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Original Research Article

In vitro antioxidant, antilipoxygenase and antimicrobial activities of extracts from seven climbing plants belonging to the Bignoniaceae

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ABSTRACT

Objectives: This study aimed to evaluate the *in vitro* antioxidant capacity, to determine the anti-inflammatory effect due to lipoxygenase inhibition and to test the antimicrobial activity of ethanolic extracts from leaves of seven climbing species belonging to the Bignoniaceae family. These species are *Adenocalymma marginatum* (Cham.) DC., *Amphilophium vauthieri* DC., *Cuspidaria convoluta* (Vell.) A. H. Gentry, *Dolichandra dentata* (K. Schum.) L. G. Lohmann, *Fridericia caudigera* (S. Moore) L. G. Lohmann, *Fridericia chica* (Bonpl.) L. G. Lohmann and *Tanaecium selloi* (Spreng.) L. G. Lohmann.
Methods: The antioxidant activity was evaluated using three methods, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power. Lipoxygenase-inhibiting activity was assayed spectrophotometrically; the result was expressed as percent inhibition. The antimicrobial activity was assessed using the agar disk diffusion method. Minimal inhibitory concentration (MIC) and minimal bactericidal/fungicidal concentration were also determined for each extract against 12 pathogenic bacterial strains of *Staphylococcus aureus* and seven fungal strains of the *Candida* genus. The identification of the major compounds present in the most promising extract was established by high-performance liquid chromatography–tandem mass spectrometry.
Results: *C. convoluta*, *F. caudigera*, and *F. chica* exhibited the best antioxidant activity by scavenging DPPH and $ABTS^{+}$ radicals and reducing Fe^{3+} ion. These extracts showed a notable inhibition of lipoxygenase. *F. caudigera* was found to have the lower MIC value against *S. aureus* strains and six *Candida* species. The extracts of *F. caudigera* and *C. convoluta* were active even against methicillin-resistant *S. aureus*. *C. convoluta* had higher total phenol content, better antioxidant activity and superior anti-inflammatory and antimicrobial activity. The main phenolic compounds found in this extract were coumaric and hydroxybenzoic acid derivatives and glycosylated and nonglycosylated flavones.
Conclusion: Most of the extracts exhibited antioxidant activity as well as *in vitro* inhibition of lipoxygenase. The excellent antimicrobial activity of *T. selloi* and *F. chica* supports their use in traditional medicine as antiseptic agents. The extracts of *F. caudigera* and *C. convoluta*, both with notable biological activities in this study, could be used as herbal remedies for skin care. In addition, this study provides, for the first time, information about phenolic compounds present in *C. convoluta*.

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1. Introduction

Members of the Bignoniaceae family are mainly used as ornamentals for their attractive flowers. However, several species are known for their bioactive compounds and pharmacological

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properties [1]. A preliminary phytochemical screening has shown the antibacterial activity of twenty tinctures from climbing species growing in the Northeast region of Argentina [2]. Seven of these species have potential as antibacterial agents because they effectively inhibited the bacteria tested and also contained phytochemical compounds with biological activity, such as tannins and phenolic compounds (mainly flavonoids). The species were *Adenocalymma marginatum* (Cham.) DC., *Amphilophium vauthieri* DC., *Cuspidaria convoluta* (Vell.) A. H. Gentry, *Dolichandra dentata* (K. Schum.) L. G. Lohmann, *Fridericia caudigera* (S. Moore) L. G. Lohmann, *Fridericia chica* (Bonpl.) L. G. Lohmann and *Tanaecium selloi* (Spreng.) L. G. Lohmann.

An exhaustive literature review showed that some of these seven species, belonging to Bignoniaceae family, also have other uses in folk medicine. *F. chica*, for example, is used in Brazil as a healing agent and is known for its anti-inflammatory properties [3]. Flavonoids are responsible for the antioxidant and photoprotective effects of *F. chica* [4]. In the same way, Ribeiro et al. [5] reported that the antioxidant activity of *F. chica* is related to its phenolic content. Keller [6] has reported the use of *A. marginatum* as a digestive agent among the Guaraní aborigines of Misiones, but there are no records of biological evidence confirming such activity. Nevertheless, studies of the chemical composition and biological activities of other species from the genus *Adenocalymma* have been found. These species showed antimicrobial and anti-inflammatory activities as well as against scab [7]. *T. selloi* is used ornamentally, and a decoction of its leaves is used as an anti-inflammatory and antiseptic agent, but there are no pharmacological or chemical studies that support these uses [8]. Recently, Torres et al. [9] showed the synergistic antibacterial effect of *C. convoluta* and *F. caudigera* extracts when they are combined with commercial antibiotics. These researchers also demonstrated the presence of three flavones with proven antimicrobial and antioxidant properties in the *F. caudigera* extracts [9].

