UNIVERSIDADE VILA VELHA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

ATIVIDADE ANTIOXIDANTE E ANTI-INFLAMATÓRIA *in vitro* DE PLANTAS ALIMENTÍCIAS NÃO CONVENCIONAIS

MARIA CAROLINA OLIVEIRA PEISINO

VILA VELHA

OUTUBRO / 2018

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Dissertação apresentada à Universidade Vila Velha como pré-requisito do Programa de Pós-graduação em Ciências Farmacêuticas, para obtenção do grau de Mestra em Ciências Farmacêuticas.

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Aprovada em 05 de outubro de 2018,

Banca Examinadora:

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Prof. Dr. Rodrigo Scherer (UVV)

Prof. Dr. Marcio Fronza (UVV)
Orientador

Dedico essa dissertação aos meus pais Joaquim e Marli, minha amada filha Ana Luisa e todos aqueles que acreditaram em mim, em especial meu orientador Marcio Fronza.

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Com carinho,

Maria Carolina O. Peisino.

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RESUMO

O consumo de plantas alimentícias não convencionais (PANCS) vem sendo estimulado devido ao seu crescimento espontâneo, livre de agrotóxicos, além da atual preocupação mundial em relação à promoção da saúde e prevenção de doenças por meio de uma alimentação saudável. O objetivo deste estudo foi investigar o perfil fitoquímico e o potencial bioativo das PANCS *Hypochaeris chillensis* (HC)*, Emília fosbergii* (EF) e *Emília sonchifolia* (ES) em relação às atividades antioxidante e anti-inflamatória *in vitro.* Os efeitos dos extratos hexânicos e metanólicos das folhas foram investigados por meio de métodos químicos e em cultura de células. Os efeitos citotóxicos foram avaliados pelo método do MTT (brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio). A atividade antioxidante foi investigada pelos métodos do DPPH, ABTS e FRAP e em cultura de células pelo método do superóxido ($O_2^{\bullet-}$) e peróxido (H_2O_2). Também em cultura de células foi avaliada sua influência sobre a produção de óxido nítrico, por meio da dosagem indireta de nitrito pelo método de Griess; a quantificação de citocinas inflamatórias pela técnica de ELISA e a determinação da atividade do NF-kB. A análise fitoquímica pelo método do LC-MS/MS revelou quantidade significativa de compostos fenólicos e a determinação de polifenóis totais, pelo método Folin-Ciocalteau, revelou significativa quantidade de fenólicos nos extratos metanólicos das três plantas, sendo o quantitativo reduzido nos extratos hexânicos. A EF apresentou maior capacidade antioxidante nos ensaios químicos, com um IC_{50} de 32.9 ± 4.8, 50.2 ± 2.3 and 24.0 ± 2.9 µg mL^{-1}, nos ensaios do ABTS, DPPH e FRAP, respectivamente. Os extratos não foram citotóxicos em nenhuma das linhagens testadas. As plantas reduziram significativamente a produção de NO e $O_2^{\bullet-}$ em macrófagos estimulados com LPS. Além disso, as PANCS reduziram a produção TNF-α e IL-6 de maneira dose-dependente. O extrato hexânico de ES apresentou 90% de inibição sobre a atividade do fator de transcrição nuclear kappa B. As PANCS apresentaram promissoras atividades bioativas, atuando no controle de radicais livres, estresse oxidativo e no controle de importantes mediadores inflamatórios. Os resultados deste estudo motivam a constante busca por produtos naturais bioativos.

Palavras chaves: plantas alimentícias não convencionais; produtos naturais; estresse oxidativo; macrófagos; inflamação.

PEISINO, MARIA CAROLINA OLIVEIRA, M.Sc, Universidade Vila Velha-ES, october of 2018. ***In vitro* antioxidant and anti-Inflammatory activities of unconventional food plants**. Advisor: Marcio Fronza.

ABSTRACT

The consumption of unconventional food plants (UFPs) has been stimulated by its spontaneous growth, free of pesticides, in addition to being present in the world in relation to health promotion and disease prevention through healthy eating. The objective of this study was to investigate the phytochemical profile and bioactive potential of PANCS *Hypochaeris chillensis* (HC), *Emília fosbergii* (EF) and *Emília sonchifolia* (ES) in relation to antioxidant and anti-inflammatory activities *in vitro*. The effects of the hexane and methanolic extracts from the leaves were investigated by chemical methods and in cell culture. Cytotoxic effects were evaluated by the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The antioxidant activity was investigated by the DPPH, ABTS and FRAP methods and in cell culture by superoxide ($O_2^{\bullet-}$) and peroxide (H_2O_2). Also in cell culture was evaluated its influence on the production of nitric oxide, through the indirect dosage of nitrite by the Griess method; quantification of inflammatory cytokines by the ELISA technique and determination of NF-kB activity. Phytochemical analysis by the LC-MS/MS method revealed a significant amount of phenolic compounds and the determination of total polyphenols by the Folin-Ciocalteau method revealed a significant amount of phenolics in the methanolic extracts of the three plants, being the quantitative reduced in the hexane extracts. EF presented higher antioxidant capacity in the chemical assays, with an IC_{50} of 32.9 ± 4.8, 50.2 ± 2.3 and 24.0 ± 2.9 μg mL^{-1}, respectively, in the ABTS, DPPH and FRAP assays, respectively. The extracts were not cytotoxic in any of the tested strains. Plants significantly reduced NO and O2 production in LPS-stimulated macrophages. In addition, UFPs reduced TNF-α and IL-6 production in a dose-dependent manner. ES hexanic extract showed 90% inhibition on the activity of nuclear transcription factor kappa B. UFPs presented promising bioactive activities, acting in the control of free radicals, oxidative stress and in the control of important inflammatory mediators. The results of this study motivate the constant search for bioactive natural products.

Keywords: unconventional food plants; natural products; oxidative stress; macrophages; inflammation.

Artigo Científico

Manuscrito em revisão na revista *Food Research International*

In vitro antioxidant and anti-Inflammatory activities of unconventional food plants

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Short Title

Health benefits of unconventional food plants

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ABSTRACT

This study aimed to investigate the *in vitro* antioxidant and anti-inflammatory activities of the Brazilian unconventional food plants (UFPs) *Hypochaeris chillensis*, *Emília fosbergii* and *Emilia sonchifolia*. Phytochemical analysis by LC-MS/MS analysis revealed significant total phenolic contents in all of the studied plants. *E. fosbergii* showed the highest antioxidant capacity in all of the chemical assays, exhibiting IC_{50} values of 32.9 ± 4.8, 50.2 ± 2.3 and 24.0 ± 2.9 μg mL^{-1} in the ABTS, DPPH and FRAP assays, respectively. The studied UFPs showed a significant intracellular reduction in nitric oxide (NO) and superoxide anion ($O_2^{\bullet-}$) production in LPS-stimulated RAW 264.7 macrophages. Additionally, UFPs suppressed the production of the pro-inflammatory cytokines TNF-α and IL-6 in a dose-dependent manner. Moreover, *E. sonchifolia* suppressed NF-κB activity. Altogether, the investigated UFPs exhibited promising bioactive compounds that were capable of neutralizing free radicals, controlling oxidative stress and modulating the inflammatory process.

Keywords: Unconventional food plants; natural products; oxidative stress; macrophages; inflammation.

HIGHLIGHTS

Unconventional food plants (UFPs) grow spontaneously and are important food sources.

The UFPs exhibited potential bioactive compounds.

The UFPs attenuated inflammation and oxidative stress *in vitro.*

The UFPs demonstrated health-promoting properties.

