determined. Eletrospray ionization with Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR MS) was applied to detail composition of *P. gymnospora* methanolic extract. No cytotoxic effect in all cell lines was detected until the maximum concentration of 110 $\mu g\,ml^{-1}$. *P. gymnospora* promoted significantly migration at the concentration of 25 $\mu g\,ml^{-1}$ ($p < 0.05$). A prominent inhibition of nitric oxide formation was achieved in a concentration of 20 $\mu g\,ml^{-1}$ of methanolic extract of *P. gymnospora* ($62.06 \pm 1.20\%$). Antibacterial activity against *S. aureus* could be demonstrated with MIC of 500 $\mu g\,ml^{-1}$. ESI-FT-ICR MS analysis indicated eleven molecules between then, linolenic, oleic and linoleic acid. *P. gymnospora* favored wound repair *in vitro* what could be related to its fatty acid composition. In addition, its antimicrobial effect, and NO inhibition activity contribute for a new approach of *P. gymnospora* as a promise natural product for treatment of cutaneous wound.

Introduction

Seaweeds have been used in traditional medicine for many centuries, and are an object of interest for the pharmaceutical and food industries (Guaratini et al., 2012). Several studies reported the great potential of seaweed and their isolated compounds for their anti-inflammatory, anti-noceptive effects (Delgado et al., 2013; Bitencourt et al., 2015) or antimicrobial activity (Yavasoglu et al., 2007). In addition, seaweeds are rich source of bioactive compounds producing a great variety of secondary metabolites with broad biological activities (Gupta and Abu-Ghanna, 2011). Nowadays many efforts for new natural therapies have pointed marine algae as a prominent source of active biochemically compounds (Torres et al., 2014).

Chronic wounds affect life quality especially in older adults' population since healing is a process related to age. In addition, cutaneous wound appears frequently in diabetic's patients (Gould et al., 2015; Henshaw et al., 2015). Therefore, new natural products would be useful as therapeutic or prevention of chronic wounds. A wound is the result of accidental damage or a surgical procedure. It is particularly susceptible to bacterial and other infections, and provides an entry point for systemic infections (Misic et al., 2014). The wound-healing process of repair that follows skin injury is dynamic, complex, and well-organized (Reinke and Sorg, 2012).

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In this process, nitric oxide has an important role related to antimicrobial properties, vasodilatory effects, inflammation response, cell proliferation, angiogenesis promotion and matrix deposition culminating with reorganization of the injury (Luo and Chen, 2005). On the other hand, nitric oxide is potentially toxic, participating in oxidative stress by generating oxygen intermediates, depressing the antioxidant system (Lin et al., 2008). High levels of NO can cause complications in many pathological processes, including inflammation (Surh et al., 2001) and carcinogenesis (Lin et al., 2008).

In addition, wounds related to *Staphylococcus aureus* remains a worldwide health concern, generating morbidity and mortality due to the development of resistance to most antibiotics (Ghasemzadeh-Moghaddam et al., 2014). Therefore, effective treatment against this bacterium remains a challenge, and new wound-healing products that possess antimicrobial effects are very desirable.

The seaweed *Padina* sp. has demonstrated an antimicrobial effect against *Bacilos cereus* (minimal inhibitory concentration, MIC 63 $\mu g\,ml^{-1}$); *S. aureus* (MIC 130 $\mu g\,ml^{-1}$); and *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella typhimurium* (MIC > 500 $\mu g\,ml^{-1}$) (Dussault et al., 2015). The compounds from the hexane fraction, 18,19-epoxyxenic-19-methoxy-18-hydroxy-4 acetoxy-6,9,13-triene and 18,19-epoxyxenic-18,19-dimethoxy-4-hydroxyl-6,9,13-triene, isolated from *Padina pavonia* presented anti-tumoral activity in H460 cells (lung carcinoma) and HepG2 cells (liver carcinoma) (Awad et al., 2008).