There are no reports in the literature about the biological activity or traditional uses for the other species. However, several species of the genus *Fridericia* have been found to have anti-inflammatory, antinociceptive and antimicrobial properties [10]. The fruits of *A. crucigerum* are used in folk medicine to treat inflammation, skin infection and headache and as a calming agent [11]. This species also showed an antioxidant potential against free radicals and antinociceptive effects [12,13]. In the same way, the extracts of *A. paniculatum* showed significant anti-inflammatory activity and analgesic, antipyretic, antioxidant and antihyperglycemic effects [14]. On the other hand, one of the most studied species of the genus *Dolichandra* is *D. unguis-cati*, which is used in folk medicine to treat dysentery, inflammation and rheumatism. Duarte et al. [15] demonstrated the antilipoxygenase and anticyclooxygenase activities of this species, and then Aboutabl et al. [16] showed a significant anti-inflammatory effect in rats. In this study, the authors isolate and identify the flavonoids present in *D. unguis-cati* and evaluate the potential cytotoxic and anti-inflammatory activities of the plant.

The promising antibacterial results and the background mentioned in the previous paragraphs indicated a close relationship between anti-inflammatory and antioxidant activities [17] and lead us to an *in vitro* study of the antioxidant capacity, the anti-inflammatory effect, through lipoxygenase (LOX) inhibition, and the antimicrobial activity of these species. We also evaluated the phytochemical constituents of the most potent extract to support the possibility of its use as a natural resource in therapeutics and to contribute to the knowledge of the chemical composition of this species.

2. Materials and methods

2.1. Chemicals

All the reagents and chemicals used in the experiments were of analytical grade. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Trolox, Folin–Ciocalteu reagent and 5-LOX of soybeans were obtained from Sigma–Aldrich, USA. Indomethacin (IM 75®, Montpellier Laboratories) was purchased from the local pharmacy.

2.2. Microorganisms

The bacterial strains were selected based on application purpose.

2.2.1. Bacteria

A total of 12 clinical isolates of *Staphylococcus aureus* were used. They were isolated locally on the mannitol salt agar slant (Britania Laboratories, Argentina) from patients hospitalized in Hospital Ramón Carrillo, Sáenz Peña, Argentina. The species were confirmed following morphological observations and biochemical tests [18]. Two strains were methicillin-resistant (Sa5637 and Sa5722). All these microorganisms were maintained on agar slants.

2.2.2. Fungi

A total of 7 strains of *Candida* were used. They were *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and clinical isolates of *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. tropicalis*. These strains were isolated from patients hospitalized in the local hospital in Colonia Aborígen, Chaco, Argentina.

2.3. Plant material

The seven selected plant species were collected in March and November 2015 from the province of Misiones, Argentina. The plants were identified by specialists from the Herbarium of Institute of Botany of the Northeast (IBONE-CONICET), Corrientes, Argentina, where the voucher specimens were deposited. The species tested were *A. marginatum* (voucher number AMG 408), *A. vauthieri* (AMG 422), *C. convoluta* (AMG 104), *D. dentata* (AMG 445), *F. caudigera* (AMG 418), *F. chica* (AMG 432) and *T. selloi* (AMG 215).

2.4. Extraction

Plant materials were dried at room temperature. The dry leaves were triturated using a mechanical mill (Dalvo®, Argentina) until particle size ranged between 1.70 mm and 710 μm, as determined by ASTM sieves. Extracts were individually prepared by macerating 20 g of each powder in 100 mL of 80% ethanol for 7 days, in a dark place at room temperature. All extracts were then filtered through Whatman No. 1 filter paper and centrifuged at 1210×*g* for 5 min. Extracts were stored at 4 °C in the dark. These extracts were used in the next assays.

2.5. Total phenolic content determination

The total polyphenol content (TPC) was analyzed using the Folin–Ciocalteu method [19]. The absorbance was measured at 765 nm (UV–VIS Spectrophotometer, Shimadzu UV-1800). The TPC in the extracts was expressed as milligram gallic acid equivalent (GAE) per gram dry extract (DE). All determinations were made in triplicate, and the data were presented as mean ± standard deviation.

2.6. Antioxidant activity

Three methods, $ABTS^+$, free radical DPPH and ferric reducing antioxidant power (FRAP), based on the reaction with electron-donating or hydrogen radical-producing compounds/antioxidants, were used [20–23]. Despite the similar mechanisms of the methods, the reagents and products are different. Trolox was used as a common standard for the calibration of the methods.