ABBREVIATIONS

ABTS – 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

DPPH – 2,2-Diphenyl-1-picrylhydrazyl

ELISA - Enzyme-linked immunosorbent assay

FRAP – Ferric reducing antioxidant power

IL – Interleukin

iNOS – Nitric oxide synthase

LPS – Lipopolysaccharide

MTT - 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

NF-kB - Nuclear transcription factor kappa B

NO – Nitric oxide

$O_2^{\bullet-}$ - Superoxide anion

UFPs – Unconventional food plants

ROS – Reactive oxygen species

TNFα - Tumour necrosis factor alpha

1. Introduction

Brazil has the highest biodiversity in the world, and this abundant variety of life translates into more than 20% of the total number of species on Earth (Brazil, 2018). Although the biodiversity of Brazilian flora is widely known, only a small part of it has been explored, meaning that it offers unprecedented opportunities for the discovery of edible plant tissues rich in nutrients and bioactive compounds (Jolly et al., 2011). It is known that the world population has based its food on more than ten thousand species over the course of millennia; however, there are currently fewer than one hundred and fifty species being cultivated. Of these, only 12 species comprise approximately 80% of all of our food needs (FAO, 2004). In this sense, many plant species that were widely consumed in the past are no longer consumed and are considered ruderal, being known as unconventional food plants (UFPs) (Kinupp & Barros, 2007). In the last decade, the consumption of these plants has increased. They grow spontaneously and free of chemical additives, and some studies have shown higher nutritional value, often more significant compared to other common food plants (Kinupp & Barros, 2008; Takeiti et al., 2009; Verdi, 2005).

Some UFPs have been used for medicinal purposes such as for wound treatment and infectious and inflammatory disease treatments (Teixeira & Mello, 2006). However, few studies have explored their bioactive potential (Gilcy & Kuttan, 2016; Nworu et al., 2012). Among potential UFPs, we can highlight *Hypochaeris chillensis* (Asteraceae), *Emília fosbergii* (Asteraceae), and *Emília sonchifolia* (Asteraceae), popularly known as radite, red serralhinha and purple serralhinha, respectively. The nutritional and medicinal use of these plants, especially their leaves, is of great interest to the food and pharmaceutical industry (Coutinho et al., 2009; Del Ré & Jorge, 2012).

Overfeeding and increased caloric intake exert deleterious effects on health and have remarkable abilities to induce oxidative damage (Burneiko et al., 2006; Tardido & Falcão, 2006; Zimmet & Thomas, 2003). Oxidative stress and the production of free radicals, especially reactive oxygen species (ROS), such as hydroxyl radical (OH), superoxide anion ($O_2^{\bullet-}$) and hydroperoxyl (ROO^-) (El-Agamey et al., 2004), have been associated with the development of many chronic and degenerative diseases, including atherosclerosis, heart disease, diabetes, obesity, neurodegenerative disorders and cancer (Banfi et al., 2008; Gottlieb et al., 2011; Green et al., 2004; Matés

et al., 1999). Their mechanism involves the oxidation of lipids and proteins, which damage cell membranes, leading to DNA damage (Roesler et al., 2007).

Overfeeding also may lead to obesity, which is considered a chronic inflammation state and directly related to alterations in endocrine and metabolic functions in the adipose tissue (Fonseca-Alaniz et al., 2006). In these individuals, tissues increase the synthesis of pro-inflammatory molecules called adipokines (Trayhurn, 2007), such as interleukin 6 (IL-6), tumour necrosis factor-alpha (TNF-α), leptin and adiponectin, which have been receiving special attention in the literature (Prado et al., 2009; Santos et al., 2016). Although the inflammatory response is considered a body defence mechanism, when it becomes exacerbated or chronic it can also lead to health damage through a cascade of biochemical events (Fullerton & Gilroy, 2016; Greaves et al., 2013). The mediators of the inflammatory response are varied, derive from plasma and cellular precursors and can be classified into vasoactive amines, vasoactive peptides, complement system cleavage products, lipid mediators, cytokines, chemokines and proteolytic enzymes. They can react as anti- or pro-inflammatory, and the knowledge of their action mechanisms is fundamental for stimulating or controlling the response (Cruvinel et al., 2010, Kopf et al., 2010).

Although food is not considered a remedy, it is believed that the consumption of functional foods that have bioactive compounds, such as phenolic compounds that mainly function in the body as antioxidants and anti-inflammatory agents, is considered important to promote some favourable health effects and reduce the risk of chronic non-communicable diseases (Bastos et al., 2009). In general, evidence has shown that dietary habits are one of the main factors for controlling or stimulating inflammation and oxidative stress in organisms (Barbosa et al., 2010).

Natural products that can neutralise or reduce these processes have been studied (Leal et al., 2018). Furthermore, foods that, in addition to their basic nutritional functions, produce health benefits are considered functional foods (Moraes & Colla, 2006; Souza et al., 2003). Therefore, to stimulate the consumption of nutritionally rich vegetables that are free of chemical additives, the objective of this study was to investigate the phytochemical profiles together with the antioxidant and anti-inflammatory *in vitro* activities of the UFPs *Hypochaeris chillensis*, *Emília fosbergii* and *Emília sonchifolia* via chemical and cell-based assays.

2. Materials and methods

2.1. Collection and identification of plant material

Hypochaeris chillensis leaves was collected in the Tucunduva city, South Brazil in January 2017 (Latitude -27.634394 and Longitude -54.408549) and *Emília sonchifolia* and *Emília fosbergii* leaves were collected in the Vila Velha city, Espírito Santo, Brazil (geographic coordinates: -20.387386, -40.319482). The plants were taxonomically identified by botanist Ms. Solange Zanotti Schneider, and a voucher specimen was deposited in the herbarium at the University Vila Velha/UVV (as UVVES-2397, UVVES-2398 and UVVES-2399).

2.2. Extraction

The leaves from each UFP species was air-dried in an oven at 50 ℃ to constant weight. Dried samples from each plant were pulverised by blending to a fine homogeneous powder prior to extraction. Ten grams of pulverised plant material was first impregnated in 100 mL of hexane and placed in an ultrasonic bath at 35 kHz frequency and 600 W power (Ultra Sonic Cleaner Unique Ultra 1440) for 30 min at room temperature. This procedure was repeated three times. Subsequently, the same material was submitted to the same procedure using methanol. The respective obtained extracts were filtrated, the fractions were combined, and the solvents were removed under a vacuum at 40 ℃ (Fisaton 801, São Paulo, Brazil). For the complete removal of the solvents from the extracts, they were subjected to lyophilization (Terroni Lyophilizer, Enterprise I) and then stored in a freezer at -20 ℃ until use.

2.3. Chemical and biochemical

Immunoenzymatic assay kits (ELISA) for the detection of IL-6 and TNF-α were obtained from eBioscience (San Diego, CA, USA). The Dual-Luciferase® Reporter Assay System 10-Pack was obtained from Promega Corporation (USA). Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4, N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME), tetrazolium nitroblue chloride (NBT), gallic acid, Np-Tosyl-L-phenylalanine chloromethylketone (TPCK), 2,2'-azino (ABTS), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) and 3-(4,5-dimethylthiazol-2-yl bromide)-2,5-diphenyltetrazolium (MTT) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and were obtained from several commercial sources.

2.4. Cell lines

L929 fibroblasts (American Type Culture Collection - ATCC® CCL-92™), RAW 264.7 murine macrophages (ATCC® TIB-71™), and Human embryonic renal cells (293.12-PTA-5554) transfected with a luciferase-expressing gene (Panomic, Fremont, CA, USA) (retroviruses) were purchased from the Cell Bank of Rio de Janeiro, Brazil. Cells were cultured in Dulbecco's-modified eagle culture medium (Sigma Aldrich® Chemical Co., St. Louis, MO, USA) or modified culture medium (DMEM - Gibco-BRL Life Biotechnologies, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS-Sigma Aldrich® Chemical Co., St. Louis, MO, USA), 100 IU mL^{-1} penicillin and 100 µg mL^{-1} streptomycin (Sigma Aldrich® Chemical Co., St. Louis, MO, USA) at 37 ° C in a humidified atmosphere containing 5% CO_2.

2.5. Determination of total phenolic content

The total phenolic content in the leaves extracts was estimated by a spectrophotometric Folin-Ciocalteu reagent assay according to Krepsky et al. (2012). An analytical curve was prepared with gallic acid (3.125 - 37.5 µg mL^{-1}). The absorbance was determined using a spectrophotometer at 715 nm. Three experiments were performed in duplicate, and the results were expressed as the mean ± standard deviation. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of crude extract.

