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Antioxidant and anti-inflammatory activities of *Frankenia triandra* (J. Rémy) extracts

R. Torres Carro [a], R.E. D'Almeida [a], M.I. Isla [a,b], M.R. Alberto [a,b,*]

[a] INQUINOA (CONICET), San Lorenzo 1469, 4000 San Miguel de Tucumán, Argentina
[b] Cátedra de Elementos de Química Orgánica y Biológica, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, San Lorenzo 1469, 4000 San Miguel de Tucumán, Argentina

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ABSTRACT

Frankenia triandra (Frankeniaceae) is a poorly studied halophyte subshrub species that grows on saline soils in the Bolivian, Argentinean and Chilean Puna, and is used by Puna inhabitants as forage and antiseptic in their folk medicine. The aim of this work is to first evaluate the antioxidant and anti-inflammatory potential (inhibitory effect on pro-inflammatory enzymes) of two hydroalcoholic extracts of *F. triandra* obtained by maceration and soxhlet extraction. Both extracts showed similar biological activity and composition (mainly polyphenolic compounds, flavonoids and phenolic acids). They exhibited an important antioxidant activity by scavenging $ABTS^{\bullet+}$ and nitrite radicals, by inhibiting β-carotene bleaching and reducing Fe^{3+}. Antioxidant properties are related to anti-inflammatory capacity, and both extracts showed a significant inhibition of hyaluronidase and two enzymes of arachidonic acid pathway (cyclooxygenase-2 and lipoxygenase). These preliminary studies are interesting since they might open the way for further studies which would allow the potential use of this plant in the treatment of chronic inflammatory diseases and as an antioxidant agent.

1. Introduction

The Puna is a highland region of the central Andes that extends through the central and Southwestern Peru, Western Bolivia, Northwestern Argentina and Northern region of Chile. The flora that grows in this region is mostly herbaceous and shrubby. A drier region can be observed in the southern area of the Argentinean and Chilean Puna, where xerophile and halophyte species are prevailing. Due to the Puna's extreme conditions (high radiation levels, extreme temperature variation during day and night, dry weather, and highly saline soils), plant species that inhabit this ecoregion have developed morphological and physiological mechanisms to survive (García and Beck, 2006). Among the physiological adaptations of plants from this eco-region, the production of secondary metabolites such as phenolic compounds, flavonoids and carotenoids can be used as defense mechanism against biotic and abiotic stress. These compounds may have remarkable benefits on human health as antioxidant, anti-inflammatory, antiseptic and antitumoral agents (Yi et al., 2005; Alberto et al., 2007; Zampini et al., 2009; Cuello et al., 2011; D'Almeida et al., 2013). This region is characterized by transitional settlements of small communities devoted to pastoralism and agropastoral activities, where plants play an important role in their daily life. Plant species are mostly used for medicinal purposes and also as forage; moreover, their use as food, firewood, tincture, among others, is also common (Villagrán et al., 2003).

Frankenia belongs to the Frankeniaceae family, which is represented by four genera of shrubby and herbaceous species that grow on highly saline soils. *Frankenia* is the most extended genera in this family and can be found in arid and semi-arid environments, growing on saline, calcareous or chalky soils (Villagrán et al., 2003; García and Beck, 2006). *Frankenia triandra* (J. Rémy), commonly known as Yareta or Yaretilla, is a halophyte subshrub species that grows on saline soils in Bolivian, Argentinian and Chilean Puna, at 3000–4500 m over sea level (m.o.s.l). This species is used by the Puna inhabitants as emergency forage, and, in folk medicine, as antiseptic (Villagrán et al., 2003). Previous studies carried out in our laboratory have demonstrated that ethanolic extract of *F. triandra* has a moderate antimicrobial activity on Gram (+) bacteria (Zampini et al., 2009). Since infection induces a localized inflammatory response in the affected tissue, we tested two extracts of *F. triandra* to determine its anti-inflammatory capacity. Moreover, studies carried out by Wided et al. (2011) on chloroformic and methanolic extracts of *Frankenia thymifolia* have shown its anti-inflammatory activity.