The state of Espírito Santo (ES), Brazil, possesses a wide coastline of 400 km, but so far, there have been few surveys of benthic communities, as well as chemical and biological studies of these species. In the Metropolitan area of Vitória (ES), there are several reefs close to beaches (Barbosa et al., 2008). However, there are no reported studies relating Brazilian algae to wound-healing properties. Therefore, the present study aimed to evaluate its wound-healing activity *in vitro*, antimicrobial activity against *S. aureus* (ATCC 25923), and NO inhibition potential and to identify the chemical composition of the methanolic extract of the brown algae *P. gymnospora* (Phaeophyta).

Materials and methods

Algae material

Padina gymmospora (Kützing) Sonder 1871 seaweed was collected in the Maguinhos area (Serra city) latitude −20.1294, Longitude −40.308. 20° 7′46″ South, 40° 18′29″ West, in the coastal region of Espírito Santo, with a permanent license number of 34342-1 in 2014 (SISBIO). The material collected was taxonomically identified by Dr Levi Pompermayer Machado and deposited by Dr. Diogia Barata in the Espirito Santo, Federal University Herbarium (VIES 36052).

The seaweed after collection was pulverized, in a knife mill and percolated with methanol 96°GL. The methanolic extract was concentrated in a rotary evaporator, with low pressure, until residue formed. The methanolic extract obtained was transferred into a flask previously weighted and maintained in a desiccator, in a vacuum, to complete removal of the solvent, for at least 48 h.

Cell lines, chemicals, and biochemicals

The cell line used for the assays was fibroblasts (L929) (ATCC® CCL 1TM), macrophage cell lines (RAW 264.7) (ATCC® TIB 71TM), and human ovarian carcinoma cell lines (OVCAR-3) (ATCC® HTB-161TM). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU ml^{-1} penicillin, and 100 $\mu g\,ml^{-1}$ streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 (all Sigma–USA). Cells were harvested by tripsinization with 0.05% Trypsin/0.02% EDTA solution from Dibco, Grand Island, NY, USA, in case of fibroblast and human ovarian carcinoma cell lines. Raw 264.7 cells were mechanically removed.

Collagen solution type I from rat tails (Sigma–Aldrich); mitomycin C (Sigma–Aldrich); platelet-derived growth factor BB (PDGF); and Prolong Gold antifade reagent (molecular probes) with 4,6-diamino-2-phenylindole (DAPI) (Sigma–Aldrich), were purchased from Sigma Chemical Co, MO, USA. All solvents and reagents were of analytical grade, purchased from Vetec (Rio de Janeiro, Brazil). Mueller-Hinton agar was purchased from Himedia Laboratories Pvt (Mumbai, India).

Assessment of in vitro *cytotoxicity*

In vitro cytotoxic activity was evaluated using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay according to Mosmann (1983). L929 fribroblasts and OVCAR-3 cells were seeded in 96-well flat-bottomed tissue culture plates at a concentration of 6×10^5 cells ml^{-1} overnight at 37 °C (5% CO_2 and 95% air). Thereafter, cells were incubated for an additional 24 h in the presence or absence of increasing concentrations (12–110 $\mu g\,ml^{-1}$) of methanol extract of the algae *P. gymnospora*. Camptothecin (10 μM) was used as a positive control (Wu et al., 2011). After incubation, 100 μl of MTT (5 mg ml^{-1} in PBS) medium (1:3) were added per well, and the plate incubated for 2 h to allow reaction of MTT by cellular mitochondrial dehydrogenases. The excess MTT was aspirated, and the formazan crystals formed were dissolved in 100 μl of dimethyl sulfoxide (DMSO). The absorbance of purple formazan, proportional to the number of viable cells, was measured at 595 nm using a microplate reader (Molecular Devices, Spectra Max 190, USA). The experiments were carried out at least in triplicate.

