UNIVERSIDADE VILA VELHA – ES

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

ALGA PARDA *Padina* sp. COMO TRATAMENTO PROMISSOR DE FERIDAS CUTÂNEAS

ALEGNA PORTES BALIANO

VILA VELHA

MAIO/2016

UNIVERSIDADE VILA VELHA – ES

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

ALGA PARDA *Padina* sp. COMO TRATAMENTO PROMISSOR DE FERIDAS CUTÂNEAS

Dissertação apresentada à Universidade Vila Velha, como pré-requisito do Programa de Pós-Graduação em Ciências Farmacêuticas para a obtenção do titulo de Mestre em Ciências Farmacêuticas.

ALEGNA PORTES BALIANO

VILA VELHA

MAIO/2016

Catalogação na publicação elaborada pela Biblioteca Central / UVV-ES

B186a Baliano, Alegna
Alga parda padina sp. como tratamento promissor de feridas cutâneas. / Alegna Balliano – 2016.
38 f.: il.

Orientador: Denise Coutinho Endringer.
Dissertação (mestrado em Ciências Farmacêuticas) - Universidade Vila Velha, 2016.
Inclui bibliografias.

1. Farmacologia e terapêutica. 2. Alga marinha. 3. Cicatrização de feridas I. Endringer, Denise Coutinho. Universidade Vila Velha. III. Título.

CDD 615

ALEGNA PORTES BALIANO

ALGA PARDA *Padina* sp. COMO TRATAMENTO PROMISSOR DE FERIDAS CUTÂNEAS

Dissertação apresentada à Universidade Vila Velha, como pré-requisito do Programa de Pós-Graduação em Ciências Farmacêuticas para a obtenção do titulo de Mestre em Ciências Farmacêuticas.

Aprovada em 31 de maio de 2016

Banca Examinadora:

Levi P. Machado

Levi Pompermayer Machado (FAESA)

Tadeu Uggere de Andrade – UVV

Denise Coutinho Endringer– UVV

Orientador

Dedico esta Dissertação à minha amada família, que é a minha base e que jamais mediu esforços para que eu alcançasse meus sonhos. Todas as minhas vitórias também são de vocês.

AGRADECIMENTOS

Agradeço primeiramente a Deus, sem Ele jamais teria conseguido chegar até aqui. Por sua presença, luz e força sempre me abençoando e me capacitando para trilhar novos caminhos.

Aos meus pais, Elisarbi e Maria Auxiliadora, e ao meu irmão Matheus que sempre me apoiaram e nunca mediram esforços para me ajudar a realizar os meus sonhos e conquistas, pela vida de dedicação, amor e trabalho. Sempre pensando no meu bem estar e na minha alegria. Eu amo muito vocês! Jamais esquecerei que vocês abdicaram de muito para eu chegar até aqui. Serei eternamente grata por isso!

A todos os meus familiares, minha madrinha querida, tios, avós, primos e amigos, obrigada por torcerem e acreditarem em mim e por entenderem as minhas ausências.

A minha querida Orientadora, Profª. Dr. Denise Coutinho Endringer, obrigada pela persistência, paciência e pela vontade de querer nos fazer jovens pessoas melhores, pela transmissão do conhecimento e por ter me acolhido tão bem. Imagino que deve ser muito difícil deixar dois lindos filhos em casa te esperando, mas mesmo assim a senhora esteve sempre presente, sempre dedicada e disposta a me ajudar. Eu jamais me esquecerei da senhora e da sua frase "brilhe jovem".

A minha amiga, Ana Claudia Hertel Pereira pela ajuda, pelo apoio, pelos incentivos positivos nos momentos de fraqueza e desespero, é a melhor amiga que alguém pode ter. Todas as aflições e sorrisos partilhados entre nós serão para sempre lembrados, com um sabor doce de um tempo de aprendizado e de muito companheirismo, jamais me esquecerei dos almoços realizados na sua casa com a presença da sua família que amo muito.

Agradeço também pela ajuda dos meus amigos de caminhada, que estiveram sempre presente nesses dois anos, sempre dispostos a me ajudar em tudo que precisei, não poderia me esquecer jamais de vocês, Jean Pierre, Bianca, Clarisse, Maria Eduarda, Professor Marcio Fronza e Professor Dominik. Não poderia deixar de mencionar o grande carinho que tenho por você Luciane, pela oportunidade de conhecê-la. Críamos uma amizade linda desde o primeiro momento que nos aproximamos. E a você Franciane tão pequenina, mas com um coração que não cabe no peito. Meninas, que possamos ter mais Atibaia nas nossas vidas. Agradeço pela amizade de vocês e espero que ela não se desfaça nunca. Vou levar essa amizade para o resto de minha vida.

Ao Laboratório de Ciências Químicas e o de Microbiologia da Universidade Vila Velha - UVV com os seus técnicos Ana Claudia, Bianca, Jean Pierre, Clarisse e Paulo sempre prestativos, e sempre cooperando nos

experimentos. Vocês foram fundamentais para o meu crescimento profissional e pessoal e souberam me acolher em cada cantinho do laboratório. Aos recém-chegados, e também muito lembrados, Mariana, Waléria, Tainã e, em especial, a Elizangela Flavia, pela dedicação e ajuda. Foram momentos de aprendizado que levarei sempre comigo. Que vocês continuem essas pessoas maravilhosas, e eu desejo tudo de bom sempre. Sucesso na vida. "Brilhem jovens!!!"

Aos professores e funcionários do PPGCF e biopráticas, à UVV, que possibilitou a execução deste trabalho.

Agradeço à FAPES, à CAPES e ao CNPq pelo apoio financeiro.

Agradeço a todos que, direta ou indiretamente, contribuíram para a realização desta pesquisa. O meu muito obrigada a todos!

“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis”.

