Antimicrobial activity of selected plant species from "the Argentine Puna" against sensitive and multi-resistant bacteria

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A R T I C L E   I N F O

A B S T R A C T

Aim: The plant species reported here are traditionally used in the "Puna" or "Altiplano" of Argentina for ailments related to bacterial infections. The aim of this study was to evaluate their antimicrobial properties against a panel of sensitive and multi-resistant Gram-positive and Gram-negative bacteria.
Materials and methods: The antimicrobial activity of tinctures and aqueous extracts (*Baccharis boliviensis*, *Chiliotrichiopsis keidelii*, *Chuquiraga atacamensis*, *Fabiana bryoides*, *Fabiana densa*, *Fabiana punensis*, *Frankenia triandra*, *Parastrephia lucida*, *Parastrephia lepidophylla*, *Parastrephia phyliciformis*, *Tetraglochin cristatum*) was determined using the agar macrodilution and broth microdilution methods recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). The antibiotic resistant clinical strains were isolated from nosocomial infection in human lesions of skin and soft parts.
Results: The ethanolic extracts of 11 plant species inhibited the growth of one or more of the following strains: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *Morganella morganii*, *Pseudomonas aeruginosa*. Ethanol extracts (tinctures) of aerial parts of *Baccharis*, *Fabiana* and *Parastrephia* showed the highest levels of antibacterial activity on methicillin, oxacillin and gentamicin resistant *Staphylococcus* with MIC values from 20 to 150 µg/ml. *Baccharis boliviensis* and *Fabiana bryoides* were more active than the other plant species on *Enterococcus faecalis* with different phenotype. The most interesting activity on multi-resistant Gram-negative strains was obtained from *Chuquiraga atacamensis*. *Parastrephia* species showed activity against *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The ethanolic extracts exhibited stronger activity and broader spectrum of action than aqueous extracts. The extracts were bactericidal in most cases.
Conclusions: The presence of antibacterial activity in Puna plant extracts against multi-resistant bacteria give support to their traditional use for treating conditions associated with microorganisms in humans and animals and consequently seems promising for the treatment of multi-resistant bacteria.

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1. Introduction

1.1. Studied region

The Puna or Altiplano in Northwest Argentina is a cold, high altitude arid environment with steppes and grassland as the two main habitats. The dominant plant life form of the Puna consists of grass and shrubs (Cabrera, 1971). In spite of high altitude and extreme climatic conditions, some 1500 plant species grow in this environment. The dominant flowering plant families include Asteraceae, Poaceae, Fabaceae, Solanaceae and Verbenaceae (Cabrera, 1957, 1971, 1978; Bonaventura et al., 1995; Malvárez, 1999; Borgnia et al., 2006; Perez, 2006; Cuello, 2006). These species have been employed as medicine, food, building and crafts material, forage, fuel and elements in spiritual activities (Villagrán and Castro, 2000; Villagrán et al., 2003; Toursarkissian, 1980; Perez, 2006). The accelerated degradation of natural ecosystems has caused the loss of some natural resources before they became known. Conservation efforts are still poor and locally concentrated in a few protected areas. The identification of useful resources, conservation status, availability and traditional management through ethnobotanical, chemical and biological studies could become an important means

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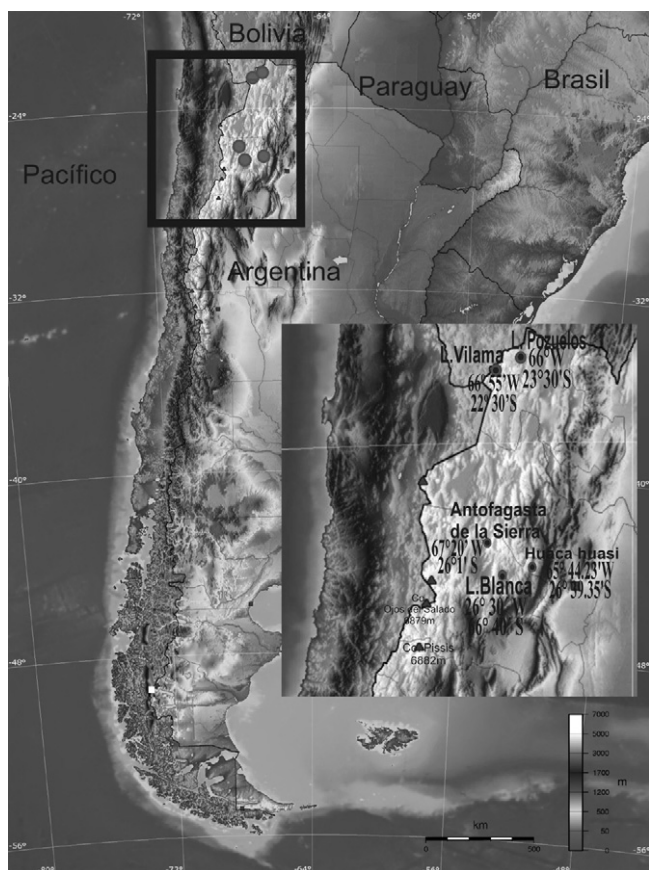*E-mail address:* misla@tucbbs.com.ar (M.I. Isla).