In vitro *cell migration assay*

The proliferation and/or migration capabilities of fibroblasts exposed to the brown algae *P. gymnospora* methanolic extracts were estimated using the *in vitro* scratch wound assay, which measures the expansion of a cell population on surfaces (Liang et al., 2007; Fronza et al., 2009). Briefly, L929 fibroblasts (2×10^6 cells ml^{-1}) were cultured in 24-well plates containing glass coverslips previously coated with collagen (40 $\mu g\,ml^{-1}$, 2 h at 37 °C) to nearly confluent cell monolayers, and an artificial linear wound was introduced. The monolayers were then, divided into two groups; both were treated for 14 h with different concentrations of the extracts (6.25, 12.5, and 25 $\mu g\,ml^{-1}$). PDGF (2 nm) was used as a positive control. One of the groups also received mitomycin C (5 mg ml^{-1}). After treatment, the cells were fixed and stained with DAPI. Pictures of the scratched areas were taken using an AxioCam MRC Zeizz camera coupled to an Axio Zeizz Vert. A1 microscope with the program Zen life 2012. SP 1. The images were saved with appropriate names to a hard disk. Cell migration into the wound area was quantified using Cellprofiler software, and the results were expressed as percentages of cells that migrate and/or proliferate into the wound area in comparison with the untreated control group.

Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells assay

The level of nitrite, the stable end product of NO, was estimated as described previously (Park et al., 2011). Briefly, RAW 264.7 cells were seeded at a density of 1×10^5 cells $well^{-1}$ and incubated in

96-well culture plates at 37 °C, 5% CO_2 in humidified air for 24 h. The cultured medium was replaced with phenol red-free medium containing various concentrations of compounds for 15 min prior to 1 μg ml^{-1} of LPS exposure for 20 h. The amount of nitrite in the cultured media was measured by using Griess reagent. Under the same experimental conditions, sulforhodamine B assays were performed to evaluate the cytotoxic effect of compounds toward RAW 264.7 cells. L-N^G-monomethyl arginine citrate (L-NMMA), a positive control of this assay, with an IC_{50} value of 25.1 μM.

Antimicrobial activity

To assess the antimicrobial activity of the brown algae *P. gymnospora* methanolic extract and to verify zones of inhibition against the strains of bacteria *Staphylococcus aureus* (ATCC 25923), 10 mg ml^{-1} of the extract was dissolved in 1 ml of dimethylsulphoxide (DMSO) and immediately diluted with saline solution (0.85%). Then, *P. gymnospora* extract in concentrations ranging from 7.8 to 500 μg ml^{-1} in 0.5% DMSO was evaluated. The final cell concentration of *S. aureus* was adjusted to 0.5 using the McFarland scale (1.5×10^8 CFU ml^{-1}), and thereafter cells were diluted with Mueller Hinton broth so that each well of the microplate presented approximately 5×10^5 CFU ml^{-1}. After this procedure, 150 μl of Mueller Hinton medium was inoculated with 150 μl of *P. gymnospora* extract. The assays were performed using a positive control, negative control, and ampicillin control. The positive controls, 75 μl of culture medium with inoculum were added to 75 μl of diluent at the concentration used. In the negative controls, 75 μl of culture medium without inoculum was added to the diluent as a sterile control. In the Ampicillin control 75 μl of culture medium with inoculum (*S. aureus*), and 75 μl were added to the 1.0 mg ml^{-1} ampicillin (Goldman and Green, 2002).

The plates were incubated at 36 °C for 24 h, and then 50 μl of the CTT indicator (solution of 2,3,5-triphenyl tetrazolium 0.5% in deionized water) were added. After 6 h of incubation, MIC was determined as the lowest concentration that inhibited visible growth of bacteria since CTT does not stain dead cells.

ESI (−) FT-ICR MS

To identify the molecules, present in methanolic extract of *P. gymnospora* under study, 10 μl of methanolic extract was dissolved in 1000 μl of methanol/water solution (50% V/V). Afterward, the solution was basified with 4 μl NH_4OH (Vetec Fine Chemicals Ltda, Brazil). Extracts were directly infused at a flow rate of 12 μl min^{-1} into the ESI(−) source.

The mass spectrometer (model 9.4 T Solarix, BrukerDaltonics, Bremen, Germany) was set to negative ion mode, ESI (−), over a mass range of m/z 150–1500. The ESI source conditions were as follows: a nebulizer gas pressure of 1.4 bar, a capillary voltage of 3.8 kV, and a transfer capillary temperature of 200 °C. Ions' time accumulation was of 0.030 s. ESI(−)FT-ICR mass spectra were acquired by accumulating 64 scans of time-domain transient signals in four mega-point time-domain data sets. All mass spectra were externally calibrated using NaTFA (m/z from 200 to 1200). A resolving power, $m/\Delta m50\% = 500{,}000$ (in which $\Delta m50\%$ is the full peak width at half-maximum peak height of m/z 400) and a mass accuracy of <1 ppm provided the unambiguous molecular formula assignments for singly charged molecular ions.