José de Alencar

RESUMO

BALIANO, Alegna, Farmacêutica, M.Sc., Universidade Vila Velha-ES. Maio de 2016. **Alga Parda *Padina* sp. como tratamento promissor de feridas cutâneas.** Orientadora: Profª. Drª. Denise Coutinho Endringer.

As algas estão relacionadas a efeitos anti-inflamatórios, antibacterianos e antinoceptivos. O Espírito Santo, costa litorânea do Brasil, possui comunidades betônicas não exploradas. Esse trabalho verifica o potencial de algas *Padina sp.* na melhora do processo de cicatrização *in vitro.* O extrato metanólico da alga *Padina sp* foi obtido por percolação. Ensaios colorimétricos de citotoxicidade sobre as linhagens celulares de fibroblastos (L929), macrófagos (RAW 264.7) e carcinoma do ovário humano (OVCAR-3) foram realizados utilizando a faixa de concentração de 12-110 $\mu g.mL^{-1}$. Para avaliar a cicatrização, monocamada de fibroblastos L929 foi lesionada in vitro. A proliferação celular foi interrompida com 5 $\mu g.mL^{-1}$ de Mitomicina C. Óxido nítrico foi quantificado usando a linhagem Raw 264.7 por meio da reação de Griess. A concentração inibitória mínima (CIM) contra *Staphylococcus aureus* (ATCC25923) foi avaliada. A composição do extrato de Padina sp. foi identificada por espectrometria de massas de altíssima resolução e exatidão com transformada de Fourier combinada à fonte de ionização por eletrospray (ESI-FT-ICR MS). Nenhum efeito citotóxico foi detectado em todas as linhas celulares testadas até a concentração de 110 $\mu g.mL^{-1}$. A concentração de 6.25 $\mu g.mL^{-1}$ do extrato de *Padina sp*. promoveu significativamente a migração ($p < 0,05$). Concentração de 25, 50 e 100 $\mu g.mL^{-1}$ apresentaram inibição do NO de 7, 21 e 37% respectivamente. O MIC para *S. aureus* foi 500 $\mu g.mL^{-1}$. Por ESI-FT-ICR MS foram identificadas 17 moléculas como ácidos láurico, esteárico e linoleico. *Padina* sp. favorece a reparação de ferida *in vitro* podendo estar relacionada com a sua composição de ácidos graxos. Além disso, seu efeito antimicrobiano, e sua atividade inibitora de NO indica a *Padina sp.* como um promissor produto natural.

Palavras-chave: *Padina* sp, cicatrização de feridas, óxido nítrico, *Staphylococcus aureus*, ácidos graxos.

ABSTRACT

BALIANO, Alegna, Farmacêutica, M.Sc., Universidade Vila Velha-ES. Maio de 2016. **Brown Seaweed *Padina Gymnospora* is a prominent natural wound-care product**. Advisor: Profª. Drª. Denise Coutinho Endringer.

Seaweeds are related to anti-inflamatory, anti-bacterial and anti-noceptive effects. The Espírito Santo, Brazil coastline, possesses a no explored benthic communities. This work verified the potential of seaweeds *Padina* sp., to improve wound healing *in vitro*. Methanolic extract was obtained by percolation (license number 343421). Colorimetric MTT cytotoxicity tests (concentration range of 12-110 $\mu g.mL^{-1}$), was performed using fibroblasts (L929), macrophages (RAW 264.7) and human ovarian carcinoma (OVCAR-3) cell lines. To evaluate healing, monolayer of fibroblasts L929 was wounded. Cell proliferation was blocked by 5 $\mu g.mL^{-1}$ Mytomycin C. Nitric oxide was quantified with Raw 264.7, Griess reaction. Minimal inhibitory concentration (MIC) against *Staphylococcus aureus ATCC25923* was evaluated. ESI-FT-ICR MS identified *Padina* sp., extract composition. No cytotoxic effect in all cell lines was detected until 110 $\mu g.mL^{-1}$.The concentration of 6.25 $\mu g.mL^{-1}$ of *Padina* sp. extract, promoted significantly migration ($p<0.05$). Concentration of 25, 50 and 100 $\mu g.mL^{-1}$ presented NO inhibition of 7, 21, and 37% respectively. *S. aureus* MIC was 500 $\mu g.mL^{-1.}$ ESI-FT-ICR MS detected 17 molecules as lauric, stearic, linoleic acid. *Padina* sp. favored wound repair *in vitro* what could be related to its fatty acid composition. In addition, its antimicrobial effect, and NO inhibition activity pointed *Padina* sp., as a promise natural product.

Key words: *Padina* sp., wound-healing, nitric oxide, *Staphylococcus aureus*, fatty acids

SUMÁRIO

CAPÍTULO

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

Alegna P. Baliano[1], Elisangela F. Pimentel[1], Aline R. Buzin[1], Tainã Z. Vieira[1], Wanderson Romão[2,3], Lilian V. Tose[2], Dominik Lenz[1], Tadeu U de Andrade[1], Marcio Fronza[1], Tamara P. Kondratyuk[4], Denise C. Endringer[1]*

[1]Pharmacy Postgraduate Program, Vila Velha University, Av. Comissário José Dantas de Melo, n°21, 29102-920 – Boa Vista, Vila Velha, ES, Brazil – Tel.: +55 27 3241-2198.

[2]Federal University of Espirito Santo, Av. Fernando Ferrari, 514 - Goiabeiras, Vitória - Espirito Santo, Vila Velha, ES, Brazil, – Tel.: +55 27 4009-2222

[3]Federal Institute of Education, Science and Technology (IFES), 29106-010 Vila Velha, ES, Brazil;

[4]University of Hawaii at Hilo, Daniel K. Inouye College of Pharmacy, 60 Nowelo Street, Ste. 101, Hilo Hawaii, 96720- Tel+ 1 8088542600

*Corresponding author DCE: endringe@gmail.com; denise.endringer@uvv.br. Pharmacy Postgraduate Program, Vila Velha University, Av. Comissário José Dantas de Melo, n°21, 29102-920 – Boa Vista, Vila Velha, ES, Brazil – Tel.: +55 27 3241-2198.

