



## Comparative study of antioxidant and anti-inflammatory activities and genotoxicity of alcoholic and aqueous extracts of four *Fabiana* species that grow in mountainous area of Argentina

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### ABSTRACT

**Ethnopharmacological relevance:** *Fabiana* species (Solanaceae family) extracts have long been used in Argentinean traditional medicine as anti-inflammatories, antiseptic, bone fractures and others diseases, but there is no scientific evidence which supports their use.

**Aim of the study:** The present study was conducted to evaluate the ability of aqueous and ethanolic extracts of four *Fabiana* species (*Fabiana bryoides* Phil., *Fabiana punensis* A.C. Arroyo, *Fabiana densa* J. Remy and *Fabiana patagonica* Speg.) to inhibit key enzymes in inflammatory processes, free radical scavenging properties and genotoxic effects.

**Materials and methods:** HPLC-DAD of aqueous and ethanolic extracts from four *Fabiana* species was established. All *Fabiana* extracts were evaluated on their ability to inhibit hyaluronidase and lipoxygenase enzymes to assess their activity against inflammatory mediators. Antioxidant capacity was determined using the 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assays and  $\beta$ -carotene-linolenic acid assay. Genotoxicity was evaluated by the Ames assay.

**Results:** The results indicated that the chromatographic patterns of four *Fabiana* species were different in quantity and absorption intensity of peaks. The alcoholic extract of *Fabiana punensis* was the most active scavenger of DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals (SC<sub>50</sub> values of  $3.85 \pm 0.24$  and  $2.56 \pm 0.10$   $\mu$ g GAE/mL, respectively). *Fabiana patagonica* extracts exhibited the highest peroxyl radical scavenging activity compared with the other three taxa (IC<sub>50</sub> values between  $1.00 \pm 0.04$  and  $4.46 \pm 0.40$   $\mu$ g GAE/mL for all extracts) and anti-lipoxygenase activity with IC<sub>50</sub> values between 12.5 and 15.5  $\mu$ g GAE/mL. The absence of mutagenicity indicates that the DNA does not seem to be a relevant target for these extracts. *Fabiana bryoides* ethanolic extract showed an interesting effect: it inhibited spontaneous mutagenesis, which could be considered as an antimutagenic effect in the TA98 (+S9) and TA100 (+S9/–S9) strains. The potency differences found between the species could be consequence of the different phytochemical pattern observed by HPLC.

**Conclusions:** The inhibitory effects on lipoxygenase and hyaluronidase, free radical scavenging activities and lack of genotoxicity of *Fabiana* extracts may support the folk use of *Fabiana punensis*, *Fabiana patagonica*, *Fabiana bryoides* and *Fabiana densa* as inhibitor of inflammatory mediators.

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## 1. Introduction

Oxidative stress aggravates a variety of chronic inflammatory diseases such as arthritis and atherosclerosis as well as other dis-

eases like cancer, diabetes, hepatitis, neurodegeneration and early aging (Niwa, 1991; Richards and Sharma, 1991; Halliwell, 1994; Aviram, 2000). In the last years, a lot of evidence has been accumulated that proves that free radicals are important components of inflammation (Kamatou et al., 2010). Lipoxygenase plays a key role in the biosynthesis of leukotrienes, the proinflammatory mediators mainly released from myeloid cells and hyaluronidase in chronic inflammations and allergic processes in humans. Thus, lipoxygenase and hyaluronidase inhibitors initially attracted attention as potential agents for the treatment of inflammatory and allergic

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diseases, but their therapeutic potential has now been expanded to certain types of cancer and cardiovascular diseases. Most lipoxigenase and hyaluronidase inhibitors are also antioxidants or free radical scavengers (Ippoushi et al., 2000; Sadik et al., 2003; Reddy et al., 2010).

### 1.1. *Fabiana* species

The South American genus *Fabiana* (Solanaceae family) grows along arid mountainous area between 16° and 51° latitude, between 1000 and 4900 m over sea level (m.o.s.l.). There are 15 species, ten are present in Argentina, seven in Chile, four in Bolivia and one in Perú. There are five species in the Altoandina, Puna, Prepuna and Monte phytogeographic provinces of the Argentinean Northwest. These species have been employed as medicine, building material, forage, fuel and elements in spiritual activities (Villagrán and Castro, 2000; Villagrán et al., 2003; Perez, 2006; Barbarán, 2008). The resinous exudates from *Fabiana* leaves and branches are used in traditional medicine to immobilize fractured extremities (Erazo et al., 2002; Perez, 2006), while the infusion and decoction are used as antiseptic and anti-inflammatory (Perez, 2006). To our knowledge, there are few reports that validate their popular use. The antimicrobial activity of *Fabiana bryoides*, *Fabiana densa*, *Fabiana punensis* against Gram positive and Gram negative bacteria have been reported (Zampini et al., 2009a). Antibacterial and diuretic activities were reported for terpenoids from *Fabiana densa* var. *ramulosa* (Erazo et al., 2002) and from *Fabiana patagonica* extracts, respectively (Alvarez et al., 2002).

Due to the lack of scientific studies on the anti-inflammatory and antioxidant activities of *Fabiana* species and toxicity, four related taxa (*Fabiana bryoides* Phil., *Fabiana punensis* A.C. Arroyo, *Fabiana densa* J. Remy and *Fabiana patagonica* Speg.) were chosen to evaluate the claims made by traditional medicine practitioners concerning the effectiveness of these plants as inhibitor of inflammatory mediators. The present paper deals with the search for anti-inflammatory mediators, antioxidant activities and genotoxicity of these plant extracts.

## 2. Materials and methods

### 2.1. General

All solvents and reagents used were analytical grade and high performance liquid chromatography (HPLC) grade, obtained from Merck, Fluka and Sigma–Aldrich Canada Ltd.

### 2.2. Plant material

The following plant species were used: *Fabiana bryoides*, *Fabiana densa*, *Fabiana patagonica* and *Fabiana punensis*. Their aerial parts were collected in March 2009 from Puna, Prepuna and Altoandina phytogeographic provinces (Cabrera, 1978), in the Argentinean Northwest mountainous area (Fig. 1A and B).

The plant materials were identified by the botanist Ana Soledad Cuello. Voucher specimens were deposited in the Fundación Miguel Lillo Herbarium (LIL) and in Instituto de Estudios Vegetales (IEV) (Table 1).

### 2.3. Preparation of *Fabiana* extracts

Maceration (tincture): ground air-dried plant material was macerated in ethanol (5 g dry tissue/100 mL ethanol 80°) for 7 days shaking at 40 cycles/min at room temperature.

Decoction: 5 g of dried and powdered plant material were extracted in 100 mL of boiling distilled water for 10 min.

Both extracts were filtered through Whatman No. 1 filter paper after cooling and the volume made up to 100 mL. Then, the ethanolic extracts were evaporated to dryness in a rotary evaporator at 40 °C and the aqueous extracts were lyophilized to obtain the dry extracts (DE). Anti-inflammatory and mutagenicity assays were realized using resuspended DE in dimethyl sulfoxide (DMSO).