2.6. LC-DAD-QTRAP analysis

The analyses of the methanolic extracts from the leaves of *H. chillensis, E. fosbergii*, and *E. sonchifolia* were performed using a UHPLC Dionex Focused Ultimate 3000 equipped with a DAD detector coupled with an LCQ Fleet Ion Trap Mass Spectrometer ThermoFisher Scientific, (Waltham, MA, USA). The chromatography conditions included an Ascentis Express C18 column (100 mm x 4.6 mm; 2.7-µm particle size) Supelco (Bellefonte, PA, USA). The eluents were 0.1% formic acid solution (A) and methanol (B) as the mobile phase at a flow rate of 0.5 mL min^{-1} and a temperature of 35 °C. The mobile phase elution was performed with a gradient mode as follows: 0 - 50 min (10 - 100% of B); 50 - 55 min (100% of B), and 55 - 60 min (10% of B). A C18 guard column (5 mm × 4.6 mm i.d., 2.7 µm particle size, Supelco (Bellefonte, PA, USA) was used to protect the analytical column. The mass spectrometry parameters were

as follows: capillary voltage 36 V, nebulization 5.5 Bar, drying gas flow 10 L min^{-1}, drying temperature 250 °C, and a collision energy of 35 eV. Signal acquisition was performed in positive ion mode at m/z between 100 to 1000 and MS2 of the base peaks ions. The results were treated using the XCalibur software ThermoFisher Scientific (Waltham, MA, USA) to obtain the chromatograms, mass and UV spectra. The samples were prepared at 2 mg mL^{-1} in HPLC grade methanol from Tedia (Fairfield, OH, USA), and 3 µL was injected.

2.7. Chemical tests for antioxidant activity

2.7.1. DPPH radical scavenging assay

The hydrogen atom donation ability of the chemical compounds in the leaves extracts was measured based on their ability to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical (DPPH) according to Scherer and Godoy (2009). Seven dilutions of the respective UFP extracts (30 µL) were prepared and mixed with 170 µL of DPPH (150 mM). After 30 min of incubation protected from light, the absorbance was measured at 517 nm using a microplate reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices Spectra, USA). The antioxidant activity was expressed as an IC_{50} value (µg mL^{-1}) obtained from three independent experiments (n=3) in triplicate.

2.7.2. Determination of ABTS cation radical scavenging assay

The $ABTS^{\bullet+}$ radical cation working solution was mixed with plant extracts at different concentrations (1.0 - 1000.0 µg mL^{-1}). After incubation for 10 min, the absorbance was measured at 734 nm using a microplate reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices Spectra, USA). The results were expressed as IC_{50} values (µg mL^{-1}) obtained from three independent experiments performed in triplicate (Re et al., 1999)

2.7.3. Ferric reducing antioxidant power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) test is based on the production of Fe^{2+} ion (ferrous form) from the reduction of Fe^{3+} ion present in the complex 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) (Antolovich et al., 2002; Benzie & Strain, 1996). The FRAP solution was mixed with different concentrations of plant extract samples and

incubated for 10 minutes. Afterwards, the optical density was measured at 595 nm using a Microplate Reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices Spectra, USA). Three experiments were performed in triplicate, and the results were expressed as IC_{50} values (µg mL^{-1}).

2.8. *In vitro* cytotoxic activity

The cytotoxic activity was investigated using the MTT colorimetric method proposed by Mosmann (1983). This method allows the determination of the mitochondrial functionality of untreated cells and has been widely used to measure cell survival; the number of living cells is directly correlated to the amount of formazan produced. L929 fibroblasts, and RAW 264.7 macrophages cells were incubated with different concentrations of samples for 24 h at 37 ºC in a 5% CO_2 atmosphere. Afterwards, the culture was incubated with MTT for 2 h, and then, 100 µL of dimethyl sulphoxide was added to dissolve the formazan crystals. The optical density was measured at 595 nm using a microplate reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices Spectra, USA). The experiments were performed in triplicate, and data are representative of at least two independent experiments.

2.9. *In vitro* biological assays for antioxidant activity

2.9.1. Reduction of superoxide anion production ($O_2^{\bullet-}$)

The superoxide assay was used to determine the inhibitory effect of the extracts on the production of superoxide radical ($O_2^{\bullet-}$) in LPS-activated RAW 264.7 macrophage culture (Choi et al., 2006; Pinho et al., 2011). Briefly, RAW 264.7 macrophages were plated at 2 x 10^5 cells mL^{-1} in 96-well plates and cultured for 24 h. Cells were then treated with different concentrations of the extracts, stimulated with 1 µg mL^{-1} LPS and incubated for 20 h. Gallic acid was used as a positive control. After incubation, the supernatant was removed, and 100 µL of tetrazolium nitrobenzyl chloride (NBT) (1 mg mL^{-1}) was added to each well for 2 h. After 2 h of incubation, the cells were washed with methanol and incubated for 20 min at 37 ºC to dry. After the 20 min incubation period, the formed formazan crystals were dissolved with dimethyl sulphoxide and 2 M potassium hydroxide (KOH). The optical density was determined at 630 nm using a microplate reader (Mults-Mode Microplate Reader, Filter Max F5, Molecular Devices

Spectra, USA). The experiments were performed in triplicate, and data are representative of at least two independent experiments.

2.9.2. Test with hydrogen peroxide (H_2O_2)

The protective effect of the extracts against the oxidative damage/toxicity of hydrogen peroxide was evaluated with a modified hydrogen peroxide assay (Adetutu et al., 2011; Annan & Houghton, 2008) using RAW 264.7 macrophages. Briefly, cells were plated and cultured overnight. After incubation, cells were exposed to H_2O_2 (500 µM) followed by the addition of different sample concentrations and incubated for 24 h. Gallic acid was used as the positive control. After incubation, the cellular viability was evaluated by the colorimetric MTT method. The optical density was measured at 595 nm using a microplate reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices Spectra, USA). The experiments were performed in duplicate, and data are representative of at least two independent experiments.

2.10. Evaluation of anti-inflammatory activity

2.10.1. Determination of nitric oxide *in vitro*

Quantification of nitric oxide was performed by determining the influence on the production of nitric oxide in LPS-activated RAW 264.7 macrophages. Macrophages were plated in 96-well cell culture plates and incubated until 70-80% confluence. Cells were exposed to different sample concentrations 60 min prior to the addition of lipopolysaccharides (LPS) (1 µg mL^{-1}). After 24 h, the cell supernatant was used to quantify nitrite concentrations, whereby the sample was mixed with the same volume of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water, 1: 1), and the nitrite concentration was calculated by linear regression using a sodium nitrite standard solution (Green et al., 1982). The absorbance was measured at 540 nm for nitrite quantification using a microplate reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices Spectra, USA). The experiments were performed in triplicate, and data are representative of at least two independent experiments.

2.10.2. Determination of cytokines *in vitro*

RAW 264.7 macrophages were plated in 96-well cell culture plates and incubated until 70-80% confluence. Cells were exposed to different sample concentrations 60 min prior to the addition of lipopolysaccharides (LPS) (1 µg mL^{-1}). After 24 h, the cellular supernatant was used for the quantification of IL-6 and TNF-α employing an enzyme-linked immunosorbent assay (ELISA). Cytokines were detected using specific antibodies (purified and biotinylated) and cytokine standards according to the manufacturer's instructions (eBioscience®). The optical density was measured at 450 nm in a microplate reader spectrophotometer. The cytokine levels were expressed in pg, and the sensitivities were >10 pg mL^{-1}. The experiments were performed in triplicate, and data are representative of at least two independent experiments.

2.10.3. Determination of nuclear factor kappa B activity

The determination of nuclear factor kappa B (NF-kB) activity was performed to evaluate the ability of the extracts to inhibit NF-kB using luciferase expression (Homhual et al., 2006; Kondratyuk et al., 2012; Morais et al., 2010). Briefly, 293 human embryonic kidney cells transfected with the addition of the NF-κB luciferase gene were seeded in a 96-well sterile plate at 2 x 10^5 cells mL^{-1} and incubated for 48 h. Cells were maintained in culture medium (DMEM) (Invitrogen Co., Carlsbad, CA, USA), supplemented with 10% foetal bovine serum (FBS), 100 IU m^{L-1} penicillin, 100 µg mL^{-1} streptomycin and 2 mM glutamine. After incubation, different extract concentrations were added to the plate, followed by the addition of TNF-α (2 ng mL^{-1}) and incubation for 6 h. The cells were then washed with PBS, and the luciferase assay was performed using the Promega Luc assay kit following the manufacturer's instructions. A TPCK curve (0.001-2.5 mM) was used as the positive control (IC_{50} = 3.8 nM). Luciferase activity was monitored by a microplate luminescence reader (Mults-Mode, Filter Max F5, Molecular Devices Spectra, USA). The results were expressed as a percentage of the NF-κB inhibitory activity.