The mass spectra were acquired and processed using Data Analysis software (BrukerDaltonics, Bremen, Germany). Elemental compositions of the compounds were determined by measuring the m/z values. The proposed structures for each formula were assigned using the Chemspider (www.chemspider.com) database. The degree of unsaturation for each molecule can be deduced directly from its DBE value according to equation DBE = $c - h/2 + n/2 + 1$, where c, h, and n are the numbers of carbon, hydrogen, and nitrogen atoms, respectively, in the molecular formula.

Statistical analysis

Statistical evaluation was carried out with prisma Software version 5.00, 2007. Data were expressed as the mean ± S.E.M. Significant differences between the treated groups and the control were determined by ANOVA test followed by Tukey test, at a level of $p < 0.05$.

Results and discussion

The extract of *P. gymnospora* did not present cytotoxicity against normal L929 mouse fibroblasts cell line and human ovarian carcinoma cell line, OVCAR-3 until the highest evaluated concentration (110 μg ml^{-1}), being consistent with the data registered for other seaweed. Studies performed with brown algae from the Japanese coast demonstrated that methanolic extract of *Cystoseira myrica*, Cystoseiraceae, presented a $IC_{50} > 1000$ μg ml^{-1} (for colon adenocarcinoma cell lines HT29 and CaCo2, T47D breast carcinoma, NIH 3T3 normal Swiss embrio fibroblast cell line). In addition, *Sargassum swartzii* presented a IC_{50} (μg ml^{-1}) > 1000 for colon adenocarcinoma cell lines HT29 and CaCo2 while of 205.21 ± 84.1 μg ml^{-1} for T47D breast carcinoma, and of 607.12 ± 2.81 μg ml^{-1} for NIH 3T3 normal cell Swiss embryo fibroblast cell line. While *Colpomenia sinuosa* presented a $IC_{50} > 1000$ μg ml^{-1} for HT29, CaCo2, T47D and IH 3T3 (Khanavi et al., 2010a,b). Studies performed with brown algae *Ecklonia cava* extract against the color cancer line CT-26 and human leukemia cell line (THP-1), demonstrated an IC_{50} higher than 100 μg ml^{-1} (Athukorala et al., 2006).

The methanolic extract of *P. gymnospora* induced proliferation and/or migration of fibroblasts (L929) represented by Fig. 1. Among the concentrations evaluated, the concentration of 25.0 μg ml^{-1} elicits higher proliferation and/or migration in comparison to the negative control group. To distinguish migration from proliferation, the same experiment was performed using mytomycin C, a cellular antimetabolite, which blocks DNA and RNA replication and

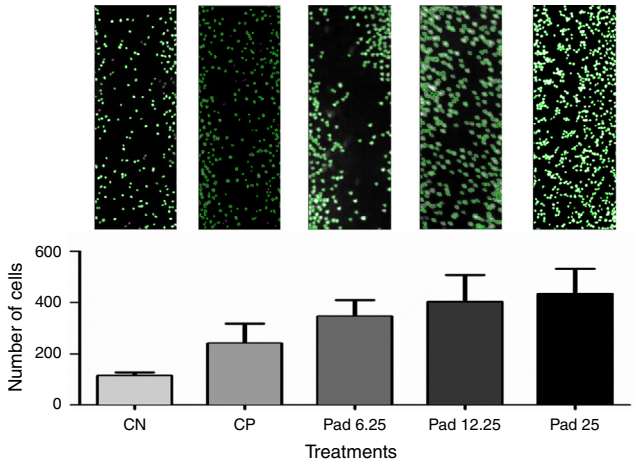

Fig. 1. *In vitro* effect of methanolic extract of algae *Padina gymnospora* in cell migration and/or proliferation in fibroblasts L929. The fibroblasts were cultivated until formed a monolayer and then the scratch were made to perform the assay. (A) Cell counting inside scratched areas. (B) Representative pictures of scratches where cultures were stimulated in five groups: (CN) negative control group untreated, (CP) positive control group stimulated by PDGF 2 nm, (PAD 6.25) group stimulated by extract of *P. gymnospora* at 6.25 μg ml^{-1}, (PAD 12.5) stimulated by extract of *P. gymnospora* at 12.50 μg ml^{-1} and (PAD 25) stimulated by extract of *P. gymnospora* at 25.00 μg ml^{-1}.*($p < 0.05$). Cell migration into the wound area was quantified using Cellprofiler software.