### 2.4. Phytochemical analysis

#### 2.4.1. Determination of total phenolic and flavonoid content in *Fabiana* extracts

Total phenolic compound contents were determined by the Folin–Ciocalteu method (Singleton et al., 1999). The reaction mixture contained 50 µL of each preparation, 2 mL of distilled water, 200 µL of Folin–Ciocalteu reagent and 800 µL of sodium carbonate (15.9%, w/v). The reaction mixture was heated at 50 °C for 5 min in a water bath. Absorbance was measured at 765 nm. Results were expressed as gallic acid equivalents/mL (GAE/mL).

Total flavonoids were estimated using the method of Woisky and Salatino (1998). To 0.1 mL of sample, 2.35 mL methanol and 50 µL of 5% AlCl<sub>3</sub> ethanolic solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. Results were expressed as quercetin equivalents/mL (QE/mL).

#### 2.4.2. HPLC fingerprints of *Fabiana* extracts

The HPLC system consisting of a Waters 1525 Binary HPLC Pumps system with a 1500 Series Column Heater, a manual injection valve with a 20 µL loop (Rheodyne Inc., Cotati, CA) and a Waters 2998 photodiode array detector (PDA) was used to analyze the extracts. An XBridge™ C18 column (4.6 mm × 150 mm, 5 µm; Waters corporation, Milford, MA) with two gradient solvent system was used.

System 1 was composed of solvent A (9% acetic acid in water) and solvent B (methanol) (conditions: 25–45% B from 0 to 10 min and kept at 45% B from 10 to 20 min, 45–70% B from 20 to 40 min, 70–75% B from 40 to 50 min, 75–100% B from 50 to 55 min) were used for separation of component from *Fabiana* ethanolic extracts. The flow rate was set at 0.8 mL/min.

System 2 was composed of solvent A (0.1% acetic acid in water) and solvent B (0.1% acetic acid in methanol) (conditions: 10–57% B from 0 to 30 min and 57–100% B from 30 to 50 min) were used for separation of component from *Fabiana* aqueous extracts. The flow rate was set at 0.5 mL/min.

A solution of 10 mg/mL was used. Data collection was carried out with Empower™ 2 software. The presence of phenolic compounds in extracts was confirmed by UV spectrometry (220–500 nm) in comparison with the standard compounds.

### 2.5. Determination of antioxidant activity

#### 2.5.1. β-Carotene-linoleic acid assay

Antioxidant activity of all extracts was determined by measuring the coupled autooxidation of β-carotene and linoleic acid (Ordoñez et al., 2006). β-Carotene was dissolved in chloroform (0.5 mg/mL), and 1 mL was added to 20 µL of linoleic acid and 184 µL of Tween 40. Chloroform was removed under vacuum and 50 mL oxygenated deionized water (50 mM) was added and mixed thoroughly. Aliquots (3 mL) of the β-carotene/linoleic acid emulsion were mixed with 100 µL of the corresponding plant extract sample (up to 25 µg GAE) or positive (butylated hydroxy-toluene, BHT up to 25 µg/mL) or negative (ethanol and water) controls and incubated in a water bath at 50 °C. Oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period.

Degradation over time is non-linear, therefore, antioxidant activity is expressed as percent inhibition relative to the control



**Fig. 1.** (A) Studied regions and plant species have been indicated in an Argentine map: (1) *Fabiana patagonica* (26°43'16.52"S; 67° 7'2.06"W), (2) *Fabiana bryoides* (26° 1'10.07"S; 67°14'21.06"W), (3) *Fabiana densa* (22°57'53.71"S; 65°29'39.45"W), (4) *Fabiana punensis* (22°35'50.29"S; 66°54'19.84"W). (B) Photograph of plant species and studied area in Antofagasta de la Sierra, Catamarca, Argentina.





Fig. 1. Continued.

after 60 min incubation using the following equation (Emmons et al., 1999).

$$AOA = \frac{100(DR_c - DR_s)}{DR_c}$$

where AOA is the antioxidant activity,  $DR_c$  is the degradation rate of the control =  $\ln(a/b)/60$ ,  $DR_s$  is the degradation rate of the sample =  $\ln(a/b)/60$ ,  $a$  is the initial absorbance at time 0, and  $b$  is the absorbance at 60 min. The  $IC_{50}$  values (concentration required to inhibit 50%  $\beta$ -carotene bleaching) were determined from the dose–response curves.

#### 2.5.2. DPPH free radical-scavenging activity

The H-donor activity of *Fabiana* extracts was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method according to Yamaguchi et al. (1998). Briefly 1.5 mL of DPPH solution (300  $\mu$ M in 95% ethanol) was incubated with the samples (up to 40  $\mu$ g GAE). The reaction mixture was shaken and incubated for 20 min at room

temperature. Absorbance was measured at 515 nm against a blank. The free radical-scavenging activity was determined by comparison with ethanol or water control. BHT was used as reference compound (up to 50  $\mu$ g/mL).

The percentage (%) of radical scavenging activity (RSA) was calculated using the following equation:

$$RSA\% = \left( \frac{A_0 - A_s}{A_0} \right) \times 100$$

where  $A_0$  is the absorbance of the control and  $A_s$  is the absorbance of the samples at 515 nm.  $SC_{50}$  values denote the concentration of sample required to scavenge 50% DPPH free radicals.

#### 2.5.3. The ABTS method

The antioxidant capacity assay was carried out by the improved ABTS method as described by Re et al. (1999). ABTS radical cation (ABTS $^{•+}$ ) was generated by reacting 7 mM 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2.45 mM

**Table 1**  
*Fabiana* species studied.

Scientific name	Province	Region	Voucher specimen	Local name	Popular use or disease treated
<i>Fabiana bryoides</i> Phil.	Catamarca	Antofagasta de la Sierra (Quebrada seca) 4278 m.o.s.l.	489618/LIL	Pata de perdiz	Antiseptic, anti-inflammatory, against distempered cattle, sheep birth and bone fractures (Villagrán et al., 2003; Perez, 2006)
<i>Fabiana patagonica</i> Speg.	Catamarca	Laguna Blanca (Peña fria) 4200 m.o.s.l.	610855/LIL	Tolilla, tola, tola-checal	Diuretic (Alvarez et al., 2002)
<i>Fabiana punensis</i> S.C. Arroyo	Jujuy	Laguna de Vilama (Ramsar Site) 4280 m.o.s.l.	081/IEV	Tolilla	Bone fractures, anti-inflammatory (Villagrán et al., 2003; Perez, 2006)
<i>Fabiana densa</i> J. Rémy	Jujuy	Abra Pampa 3400 m.o.s.l.	487800/LIL	Tolilla	The leaves resinous exudates are used to immobilize fractured limbs and as an infusion against lung disease and cough (Erazo et al., 2002)

potassium persulfate after incubation at room temperature (23 °C) in the dark for 16 h. ABTS<sup>•+</sup> solution (1 mL; absorbance of  $0.7 \pm 0.02$  at 734 nm) was added to 1–10 µg GAE of each tested sample and mixed thoroughly. The reactive mixture was allowed to stand at room temperature and the absorbance was recorded at 734 nm, 1 min after initial mixing and up to 6 min. Results were expressed in terms of percentage (%) of radical scavenging activity (RSA) at 6 min and SC<sub>50</sub> values denote the sample concentration required to scavenge 50% ABTS free radicals. BHT was used as reference compound (up to 25 µg/mL).