2.6.1. DPPH assay

DPPH quantification followed the procedure described by Lim et al. [20]. The absorbance was measured at 517 nm. The DPPH radical-scavenging activity was presented as Trolox equivalent antioxidant capacity (TEAC), which measures the ability of antioxidants to quench a radical (DPPH or $ABTS^+$), expressed in a unit called Trolox equivalent (TE). Due to the difficulties in measuring individual antioxidant components from a complex mixture (such as plant extracts), Trolox equivalence is used as a benchmark for the antioxidant capacity in these cases. The results were expressed as TEAC value in μmol TE/g DE and as 50% inhibition concentration (IC_{50}; μg/mL).

2.6.2. $ABTS^+$ decolorization assay

ABTS decolorization was estimated according to the procedure of Re et al. [21]. Briefly, ABTS was dissolved to a 7 mmol/L concentration. $ABTS^+$ was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Afterward, the $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. One milliliter of diluted $ABTS^+$ solution was mixed with 10 μL of samples and incubated for 6 min before taking the absorbance reading. The results were expressed as a TEAC value in μmol TE/g DE and as IC_{50} (μg/mL).

2.6.3. FRAP assay

FRAP was quantified following the procedure of Benzie and Strain [22] with some changes [23]. The FRAP reagent was freshly prepared daily and was warmed to 37 °C before use. Plant extracts (150 μL) and FRAP reagent (2850 μL) were allowed to react for 4 min at 37 °C, and the absorbance was measured at 593 nm. The results were expressed as μmol TE/g DE.

2.7. Anti-inflammatory activity (5-LOX assay)

The LOX-inhibiting activity was assayed spectrophotometrically as described by Taraporewala and Kauffman [24] with minor modifications. Briefly, 100 μL of the enzyme solution (at the final concentration of 200 U/mL) was prepared in boric acid buffer (0.2 mol/L; pH: 9), mixed with 10 μL of extract solution (0.04 mg/mL) and then incubated at room temperature for 3 min. The reaction was subsequently initiated by the addition of the substrate solution (linoleic acid, 250 μmol/L), and absorbance was recorded for 6 min at 234 nm. Indomethacin was used as the positive control. The percent inhibition for each inhibitor was calculated using the following equation:

$$\text{Percent inhibition } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.8. Antimicrobial activity

The first step in this process was to determine the qualitative antimicrobial activity of the extracts. This was assessed by the agar disk diffusion method. Minimal inhibitory concentration (MIC) and minimal bactericidal/fungicidal concentration (MBC/MFC) were also determined for the active extracts. The MIC, MBC and MFC of Bignoniaceae extracts were evaluated at different concentrations against 12 pathogenic bacterial strains of *S. aureus* and seven fungal strains of the genus *Candida*.

2.8.1. Agar disk diffusion assay

The antimicrobial activity of the crude extracts was qualitatively determined using the agar diffusion assay [25,26]. Briefly, Petri dishes were prepared with a base layer of 20 mL Müeller–Hinton agar (Britania Laboratories, Argentina) and inoculated with each microbial suspension (1×10^6–5×10^6 colony-forming units per milliliter, CFU/mL). Sterile filter disks 6 mm in diameter (Oxoid, UK) were permeated with each extract (30 μg of phenolic compounds/disk) and placed on the inoculated plate. The treated Petri dishes were incubated at 37 °C for 18 h for bacteria and 24–48 h for yeast. Standard disks of ampicillin (10 μg; Britania Laboratories, Argentina) and fluconazole (25 μg) were used as positive antimicrobial controls. Disks with 20 μL of 80% ethanol were used as negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the disks. Extracts with halo values greater than 10 mm were selected for determination of MIC. The growth inhibition diameter was an average of four measurements, taken in four different directions. All tests were performed in triplicate.

2.8.2. Microdilution assay

This test was performed in sterile 96-well microplates [27,28]. The extracts were transferred to individual microplate wells, and serial dilutions of original extract (31.25–1000 μg GAE/mL) were made on the plates. The stock solutions of plant extracts were dissolved in dimethyl sulfoxide (DMSO). Bacterial or fungal inocula (100 μL) containing 5×10^5 CFU/mL and 5×10^3 CFU/mL, respectively, were added to each. A number of wells in each plate were reserved for sterile controls (no inoculum added), inoculum viability (no extract added), positive control (ampicillin and fluconazole) and solvent control (DMSO). Plates were aerobically incubated at 37 °C. After incubation for 16–20 h for bacteria, or 24–48 h for yeasts, microbial growth was indicated by the presence of turbidity in the fluid or a pellet on the bottom of the well. MIC was defined as the lowest concentration of extract that had restricted growth to a non-macroscopically visible level.