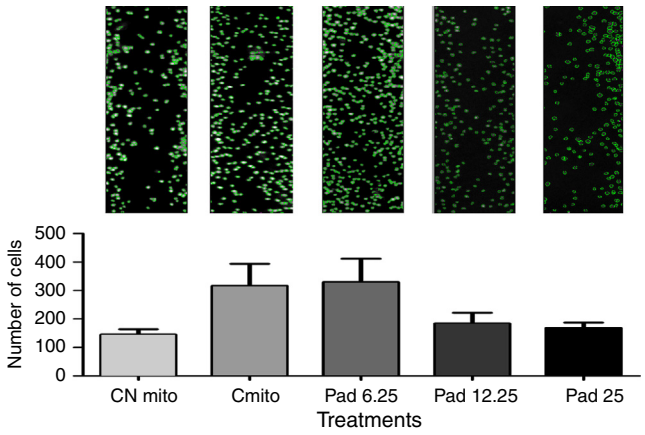

Fig. 2. *In vitro* effect of methanolic extract of *Padina gymnospora* in cell migration in fibroblasts L929. The fibroblasts were cultivated until formed a monolayer and then the scratch were made to evaluate cell migration in the area. To analyze only the cell migration, all groups were added mitomycin C. (A) Cell counting inside scratched areas. (B) Representative pictures of scratches where cultures were stimulated with (CN mito) negative control group untreated with mitomycin C, (C mito) positive control group stimulated by PDGF with mitomycin C, (PAD 6.25) group stimulated by extract *P. gymnospora* at 6.25 μg ml^{-1}, (PAD 12.5) stimulated by extract of *P. gymnospora* at 12.50 μg ml^{-1} and (PAD 25) stimulated by extract of *P. gymnospora* at 25.00 μg ml^{-1}.*($p < 0.05$) Cell migration into the wound area was quantified using Cellprofiler software.

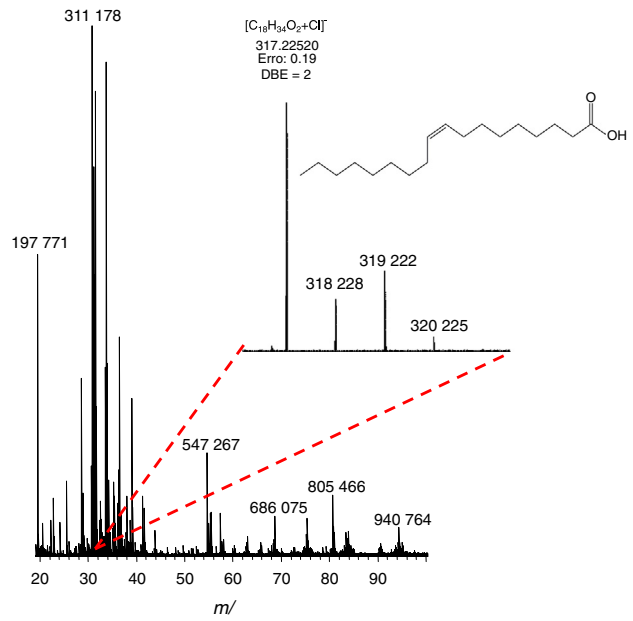

Fig. 3. *Padina gymnospora* methanolic extract ESI(−)-FT-ICR MS profile for identified oleic acid Chloride Isotopic *m/z* 317.