## 2.6. Determination of anti-inflammatory activity

### 2.6.1. Lipoyxygenase enzyme assay

Lipoyxygenase activity was determined spectrophotometrically according to Taraporewala and Kauffman (1990). It is based on the enzymatic oxidation of linoleic acid to the corresponding hydroperoxide. To determine hydroperoxide, soy lipoyxygenase-1 (948 U) was incubated with linoleic acid (50 µM) in sodium borate buffer (200 mM, pH 9.0) for 4 min at 25 °C. The absorbance at 234 nm was measured on a Spectronic Unicam (Genesys) spectrophotometer. The assay to obtain the 100% of lipoyxygenase activity was carried out with DMSO as solvent control. The inhibitory assays were performed in presence of extracts in different concentrations (up to 25 µg GAE/mL). The anti-inflammatory effect was evaluated by calculating percentage inhibition of hydroperoxide production from the ΔOD (optical density) values at 234 nm at the end of 3 min incubation. The test compound concentration causing 50% inhibition of hydroperoxide-release (IC<sub>50</sub>) was calculated from the concentration–inhibition response curve by regression analysis. The extinction coefficient of  $25 \text{ mM}^{-1} \text{ cm}^{-1}$  was used for quantification of lipid hydroperoxides. Caffeic acid (up to 100 µg/mL) and naproxen (up to 25 µg/mL) were employed as reference.

### 2.6.2. Hyaluronidase enzyme assay

Hyaluronidase activity was assayed by estimating the amount of N-acetyl glucosamine (NAGA) released according to the method of Lee et al. (1993). The reaction mixture had 0.2 M sodium acetate buffer pH 4.5 containing hyaluronidase (57 U) pre-incubated for 10 min at 37 °C with CaCl<sub>2</sub> (0.125 M). Potassium hyaluronidate (0.066 mg) was then added in presence and absence of different extract concentrations (up to 228 µg GAE/mL) and incubated for 30 min at 37 °C. Activity in the absence of extract with DMSO as solvent control was considered 100%. The change in absorbance was monitored at 585 nm. Activity was expressed as µmoles of NAGA released during 30 min incubation at 37 °C. Naproxen and hesperetin were employed as reference (up to 171 µg/mL). The concentrations inhibiting the enzymatic activity by 50% (IC<sub>50</sub>) were calculated by graphic interpolation of the concentration–enzyme activity curves.

### 2.7. Salmonella mutagenicity assay

The Ames test is a well-known bacterial mutagenicity test. In this test reverse His<sup>−</sup> → His<sup>+</sup> mutations are visualized by plating *Salmonella typhimurium* bacteria in a histidine poor growth medium. In this medium only His<sup>+</sup> mutants are able to form visible colonies. Different bacterial strains are available to identify different types of mutations. Strains TA98 and TA100 are currently most often used as they detect the great majority of mutagens. Strain TA98 gives an indication of frame-shift mutations, while a positive response from strain TA100 indicates base-pair substitution.

The mutagenicity assay with *Salmonella typhimurium* was performed as described by Maron and Ames (1983). The experiments were performed with and without an exogenous metabolic system, the S9 fraction in S9-mix. The S9-mix was freshly prepared before

each test from an Aroclor-1254-induced rat liver fraction purchased (lyophilized) from Molttox – Molecular Toxicology Inc.

Three different dilutions (100, 500, 1000 µg GAE/plate) of the ethanolic and aqueous extracts, obtained from the aerial parts of *Fabiana patagonica*, *Fabiana punensis*, *Fabiana densa*, and *Fabiana bryoides* were evaluated in this assay. One hundred microliters of an overnight culture of bacteria (cultivated for 16 h at 37 °C, approximate cell density ( $2\text{--}5 \times 10^8$  cells/mL), the different concentrations of each extract and 500 µL of sodium phosphate buffer (0.2 M, pH 7.4 for assay without S9-mix) or 500 µL of S9-mix were added to 2 mL aliquots of top agar (supplemented with 0.5 mM L-histidine and 0.5 mM D-biotine). The resulting complete mixture was poured on minimal agar plates prepared as described by Maron and Ames (1983). The plates were incubated at 37 °C for 48 h and the revertant bacterial colonies of each plate were counted. An extract was considered mutagenic if the number of revertants per plate was more than twice the number of colonies produced on the solvent control plates (spontaneous revertant frequency) (Maron and Ames, 1983). Samples were tested in triplicate with two replicates. DMSO was used as the negative control, 100 µL/plate and the positive controls employed were 4-nitro-o-phenylenediamine (4-NPD), 20 µg/plate and 2-aminofluorene (2-AF), 10 µg/plate.

To discriminate cytotoxicity, the number of surviving cells was determined by plating appropriate dilutions of treated bacterial suspension onto complete agar plates.

## 2.8. Statistical analysis

Results are mean values obtained from at least three independent experiments. The correlation between two variants was analyzed by Pearson test using GraphPad Prism 5.0 software, with the level of significance set at  $p < 0.05$ .

## 3. Results and discussion

Two extract preparations (tincture and decoction) of four *Fabiana* species (*Fabiana densa*, *Fabiana bryoides*, *Fabiana patagonica*, and *Fabiana punensis*), traditionally used as medicine, were investigated for its phytochemical profile and their antioxidant properties, inhibitory capacity of enzymes (lipoyxygenase/hyaluronidase) and mutagenicity.

### 3.1. Phytochemical analysis

From bibliographical information it was possible to conclude that there is meager knowledge about the chemical composition and biological properties of *Fabiana* species.

The *Fabiana bryoides*, *Fabiana densa*, *Fabiana patagonica* and *Fabiana punensis* extracts were analyzed for total phenolic compounds, flavonoid contents and soluble principle yields (Table 2). Extraction yield of soluble compounds in ethanol was generally two times higher than the soluble principle in water. *Fabiana punensis* alcoholic extract gave the best yields of total phenolic compounds and flavonoid contents per gram of dry plant material followed by *Fabiana densa* and then, *Fabiana patagonica* and *Fabiana bryoides*, that not showed significant differences in phenolic content between them. Otherwise, *Fabiana punensis* aqueous extracts showed the higher polyphenol content than the other aqueous extractions ( $p < 0.05$ ). Total phenolic content varied from 22.7 mg GAE/g plant dry weight (DW) to 53.7 mg GAE/g DW for alcoholic extracts and from 7.8 mg GAE/g DW to 30.9 mg GAE/g DW for aqueous extracts, while flavonoid content went from 6.16 mgQE/g DW to 20.64 mgQE/g DW for alcoholic extracts and from 0.82 mgQE/g DW to 4.68 mgQE/g DW for aqueous extracts of the four *Fabiana* species.

The chromatographic patterns by HPLC-DAD of extracts of four *Fabiana* species were different in quantity and absorption

**Table 2**

Yield of dry extract, total phenolics and flavonoid contents of *Fabiana bryoides*, *Fabiana patagonica*, *Fabiana punensis* and *Fabiana densa* extracts. Values (mean  $\pm$  SD,  $n = 4$ ) in the same column followed by a different letter are significantly different ( $p < 0.05$ ).