ABSTRACT

Seaweeds are related to anti-inflammatory, anti-bacterial and anti-noceptive effects. This work aimed to verify the potential of seaweed *Padina gymnospora* 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 $\mu g \cdot ml^{-1}$. To evaluate *in vitro* wound healing, monolayer of fibroblasts L929 was seeded and artificial wounded. Cell proliferation was blocked by $5\mu g \cdot ml^{-1}$ 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 $\mu g \cdot ml^{-1}$ ($p<0.05$). A prominent inhibition of nitric oxide formation was achieved in a concentration of 20 $\mu g \cdot ml^{-1}$ of methanolic extract of *P. gymnospora* (62.06 ± 1.20 %). Antibacterial activity against *S. aureus* could be demonstrated with MIC of 500 $\mu g \cdot ml^{-1}$. ESI -FT-ICR MS analysis indicated 11 molecules between then, linolenic, oleic and linoleic acid. *Padina 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.

Key words: *Padina gymnospora*, wound-healing, nitric oxide, *Staphylococcus aureus*, fatty acids.

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 (BITENCOURT et al., 2015; DELGADO et al., 2013) 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-GHANNAM, 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). 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, of 63µg·ml^{-1}); *Staphylococcus aureus* (MIC = 130 µg·ml^{-1}); and *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella typhimurium* (MIC> 500 µg·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 (1) and 18,19-epoxyxenic-18,19-dimethoxy-4-hydroxyl-6,9,13-triene (2), isolated from *Padina pavonia* (L.) Gaill 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 *Padina gymnospora* (Phaeophyta).

MATERIALS AND METHODS

Algae Material

Padina gymmospora 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 48h.

Cell lines, Chemicals, and Biochemicals

The cell line used for the assays were fibroblasts (L929) (ATCC® CCL1TM), macrophage cell lines (RAW 264.7) (ATCC® TIB71TM), 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 $IU \cdot ml^{-1}$ penicillin, and 100 $\mu g \cdot ml^{-1}$ 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 ºC (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 µg. ml^{-1}) of methanol extract of the algae *Padina 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 (FRONZA et al., 2009; LIANG et al., 2007). Briefly, L929 fibroblasts (2 10^6 cells·ml^{-1}) were cultured in 24-well plates containing glass coverslips previously coated with collagen (40 µg·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 $\mu g \cdot ml^{-1}$). PDGF (2 nM) was used as a positive control. One of the groups also received mitomycin C (5 $mg \cdot ml^{-1}$). 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 disc. 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 $1 \cdot 10^5$ $cells \cdot well^{-1}$ 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 $\mu g \cdot ml^{-1}$ 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 $mg \cdot ml^{-1}$ of the extract was dissolved in 1 ml of dimethylsulphoxide (DMSO) and immediately diluted with saline solution (0.85%). Then, *Padina gymnospora* extract in concentrations ranging from 7.8 to 500 $\mu g \cdot ml^{-1}$ in 0.5% DMSO were evaluated. The final cell concentration of *S. aureus* was adjusted to 0.5 using the McFarland scale ($1.5 \cdot 10^8$ $CFU \cdot ml^{-1}$), and thereafter cells were diluted with Mueller Hinton broth so that each well of the microplate presented approximately $5 \cdot 10^5$ $CFU \cdot ml^{-1}$. After this procedure, 150 µl of Mueller Hinton medium were 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 were 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 $mg \cdot ml^{-1}$ 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 $\mu l \cdot min^{-1}$ 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 200ºC. 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/Δm50% = 500,000 (in which Δm50% 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 $\mu g \cdot ml^{-1}$), 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 $\mu g \cdot ml^{-1}$ (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} ($\mu g \cdot ml^{-1}$) >1000 for colon adenocarcinoma cell lines HT29 and CaCo2 while of 205.21 ±84.1 $\mu g \cdot ml^{-1}$ for T47D breast carcinoma, and of 607.12 ±2.81 $\mu g \cdot ml^{-1}$ for NIH 3T3 normal cell Swiss embryo fibroblast cell line. While *Colpomenia sinuosa* presented a IC_{50} > 1000 $\mu g \cdot ml^{-1}$ for HT29, CaCo2, T47D and IH 3T3 (KHANAVI et al., 2010). 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 $\mu g \cdot ml^{-1}$ (ATHUKORALA et al., 2006).

The methanolic extract of *P. gymnospora* induced proliferation and/or migration of fibroblasts (L929) represented by Figure 1. Among the concentrations evaluated, the concentration of 25.0 $\mu g \cdot ml^{-1}$ 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 by that inhibits protein synthesis (5 $\mu g \cdot ml^{-1}$). The concentration of 6.25 $\mu g \cdot ml^{-1}$ methanolic extract of *P. gymnospora* significantly ($p<0.05$) promoted more migration of cells when compared to the negative control group (Figure 2). *P. gymnospora* showed an anticoagulant activity being glucuronofucan apparently responsible for this activity (SILVA et. al.; 2005, NADER et al., 2001; HAROUN-BOUHEDJA, 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) (MAXSON et al., 2012; GUO and DIPIETRO, 2010). 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 $\mu g \cdot ml^{-1}$ (77.5%); moreover, ampicillin presented an inhibition of 90% at a concentration of 1000 $\mu g \cdot ml^{-1}$. ALIGIANNIS et al. (2001) classified extracts with inhibitory activity in concentrations $\leq$ 500 $\mu g \cdot ml^{-1}$ 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 8.0 $mg \cdot ml^{-1}$ and greater than 2.0 $mg \cdot ml^{-1}$. 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 $\mu g \cdot ml^{-1}$ 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 11 organic compounds between then oleic, linolenic and linoleic, arachidonic acids (Figure 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 Figure 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, 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 *Escherichia coli* (NCMB 11943), *Pseudomonas aeruginosa* (NCMB 8295), *Salmonella typhimurium* (NCMB 74), *Shigella boydii* (ATCC 9207), *Staphylococcus aureus* (NCMB 6571) and actinobacteria *Streptomyces 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, 2010). This acid is 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 *Padina 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 $\mu g \cdot ml^{-1}$) confers a synergistic therapeutic advantage to *Padina gymnospora*. However, further investigations should be conducted to elucidate the mechanisms of action of the *Padina gymnospora* extract, expanding the knowledge of the genre.