Fig. 1. Studied regions have been indicated in an Argentine map.

to improve the living conditions of the residents and ensure availability of natural resources.

As part of our search directed towards the evaluation of the popular use of Argentine medicinal plants that grow 3000 m above sea level (masl), study of the antimicrobial activity of some plants from the Argentine Puna was undertaken. The target regions were: Laguna Blanca, Punta de la Peña and Quebrada Seca (Catamarca), Laguna de Pozuelos and Laguna de Vilama (Jujuy) and Huaca Huasi (Tucumán) (Fig. 1). Table 1 shows the characteristic plant species of this environment. We chose 11 plant species used traditionally by local inhabitants of the Argentine Puna for treating skin and soft tissue infections and inflammatory processes in humans and animals. The relevance of this study is the possibility to use multi-resistant antibiotic bacteria obtained from skin and soft tissue infections local hospital patients in comparison with sensitive antibiotic clinical isolates and with American Type Culture Collection (ATCC) strains.

1.2. Selected microorganisms

Staphylococcus aureus is the most important cause of skin infections such as boils, abscesses, carbuncles and wound sepses. Another species associated with skin infections is *Pseudomonas aeruginosa*. Both strains are biofilm producers and show a better ability to attach themselves to mucosal surfaces and cause infection than non-biofilm producer strains (Clutterbuck et al., 2007; Quave et al., 2008). Bacterial resistance to antimicrobial agents has become a widespread medical problem, especially in hospitals. It becomes evident in the permeability changes of the cell envelopes, chemical modifications of the antimicrobial agent, enzymatic antibiotic degradation and the presence of membrane efflux systems which pump out the antimicrobial agents from the cytosol. Acquired resis-

tance to antimicrobial agents results from generic cell changes and arises either by mutation or by the acquisition of genetic material (i.e. plasmids) from another cell.

Methicillin resistant *Staphylococcus aureus* strains, multi-resistant *Enterococcus* spp. and *Pseudomonas aeruginosa*, as well as members of the Enterobacteriaceae family such as *Proteus mirabilis*, *Morganella morganii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli* (resistant on five or more antibiotics), are the most common bacterial isolates in Argentine nosocomial infections (Zampini et al., 2007). At present, the pharmaceutical arsenal available to control antibiotic-resistant bacteria is limited. Hence, it is very important to detect natural products with antimicrobial activity. Medicinal plants offer a significant potential for the development of novel antibacterial therapies and adjunct treatments. However, the investigations should not only use modern methodology with internationally recognized protocols and ATCC strains but also drug multi-resistant clinical strains, isolated nationally or regionally.

1.3. Reported activities

To our knowledge, there are few reports related to antimicrobial activities for the selected species. Antibacterial activity was reported for terpenoids from *Fabiana densa* var. ramulosa and *Baccharis boliviensis* (Erazo et al., 2002a,b).

Allelopatic, anti-*Trypanosoma cruzi* and antioxidant activities were reported for *Baccharis boliviensis* (González et al., 1990; Cazon et al., 2000; Abad and Bermejo, 2007; Zampini et al., 2008). Acaricide activity was demonstrated in chloroformic, ethanolic and aqueous extracts of *Parastrephia lucida* (Ayma et al., 1995). There are no reports about biological activities for *Chiliotrichiopsis keidelii*, *Chuquiraga atacamensis*, *Fabiana bryoides*, *Fabiana densa*, *Fabiana punensis*, *Frankenia triandra*, *Parastrephia lepidophylla*, *Parastrephia phyliciformis* and *Tetraglochin cristatum*.

1.4. Previously isolated constituents

Terpenoids and flavonoids from *Baccharis boliviensis* (Morales et al., 1990; Verdi et al., 2005), triterpenoids, flavonoids and other phenolic compounds from *Chuquiraga atacamensis* (Hoeneisen et al., 2000; Mendiondo et al., 2000; Juarez and Mendiondo, 2002), and two diterpenoids from *Fabiana densa* (Erazo et al., 2002b) and a tremeton derivate from *Parastrephia lepidophylla* (Bohlmann et al., 1979) were previously isolated. There are no studies about the chemical composition for the other assayed species.