Samples	Dry extract yield (% w/w) Alcoholic	Total phenolics (mg GAE/g DW) <sup>A</sup>	Flavonoids (mg QE/g DW)	Dry extract yield (% w/w) Aqueous	Total phenolics (mg GAE/g DW)	Flavonoids (mg QE/g DW)
<i>Fabiana patagonica</i>	31.74 $\pm$ 0.48 <sup>d</sup>	22.80 $\pm$ 1.90 <sup>c</sup>	2.33 $\pm$ 0.06 <sup>c</sup>	20.00 $\pm$ 3.20 <sup>c</sup>	7.80 $\pm$ 0.40 <sup>d</sup>	0.82 $\pm$ 0.04 <sup>c</sup>
<i>Fabiana bryoides</i>	47.52 $\pm$ 0.50 <sup>a</sup>	22.70 $\pm$ 1.80 <sup>c</sup>	6.30 $\pm$ 0.20 <sup>b</sup>	22.33 $\pm$ 3.00 <sup>b</sup>	22.70 $\pm$ 1.10 <sup>c</sup>	2.14 $\pm$ 0.04 <sup>b</sup>
<i>Fabiana densa</i>	34.80 $\pm$ 0.53 <sup>c</sup>	38.80 $\pm$ 1.90 <sup>b</sup>	6.16 $\pm$ 0.08 <sup>b</sup>	19.60 $\pm$ 2.90 <sup>c</sup>	27.22 $\pm$ 1.00 <sup>b</sup>	4.68 $\pm$ 0.15 <sup>a</sup>
<i>Fabiana punensis</i>	38.43 $\pm$ 0.40 <sup>b</sup>	53.70 $\pm$ 2.40 <sup>a</sup>	20.64 $\pm$ 1.51 <sup>a</sup>	23.86 $\pm$ 4.30 <sup>a</sup>	30.90 $\pm$ 2.20 <sup>a</sup>	2.42 $\pm$ 0.03 <sup>b</sup>

<sup>A</sup> Plant dry weight (DW).

intensity of peaks (Fig. 2A). The tinctures of *Fabiana punensis* showed ten peaks (retention time of 9.6 (1), 11.0 (2), 14.2 (3), 14.7 (4), 22.2 (5), 30.0 (6), 30.7 (7), 38.4 (8), 40.5 (9), 42.4 (10) min) with UV spectra characteristic of flavonoids (Mabry et al., 1970) while that the other tinctures showed minor number of chemical constituents. These results reveal major structural diversity (Fig. 2A) and total flavonoid content for *Fabiana punensis* (Table 2). The peak at 9.6 min was identified as quercetin. The others compounds have not been identified and are under investigation.

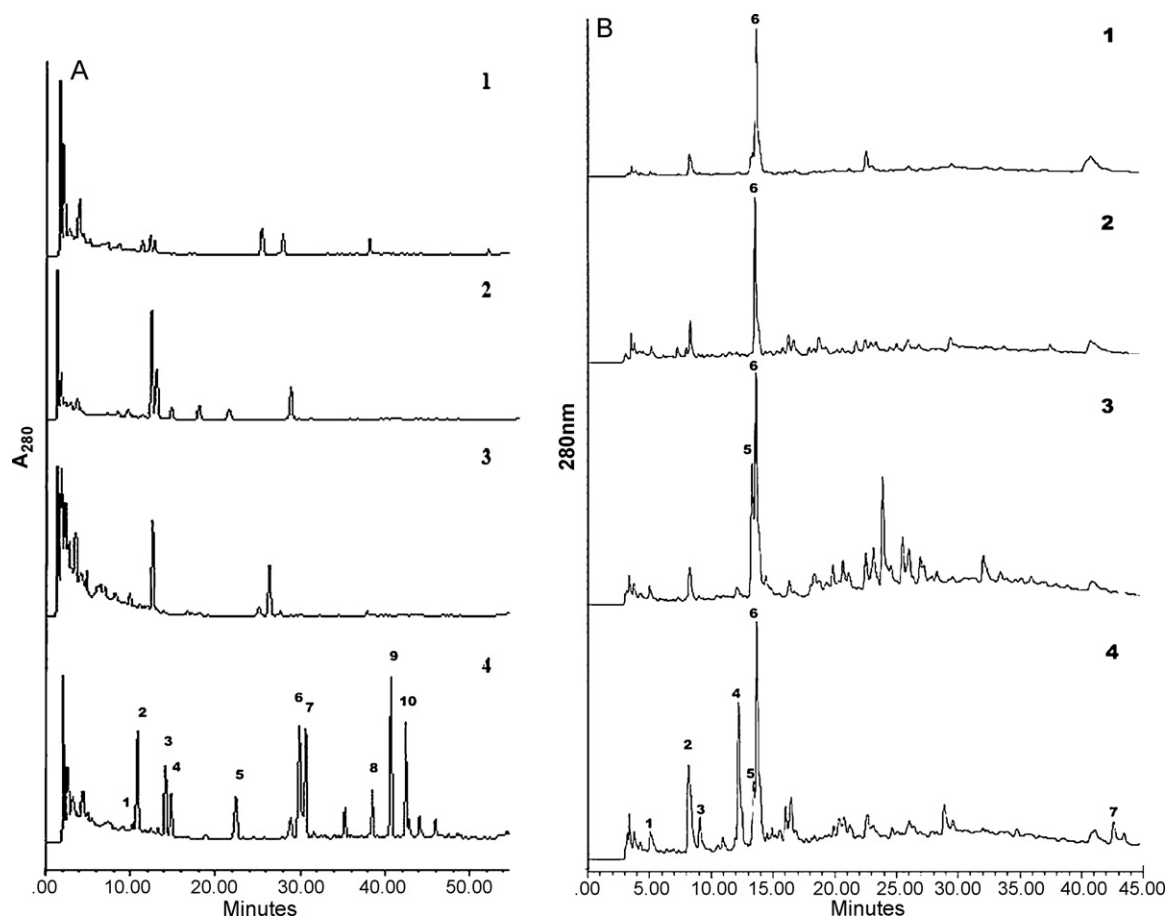
The *Fabiana punensis* and *Fabiana densa* aqueous extracts showed higher peak numbers than *Fabiana patagonica* and *Fabiana bryoides* aqueous extracts. The peaks with retention time of 13.3 (5), 29.4 and 42.6 (7) min was identified as chlorogenic acid (*Fabiana punensis* and *Fabiana densa*), rutin (in *Fabiana bryoides*) and quercetin (*Fabiana punensis*), respectively. The peak with retention time of 13.6 (6) min and UV bands characteristic for flavonoids was

detected in all aqueous extracts (Fig. 2B). Chlorogenic acid is one of the major components of the polar extracts of *Fabiana imbricata* (Schmeda-Hirschmann et al., 2004). *Fabiana imbricata* also has been demonstrated the presence of alkaloid, sesquiterpenes, triterpene (oleanolic acid), p-hydroxyacetophenone, anthraquinones, and phenolic compounds like coumarins (scopoletin) and flavonoids (Schmeda-Hirschman and Papastergiou, 1993; Brown, 1994; Ngo and Brown, 1999; Schmeda-Hirschmann et al., 2004).