AKNOWLEDGMENTS

The authors thank FAPES (TO 550/2015) and CNPq (401409/2014-7 and 472141/2013-5) for their financial support. CNPq, CAPES, FAPES and UVV are acknowledged for the fellowship.

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.

REFERENCES

ADETUTU. A.; MORGAN, W.A.; CORCORAN, O. Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extracts of *Bridelia ferruginea* leaf, a wound-healing plant of Nigeria. **Journal of Ethnopharmacology**. v. 133, p. 116-119, 2011.

ALIGIANNIS. N.; KALPOUTZAKIS, E.; MITAKU, S.; CHINOU, I.B. Composition and antimicrobial activity of the essential oils of two Origanum species. **Journal of Agricultural and Food Chemistry**. v. 40, p. 4168-4170, 2001.

ATHUKORALA. Y.; KIM, K.N.; JEON, Y.L. South Korea Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. **Food and Chemical Toxicology**. v. 44, p. 1065-1074, 2006.

AWAD. N.E.; SELIM, M.A.; METAWE, H.M.; MATLOUB, A.A. Cytotoxic Xenicane Diterpenes from the Brown Alga *Padina pavonia* (L.) Gaill. **Phytotherapy Research**. v. 22, p. 1610-1613, 2008.

BARBOSA. S.O.; FIGUEIREDO, M.A.G.; TESTA, V. Estrutura e dinâmica de comunidades betonicas dominadas por macrofitas na zona intramareal da Praia de Jacaraipe, Espirito Santo. Brasil. (Structure and dinamic of bentonics communities dominated by by macrophytes in the intertidal zone of Jaracaipe beach, Espirito Santo, Brazil.). **Hoehnea**. v. 35, p. 563-575, 2008.

BITENCOURT. M.A.O.; SILVA, H.M.D.; ABÍLIO, G.M.F.; MIRANDA, G.E.C.; MOURA, A.M.A. Anti-inflammatory effects of methanolic extract of green algae *Caulerpa mexicana* in a murine model of ulcerative colitis. 2015. **Revista Brasileira de Farmacognosia**. v. 25, p. 677-682, 2015.

DAWCZYNSKI. C.; RAINER, S.; JAHREIS, G. Amino acids, fatty acids, and dietary fiber in edible seaweed products. **Food Chemistry**. v. 103, p. 891-899, 2007.

DELGADO. N.G.; VAZQUEZ, A.I.; SANCHEZ, C.H.; SOTO DEL VALLE, R.M.; GOMEZ, Y.S.; ALFONSO, A.M.S. Anti-inflammatory and antinociceptive activities of

methanolic extract from red seaweed *Dichotomaria obtusata*. **Brazilian Journal of Pharmaceutical Sciences**. v. 49, p. 65-74, 2013.

DUSSAULT. D.; VU, K.D.; VANSACH, T.; DAVID, H.F.; LACROIX, M. Antimicrobial effects of marine algal extracts and cyanobacterial pure compounds against five foodborne pathogens. **Food Chemistry**. v. 199, p. 114-118, 2015.

El SHOUBAKY. G.A.; El RAHMAN SALEM, E.A. Active ingredients fatty acids as antibacterial agent from the brown algae *Padina pavonica* and Hormophysa triquetra. **Journal of Coastal Life Medicine**. v. 2, p. 535-542, 2014. Doi:10.12980/JCLM.2.2014JCLM-2014-0025.

FRONZA. M.; HEINZMANN, B.; HAMBURGER, M.; LAUFER, S.; MERFORT, I. Determination of the wound healing effect of Calendula extracts using the scratch assay with 3T3 fibroblasts. **Journal of Ethnopharmacology**. v. 126, p. 463-467, 2009.

GHASEMZADEH-MOGHADDAM, H.; BELKUM, A.; HAMAR, R.A.; WAMEL, W.; NEELA, V. Methicillin-susceptible and -resistant *Staphylococcus aureus* with high-level antiseptic and low-level mupirocin resistance in Malaysia. **Microbial Drug Resistance**. v. 20, p. 472-477, 2014.

GOULD. L.; ABADIR, P.; BREM, H.; CARTER, M.; CONNER-KERR, T.; DAVIDSON, J.; DI PIETRO, L.; FALANGA, V.; FIFE, C.; GARDNER, S.; GRICE, E.; HARMON, J.; HAZZARD, W.R.; HIGH, K.P.; HOUGHTON, P.; JACOBSON, N.; KIRSNER, R.S.; KOVACS, E.J.; MARGOLIS, D.; HORNE, F.F.; REED, M.J.; SULLIVAN, D.H.; THOM, S.; CANIC, M.T.; WALSTON, J.; WHITNEY, J.A.; WILLIAMS, J.; ZIEMAN, S.; SCHMADER, K. Chronic Wound Repair and Healing in Older Adults: Current Status and Future Research. **Wound Repair and Regeneration**. v. 63, p.427-438, 2015.

JASWIR. I.; NOVIENDRI, D.; SALLEH, H.M.; TAHER, M.; MIYASHITA, K. Isolation of fucoxanthin and fatty acids analysis of *Padina australis* and cytotoxic effect of fucoxanthin

on human lung cancer (H1299) cell lines. **African Journal of Biotechnology**. v. 10, p. 18855-18862, 2011.

GOLDMAN. E.; GREEN, L.H. Practical Handbook of Microbiology. New York: **Boca Raton**, 2002.