2. Materials and methods

2.1. Plant material

The selected plant species were collected in the montainous area (Puna and high Andean phytogeographic provinces, Cabrera, 1978) from Northwest Argentina (Catamarca, Jujuy and Tucumán) at different altitudinal levels, between 3700 and 4800 masl. The botanical identification of the plants was done by the botanist Ana Soledad Cuello and the voucher specimens are conserved in the Fundación Miguel Lillo Herbarium (LIL) and the Instituto de Estudios Vegetales (IEV) (Table 2). The aerial parts of the plants were used.

2.2. Preparation of plant extracts

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

Table 1
Characteristic plant species of the Argentine Puna.

Region	Province	Geographical coordinates and altitudinal levels	Plant communities
Laguna Blanca (Biosphere Reserve)	Catamarca	26°30′W, 66°40′S, 4000 masl	*Acantholippia* sp.; *Fabiana densa*; *Stipa* spp.; *Festuca* spp.; *Panicum chloroleucum*; *Baccharis boliviensis*; *Baccharis incarum*; *Parastrephia lucida*; *Parastrephia phyliciformis*; *Senecio* spp; *Adesmia* sp.; *Acantholippia salsoloides* (Borgnia et al., 2006)
Quebrada Seca Antofagasta de la Sierra	Catamarca	67°14′W, 26°1′S, 4280 masl	*Stipa chrysophylla*; *Festuca hortophylla*; *Adesmia* sp.; *B. incarum*; *Baccharis boliviensis*; *Parastrephia quadrangularis*; *Fabiana bryoides* (Cuello, 2006); *Nardophyllum armatum*
Punta de la Peña Antofagasta de la Sierra	Catamarca	67°20′W, 26°1′S, 3600 masl	*Fabiana punensis*; *Chuquiraga atacamensis*; *Cotula mexicana*; *Hypochoeris chondrilloides*; *Hypochoeris eremophila*; *Parastrephia lucida*; *Acantholippia desertícola* (Cuello, 2006)
Laguna de Vilama (Sitio Ramsar)	Jujuy	66°55′W, 22° 30′S, 4500 masl	*Festuca* spp; *Azorella compacta*; *Parastrephia lepidophylla*; *Parastrephia lucida*; *P. quadrangularis*; *Frankenia trianda*; *Oxychloe* spp. (Malvárez, 1999); *Nardophyllum armatum* (Cabrera, 1978); *Chiliotrichiopsis keidelii*
Laguna de Pozuelos (Sitio Ramsar o Biosphere Reserve)	Jujuy	66°W, 23°30′S, 3600 masl	*Polylepis tomentella*; *Colletia spinosissima*; *Chersodoma argentina*; *B. incarum*; *Baccharis boliviensis*; *Fabiana densa*; *Parastrephia lepidophylla*; *Parastrephia lucida*; *Menodora pulchella*; *Alternanthera* sp.; *Evolvulus sericeus*; *Dalea hofstenii*; *Hypochoeris elata*; *Lepidium elata*; *Dichondra argéntea*; *Eryoneuron avenaceu*; *Maihueniopsis glomerata*; *Stipa bomani*; *Festuca crysophylla*; *Oreocereus trollii*; *Opuntia soehrensii* (Bonaventura et al., 1995); *Tetraglochin cristatum*; *Chuquiraga atacamensis*
Huaca Huasi	Tucumán	65°44.23′W, 26°39.35′S, 4300 masl	*Festuca orthophylla*; *Pycnophyllum tetrastichum*; *Mullinun axilariflorum*; *B. incarum*; *Parastrephia phyliciformis*; *Tetraglochin cristatum*; *Senecio graveolens*; *Silene mandonii*; *Calceolaria glacialis*; *Nothotriche caecia*

Decoction: ground air-dried plant material was heated in distilled water (5 g of dry tissue per 100 ml of water) for 10 min at 100 °C.

Then, both extracts were filtered through Whatman no. 1 filter paper. Prepared extracts were stored at 4 °C in the dark.

2.3. Phytochemical screening

2.3.1. Phenolic compound determination

Total phenolic compound content was determined using the Folin–Ciocalteau method (Singleton et al., 1999). Results were expressed as gallic acid equivalents (GAE). Flavonoid content was determined according to Woisky and Salatino (1998). Results were expressed as quercetin equivalents.

2.3.2. Thin layer cromatography

The components of the different extracts (10 μg) were separated by TLC (Kieselgel 60 F254 0.2 mm, Merck). Chloroform:methanol (8:2) was used as development solvent. The separated components were visualized under ultraviolet light (254 and 365 nm, UV Lamp Model UV 5L-58 Mineralight Lamp). Phenolic compounds were detected with Natural Products reagent (NP – 1% methanolic solution of diphenylboric acid aminoethyl ester) or aluminium chloride (Wagner et al., 1984). Methanolic potassium hydroxide was used for coumarins (Harbone, 1973), Dragendorff's reagent for alkaloids and anisaldehyde/sulfuric acid for steroids and terpenes (Krebs et al., 1969).