### 3.2. Antioxidant activity

Antioxidant activity results are displayed in Table 3. Our findings showed that the *Fabiana* ethanolic extracts had an interesting antioxidant activity, being more active than the aqueous extracts.

The alcoholic extract of *Fabiana punensis* was the most active scavenger of DPPH radical followed by *Fabiana densa*, *Fabiana patagonica* and *Fabiana bryoides* ethanolic extracts, respectively. The SC<sub>50</sub>



**Fig. 2.** High performance liquid chromatography profiles of aqueous and ethanolic extracts at 280 nm (A) tincture and (B) decoction of *Fabiana patagonica* (1), *Fabiana bryoides* (2), *Fabiana densa* (3) and *Fabiana punensis* (4).



**Table 3**  
Antioxidant activities of alcoholic and aqueous extracts of four *Fabiana* species. Values (mean  $\pm$  SD,  $n = 4$ ) in the same column followed by a different letter are significantly different ( $p < 0.05$ ).

Samples	$\beta$ -Carotene assay (IC <sub>50</sub> values in $\mu$ g GAE/mL) Alcoholic	DPPH assay (SC <sub>50</sub> values in $\mu$ g GAE/mL)	ABTS assay (SC <sub>50</sub> values in $\mu$ g GAE/mL)	$\beta$ -Carotene assay (IC <sub>50</sub> values in $\mu$ g GAE/mL) Aqueous	DPPH assay (SC <sub>50</sub> values in $\mu$ g GAE/mL)	ABTS assay (SC <sub>50</sub> values in $\mu$ g GAE/mL)
<i>Fabiana patagonica</i>	1.00 $\pm$ 0.04 <sup>ab</sup>	9.26 $\pm$ 0.70 <sup>a</sup>	3.48 $\pm$ 0.24 <sup>b</sup>	1.14 $\pm$ 0.11 <sup>c</sup>	14.14 $\pm$ 0.42 <sup>b</sup>	3.32 $\pm$ 0.06 <sup>bc</sup>
<i>Fabiana bryoides</i>	1.48 $\pm$ 0.13 <sup>ab</sup>	9.44 $\pm$ 0.20 <sup>a</sup>	4.51 $\pm$ 0.09 <sup>a</sup>	3.58 $\pm$ 0.36 <sup>b</sup>	23.33 $\pm$ 1.42 <sup>a</sup>	6.49 $\pm$ 0.13 <sup>a</sup>
<i>Fabiana densa</i>	1.92 $\pm$ 0.18 <sup>a</sup>	7.75 $\pm$ 0.08 <sup>b</sup>	3.69 $\pm$ 0.34 <sup>b</sup>	1.25 $\pm$ 0.04 <sup>c</sup>	11.50 $\pm$ 0.43 <sup>c</sup>	3.11 $\pm$ 0.13 <sup>c</sup>
<i>Fabiana punensis</i>	1.14 $\pm$ 0.07 <sup>b</sup>	3.85 $\pm$ 0.24 <sup>c</sup>	2.56 $\pm$ 0.10 <sup>c</sup>	4.46 $\pm$ 0.40 <sup>a</sup>	7.83 $\pm$ 0.37 <sup>d</sup>	3.42 $\pm$ 0.09 <sup>b</sup>
BHT	4.00 $\pm$ 0.43	21.63 $\pm$ 0.67	5.00 $\pm$ 0.10			

values of *Fabiana punensis* were sixfold lower than the SC<sub>50</sub> value of BHT, a known antioxidant compound used as control.

The *Fabiana punensis* alcoholic extract also showed the best antioxidant capacity (2.56  $\mu$ g GAE/mL) to quench ABTS<sup>•+</sup> compared to *Fabiana patagonica*, *Fabiana densa*, and *Fabiana bryoides* ethanolic extracts. The SC<sub>50</sub> values were lower than BHT (5.00  $\mu$ g/mL).

In the  $\beta$ -carotene assay, except for the aqueous extract of *Fabiana punensis*, all extracts showed IC<sub>50</sub> values (from 1.00 to 3.58  $\mu$ g GAE/mL) lower than the BHT (4.00  $\mu$ g/mL) and lower than those obtained in DPPH and ABTS assay. In the  $\beta$ -carotene assay, *Fabiana patagonica* extracts exhibited the highest activity compared with the other three taxa.

The aqueous extracts showed antioxidant capacities with SC<sub>50</sub> and IC<sub>50</sub> values between 1.14 and 23.33  $\mu$ g GAE/mL for all assays. These results are very interesting because the decoctions are used in traditional medicine (Perez, 2006).

There was a positive correlation between phenolic and flavonoid content with the antioxidant capacity of alcoholic extracts in DPPH<sup>•</sup> assay ( $R^2 = 0.8478$  and  $R^2 = 0.9711$ , respectively) and ABTS<sup>•+</sup> scavenging activity ( $R^2 = 0.9107$  and  $R^2 = 0.9205$ , respectively) (Fig. 3A and C). Furthermore, the same effect was observed between the phenolic content of aqueous extracts with the DPPH and ABTS scavenging activities ( $R^2 = 0.6885$  and  $R^2 = 0.8376$ , respectively) (Fig. 3B and D). The antioxidant activity in  $\beta$ -carotene assay showed a positive correlation between alcoholic or aqueous extracts and flavonoids ( $R^2 = 0.6893$  and  $R^2 = 0.9002$ , respectively) (Fig. 3E and F). According with our results the antioxidant properties of the all preparations depend, principally, on their intrinsic electron-donor capacity determined by the flavonoid and polyphenol content. The phenolic compounds, in particular flavonoids have long been considered beneficial for health due to their “antioxidative” effect (Zhang and Björn, 2009).

The antioxidant activity found in *Fabiana* species was similar to the obtained activity of other shrubs species that grow in mountainous area of Argentina such as *Baccharis incarum* (Zampini et al., 2009b), *Chuquiraga atacamensis*, *Parastrephia lucida* (Zampini et al., 2008) and *Zuccagnia punctata* (Morán Vieyra et al., 2009). These plants are characterized by a high physiological plasticity not only for their salt tolerance limits, but also for the UV-radiations. They are equipped with a powerful antioxidant system that includes enzymatic and non-enzymatic components (Alonso Amelot, 2008). Enhanced synthesis of determined secondary metabolites under stressful conditions is believed to protect the cellular structures from oxidative effects and these compounds are principally polyphenols and are considered as natural antioxidants (Zhang and Björn, 2009).

### 3.3. Anti-inflammatory activity

Compounds that have free radical scavenging activities and/or suppressive activities on lipid peroxidation may thus be expected to have therapeutic potentials for several skin inflammatory diseases.

In our present study, the anti-inflammatory activities of *Fabiana* extracts on inflammatory mediators were measured against isolated enzymes (soybean lipoxygenase and hyaluronidase).