MBC was determined by transferring 10 μL of each culture medium from each well with no visible growth to Mueller–Hinton agar plates for bacteria, or Sabouraud glucose agar plates for yeasts. After 16–20 h (bacteria) or 48 h (yeasts) of aerobic incubation at 37 °C, the number of surviving organisms was determined. MBC and MFC were defined as the lowest extract concentration at which 99.9% of the bacteria or fungi had been killed. For example, experimentally, MFC was the lowest concentration of the antifungal agent in which fewer than three colonies grew.

2.9. Determination of the main polyphenols in the most active extract

The phenolic compound profile was achieved by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) in the Research and Development Center in Chemistry of National Institute of Industrial Technology, Buenos Aires, Argentina. The analyses were performed in negative mode, and the identification of compounds was carried out on the basis of the *m*/*z* ratio of the quasimolecular ion, fragmentation patterns and data from the literature [29–35].

The detection was performed using a Quattro Premier XE tandem quadrupole mass spectrometer (Waters, Manchester, UK). The separations were conducted at a temperature of 35 °C on

ACE 3C-18 (ACE, UK) 3 μm particle size column (50 mm × 2.1 mm). The injection volume was 40 μL, and the flow rate was 0.3 mL/min. Gradient elution was carried out with a binary system consisting of water/formic acid 0.1% v/v (A) and methanol (B). The gradient elution was modified as follows: 0–5 min 20% B, 5–15 min 50% B, 15–21 min 70% B, and 21–41 min 20% B. Ultraviolet detection was performed at 254 nm. The conditions of electrospray ionization were as follows: drying and nebulizer gas (N_2) flow rate and pressure, 8 L/min and 4.0 bar; drying temperature, 180 °C. N_2 and Ar gases were used as nebulizer and collision gas, respectively. The system was calibrated in the negative ion mode: a capillary voltage of 4500 V at m/z ranging from 100 to 800.

2.10. Statistical analysis

Data are presented as mean ± standard deviation. Statistical analysis was performed using a one-way analysis of variance, followed by Tukey's post-hoc test using the SPSS 21.0 statistical package (IBM Corp, Armonk, NY, USA). A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Total phenolic content

The extract of *C. convoluta* presented the highest TPC followed by the extracts of *F. caudigera* and *F. chica* (Table 1).

3.2. Antioxidant activity

In this study, all extracts showed some DPPH-scavenging activity (Table 2). IC_{50} values varied from (57.84 ± 2.17) to (256.22 ± 1.99) μg/mL. *F. chica* and *C. convoluta* had the lowest IC_{50} values. The IC_{50} values of ABTS radical-scavenging activity ranged from (22.94 ± 1.98) to (93.71 ± 1.13) μg/mL (Table 2). Again, *F. chica* and *C. convoluta* had the lowest IC_{50} values. The ferric reducing abilities of these extracts are also shown in Table 2. All species demonstrated antioxidant activity. *C. convoluta* and *F. caudigera* had the best FRAP values.

Table 1
Total phenolic content of extracts.

Species	Total phenolic content (mg GAE/g DE)
Adenocalymma marginatum	18.91 ± 1.37$^{\lambda}$
Amphilophium vauthieri	11.19 ± 0.95$^{\alpha}$
Cuspidaria convoluta	42.03 ± 1.53*
Dolichandra dentata	18.75 ± 2.04$^{\lambda}$
Fridericia caudigera	36.05 ± 1.37$^{\#}$
Fridericia chica	33.71 ± 0.56$^{\#}$
Tanaecium selloi	24.15 ± 1.13$^{\phi}$

Results are expressed as mean ± standard deviation. Values with different symbols in superscript indicate significant difference from each other at $P < 0.05$. GAE: gallic acid equivalent; DE: dry extract.

3.3. Anti-inflammatory activity

The ability of Bignoniaceae extracts to inhibit LOX activity was expressed as percent inhibition and is shown in Fig. 1. All extracts had some inhibitory activity at the concentration tested (0.04 mg/mL). The amount of inhibited LOX varied from 90.61% ± 1.12% to 38.87% ± 0.79%. The highest percentage of inhibition was obtained with *F. caudigera* extract and the lowest with *A. marginatum*. *C. convoluta*, *F. caudigera* and *F. chica* strongly inhibited LOX activity, values of which were very close to the positive control, indomethacin.