GUARATINI. T.; LOPES, N.P.; MARINHO-SORIANO, E.; COLEPICOLO, P.; PINTO, E. Antioxidant activity and chemical composition of the non polar fraction of *Gracilaria domingensis* (Kützing) *Sonder ex Dickie* and *Gracilaria birdiae*. **Revista Brasileira de Farmacognosia.** v. 22, p. 724-729, 2012.

GUO. S.; DIPIETRO, L.A. Factors affecting wound healing. **Journal of Dental Research.** v. 89, p. 219-229, 2010.

GUPTA. S.; ABU-GHANNA, N. Bioactive potential and possible health effects of edible brown seaweeds. **Trends in Food Science & Techology**. p. 1-12, 2011. Doi:10.1016/j.tifs.2011.03.011

KHANAVI. M.; NABAVI, M.; SADATI, N.; ARDEKANI, M.S.; SOHRABIPOUR, J.; NABAVI, S.M.B.N.; GHAELI, P.; OSTAD, S.N. Cytotoxic activity of some marine brown algae against cancer cell lines. **Biological Research**. v. 43, p. 31-37, 2010.

HATANAKA. E.; CURI, R. Ácidos graxos e cicatrização: uma revisão. (Fat acids and healing: a review). **Revista Brasileira de Farmacognosia**. v. 88, p. 53-58, 2007.

Henshaw, F.R.; Boughton, P.; Lo, L.; McLennan, S.V.; Twigg, S.M. Topically Applied Connective Tissue Growth Factor/CCN2 Improves Diabetic Preclinical Cutaneous Wound Healing: Potential Role for CTGF in Human Diabetic Foot Ulcer Healing. **Journal of Diabetes Research**. p. 1-10, 2015. Doi: 10.1155/2015/236238

HAROUN-BOUHEDJA. F.; MOUSTAFA, E.; SINQUIN, C.; BOISSON-VIDAL, C. Relation between sulfate groups and biological activities of fucans. **Thrombosis Research**. v. 100, p. 453-459, 2000.

KHAN. M.N.; LEE, M.C.; KANG, J.Y.; PARK, N.G.; FUJII, H.; HONG, Y.K. Effects of the brown seaweed *Undaria pinnatifida* on erythematous inflammation assessed using digital photo analysis. **Phytotherapy Research**. v. 22, p. 634-639, 2008.

KHANAVI. M.; NABAVI, M.; SADATI, N.; ARDEKANI, M.S.; SOHRABIPOUR, J.; NABAVI, S.M.B.; GHAELI, P.; OSTAD, S.N. Cytotoxic activity of some marine brown algae against cancer cell lines. **Biological Research.** v. 43, p. 31-37, 2010.

LIANG C.C.; PARK, A.Y.; GUAN, J.L. *In vitro* scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. **Nature Protocols**. v. 2, p. 329-333, 2007.

LIN. C.T.; CHEN, C.J.; LIN, T.Y.; TUNG, J.C.; WANG, S.Y. Anti-inflammation activity of fruit essential oil from *Cinnamomum insularimontanum hayata.* **Bioresource Technology**. v. 99, p. 8783-8787, 2008.

LUO. L.D.; CHEN, A.F. Nitric oxide: a newly discovered function on wound healing. **Acta Pharmacologica Sinica.** v. 26, p. 259-264, 2005.

MATANJUN. P.; MOHAMED, S.; MUSTAPHA, M.N.; MUHAMMAD, K. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii, Caulerpa lentillifera* and *Sargassum polycystum*. **Journal of Applied Phycology**. v. 1, p. 75-80, 2009.

MAXSON. S.; ERASMO, A.L.; YOO, D.; DANILKOVITCH-MIAGKOVA, A.; LEROUX, M., A. Concise Review: Role of Mesenchymal Stem Cells in Wound Repair**. Stem Cells Translational Medicine**. v. 1, p. 142-149, 2012. Doi: 10.5966/sctm.2011-0018.

McCUSKER. M.M.; GRAN-KELS, M.J. Healing fats of the skin: the structural and immunologic roles of the ω-6 and ω-3 fatty acids. **Clinics in Dermatology**. v. 28, p. 404-451, 2010.

MISIC. A.M.; GARDNER, S.E.; GRICE, E.A. The wound microbiome: Modern approaches to examining the role of microorganisms in impaired chronic wound healing. **Advances in Wound Care**. v. 3, p. 502-510, 2014.

MOSMANN. T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. **The Journal of Immunology Methods**. v. 65, p. 55-63, 1983.

MURUGAN. K.; IYER, V.V. Antioxidant activity and gas chromatographic-mass spectrometric analysis of extracts of the marine algae, *Caulerpa peltata* and *Padina gymnospora*. **Indian Journal of Pharmaceutical Sciences**. v. 76, p. 548-552, 2014.

NADER. H.B.; PINHAL, M.A.S.; BAÚ, E.C. Development of new heparin-like compounds and other antithrombotic drugs and their interaction with vascular endothelial cells. **Brazilian Journal of Medical and Biological Research**. v. 34, p. 699-709, 2001.

NIH. National institute of Health. Drugs that delay wound healing, **Prescrire international**. v. 137, p. 94-98, 2013. Available in: http://www.ncbi.nlm.nih.gov/pubmed/23662318, accessed Jun 2016.

PARK. E.J.; KONDRATYUK, T.P.; MORRELL, A.; KISELEV, E.; CONDA-SHERIDAN, M.; CUSHMAN, M.; AHN, S.; CHOI ,Y.; WHITE, J.J.; VAN BREEMEN, R.B.; PEZZUTO, J.M. Induction of retinoid X receptor activity and consequent upregulation of p21WAF1/CIP1 by indenoisoquinolines in MCF7 cells. **Cancer Prevention Research (Phila).** v. 4, p. 592-607, 2011.

REINKE. J.M.; SORG, H. Wound Repair and Regeneration. **European Surgical Research**. v. 49, p. 35-43, 2012.