2.11. Statistical analysis

Statistical analyses were performed using GraphPad software (San Diego, CA, USA 176). Data were expressed as the mean ± standard error of the mean (SEM) and/or standard deviation (SD). Statistical variations between groups were determined using multifactorial analysis and/or the t-test, with values of $p<0.05$ considered significant.

3. Results and discussion

3.1. Phytochemical analysis

Plant extracts rich in polyphenols are important for the preparation of medicines, as polyphenols comprise a wide range of chemical structures, are easily obtained from natural sources and exhibit many biological properties (Gorzynik-Debicka et al., 2018). In the present study, we detected total polyphenol contents in samples ranging from 7.65 ± 0.22 in the hexane leaf extract from *Hypochaeris chillensis* to 223.42 ± 6.98 in the methanolic leaf extract from *Emília fosbergii* (**Table 1**). There are many important steps to consider in obtaining polyphenolic-rich extracts from plants. Among them, the solvent used is one of the main steps. Polar solvents are frequently used for recovering polyphenols from different plant matrices due to their polar chemical characteristics (Do et al., 2014). In this work, the total phenolic contents in the methanolic extracts from all of the UFPs revealed significantly higher amounts of phenolics than those in the hexane extracts in the literature.

Table 1. Quantification of total phenolics in the methanolic leaf extracts from *H. chillensis, E. fosbergii* and *E. sonchifolia.*

Plant	Extraction solvent	Total phenolics (mg g^{-1}) *
Hypochaeris chillensis	Hexane	7.65 ± 0.22[a]
	Methanol	202.26 ± 4.33[b]
Emília fosbergii	Hexane	8.57 ± 0.52[a]
	Methanol	223.42 ± 6.98[b]
Emília sonchifolia	Hexane	9.56 ± 0.59[a]
	Methanol	89.57 ± 5.86[c]

* results are expressed in mg of gallic acid equivalents per gram of crude extract. Different letters within the same columns indicate significantly different mean values ($p < 0.05$).

The LC-MS/MS analysis for the *H. chillensis* methanolic leaf extract revealed the presence of chlorogenic acid (1), quercetin O-hexoside (2) and quercetin (3) (Valli et al., 2013, Clifford et al., 2003) as major secondary metabolites (**Table 2 and Figure 1**).

Table 2. The substances identified in the *H. chillensis* methanolic leaf extract.

Compounds	t_R (min)	$[M + H]^+$	Fragment ions	λ_{MAX} (nm)
1. Chlorogenic acid	10.18	355	163	244; 326
2. Quercetin O-hexoside	19.85	465	303	258; 356
3. Quercetin	22.3	303	285; 257; 229	258; 349

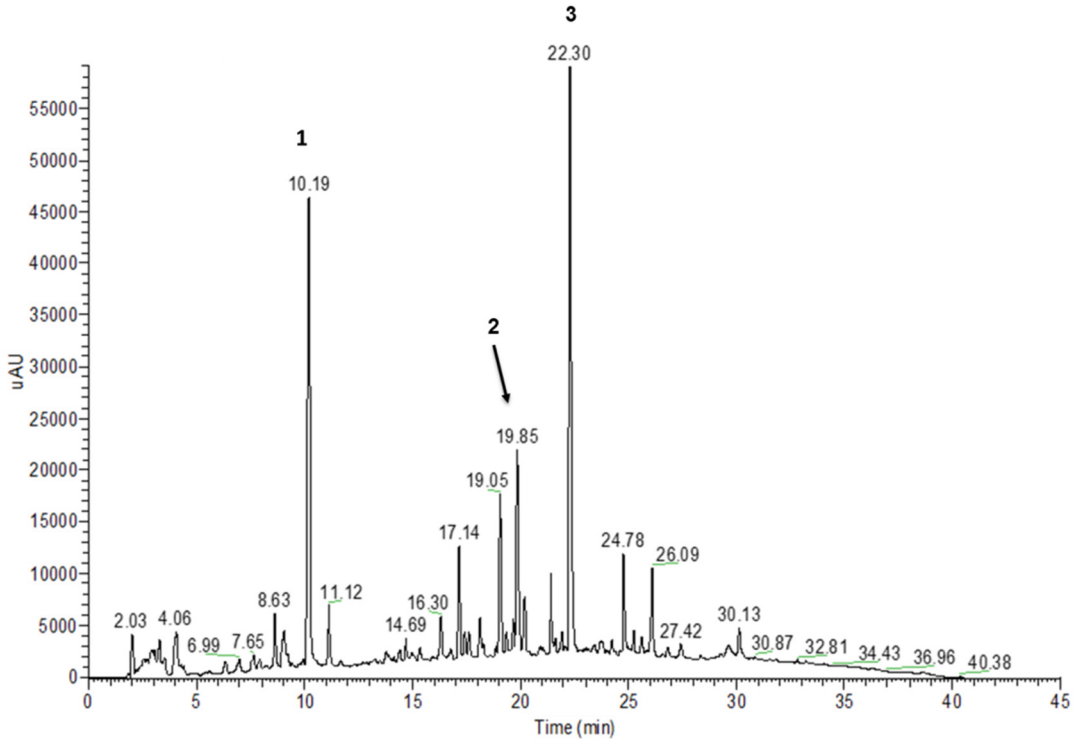

Fig. 1. Representative chromatogram of *H. chillensis* methanolic leave extract. 1 Chlorogenic acid, 2 Quercetin O-hexoside and 3 Quercetin.

Analysis of the *E. fosbergii* methanolic leaf extract revealed the presence of chlorogenic acid (1), quercetin-pentoside-hexoside (2), quercetin-hexoside-hexoside (3), luteolin glucoronide (4), luteolin O-hexoside (5) and quercetin O-hexoside (6) (Valli et al., 2013, Clifford et al., 2003, March et al., 2006, Chen et al., 2012, Hossain et al., 2014) (**Table 3 and Figure 2**).

Table 3. The major substances identified in the *E. fosbergii* methanolic leaf extract.

Compounds	t_R (min)	$[M + H]^+$	Fragment ions	λ_{MAX} (nm)
1. Chlorogenic acid	10.18	355	163	247; 327
2. Quercetin-pentoside-hexoside	13.27	597	435; 303	261; 357
3. Quercetin-hexoside-hexoside	13.52	627	303	261; 357
4. Luteolin glucuronide	19.35	463	287	259; 350
5. Luteolin O-hexoside	19.52	449	287	257; 350
6. Quercetin O-hexoside	19.71	465	303	261; 357

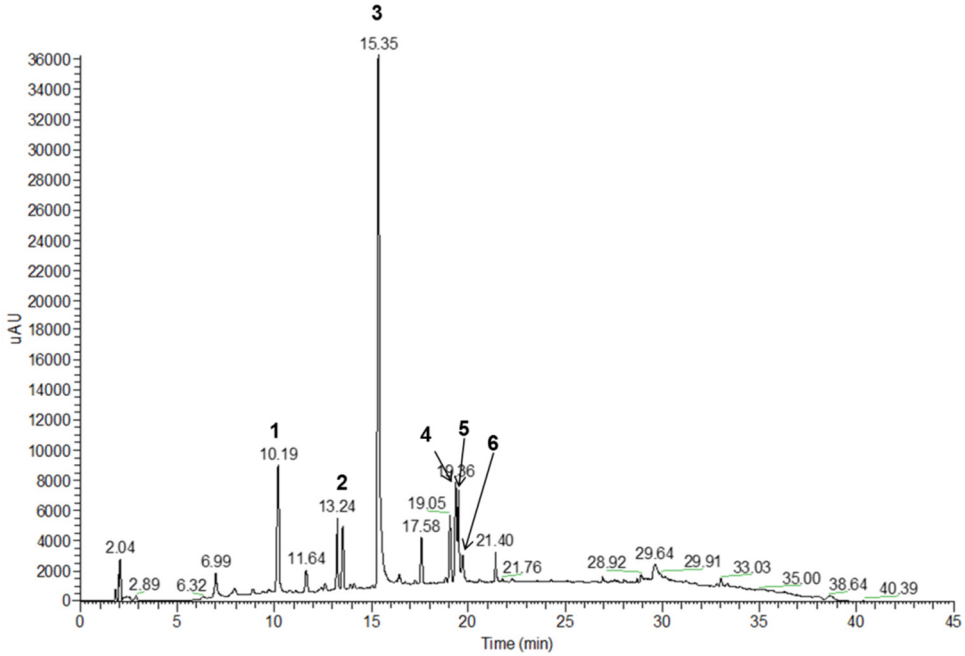

Fig. 2. Representative chromatogram of the *E. fosbergii* methanolic leaf extract.