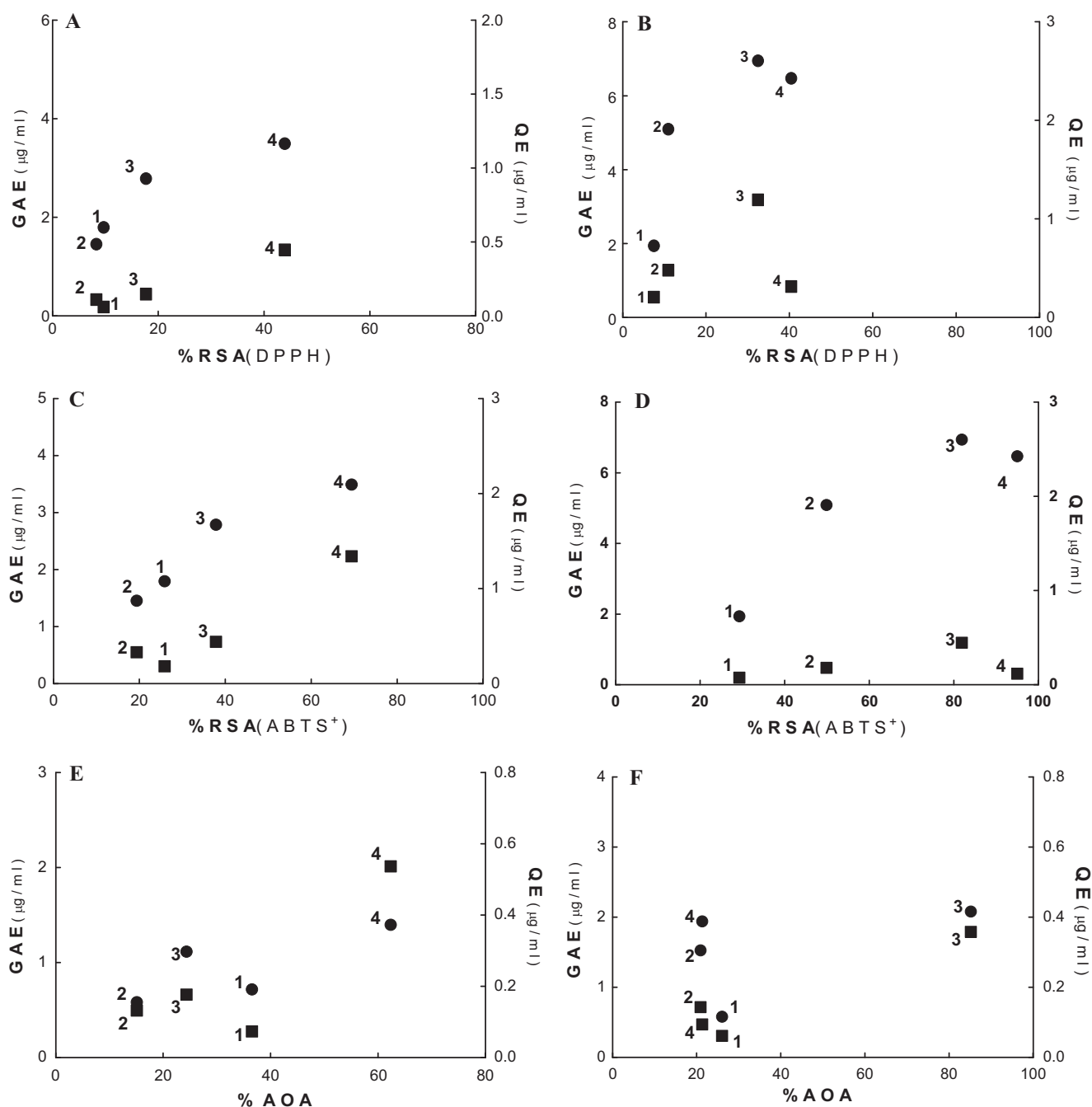
Fig. 4 shows the hydroperoxide production by lipoxygenase-catalyzed reaction in presence of different total phenolic compound concentrations of *Fabiana* extracts.

All *Fabiana* extracts inhibited the lipoxygenase enzyme. The inhibitory effects increase with the extract concentrations. The aqueous extract of *Fabiana patagonica* exhibited the highest activity (IC<sub>50</sub> 12.5  $\mu$ g GAE/mL) compared with the aqueous extract of *Fabiana punensis* and *Fabiana bryoides* and *Fabiana densa* (IC<sub>50</sub> 15.5; 19.3 and >25  $\mu$ g GAE/mL, respectively), while the IC<sub>50</sub> values for alcoholic extracts were 30.0, 25.0, 15.5 and 14.2  $\mu$ g GAE/mL for *Fabiana densa*, *Fabiana bryoides*, *Fabiana patagonica*, and *Fabiana punensis*, respectively. In our study, the alcoholic extract of *Fabiana punensis* and the aqueous extract of *Fabiana patagonica*, which exhibited potent antioxidant activity, displayed the major anti-inflammatory activity in the lipoxygenase assay. The hydroperoxide released was almost completely inhibited by 25  $\mu$ g GAE/mL of these extracts. The anti-lipoxygenase activity was similar to obtained with naproxen (IC<sub>50</sub> value of 14  $\mu$ g/mL) an anti-inflammatory commercial drug, and higher than the obtained with caffeic acid (IC<sub>50</sub> value of 57  $\mu$ g/mL), a natural anti-inflammatory.

Fig. 5 shows the time course of lipoxygenase-catalyzed reaction in presence of 17  $\mu$ g GAE/mL of *Fabiana* extracts. At the same phenolic compound concentration, the alcoholic and aqueous extract of *Fabiana punensis* and *Fabiana patagonica* were more effective than the other *Fabiana* species. Lipoxygenase are sensitive to antioxidants, and they commonly inhibit lipid hydroperoxide formation due to scavenging of lipid-oxy- or lipid-peroxy-radicals formed in the course of enzymatic peroxidation. This could limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of lipoxygenase.

The other key enzyme involved in local tissue damage and inflammation is hyaluronidase. The IC<sub>25</sub> values for alcoholic extracts were 192.5, 194.0, 190.5, 230.5  $\mu$ g GAE/mL for *Fabiana patagonica*, *Fabiana bryoides*, *Fabiana punensis* and *Fabiana densa*, respectively. In general, decoctions were more active than tinctures. The IC<sub>25</sub> values for aqueous extracts were 157.7, 165.2, 132.0 and 184.5  $\mu$ g GAE/mL for *Fabiana patagonica*, *Fabiana bryoides*, *Fabiana punensis*, and *Fabiana densa*, respectively. All the extracts exhibited good activity compared with the controls naproxen and hesperetin with IC<sub>25</sub> values of 102 and 66  $\mu$ g/mL. The drugs may exert a portion of their anti-inflammatory activity by preventing the generation of small hyaluronic acid fragments, because these fragments are potent inducers of inflammatory cytokine release (Khanum et al., 2005).