by that inhibits protein synthesis (5 μg ml^{-1}). The concentration of 6.25 μg ml^{-1} methanolic extract of *P. gymnospora* significantly ($p < 0.05$) promoted more migration of cells when compared to the negative control group (Fig. 2). *P. gymnospora* showed an anticoagulant activity being glucuronofucan apparently responsible for this activity (Silva et al., 2005; Nader et al., 2001; Haroun-Bouhedja et al., 2000). It could explain why by proliferation blocking by mytomycin C, no better migration was achieved when concentration was enhanced. Indeed, it is know that *in vivo* the clot and surrounding wound tissue release pro-inflammatory cytokines and growth factors (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) (Guo and Dipietro, 2010; Maxson et al., 2012). Therefore, anticoagulation activity could retard clot and healing process imitating the effect of some drugs classes (National Institutes of Health, 2013). To elucidate concentration reason, anticoagulation effect and healing delay relation, *in vivo* tests should be performed.

The extract of *P. gymnospora* collected in the coastal area of Espírito Santo, Brazil, may be considered a prosperous antimicrobial agent with a minimal inhibitory concentration against *S. aureus* of 500 μg ml^{-1} (77.5%); moreover, ampicillin presented an inhibition of 90% at a concentration of 1000 μg ml^{-1}. Aligiannis et al. (2001) classified extracts with inhibitory activity in concentrations ≤500 μg ml^{-1} to present a significant antimicrobial activity against some bacterial pathogens commonly found in cutaneous wounds, like *S. aureus*. Adetutu et al. (2011) also stated that, for an extract be designated an antimicrobial agent with good pharmacologic relevance, it is necessary that the MIC value be less than 8 mg ml^{-1} and greater than 2 mg ml^{-1}. The compounds have to be isolated to be identified responsible for the antimicrobial activity.

In the nitric oxide inhibition assay by the Griess reaction and in the quantification of nitrite (NO_2^-) e nitrate (NO_3^-) in cell culture medium, for the macrophage cells (RAW), a prominent response was achieved in the concentration of 20 μg ml^{-1} of methanolic extract of *P. gymnospora* which inhibited 62.06 ± 1.20% of nitric oxide formation. L-N^G-monomethyl arginine citrate (L-NMMA) was used as a positive control, with an IC_{50} value of 25.1 μM. This effect could contribute directly or indirectly to modulation of inflammatory responses (Surh et al., 2001). In addition, NO is also related to cell growth, cell morphology, F-actin organization disruption, and the decreased expression of the focal adhesion-related molecules integrin α5 and paxillin (Xing et al., 2015). Therefore, NO inhibition associated with cell proliferation and migration demonstrated by *P. gymnospora* could contribute to accelerated wound repair.

The chemical analysis using ESI(−)-FT-ICR-MS is a powerful analytical tool and was used to access the chemical composition of the *P. gymnospora* methanolic extract suggesting the presence of eleven organic compounds between then oleic, linolenic and linoleic, arachidonic acids (Fig. 3, Table 1).

Many of these compounds are found with a chloride added to its chemical structure justified by its marine source. ESI(−)-FT-ICR MS profile of the methanolic extract of *P. gymnospora* represented by Fig. 3 confirms the detection of organic acids as chloride adduct, $[M+Cl]^-$. A magnification in *m/z* 317 region highlights the isotopic pattern of oleic acid, detected as $[C_{18}H_{34}O_2+Cl]^-$ ion of *m/z* 317.22522, DBE = 2 and mass error of 0.19 ppm. Note that the isotopic pattern of a typical chloride adduct shows for the $[M+^{37}Cl]^+$ isotope of *m/z* 319 a relative intensity of 1/3 of $[M+^{35}Cl]^+$ ion. The *m/z* values of all molecules identified are represented in the Table 1. Linoleic acid, α-linolenic and oleic acids were identified by the *Padina pavonica* as well as palmitic, myristic and stearic fatty acids (El Shoubaky and El Rahman Salem, 2014). Wahbeh (1997) described that *P. pavonica* contained fatty acids with 12–22 carbon atoms, similar to other brown algae. The crude extract of *P. pavonica* showed antibacterial activity against *E. coli* (NCMB 11943), *Pseudomonas aeruginosa* (NCMB 8295), *S. typhimurium* (NCMB 74), *Shigella boydii* (ATCC 9207), *S. aureus* (NCMB 6571) and actinobacteria *S. antibioticus* (wild type). While fatty acids extract of *P. pavonica* presented no inhibition effect against *E. coli* and *S. aureus*. Besides, the use of fatty acids as wound-healing agents is related to the fact that they are part of the biological membrane, act as intracellular messengers, and are oxidized, generating ATP, and may then interfere in the inflammatory process, as the triggered events occur in the pathway derived from arachidonic acid (Hatanaka and Curi, 2007). In addition, they inhibit pro-inflammatory eicosanoids and cytokines (tumoral necrose factor-α, interferon-γ, and interleucin-12). Linoleic acid is appointed as a promised adjuvant in safe treatments for skin diseases and wound-healing promotion (McCusker and Gran-Kels, 2010). This acid is