Analysis of the methanolic extract from *E. sonchifolia* leaves revealed the presence of 1 chlorogenic acid (1) and apigenin-di-C-glucoside (2) (Valli et al., 2013, Clifford et al., 2003, Zheng et al., 2013) (**Table 4 and Figure 3**).

Table 4. The substances identified in methanolic extract from *E. sonchifolia* leaves.

Compounds	t_R (min)	$[M + H]^+$	Fragment ions	λ_{MAX} (nm)
1 Chlorogenic acid	10.18	355	163	247; 327
2 Apigenin-di-C-glucoside	14.74	595	577, 559, 541, 499,457, 415, 409, 391, 379, 365, 325	270; 335

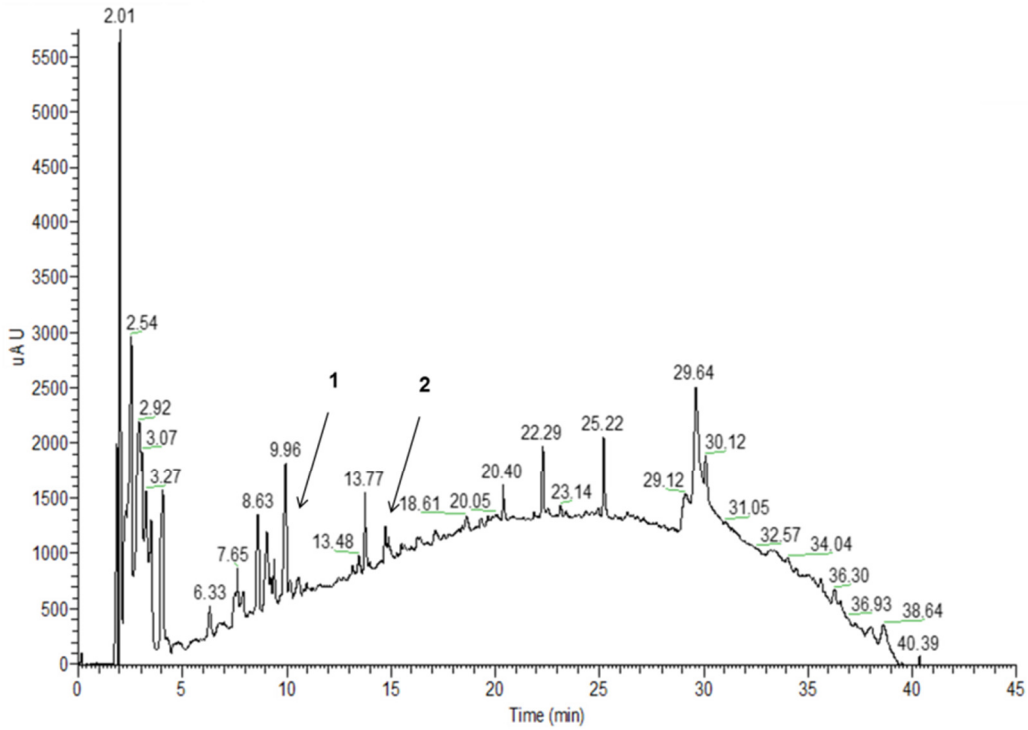

Fig. 3. Representative chromatogram of *E. sonchifolia* methanolic leaf extract. 1 Chlorogenic acid and 2 Apigenin-di-C-glucoside.

In agreement with our previous study, we also identified the presence of polyphenols and flavonoids together with saponins, alkaloids and terpenes in the leaf extract from *E. sonchifolia*. Quantitative HPLC analysis revealed eleven major peaks, including alkaloids (11.09%), quercetin O- and C-glycoside derivatives (10.83%), quetzrin (15.94%), the C-glycoside derivatives kaempferol (4.25%) and chlorophyll (11.73%), carotenoid derivatives (10.64%), triterpenoids (1.54%), and caffeic acid derivatives (33.88%) (Nworu et al., 2012). Quercetin and other flavonoid-related compounds were identified in the *E. sonchifolia* hydroethanolic leaf extract (Couto et al., 2011).

To date, no phytochemical studies describe the chemical composition of *E. fosbergii* and *H. chillensis*. Preliminary phytochemical analysis with *H. radicata*, from the same family and genus as *H. chillensis,* showed that the *H. radicata* leaf and root aqueous extracts contained alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids (Senguttuvan et al., 2014).

Polyphenols are secondary metabolites naturally occurring in almost all plants but are particularly abundant in acidic-tasting fruits. Many important physiological properties, such as anti-inflammatory, antimicrobial, antidiabetic, antithrombotic, antiatherogenic and cardioprotective features, are attributed to phenolic compounds, and these effects are highly correlated with their antioxidant properties (Balasundram et al., 2006). The antioxidant activity of phenolic compounds is associated with their ability to scavenge free radicals, chelate transition metals and disrupt free radical propagation reactions in lipids (Kyungmi & Ebeler, 2008; Podsedek, 2007). They have also been shown to modify prostaglandin metabolic pathways (Valko et al., 2007), block the actions of specific enzymes that cause inflammation (Moreira & Mancini-Filho, 2004), protect against platelet aggregation and inhibit the activation of carcinogens (Liu, 2005).

3.2. Chemical methods for determining antioxidant activity

To obtain preliminary information on the antioxidant capacity of *H. chillensis*, *E. fosbergii* and *E. sonchifolia* methanolic leaf extracts, three analytical methods were used, including the ABTS cation radical ($ABTS^{\bullet+}$), ferric reducing/antioxidant power (FRAP), and DPPH radical scavenging activity assays. As presented in Table 5, *H. chillensis and E. fosbergii* demonstrated higher antioxidant activities. *E. fosbergii* showed better antioxidant capacities in all the chemical assays, exhibiting IC_{50} values of 32.9 ± 4.8, 50.2 ± 2.3 and 24.0 ± 2.9 µg mL^{-1} in the ABTS, DPPH and FRAP assays, respectively.

Table 5. Antioxidant activities of *H. chillensis*, *E. fosbergii* and *E. sonchifolia* methanolic leaf extracts determined by chemical methods, including the ABTS cation radical scavenging assay, DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP).

Plant	**Antioxidant activity (IC_{50} µg mL^{-1})**

	Extraction solvent	ABTS	DPPH	FRAP
H. chillensis	Hexane	> 500	> 500	> 500
	Methanol	45.5 ± 2.3[a]	79.9 ± 4.2 [a]	36.5 ± 1.4 [a]
E. fosbergii	Hexane	> 500	> 500	> 500
	Methanol	32.9 ± 4.8[b]	50.2 ± 2.3 [b]	24.0 ± 2.9 [b]
E. sonchifolia	Hexane	> 500	> 500	> 500
	Methanol	276.7 ± 15.1[c]	> 500	353.5 ± 20.6 [c]
Gallic acid		1.4 ± 0.5[d]	4.1 ± 0.6 [c]	0.1 ± 0.04 [d]

Different letters within the same columns indicate significantly different mean values ($p < 0.05$).

Our results are in agreement with the current literature that has provided substantial evidence indicating the relationship between antioxidant activity and the presence of phenolic compounds, showing that they contribute to the antioxidant activity of the plant extracts (Saeed et al., 2012; Vittorazzi et al., 2016).

Oxidative stress arises from an imbalance between oxidant compounds and antioxidants, resulting in excessive free radical production or even due to detriments in free radical removal. This process leads to the oxidation of biomolecules with the consequent loss of their biological functions and/or homeostatic imbalance, whose manifestation is potential oxidative damage against cells and tissues (Halliwell & Whiteman, 2004). The non-enzymatic defence against the oxidative stress system includes antioxidant compounds of dietary origin, with emphasis on hydrophobic vitamins, minerals and phenolic compounds (Barbosa et al., 2010).