3.4. Antimicrobial activity

For the qualitative antibacterial test, all extracts had an inhibition halo greater than 10 mm in the agar disk diffusion assay. The MIC and MBC values of the seven plant extracts on *S. aureus* isolates are presented in Table 3. *F. caudigera* with MIC values ranging from 62.5 to 500 μg GAE/mL was found to have the lowest values of MIC on almost all of the tested strains. The extracts of *F. caudigera* and *C. convoluta* were active even against Sa5637 and Sa5722, methicillin-resistant strains (MRSA). It is important to note that five of the strains used were resistant to ampicillin (positive control).

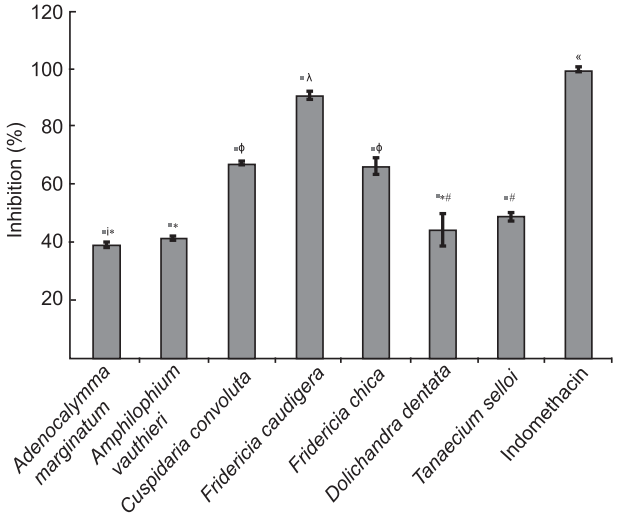

Fig. 1. Percent inhibition of lipoxygenase activity. Values are expressed as mean ± standard deviation. Different symbols indicate significant difference at $P < 0.05$ from each other.

Table 2
Antioxidant results of extracts measured by DPPH, ABTS and FRAP assay.

Species	DPPH		ABTS		FRAP (μmol TE/g DE)
	IC_{50} (μg/mL)	TEAC	IC_{50} (μg/mL)	TEAC	
Adenocalymma marginatum	126.17 ± 2.11$^{\phi}$	32.06 ± 1.63	51.40 ± 0.56$^{\phi}$	959.89 ± 21.34	4,441.00 ± 54.87$^{\phi}$
Amphilophium vauthieri	256.22 ± 1.99$^{\alpha}$	30.65 ± 0.87	93.71 ± 1.13$^{\lambda}$	921.62 ± 17.76	2,016.81 ± 43.27$^{\square}$
Cuspidaria convoluta	77.93 ± 8.07$^{\#}$	25.71 ± 1.64	22.94 ± 1.98*	773.71 ± 20.44	6,643.96 ± 15.00*
Dolichandra dentata	129.87 ± 1.61$^{\phi}$	29.33 ± 2.55	48.06 ± 2.02$^{\phi}$	900.51 ± 18.75	4,465.87 ± 98.76$^{\phi}$
Fridericia caudigera	130.18 ± 6.68$^{\phi}$	31.35 ± 0.75	33.43 ± 0.74$^{\#}$	970.71 ± 25.63	5,680.95 ± 6.50$^{\#}$
Fridericia chica	57.84 ± 2.17*	19.96 ± 2.34	23.17 ± 1.39*	597.52 ± 20.38	3,427.29 ± 63.27$^{\alpha}$
Tanaecium selloi	155.27 ± 3.47$^{\lambda}$	34.28 ± 1.73	35.38 ± 1.36$^{\#}$	924.07 ± 35.28	4,122.73 ± 28.94$^{\lambda}$

Values with different superscripts in the same column for each parameter are significantly different ($P < 0.05$). DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: ferric reducing antioxidant power; TEAC: Trolox equivalent antioxidant capacity; IC_{50}: 50% inhibition concentration; TE: Trolox equivalent; DE: dry extract.

Table 3
Antibacterial activity (MIC and MBC values of extracts against *Staphylococcus aureus* strains).