The main targets of anti-inflammatory drugs are inducible enzymes that produce a large number of pro-inflammatory mediators such as the enzymes from the arachidonic acid pathway (phospholipase,

* Corresponding author at: INQUINOA-CONICET, Universidad Nacional de Tucumán, San Lorenzo 1469, T4000INI San Miguel de Tucumán, Tucumán, Argentina. Tel.: +54 381 4203062; fax: +54 381 4248169.
E-mail address: mralberto@fbqf.unt.edu.ar (M.R. Alberto).

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cyclooxygenase and lipoxygenase), and hyaluronidase. Non-steroidal and steroidal anti-inflammatory drugs exert their action by inhibiting these enzymes through different mechanisms (Vane and Botting, 1998). Another action mechanism of anti-inflammatory drugs is the inhibition of the reactive oxygen species generation or the scavenging of them (Werz and Steinhilber, 2005). Different studies have demonstrated a strong bond between antioxidant and anti-inflammatory properties (Rodríguez et al., 2006; Mateo Anson et al., 2011; D'Almeida et al., 2013). Nevertheless, a great amount of secondary effects have been associated to the consumption of anti-inflammatory drugs over a prolonged period of time, which increases the costs in healthcare. Hence, searching for new natural alternative sources of drugs to treat chronic inflammatory pathologies is of great interest.

Natural products play a significant role in human health in relation to the prevention and treatment of inflammatory conditions. The aim of this work is to evaluate the anti-inflammatory potential (inhibitory effect of pro-inflammatory enzymes) and antioxidant activity of two hydroalcoholic extracts of *F. triandra* obtained by different extraction methods.

2. Material and methods

2.1. Chemicals reagents

Soy lipoxygenase, 2,4-dinitrophenylhydrazine (DNPH), diphenylboric acid-ß-ethylamino ester (NP), caffeic acid, Folin–Ciocalteau reagent, aluminum chloride, quercetin dihydrate, naringenin, gallic acid, dimethylaminocinnamaldehyde (DMAC), caffeic acid, ethylenediaminetetraacetic acid (EDTA), 2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ß-carotene and Griess reagent were purchased from Sigma-Aldrich (MO, USA). Hyaluronidase, linoleic acid and dimethyl sulfoxide (DMSO) were obtained from Merck (Germany). Potassium hyaluronidate was bought from Calbiochem, (USA). Triton X-100 and procyanidin B_2 were supplied by Fluka Chemical Corp. (USA). 1,2-Diheptanoilthio-glycerophosphocholine (1,2 dHGPC) and secretory phospholipase A_2 ($sPLA_2$) were obtained from bee venom and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) from Cayman Chemical Co. (MI, USA). Other chemicals were purchased from local commercial sources and they were of analytical grade quality.

2.2. Plant material

F. triandra was collected in the Laguna de Vilama (4500 m.o.s.l), in the Argentinian province of Jujuy (22°30′S 66°55′O). Botanical identification was done by Dr. *Ana* Soledad Cuello and the voucher specimen was conserved in the Fundación Miguel Lillo (487,801/LIL), its aerial parts being used in all the experiments.

2.3. Preparation of plant extracts

For ethanolic extract, 20 g of air-dried aerial parts of *F. triandra* was macerated in 100 ml of ethanol (EtOH) 80% for 7 days by shaking (40 cycles/min) at room temperature. Afterwards, extracts were filtered by using a Whatman No 1 filter paper (Sigma-Aldrich). The filtrate was dried under reduced pressure and the solid remnant was used to obtain stock solutions of 50 mg/ml in DMSO and stored at 4 °C in the dark. As for the soxhlet extract, air-dried aerial parts of *F. triandra* (65 g) were mixed with 1 l of EtOH 96%:water (1:1; *v*/v) as solvent system in a soxhlet equipment. An eight-cycle extraction was carried out, and then, the extract obtained was centrifuged for 25 min at 24,000 ×*g*. The supernatant was dried under reduced pressure to obtain a solid remnant, which was then dissolved with DMSO to obtain stock solutions of 50 mg/ml and stored at 4 °C in the dark. The extraction yields of both extracts were calculated as the ratio of the mass of the dried extract to the mass of the ground plant sample, and expressed as mg of soluble principles per g of dry weight of plant material (mg SP/g DW).