Although chemical tests have provided us with important preliminary data, they are considered relatively ineffective for determining the antioxidant capacity, as they are not representative of the cellular conditions within an organism (Alves et al., 2010). Because products with antioxidant properties play an important role in medicine and related industries, it is necessary that this information is confirmed in biological systems. Therefore, the observed antioxidant properties of the UFPs in the chemical methods were further verified using various intracellular methods.

3.3. *In vitro* cytotoxic activity studies

When the target of a study involves cell biology, cellular viability assessments are mandatory to determine whether the tested concentrations negatively affect cell

viability. Thus, the *in vitro* cytotoxic effects of the hexane and methanolic leaf extracts from the UFPs were evaluated in different cell lines, including L929 fibroblasts and RAW 264.7 macrophages, using the MTT colorimetric method. The results demonstrated that none of the extracts tested at different concentrations presented significant cytotoxic effects in any of the cell lines up to 100 µg mL^{-1} (data not shown).

3.4. Biological assays for antioxidant activity

3.4.1. Reduction of superoxide anion production ($O_2^{\bullet-}$)

The intracellular antioxidant activities of the extracts were evaluated according to their ability to inhibit the production of superoxide anion in RAW 264.7 macrophages stimulated with LPS. As can be observed in Figures 4, 5 and 6, LPS presented a significant increase in the production of radicals compared to the unstimulated control, certifying that the cells were properly stimulated. The *H. chillensis* hexane leaf extract showed concentration-dependent (1.0 - 100.0 µg mL^{-1}) inhibition of superoxide anion generation in the LPS-stimulated macrophages. In contrast, the methanolic extract did not influence the intracellular production of superoxide anion ($O_2^{\bullet-}$) in LPS-stimulated RAW 264.7 macrophage cells (**Fig. 4**).

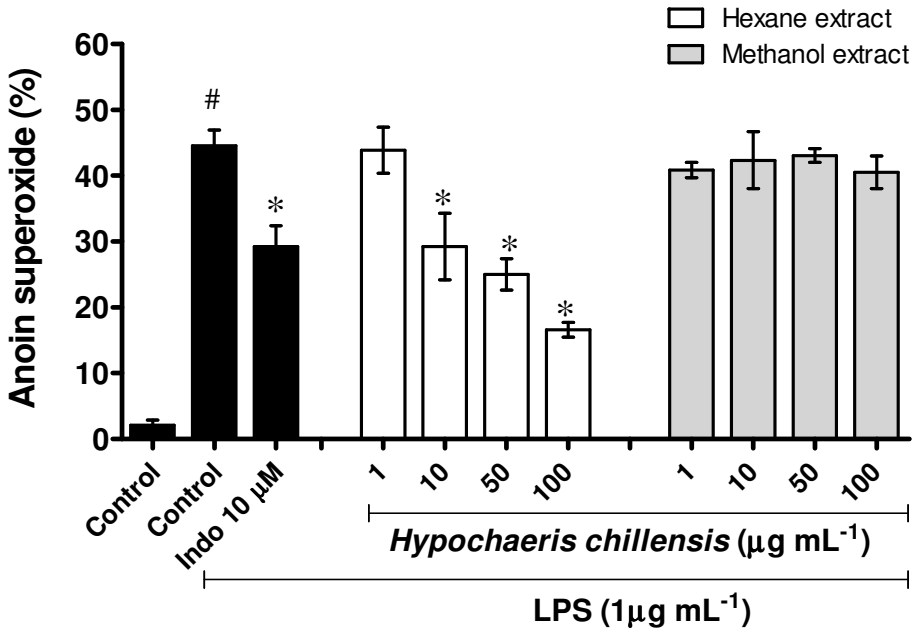

Fig. 4. Effect of *Hypochaeris chillensis* leaf extracts at concentrations of 1, 10, 50 and 100 µg mL^{-1} on the intracellular production of superoxide anion ($O2^{\bullet-}$) in LPS-stimulated RAW 264.7 macrophages after 24 h of exposure. The $O2^{\bullet-}$ concentration was determined by the nitroblue tetrazolium (NBT) reduction

assay. Indomethacin (10 µM) was used as a positive control. The results are expressed as the mean ± SD of two independent experiments. *($p < 0.05$) indicates significant differences compared with the LPS-treated cells.

The leaves from *H. chillensis* showed a significant reduction in $O_2^{\bullet-}$ production in the cells protected with hexane extracts at the concentrations of 10, 50, 100 µg mL^{-1}, as well as with indomethacin at 10 µM. The methanolic extracts showed no significant differences at any of the concentrations tested.

The leaves from *Emília fosbergii* did not influence the production of $O_2^{\bullet-}$; neither the hexane nor methanolic extract showed any effects at any of the tested concentrations (**Fig. 5**). *Emilia sonchifolia* leaves showed a significant reduction in O2•- production in the cells protected with the hexane extract at 100 µg mL^{-1}, as well as with the indomethacin standard at 10 µM. The methanolic extracts showed no significant differences at any of the concentrations tested (**Fig. 6**).

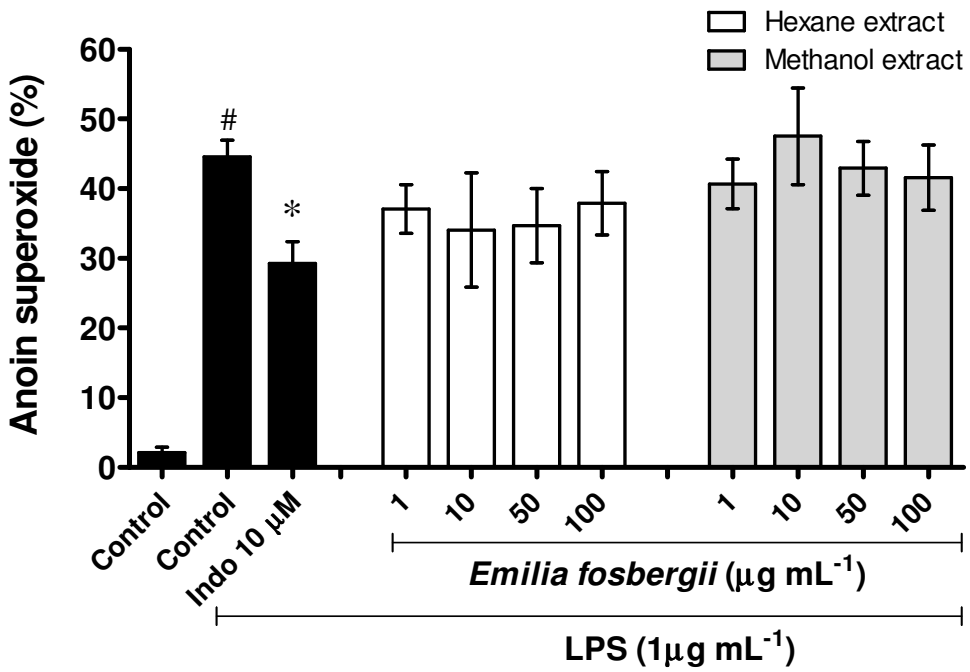

Fig. 5. Effect of *Emília fosbergii* leaf extracts at concentrations of 1, 10, 50 and 100 µg mL^{-1} on the intracellular production of superoxide anion ($O_2^{\bullet-}$) in LPS-stimulated RAW 264.7 macrophages after 24 h of exposure. The $O_2^{\bullet-}$ concentration was determined by the nitroblue tetrazolium (NBT) reduction assay. Indomethacin (10 µM) was used as a positive control. The results are expressed as the mean ± SD of two independent experiments. *($p < 0.05$) indicates significant differences compared with the LPS-treated cells.