Table 1
Molecules identified by ESI-FT-ICR MS in methanolic extract of *Padina gymnospora.*

$m/z_{mesured}$	$m/z_{theoretical}$	Error[a]	DBE	$[M-H]^-$	Proposed compound	References
311.17824	311.17833	0.30	5	$[C_{18}H_{28}O_2+Cl]^-$	Stearidonic acid	Khan et al. (2008)
329.18888	329.18890	0.05	4	$[C_{18}H_{30}O_3+Cl]^-$	9-Oxo-10,12-octadecadienoic acid	No reference
363.23079	363.23076	−0.07	2	$[C_{19}H_{36}O_4+Cl]^-$	Nanodecanoic acid	No reference
317.22522	317.22528	0.19	2	$[C_{18}H_{34}O_2+Cl]^-$	Oleic acid	Dawczynski et al. (2007)
315.20957	315.20963	0.20	3	$[C_{18}H_{32}O_2+Cl]^-$	Linoleic acid	Murugan and Iyer (2014)
347.27224	347.27223	0.01	1	$[C_{20}H_{40}O_2+Cl]^-$	Eicosanolic acid	Shanab Sanaa (2007)
313.19393	313.19398	0.15	4	$[C_{18}H_{30}O_2+Cl]^-$	Linolenic acid	Matanjun et al. (2009)
339.20957	339.20963	0.17	5	$[C_{20}H_{32}O_2+Cl]^-$	Arachidonic acid	Sánchez-Machado et al. (2004)
337.19396	337.19398	0.07	6	$[C_{20}H_{30}O_2+Cl]^-$	Eicosapentaenolic acid	Jones and Harwood (1992)
389.24639	389.24641	0.06	3	$[C_{21}H_{38}O_4+Cl]^-$	Linolenic acid	Jaswir et al. (2011)
289.19391	289.19398	0.25	2	$[C_{16}H_{30}O_2+Cl]^-$	Palmitoleic acid	Rohani-Ghadikolaei et al. (2012)

[a] Error (ppm) = $[(m/z_{mesured}/m/z_{theoretical})/m/z_{mesured}] \times 10^6$.

proposed as one component of the methanolic extract of *P. gymnospora* evaluated in the present study.

Conclusion

It is possible to relate the favorable effect of *in vitro* wound healing of the methanolic extract of *P. gymnospora* seaweed to its chemical composition as fatty acids, and principally related to NO inhibition, which directly affects repair processes. In addition, the antimicrobial effect (MIC against *S. aureus* was 500 $\mu g\,ml^{-1}$) confers a synergistic therapeutic advantage to *P. gymnospora*. However, further investigations should be conducted to elucidate the mechanisms of action of the *P. gymnospora* extract, expanding the knowledge of the genre.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

APB contributed with the acquisition of the data; analysis and interpretation of the data. EFP contributed with the acquisition of the data; analysis and interpretation of the data, and drafting of the article. ARB contributed with Cell Profile Analysis. TZV contributed with mass spectrometry data analysis. WR, LVT contributed with mass spectrometry acquisition data, chemical composition analysis. DL contributed with Cell Profile Analysis. TUA contributed with critical revision of the manuscript. MF contributed with scratch assay methodology. TPK contributed with Inhibition of nitric oxide (NO) cell assay. DCE contributed to the concept, design and supervision of the study and interpretation of data.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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