ROHANI-GHADIKOLAEI. K.; ABDULALIAN, E.; WING-KEONG, N. Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. **Journal of Food Science and Technology**. v. 49, p. 774-780, 2012.

SÁNCHEZ-MACHADO, D.I.; LÓPEZ-CERVANTES, J.; LÓPEZ-HERNÁNDEZ, J.; PASEIRO-LOSADA, P. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. **Food Chemistry**. v. 85, p. 439-444, 2004.

SILVA. T.M.A.; ALVES, L.G.; QUEIROZ, K.C.S.; SANTOS, M.G.L.; MARQUES, C.T.; CHAVANTE, S.F.; ROCHA, H.A.O.; LEITE, E.L. Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. **Brazilian Journal of Medical and Biological Research**. v. 38, p. 523-533, 2005.

SHANAB S.M.M. Antioxidant and antibiotic activities of some seaweeds (Egyptian isolates). **International Journal of Agricultural and Biology**. v. 9, p. 220-225, 2007.

SURH. Y.J.; CHUNA, K.S.; CHAA, H.H.; HANA, S.S.; KEUMA, Y.S.; PARK, K.K.; LEE, S.S. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NFκB activation. **Mutation Research**. v. 480-481, p. 9243-9268, 2001.

TORRES. F.A.E.; PASSALACQUA, T.G.; VELÁSQUEZ, A.M.A.; SOUZA R.A.; COLEPICOLO, P.; GRAMINHA, M.A.S. New drugs with antiprotozoal activity from marine algae: a review. **Revista Brasileira de Farmacognosia**. v. 24, p. 3, 2014. Doi: http://dx.doi.org/10.1016/j.bjp.2014.07.001

YAVASOGLU. N.U.K.; YAVASOGLU, A.S.G.; HORZUM, Z. Antimicrobial Activity of Volatile Components and Various Extracts of the Red Alga *Jania rubens*. **Phytotheraphy Research**. v. 21, p. 153-156, 2007.

WAHBEH. M.I. Amino acid and fatty acid profiles of four species of macroalgae from Aqaba and their suitability for use in fish diets. **Aquaculture**. v. 159, p. 101-109, 1997.

WU. C.D.; KUO, Y.S.; WU, H.C.; LIN, C.T. MicroRNA-1 induces apoptosis by targeting prothymosin alpha in nasopharyngeal carcinoma cells. **Journal of Biomedical Science.** v. 18, p. 80, 2011.

XING. Q.; ZHANG, L.; REDMAN, T.; QI, S.; ZHAO, F. Nitric oxide regulates cell behavior on an interactive cell-derived extracellular matrix scaffold. **Journal of Biomedical Materials Research**. v. 103, p. 3807-3814, 2015.

FIGURE 1

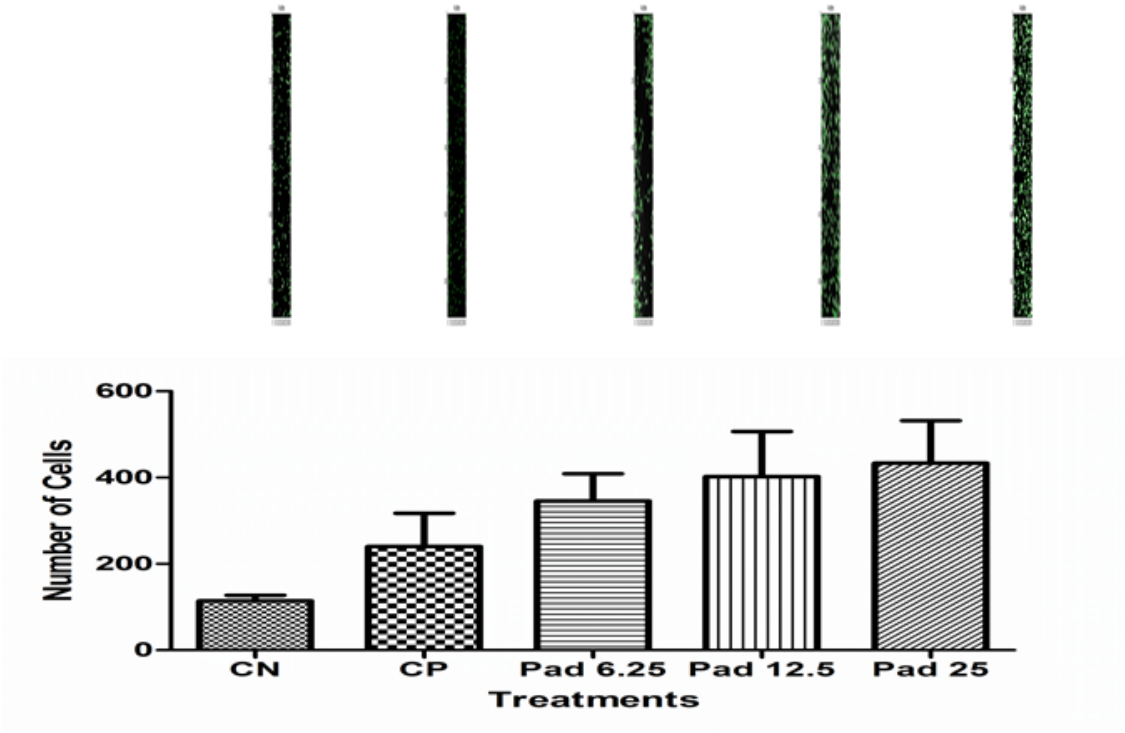

Figure 1. In vitro effect of methanolic extract of algae *Padina gymnospora* in cell migration and/or proliferation in fibroblasts L929. The fibrobl asts were cultivated until formed a monolayer and then the scratch were made to perform the assay. (A) Cell counting inside scratched areas. (B) Representative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