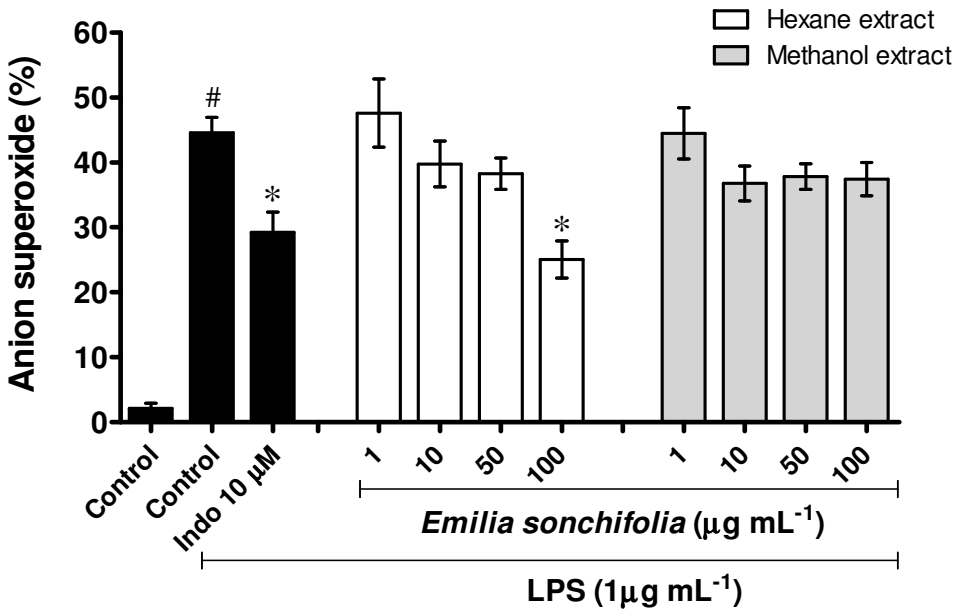

Fig. 6. Effect of *Emília sonchifolia* leaf extracts at concentrations of 1, 10, 50 and 100 µg mL^{-1} on the intracellular production of superoxide anion ($O_2^{\bullet-}$) in LPS-stimulated RAW 264.7 macrophages after 24 h of exposure. The $O_2^{\bullet-}$ concentration was determined by the nitroblue tetrazolium (NBT) reduction assay. Indomethacin (10 µM) was used as a positive control. The results are expressed as the mean ± SD of two independent experiments. *($p < 0.05$) indicates significant differences compared with the LPS-treated cells.

The intracellular results are in contrast to those observed in the chemical assays, in which the methanolic extracts presented greater antioxidant actions. However, in the biological assays, the hexane extracts showed a significant reduction in the intracellular production of superoxide anion (O2•-) in LPS-stimulated RAW 264.7. This phenomenon may occur due to the mechanism of action of each method. In the chemical methods, the main activity evaluated is the radical scavenger ability, which is known to be exerted by the phenolic compounds present mainly in the methanolic extracts and as observed in the phytochemical analysis. In the biological assays, other compounds may be involved in the antioxidant system, suggesting a different or potential enzymatic mechanism of action.

The enzymatic antioxidant system includes the superoxide dismutase, catalase and glutathione peroxidase enzymes. These enzymes act by preventing and/or controlling the formation of free radicals and non-radical species involved in the initiation of chain reactions that culminate in the propagation and amplification of the process and, consequently, the occurrence of oxidative damage (Schneider & Oliveira, 2004).

3.4.2. Test with hydrogen peroxide (H_2O_2)

The protective effects of the *H. chillensis, E. fosbergii* and *E. sonchifolia* methanolic leaf extracts against hydrogen peroxide (H_2O_2)-induced damage were tested in RAW 264.7 macrophages. Despite the antioxidant effects demonstrated in the other tests, the extracts showed no protective actions against oxidative damage at any of the tested concentrations using this method (data not shown).

3.5. Evaluation of intracellular anti-inflammatory activity

3.5.1. Determination of nitric oxide *in vitro*

UFPs showed significant anti-inflammatory activities in all samples tested, as depicted by their ability to suppress nitric oxide production in LPS-stimulated macrophages (**Table 6**). Increased NO production, a typical phenomenon that occurs in LPS-stimulated macrophages, was significantly reduced by all of the tested extracts. The results showed that *H. chillensis, E. fosbergii* and *E. sonchifolia* methanolic leaf extract exposure can lead to a decrease in NO production in RAW 264.7 cells in a concentration-dependent manner. The inhibitory activity of all of the extracts was more potent than that measured for gallic acid. In this assay, L-NAME and L-NIL, selective inhibitors of inducible nitric oxide synthase (iNOS), were used as positive controls. Their IC_{50} values are present in Table 6, which also shows that among the UFPs, the *E. fosbergii* hexane and methanolic extracts were the most potent NO production inhibitors, with IC_{50} values of 19.5 ± 3.1and 8.4 ± 0.1 **µg mL^{-1}**, respectively.

Table 6. Inhibition of *H. chillensis, E. fosbergii* and *E. sonchifolia* methanolic leaf extract on nitric oxide produced by LPS-stimulated RAW 264.7 cells.

Plant	Extraction solvent	NO (IC_{50} µg mL^{-1})
H. chillensis	Hexane	39.4 ± 2.9 ^{a}
	Methanol	42.8 ± 5.2 ^{a}
E. fosbergii	Hexane	19.5 ± 3.1 ^{b}
	Methanol	8.4 ± 0.1 ^{c}
E. sonchifolia	Hexane	51.4 ± 3.5 ^{d}
	Methanol	24.8 ± 4.7 ^{be}
Gallic acid		82.8 ± 4.0^{f}
L-NAME		313.6 ± 19.0^{g}
L-NIL		33.8 ± 11.1^{ab}

L-NAME – Nω-Nitro-L-arginine methyl ester hydrochloride.

L-NIL – N6-(1-iminoethyl)-L-lysine, dihydrochloride.
Different letters within the same columns indicate significantly different mean values ($p < 0.05$).

Consistent with our findings, a previous study by Nworu et al. (2012) with *E. sonchifolia* leaf extract revealed that cells pretreated with the plant at concentrations of 20 and 100 µg mL^{-1} showed a significant reduction in the production of nitric oxide in BMDM cells stimulated with LPS. Reductions of 46.69 and 56.45% were observed at 20 and 100 µg mL^{-1}, respectively (Nworu et al., 2012).

The amount of NO produced can determine protective or toxic functions. Small amounts are necessary, since this molecule mediates several phenomena, such as endothelium-dependent vessel relaxation, adhesion and platelet aggregation, regulation of basal blood pressure, synaptic depression, potentiation of synaptic transmission, and medullary and glomerular microcirculation, among others (Durán et al., 2010; Hardingham et al., 2013). NO is also considered a potent cellular toxin and protects the body against invading pathogens or tumour cells. However, NO works as a free radical with pro-oxidant actions, as it can combine with superoxide anion to form peroxynitrite (ONOO-), a potent free radical that is more toxic and has a longer survival time than NO or $O_2^{\bullet-}$ (Molavi & Mehta, 2004).

The inducible form of NOS is involved in the inflammatory process, is induced by bacterial products and inflammatory cytokines and is released by macrophages and other cells (Coutinho et al., 2009). Thus, the promising effectiveness of UFPs to inhibit the expression or activity of iNOS highlights their potential anti-inflammatory properties (Sautebin, 2000).

3.5.2. Determination of cytokine production *in vitro*

The ability of *H. chillensis, E. fosbergii* and *E. sonchifolia* leaf extracts to inhibit the production of the pro-inflammatory cytokines TNF-α and IL-6 was determined in LPS-induced inflammation in RAW 264.7 macrophages. As observed in Figure 7, LPS significantly increased the production of the studied cytokines compared to non-stimulated cells, demonstrating that the cells were effectively induced. However, the LPS-induced release of inflammatory mediators was significantly inhibited by treatment with the hexane and methanolic leaf extracts from *H. chillensis, E. fosbergii* and *E. sonchifolia* in a concentration-dependent manner.