Staphylococcus aureus strain	*Adenocalymma marginatum*		*Amphilophium vauthieri*		*Cuspidaria convoluta*		*Dolichandra dentata*		*Fridericia caudigera*		*Fridericia chica*		*Tanaecium selloi*		Ampicillin*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3200	125	500	250	1000	250	500	500	1000	62.5	250	250	1000	500	1000	1.6	3.2
3300	125	500	250	1000	250	500	500	1000	62.5	250	250	1000	500	1000	1.6	6.4
5246	250	1000	250	1000	250	500	500	1000	125	500	500	1000	500	1000	202.8	R
5289	250	1000	125	1000	250	500	500	1000	62.5	250	500	1000	500	1000	3.2	12.8
5307	125	500	250	1000	125	500	250	1000	125	250	1000	1000	500	1000	202.8	R
5357	250	1000	250	1000	250	500	500	1000	125	500	250	500	500	1000	25.6	101.4
5377	250	1000	125	1000	125	500	250	1000	62.5	250	500	1000	250	1000	51.2	202.8
5621	250	1000	125	1000	250	500	500	1000	125	250	500	1000	250	1000	12.8	51.2
5627	125	500	250	1000	250	1000	250	1000	125	500	250	1000	500	1000	6.4	25.6
5632	250	1000	250	1000	250	500	500	1000	125	500	500	1000	500	1000	25.6	R
5637	500	R	500	R	250	1000	500	R	125	500	500	R	1000	R	202.8	R
5722	500	R	1000	R	250	1000	1000	R	125	500	1000	R	1000	R	202.8	R

MBC: minimal bactericidal concentration; MIC: minimal inhibitory concentration; R: resistant, not detected within the tested concentrations (62.5–1000 μg of phenolic compounds/mL). Values are expressed in μg of phenolic compounds/mL.
* Values expressed in μg/mL, range of concentrations from 0.8 to 202.8 μg/mL.

In addition, we have observed that *A. vauthieri*, *C. convoluta*, *F. caudigera* and *T. selloi* have exhibited anticandidal activity with MIC values ranging from 125 to 1000 μg GAE/mL (Table 4). The other species did not show pronounced activity, so their activity is not shown in the table. *C. albicans* strains and the clinical isolates of *C. parapsilosis* were found resistant to fluconazole (MIC $\geq$ 64 μg/mL). *C. parapsilosis* ATCC 22019 was the most sensitive species, and the *F. caudigera* extract was the one that had the lowest MIC values. In this study, the MFC values of the extracts ranged from 250 to > 1000 μg GAE/mL. The MFC/MIC ratio in all the cases was $\leq$ 4, which was considered fungicidal.

3.5. Identification of polyphenols

Further investigation of the chemical composition of *C. convoluta* was conducted because it had excellent antioxidant activity and strong anti-inflammatory and antimicrobial effects. Fig. 2 presents a representative total ion chromatogram of the ethanolic extract; the peaks corresponding to the compounds tentatively identified by HPLC-MS/MS are described in Table 5.

Five main compounds were detected and tentatively identified. They were coumaric and hydroxybenzoic acid derivatives, and glycosylated and nonglycosylated flavones.

The *p*-coumaric acid produces MS/MS spectra due to a loss of a CO_2 group from the carboxylic acid functional group, which has fragment ions at *m*/*z* 119, [(M−H)−44]. The fragment 119 was detected in compound 1, which indicates that it could be a *p*-coumaric acid derivative.

Peak 2 presented a pseudomolecular ion [M−H] at *m*/*z* 563. The MS/MS fragmentation pattern of the compound observed in our samples points to the pentosyl residue O-attached to a C-glycosylating hexose. The fragment ion at *m*/*z* 443 ([M−H]−120]), and the simultaneous absence of ion ([M−H]−60) supported the presence of a C-attached hexose. The ions at *m*/*z* 443 and 473 especially indicated the presence of substituted pentose. This suggests that peak 2 corresponds to apigenin-*O*-pentoxyl-hexoside.

Peak 3 was identified as luteolin. The molecular ion at *m*/*z* 285 and the base peak at *m*/*z* 133 are consistent with this. Other typical fragment ions were *m*/*z* 175 and 151.

Peak 4 showed a pseudomolecular ion at *m*/*z* 299. The fragment ion at *m*/*z* 137 from loss of one sugar unit ([M−H]−162]) represents *p*-hydroxybenzoic acid. This compound was tentatively identified as a hydroxybenzoic acid sugar derivative.

Peak 5 displayed [M−H] ion at *m*/*z* 329 and one fragment at *m*/*z* 299 by loss of two methyl groups, which is corresponding to cirsiliol (6-hydroxyluteolin-6,7-dimethyl ether).

4. Discussion

The comparison between IC_{50} values of the ABTS and DPPH assays suggests that the mechanism of the antioxidant activity is mainly based on single electron transfer. Halvorsen et al. [36] suggested that most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP assay. Regarding extracts prepared from leaves of *F. chica,* other researchers have already demonstrated their potential antioxidant activity [3–5,37]. Such activity was attributed to the presence of phenolic compounds [38], which are capable of interrupting chain reactions caused by free radicals due to their ability to donate hydrogen atoms [39]. In our work, the IC_{50} of this extract was 57.84 μg/mL, which is higher than the values found by other authors [3,4,37] who described significant antioxidant activity (IC_{50} = 13–16 μg/mL). These differences may be due to the source and environment in which the plants were gathered. The plants used in other works were collected from Southern and Amazonian regions of

Table 4
Anticandidal activity (MIC and MFC values of extracts against *Candida* species).