FIGURE 2

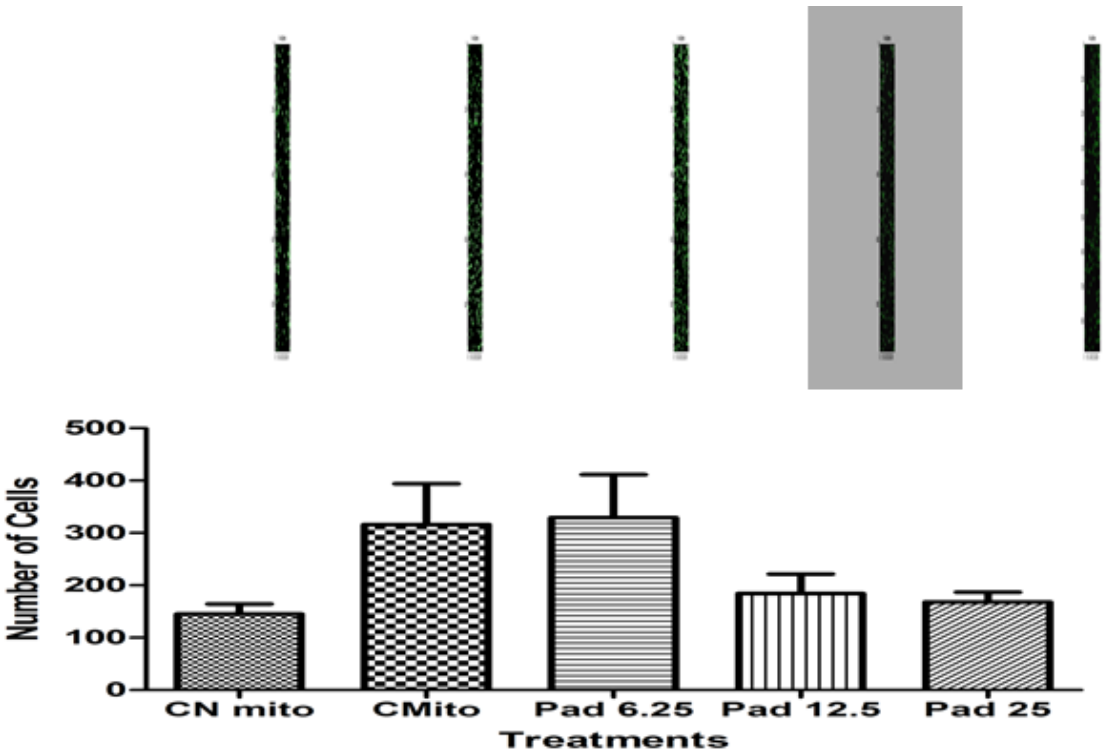

Figure 2: In vitro effect of methanolic extract of *Padina gymnospora* in cell migration in fibroblasts L929. The fibroblasts were cultivated until formed a monolayer and then the scratch were made to evaluate cell mig ration in the area. To analyse only the cell migratio n, all groups were added mitomycin 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 estimu lated by PDGF with mitomycin C, (PAD 6.25) group estimulated by extract *P. gymnospora* at 6.25 µg/ml, (PAD 12.5) estimulated by extract of *P. gymnospora* at 12.50 µg/ml and (PAD 25) estimulated by extract of *P. gymnospora* at 25.00 µg/ml.*($p<0.05$) Cell migrat ion into the wound area was quantified using Cellprofiler software.

FIGURE 3

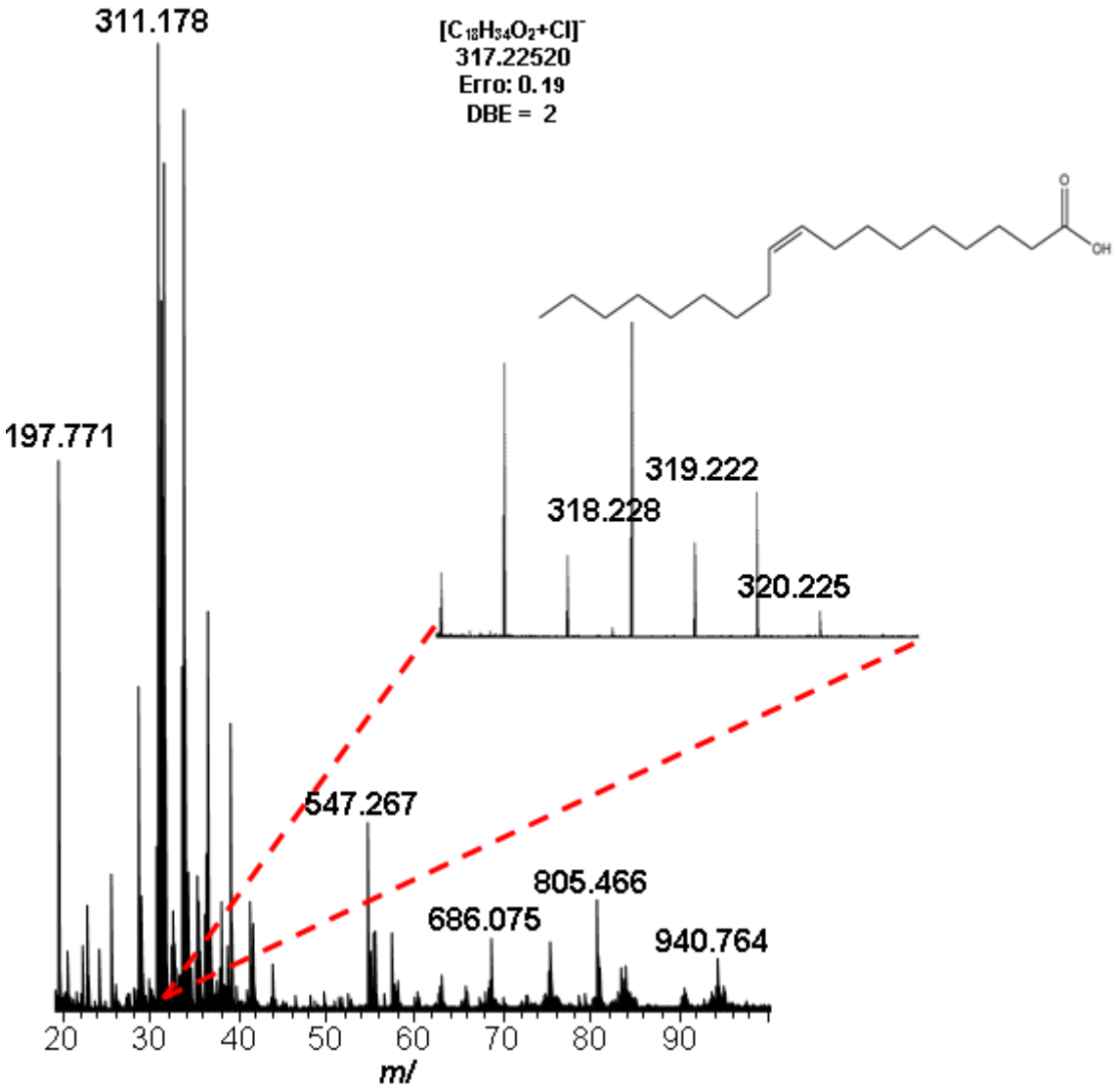
311.178
[C18H34O2+Cl]-
317.22520
Erro: 0.19
DBE = 2
197.771
319.222
318.228
320.225
547.267
805.466
686.075
940.764
20
30
40
50
60
70
80
90
m/