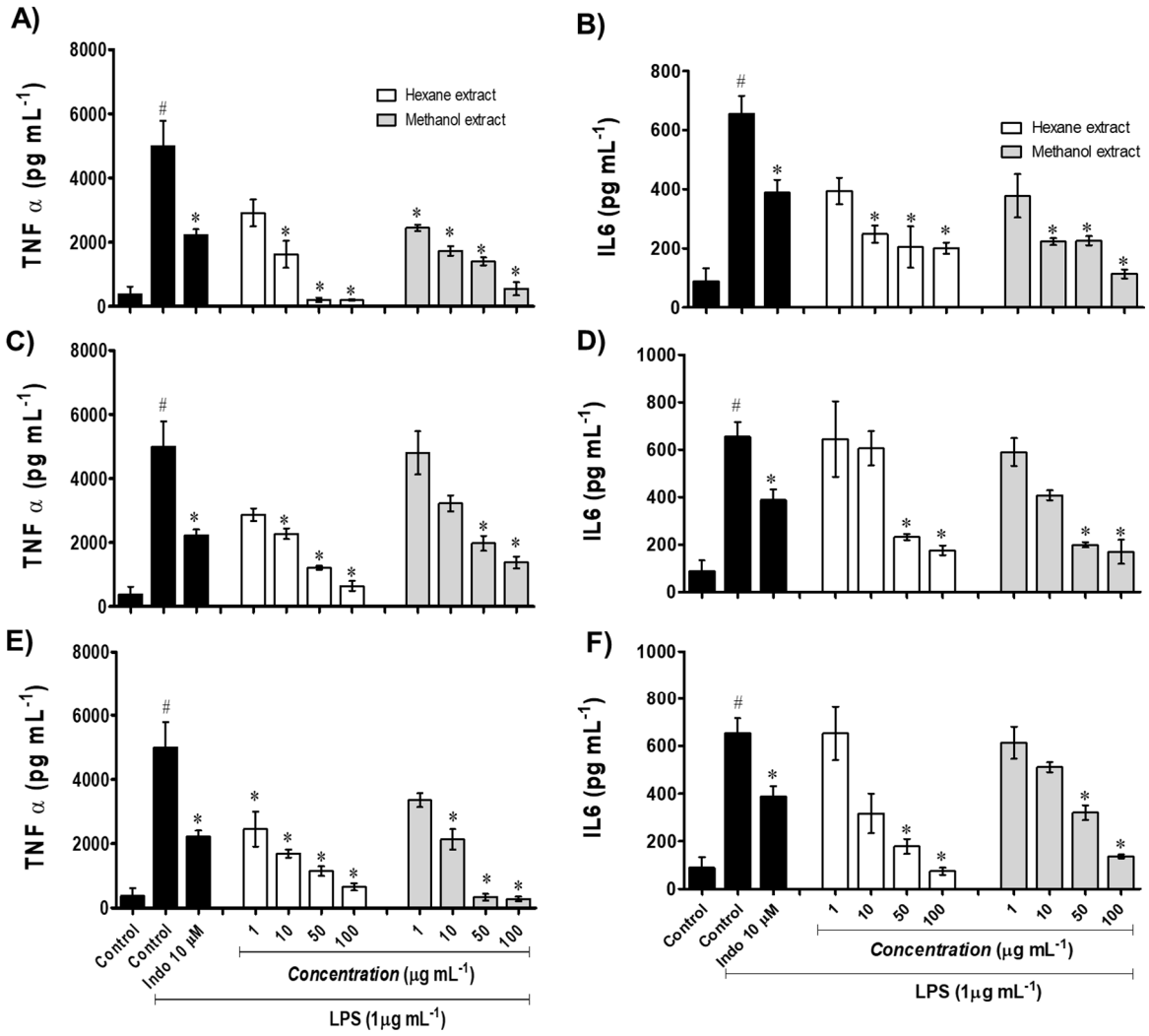

Fig. 7. Effect of UFP leaf extracts at concentrations of 1, 10, 50 and 100 µg mL^{-1} on the concentration of the pro-inflammatory cytokines tumour necrosis factor (TNF-α) and interleukin-6 (IL-6) in RAW 264.7 macrophages stimulated with LPS (1 µg mL^{-1}) after 24 h of treatment. (A) and (B) *H. chillensis,* (C) and (D) *E. fosbergii* and (E) and (F) *E. sonchifolia*. The results are expressed as the mean ± SD of two independent experiments performed in duplicate. * Significant at $p < 0.05$ compared to control + LPS.

Cytokines are considered the key regulators of the immune response that are necessary to drive the inflammatory response to infection and injury sites. At the top of the inflammatory cytokine cascade are molecules such as TNFα and IL-6, which are secreted mainly by myeloid cells (Kopf et al., 2010). Cytokines bind to specific receptors, activating intracellular messengers that regulate gene transcription and influencing the activity, differentiation, proliferation and survival of immune cells, as well as regulating the production and activity of other cytokines, which may increase or attenuate the inflammatory response (Kopf et al., 2010).

To date, no previous studies have revealed the anti-inflammatory activities of *H. chillensis* and *E. fosbergii*. However, preliminary studies conducted with *E. sonchifolia* have demonstrated its potential anti-inflammatory activity, reductions in serum vascular endothelial growth factor and the pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and granulocyte-macrophage colony-stimulating factor, and reductions in the production of inducible nitric oxide (Gilcy et al., 2016; Muko & Ohiri, 2000; Nworu et al., 2012).

In this study, we demonstrated that the production of IL-6 and TNF-α by LPS-simulated macrophages was significantly suppressed by UFPs. Thus, the observed capability of the studied UFPs to suppress the overproduction and activity of these pro-inflammatory cytokines is considered an important event to impede and control chronic inflammatory diseases and therefore promote favourable health effects on the consumers of these plants.

3.5.3. Determination of nuclear factor activity kappa B

Nuclear factor-κB (NF-κB) is a transcription factor that has been connected to a wide range of biological functions, including proliferation, survival, and inflammation (Park & Hong, 2016). Interestingly, the suppression of NF-κB activation by *E. sonchifolia* hexane leaf extract reached 90.3 ± 10.5% at 100 µM (**Table 7**). Although other extracts from UFPs exhibited significant inhibitory activity on pro-inflammatory mediators such as TNF-α, IL-6 and NO production, they did not inhibit NF-κB.

Table 7. Effects of UFP leaf extracts at the concentration of 100 µg mL^{-1} on nuclear factor kappa B transcriptional activity in HEK 293 cells.

Plant	Extraction solvent	NF-kB Inhibition (%)	Survival (%)
H. chillensis	Hexane	6.3 ± 4.6	118.1 ± 1.1
	Methanol	-	114.4 ± 2.2
E. fosbergii	Hexane	6.1 ± 0.1	114.3 + 5.5
	Methanol	3.5 ± 6.2	114.1 + 5.0
E. sonchifolia	Hexane	90.3 ± 10.5	110.9 + 0.1
	Methanol	-	111.5 + 3.9

NF-κB plays a key role in the expression of stress-responsive genes, including those that encode pro-inflammatory cytokines and are involved in modulating cellular

sensitivity to oxidative injury (Haddad, 2002). NF-κB is inactive in the cell cytoplasm; however, in the presence of inducing stimuli, it is activated and translocates to the nucleus, where it binds to specific genes that will be activated. Among these genes are those responsible for the production of cytokines and growth factors, cytokine receptors, stress proteins, adhesion molecules and immunomodulators (Park & Hong, 2016). FTs can be modulated by oxidative stress, redox states (reduction/oxidation processes) and inflammatory and anti-inflammatory agents. Additionally, through the activation of NF-κB, inflammatory cytokines can stimulate iNOS and greatly increase NO production (Molavi & Mehta, 2004).

Therefore, as observed for many natural compounds, including phenolics that have been proven to have anti-inflammatory properties by suppressing the production of pro-inflammatory cytokines and by inhibiting NF-κB signalling, the present study suggests that the hexane extract from *E. sonchifolia* leaves may possess similar immunomodulatory mechanisms and anti-inflammation activities.

4. Conclusion

The studied unconventional food plants exhibited promising antioxidant and anti-inflammatory activities. These results suggest that the leaf extracts have bioactive compounds capable of blocking free radicals and/or inhibiting their actions at the enzymatic level, as well as the capacity to inhibit the production of important mediators of the inflammatory process such as nitric oxide, TNF-α, and IL-6 and to inhibit the activation of the transcription factor NF-kB. Considering the obtained results and the promising health benefits of these plants, our study provides a great field of exploration into the chemical constituents, as well as the biological potential, not only in these plants but also among the great diversity of UFPs found in nature.

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Conflict of Interest

The authors report no declarations of interest.

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ANEXOS

ANEXO 1: Comprovante de submissão à revista

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