There was a positive correlation between phenolic and flavonoid content and anti-lipoxygenase capacity of alcoholic extracts ( $R^2 = 0.9565$  and  $R^2 = 0.7397$ , respectively) (Fig. 6C). Anti-hyaluronidase activity of alcoholic extracts showed a positive correlation with phenolic ( $R^2 = 0.9668$ ) and flavonoid



**Fig. 3.** Linear regression plots of percentage of antioxidant activity (AOA%) relative to control using the  $\beta$ -carotene bleaching assay and percentage of radical scavenging activity (%RSA) on DPPH and ABTS $^{\bullet\bullet}$  radical assays with respect to total phenols (●) and total flavonoids (■) of *Fabiana* spp. Alcoholic (A, C and E) and aqueous (B, D and F) extracts of (1) *Fabiana patagonica*, (2) *Fabiana bryoides*, (3) *Fabiana densa*, (4) *Fabiana punensis*. The same quantities of dry extract were used in each assay for the different species: 10  $\mu$ g DW for  $\beta$ -carotene bleaching assay and DPPH scavenging activity and 25  $\mu$ g DW for ABTS scavenging activity.

( $R^2 = 0.6815$ ) content (Fig. 6A). However, anti-lipoxygenase and anti-hyaluronidase activities of aqueous extracts did not show any significant correlation with phenolic nor flavonoids content (Fig. 6B and D). In a previous paper, we have demonstrated that, *Baccharis incarum*, *Baccharis boliviensis*, *Chuquiraga atacamensis* and *Parastrephia lucida* that grow in the Argentine Puna (3800 m.o.s.l.) and that are used to reduce oxidative stress and alleviate gout and arthritic pain are able to inhibit cyclooxygenase enzyme (Alberto et al., 2009) as well as *Fabiana* species shows capacity to inhibit other enzyme of inflammatory pathway. Our results justify the popular use of *Fabiana* species.

### 3.4. Mutagenicity activity

*Fabiana patagonica*, *Fabiana punensis*, *Fabiana densa*, and *Fabiana bryoides* are medicinal plants used by the Argentine population to treat different diseases, but little is known about the risks in consuming these preparations. In this study, mutagenicity was evaluated by the Ames assay. In a series of experiments preceding the mutagenicity studies, it was ascertained that the different amounts of extracts of *Fabiana patagonica*, *Fabiana punensis*, *Fabiana densa* and *Fabiana bryoides* added to the indicator bacteria do not influence their viability (data not shown). Table 4 shows the



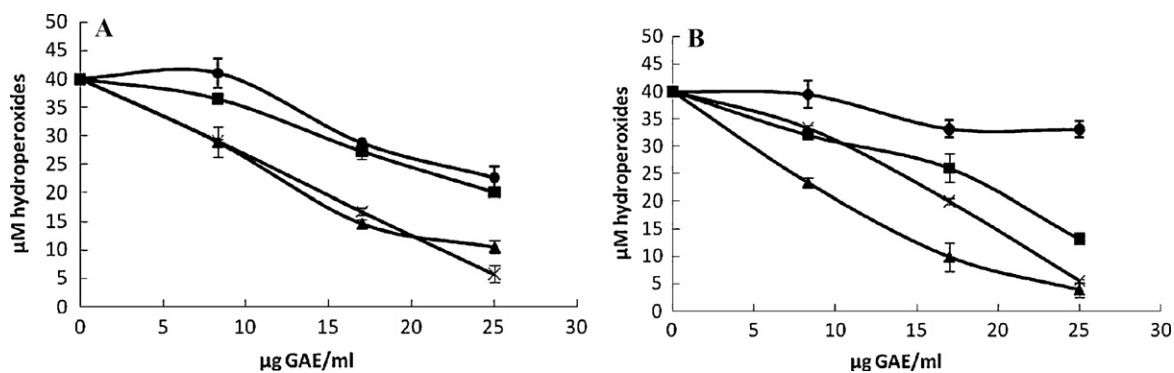


Fig. 4. Hydroperoxide production by lipoxygenase catalyzed reaction in presence of different total phenolic compound concentration of alcoholic (A) or aqueous (B) extracts of *Fabiana bryoides* (■), *Fabiana patagonica* (▲), *Fabiana punensis* (×) and *Fabianadensa* (●).

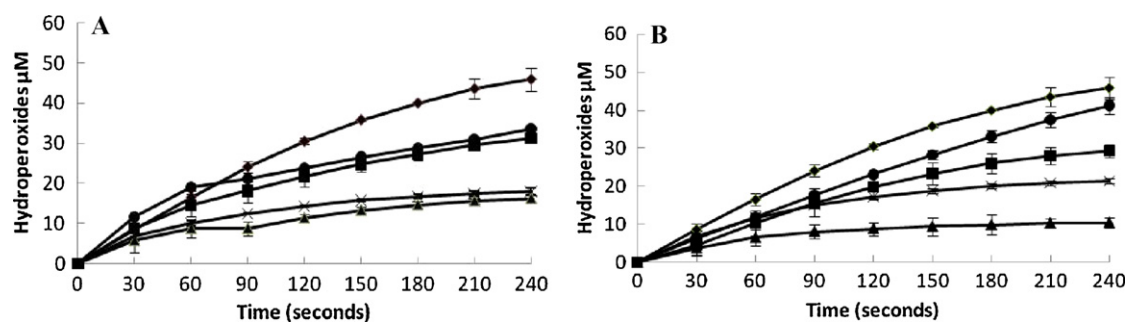


Fig. 5. Time course of lipoxygenase catalyzed reaction without extracts (♦) and in presence of 17 μg GAE/mL alcoholic (A) or aqueous (B) extracts of *Fabiana bryoides* (■), *Fabiana patagonica* (▲), *Fabiana punensis* (×) and *Fabiana densa* (●).