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

$m/z_{measured}$	$m/z_{theoretical}$	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 et al., 2014.
347.27224	347.27223	0.01	1	$[C_{20}H_{40}O_2+Cl]^-$	Eicosanolic acid	Shanab and 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	Jonnes et. al., 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.

.

ANEXO 1

Fwd: Your Submission - BJP-D-16-00117R1

Ms. Ref. No.: BJP-D-16-00117R1
Title: Brown seaweed Padina gymnospora is a prominent natural wound-care product
Revista Brasileira de Farmacognosia - Brazilian Journal of Pharmacognosy

Dear Prof. endringer,

I am pleased to inform you that your paper "Brown seaweed Padina gymnospora is a prominent natural wound-care product" has been accepted for publication in Revista Brasileira de Farmacognosia - Brazilian Journal of Pharmacognosy.

Below are comments from the editor and reviewers.

Thank you for submitting your work to Revista Brasileira de Farmacognosia - Brazilian Journal of Pharmacognosy.

Yours sincerely,

Maique Weber Biavatti
(Associate editor)
Revista Brasileira de Farmacognosia - Brazilian Journal of Pharmacognosy

Comments from the editors:

ANEXO 2

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Licença permanente para coleta de material zoológico

Número: 34342-1	Data da Emissão: 16/05/2012 17:34

Dados do titular

Nome: Diógina Barata	CPF: 079.232.047-64
Nome da Instituição : UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO	CNPJ: 32.479.123/0001-43

Observações e ressalvas

1	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
2	A licença permanente não é válida para: a) coleta ou transporte de espécies que constem nas listas oficiais de espécies ameaçadas de extinção; b) manutenção de espécimes de fauna silvestre em cativeiro; c) recebimento ou envio de material biológico ao exterior; e d) realização de pesquisa em unidade de conservação federal ou em caverna. A restrição prevista no item d não se aplica às categorias Reserva Particular do Patrimônio Natural, Área de Relevante Interesse Ecológico e Área de Proteção Ambiental constituídas por terras privadas.
3	O pesquisador titular da licença permanente, quando acompanhado, deverá registrar a expedição de campo no Sisbio e informar o nome e CPF dos membros da sua equipe, bem como dados da expedição, que constarão no comprovante de registro de expedição para eventual apresentação à fiscalização;
4	Esta licença permanente NÃO exime o pesquisador titular da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal.
5	Esta licença permanente não poderá ser utilizada para fins comerciais, industriais ou esportivos ou para realização de atividades integrantes do processo de licenciamento ambiental de empreendimentos.
6	Este documento NÃO exime o pesquisador titular da necessidade de atender ao disposto na Instrução Normativa Ibama nº 27/2002, que regulamenta o Sistema Nacional de Anilhamento de Aves Silvestres.
7	O pesquisador titular da licença permanente será responsável pelos atos dos membros da equipe (quando for o caso)
8	O órgão gestor de unidade de conservação estadual, distrital ou municipal poderá, a despeito da licença permanente e das autorizações concedidas pelo ICMBio, estabelecer outras condições para a realização de pesquisa nessas unidades de conservação.
9	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
10	O titular da licença permanente deverá apresentar, anualmente, relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias após o aniversário de emissão da licença permanente.
11	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio e o material biológico coletado apreendido nos termos da legislação brasileira em vigor.
12	A licença permanente será válida enquanto durar o vínculo empregatício do pesquisador com a instituição científica a qual ele estava vinculado por ocasião da solicitação.
13	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.

Táxons autorizados

#	Nível taxonômico	Táxon(s)
1	FILO	Rhodophyta, Ochrophyta, Chlorophyta
2		

Destino do material biológico coletado

#	Nome local destino	Tipo Destino
1	UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO	coleção

Este documento (Licença permanente para coleta de material zoológico) foi expedido com base na Instrução Normativa nº154/2007. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 22193665

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Licença permanente para coleta de material zoológico

Número: 34342-1	Data da Emissão: 16/05/2012 17:34

Dados do titular

Nome: Diógina Barata	CPF: 079.232.047-64
Nome da Instituição : UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO	CNPJ: 32.479.123/0001-43

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº154/2007, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de amostra	Qtde.	Data

* Identificar o espécime no nível taxonômico possível.

Este documento (Licença permanente para coleta de material zoológico) foi expedido com base na Instrução Normativa nº154/2007. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 22193665