2.4. Phytochemical screening

Total phenolic compound content was determined according to Folin–Ciocalteau method (Singleton et al., 1999). Results were expressed as μg of gallic acid equivalents per mg of dry weight (μg GAE/mg DW). Non-flavonoid phenols were measured by determining the total phenol content remaining after precipitation of the flavonoids with acidic formaldehyde (Zoecklein et al., 1990). Results were expressed as μg GAE/mg DW. Flavones and flavonol content were determined according to Popova et al. (2005) method, using a 5% $AlCl_3$ solution. Flavonoid content was expressed as μg quercetin equivalents per mg of dry weight (μg QE/mg DW). Content of flavanones and dihydroflavonols was measured according to Nagy and Grançai (1996), using 1% DNPH and 70% methanol. Naringenin was used as standard and results were expressed as μg of naringenin equivalent per mg of dry weight (μg NE/mg DW). The total condensed tannin (proanthocyanidins) content was determined with DMAC according to Prior et al. (2010). Procyanidin B_2 was used as standard, and results were expressed in μg of procyanidin B_2 equivalents per mg of dry weight (μg PB_2E/mg DW).

2.5. Antioxidant activity

2.5.1. Free-radical ABTS scavenging activity

The antioxidant capacity assay was carried out according to an improved $ABTS^{\bullet+}$ method described by Re et al. (1999). One hundred microliters of an $ABTS^{\bullet+}$ solution was added to different concentrations of the extracts (25–75 μg/ml) to a final volume of 200 μl. The inhibition percentage was measured after 1 min reaction. The SC_{50} is defined as the concentration of extracts, in micrograms per milliliter (μg/ml) necessary to scavenge 50% of the $ABTS^{\bullet+}$. Quercetin was used as positive control (7–25 μg/ml).

2.5.2. β-Carotene-linoleic acid assay

Antioxidant activity of *F. triandra* extracts was determined by Wang et al. (2008) method. Different concentrations of both extracts (up to 50 μg/ml) were mixed with a β-carotene emulsion and incubated at 50 °C. The oxidation was monitored spectrophotometrically at 470 nm during 120 min. Results were compared to the control without extract. Quercetin (1.18–27.3 μg/ml) and BHT (2.3–9.1 μg/ml) were used as positive control. The concentration necessary to inhibit 50% of β-carotene bleaching (IC_{50}), expressed in micrograms per milliliter (μg/ml), was determined.

2.5.3. Nitric oxide scavenging assay

The capacity of *F. triandra* extracts to scavenge the nitric oxide released by sodium nitroprusside was determined spectrophotometically according to the method described by Govindarajan et al. (2003). Different concentrations of the extracts (100–400 μg/ml) were mixed with sodium nitroprusside (100 mM) and sodium phosphate buffer (0.2 M; pH 7.4). The reaction mixture was incubated for 60 min at 37 °C in the light. After 60 min, Griess reagent was added and incubated for 15 min in the dark. The absorbance of the formed chromophore was measured at 550 nm. SC_{50} was defined as the extract concentration necessary to scavenge 50% of nitric oxide. Ascorbic acid was used as positive control (10–100 μg/ml).

2.5.4. Iron III to iron II reductive capacity

The capacity of *F. triandra* extracts to reduce Fe (III) to Fe (II) was assessed spectrophotometrically according to Oyaizu (1986). Potassium ferricyanide 1% was mixed with different concentrations of *F. triandra* extracts, and sodium phosphate buffer (0.1 M; pH 6.3). The reaction mixture was incubated for 20 min at 50 °C, and then trichloroacetic acid 10% was added to stop the reaction. An aliquot was mixed with $FeCl_3$ 0.1%. Absorbance values at 700 nm were used to determine the concentration at which the absorbance is 0.5 for reducing power