Drug	*Candida albicans* ATCC 10231		*Candida albicans*		*Candida parapsilosis* ATCC 22019		*Candida parapsilosis*		*Candida glabrata*		*Candida tropicalis*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Amphilophium vauthieri	250	500	500	1000	500	R	1000	R	250	1000	250	1000
Cuspidaria convoluta	500	1000	500	1000	125	250	250	500	500	1000	250	1000
Fridericia caudigera	250	500	250	500	125	250	250	500	500	1000	250	500
Tanaecium selloi	500	R	1000	R	250	500	1000	R	1000	R	500	R
Fluconazole	> 64	> 64	> 64	> 64	8	16	64	> 64	32	64	2	4

Values are expressed in μg of phenolic compounds/mL. R: Resistant, not detected within the tested concentrations (62.5–1000 μg of phenolic compounds/mL); MFC: minimal fungicidal concentration; MIC: minimal inhibitory concentration.

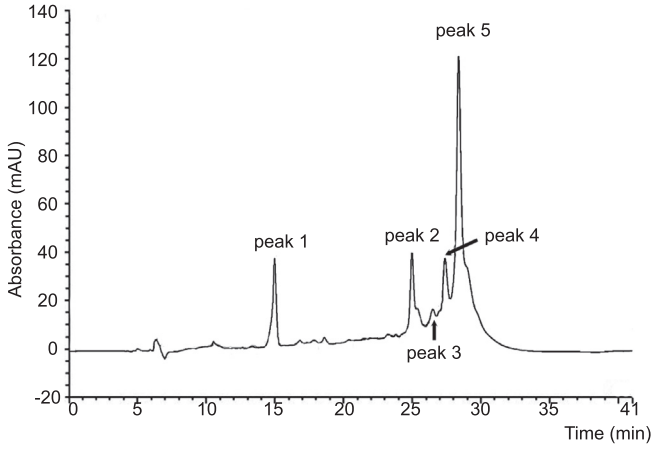

Fig. 2. High-performance liquid chromatography-diode array detector chromatogram of ethanolic extract of *Cuspidaria convoluta*, λ = 254 nm. Peak identities are numbered in Table 5.

Brazil, whereas our assays were made with plants growing in the Northern of Argentina. In reviewing the literature, no data were found for the IC_{50} of other species tested in this study.

The oxidant substances are important in inflammation because they contribute to oxidative stress. This stress is caused by an imbalance between the cellular production of reactive oxygen species (ROS) and the ability of a biological system to rapidly decode the intermediate reactants or repair the resulting damage. ROS, such as superoxide radical, peroxynitryl, hydroxyl radical and hydrogen peroxide, is continuously produced in living systems as a result of metabolic reactions. The antioxidant activity of phenolic compounds present in plants is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential [40]. The processes associated with inflammatory responses are complex and often involve ROS.

The anti-inflammatory activity results show that these plants have some phytochemical constituents which may be active against the LOX enzyme. Potent inhibition of the LOX enzyme by these extracts makes them attractive materials for further biological and phytochemical examination. Extracts from *F. chica* leaves have shown anti-inflammatory activities in other research [3,41,42], but to date, there are no reports about the other species.

The presence of phenolic compounds may explain the antioxidant activities and the LOX inhibition [43,44]. Such inhibition could be attributed to the antioxidant activities of these extracts because the most mechanism of action involves inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy- or lipidperoxy-radicals formed in the course of enzymatic peroxidation [45]. This inhibition can limit the availability of lipid hydroperoxide substrate, which is necessary for the catalytic cycle of LOX [46]. Inhibition of the LOXs by antioxidants can also be attained via chelation of its nonheme bound iron [47] or by reduction in its ferric form [48]. Hence, the anti-inflammatory activities of these plant extracts could be explained by the potent inhibitory effects of their phenolic compounds on arachidonic acid metabolism through the LOX pathway [40].

MICs' values of all the active plant extracts were almost fourfold lower than their MBC values. In general, MBC/MIC ratio less than or equal to four signifies a bactericidal effect of the test substance [49]. In other research, these species have demonstrated antibacterial activity against Gram-positive bacteria, but it is the first time that they show activity against MRSA [2]. *Fridericia caudigera* and *C. convoluta* also increased the antibacterial effect of oxacillin against MRSA [9]; Höfling et al. [50] demonstrated the antimicrobial potential of the leaf extract of *F. chica*.