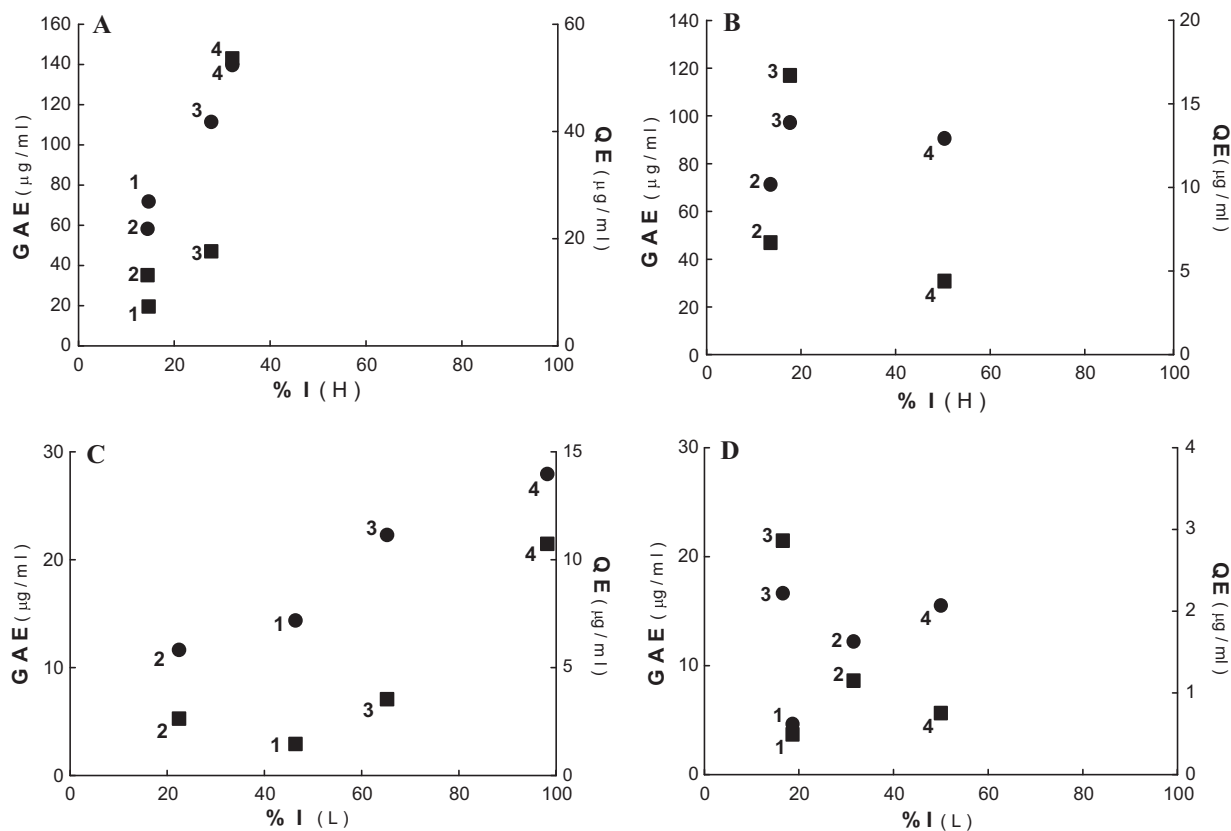


Fig. 6. Linear regression plots of percentage of inhibition (%I) of lipoxygenase (L) and hyaluronidase (H) activities with respect to total phenols (●) and total flavonoids (■) of alcoholic (A and C) and aqueous (B and D) extracts of (1) *Fabiana patagonica*, (2) *Fabiana bryoides*, (3) *Fabiana densa*, (4) *Fabiana punensis*.

**Table 4**

Revertant/plate in the strains TA98 and TA100 of *Salmonella typhimurium* after treatment with various doses of the alcoholic and aqueous extracts of *Fabiana bryoides*, *Fabiana patagonica*, *Fabiana punensis* and *Fabiana densa* with (+S9) and without (–S9) metabolic activation.

Extracts	Treatment (μg GAE/plate)		No. revertant/plate <sup>a</sup>			
			TA98		TA100	
			–S9	+S9	–S9	+S9
<i>Fabiana bryoides</i>	Aqueous	100	35 ± 1	30 ± 2	121 ± 6	113 ± 8
		500	33 ± 5	33 ± 6	148 ± 5	121 ± 1
		1000	43 ± 2	29 ± 3	127 ± 8	134 ± 1
	Ethanollic	100	20 ± 2	39 ± 3	81 ± 6	94 ± 2
		500	19 ± 2	37 ± 7	52 ± 9	96 ± 9
		1000	21 ± 3	14 ± 4	28 ± 10	54 ± 7
<i>Fabiana patagonica</i>	Aqueous	100	21 ± 1	40 ± 5	129 ± 14	135 ± 18
		500	30 ± 10	46 ± 3	135 ± 20	160 ± 28
		1000	25 ± 12	41 ± 7	145 ± 7	125 ± 24
	Ethanollic	100	26 ± 3	40 ± 3	98 ± 2	101 ± 3
		500	29 ± 8	66 ± 2	110 ± 3	109 ± 2
		1000	30 ± 6	54 ± 2	95 ± 3	95 ± 8
<i>Fabiana punensis</i>	Aqueous	100	30 ± 5	31 ± 4	146 ± 3	110 ± 9
		500	34 ± 9	40 ± 3	152 ± 1	123 ± 13
		1000	35 ± 10	36 ± 2	163 ± 6	143 ± 13
	Ethanollic	100	28 ± 8	63 ± 4	99 ± 3	98 ± 6
		500	29 ± 12	60 ± 3	70 ± 18	151 ± 15
		1000	32 ± 15	61 ± 7	85 ± 14	116 ± 4
<i>Fabiana densa</i>	Aqueous	100	31 ± 5	31 ± 4	90 ± 3	108 ± 9
		500	39 ± 1	32 ± 8	96 ± 8	137 ± 13
		1000	33 ± 6	56 ± 5	toxic	toxic
	Ethanollic	100	40 ± 2	30 ± 6	94 ± 10	110 ± 6
		500	41 ± 5	34 ± 3	73 ± 5	94 ± 17
		1000	33 ± 8	36 ± 4	86 ± 10	100 ± 9
Negative control <sup>b</sup>	DMSO		23 ± 3	34 ± 9	92 ± 3	102 ± 20
Positive control <sup>c</sup>			1438 ± 114	780 ± 34	990 ± 90	1150 ± 50

(–S9) without and (+S9) with metabolic activation.

<sup>a</sup> Mean number of revertants (Mean of three plates ± SD).

<sup>b</sup> The number of spontaneous revertants was determined in a assays without sample.

<sup>c</sup> Mean number of revertants induced by reference mutagens [2-AF, 2-aminofluorene (10 μg/plate), positive control for +S9; 4-NPD, 4-nitro-o-phenylenediamine (20 μg/plate) positive control for –S9].

number of revertants/plate after the treatments with the extracts in the two different strains of *Salmonella typhimurium*, with or without metabolic activation. None of the eight plant extracts (aqueous and ethanollic) were mutagenic in strains TA98 or TA100 under the conditions used in this assay which indicates the inexistence of compounds in the extracts that cause base substitution (detected in TA100) and frameshift (detected in TA98) mutations. The absence of mutagenicity for the different extracts studied in the *Salmonella* tested strains indicates that DNA does not seem to be a relevant target for these extracts. *Fabiana bryoides* ethanollic extract showed an interesting effect: it inhibited spontaneous mutagenesis in strains TA98 (+S9) and TA100 (+S9/–S9). At the highest tested concentration (1000 μg/plate), it decreased the number of revertants by about 50% without any effect on cell viability, which could be considered antimutagenic.

The decrease of a spontaneous mutation rate by *Fabiana bryoides* indicates that this plant species might suppress mucAB-facilitated replication over DNA damage. The idea is supported by previous studies showing that some natural products reduced spontaneous mutagenesis in the pKM101 plasmid harbouring strain (Stajkovic et al., 2007; Beric et al., 2008; Mitić-Ćulafić et al., 2009). *Fabiana bryoides* could be a promising candidate for further studies designed to obtain more evidence on its components with potential chemopreventive activity.

#### 4. Conclusion

*Fabiana* species are traditionally employed like antiseptic, anti-inflammatory and diuretic in the Argentine Northwest to treat many diseases (lung, cough and bone fractures). To our knowledge, this is the first report on activity against inflammatory mediators,

free radical scavenging activities and lack of genotoxicity of *Fabiana* extracts. These findings promote the study of these plants to provide a scientific basis that supports the continued traditional use of medicinal plants. Further investigations are being carried out in our laboratory to isolate and characterize the active components of the plant extracts.

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