Candida albicans is the most common fungal pathogen. This species causes invasive infections, and it is a severe problem, mainly in immunosuppressed patients [51,52]. However, the epidemiology of yeast infections is rapidly evolving, and non-*albicans Candida* species and other rare yeasts have emerged as major opportunistic pathogens [53]. In recent years, the investigation of non-*albicans Candida* species has received particular attention. Several species of this group are commonly associated with oral mucosa and are identified as commensals for a minority of healthy individuals [54]. The secondary metabolites of plants are a natural source of a wide range of chemical compounds with antifungal properties, promoted by the intense evolutionary pressure exerted by microbial pathogens in the environment [55]. Notably, the results of the tests for both *C. albicans* and *C. parapsilosis* were promising and may help to develop new antimicrobial tools against both

Table 5
Main phenolic compounds obtained from the ethanolic extract of *Cuspidaria convoluta*.

Peak No.	t_R (min)	*m/z* [M−H]	MS/MS fragmentation, [M−H] *m/z*	Tentative identification
1	14.6	419	119, 141	Coumaric acid derivative
2	24.9	563	443, 473	Apigenin-*O*-pentoxyl-hexoside
3	26.5	285	133, 151, 175	Luteolin
4	27.3	299	137, 212, 228	Hydroxybenzoic acid sugar derivative
5	28.1	329	299	Cirsiliol

yeasts which are resistant to fluconazole. According to Aligiannis et al. [56] and Ferreira et al. [57], the inhibitory activity of compounds or natural products can be classified as strong (MICs up to 500 μg/mL), moderate (MICs between 500 and 1500 μg/mL) or weak (MICs above 1500 μg/mL). By this standard, the extract of *F. caudigera* provided strong inhibition against all *Candida* species evaluated. This result is relevant because the extract could be an alternative to fluconazole, which is the reference antifungal drug for candidiasis treatment.

This is the first report on phytochemical composition of *C. convoluta* extracts. Several compounds found in this extract may be associated with its biological activities due to phenolic compounds that are linked to antioxidant, anti-inflammatory and antimicrobial activities, among many other biological effects [58,59].

The biological activities of *p*-hydroxy benzoic acid and its derivatives are known, and are summarized in the review by Manuja et al. [60]. Among the activities potentially related to human health, this review highlights antimicrobial, antimutagenic, antiestrogenic, hypoglycemic, anti-inflammatory, anti-platelet-aggregating, nematicidal, antiviral and antioxidant activity.

Flavonoids and phenolic acids have a protective role against inflammation and have high antioxidant capacity [61]. They interact with various enzymatic systems. The inhibition of the enzymes cyclooxygenase and lipooxygenase results in their anti-inflammatory activity [62]. Many other biological activities are attributed to flavonoids and phenolic acids: antiviral, antimicrobial, antioxidant, antihepatotoxic, antiosteoporotic, antiulcer, immunomodulatory, antiproliferative and apoptotic activity [31].

Apigenin O-pentosyl hexoside showed an inhibitory effect in the β carotene–linoleic acid system, confirming its antioxidant effect [63]. Torres et al. [9] showed the antimicrobial effect of luteolin isolated from *F. caudigera*; this compound also has anti-inflammatory and antioxidant activity. It is interesting to note that, according to our knowledge, this is the second time that cirsiliol has been found in the Bignoniaceae family [64]. This flavone is a potent inhibitor of arachidonate 5-LOX, an enzyme responsible for leukotriene biosynthesis [65]; Shoeb et al. [66] demonstrated its antioxidant activity.

On the whole, most of the extracts exhibited antioxidant activity (as radical-scavenging and reducing abilities) as well as *in vitro* inhibition of LOX. The extracts of *F. caudigera* and *C. convoluta* were active even against MRSA. Moreover, *A. vauthieri, C. convoluta*, *F. caudigera* and *T. selloi* have exhibited anticandidal activity. These promising findings of antimicrobial activity by *T. selloi* and *F. chica* extracts support their use in traditional medicine as antiseptic agents. This study has also shown that the extracts of *C. convoluta* and *F. caudigera*, both with notable biological activities, could be used as herbal remedies for skin care with antioxidant, antibacterial and antifungal activities.

In addition, this study provides, for the first time, information about phenolic compounds present in *C. convoluta*. These compounds may be associated with its biological activities. Future research will be needed to elucidate the antioxidant and anti-inflammatory mechanisms *in vivo*, as well as the bioavailability and metabolic pathways involved.

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Conflict of interest

No conflict of interest declared.

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