2.4. Antimicrobial assays

2.4.1. Culture media and microbial identification

Clinical isolates of *Staphylococcus aureus* ($n = 8$), *Enterococcus faecalis* ($n = 8$), *Escherichia coli* ($n = 2$), *Klebsiella pneumoniae* ($n = 1$), *Proteus mirabilis* ($n = 1$), *Enterobacter cloacae* ($n = 1$), *Morganella morganii* ($n = 1$), and *Pseudomonas aeruginosa* ($n = 1$) were obtained from clinical samples from Hospital Dr. Nicolás Avellaneda, San Miguel de Tucumán, Tucumán, Argentina. The following reference strains were included in the study: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 35218, and *Klebsiella pneumoniae* ATCC 700603.

The strains were identified by the use of biochemical profiles according to the recommendations of the Manual of Clinical Microbiology (Murray et al., 1999). All organisms were maintained in brain–heart infusion (BHI medium) containing 30% (v/v) glycerol at −20 °C. Before testing, the suspensions were transferred to trypticase soy agar supplemented with 5% sheep blood (Difco) and aerobically grown overnight at 35 °C. Individual colonies were isolated and suspended in 5 ml of 0.9% NaCl solution. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standard and diluted in CAMHB (cation-adjusted Müeller–Hinton broth) in order to achieve the adequate inocula in each case.

The cell number in CAMHB was estimated using a serial dilution technique according to the recommendations of the CLSI, 2006 (Clinical Laboratory Standards Institute) for each assay.

2.4.2. Sensitivity test
2.4.2.1. Bioautography. Developed TLC plates were dried overnight in a sterile room. Then, plates were covered with 2 ml of soft

Table 2
Ethnobotanical data of the studied plants.

Scientific name	Family	Local name	Voucher specimen	Popular use or disease treated
Baccharis boliviensis (Wedd.) Cabr.	Asteraceae	*Lejia, tola chica, tolita, tolilla*	607936/LIL	Liver function helper, gastric protector, muscle pain reliever, anti-inflammatory, antiseptic (Villagran et al., 2003)
Chuquiraga atacamensis Kuntze	Asteraceae	*Lengua de gallina, azafrán, quebrolla, San Pedro*	607929/LIL	Common cold, dry cough with sticky phlegm, blood purifier, antiseptic (Villagran et al., 2003)
Parastrephia lucida (Meyen) Cabrera	Asteraceae	*Romero, tola, chachakoa, tola de rio, tola de agua*	607923/LIL	Antiodontalgic by applications of leaves. Bone fractures and haematomas (Villagran et al., 2003)
Parastrephia phyliciformis (Meyen) Cabrera	Asteraceae	*Tola, tola de rio*	487802/LIL	The leaves resinous exudates are used to immobilize fractured limbs and as anti-inflammatory, antiseptic, anti-dandruff and anti-baldness drug (Villagran et al., 2003)
Parastrephia lepidophylla (Wedd.) Cabrera	Asteraceae	*Tola, tola vaca, tola*	68979/LIL	The leaves resinous exudates are used to immobilize fractured limbs and as anti-inflammatory, antiseptic, anti-dandruff and anti-baldness drug (Villagran et al., 2003)
Chiliotrichiopsis keidelii Cabrera	Asteraceae	*Tola viscacha, viscachera tola*	0004500/IEV	Antiseptic
Fabiana bryoides Phil.	Solanaceae	*Pata de perdiz, leña de perdiz*	489618/LIL	Antiseptic, anti-inflammatory, against distempered cattle, sheep birth and bone fractures (Perez, 2006)
Fabiana punensis S.C. Arroyo	Solanaceae	*Tolilla*	607771/LILL	Bone fractures (Perez, 2006), vulnerary
Fabiana densa J. Rémy	Solanaceae	*Tolilla*	607770/LIL	The leaves resinous exudates are used to immobilize fractured limbs and as an infusion against lung disease and cough (Erazo et al., 2002b)
Frankenia triandra J. Rémy	Frankeniaceae	*Yaretilla*	487801/LIL	Vicuna, sheep and llama forage (Villagran and Castro, 2000). In the XVII century, it was used to obtain sodium salts which crystallize on leaves and stems by means of excretory glands. Antiseptic
Tetraglochim cristatum (Britton) Rothn	Rosacea	*Horizonte*	0003600/IEV	Antiseptic

medium (BHI with 0.6% agar) containing 10^5 colony forming units (CFU) of *Staphylococcus aureus* (F7), *Enterococcus faecalis* (F213), *Pseudomonas aeruginosa* (F305) and *Escherichia coli* (F301), incubated at 35 °C for 16–20 h and sprayed with a 2.5 mg/ml MTT solution (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) in PBS (10 mM sodium phosphate buffer, pH 7, with 0.15 M NaCl). Plates were incubated at 35 °C for 1 h in the dark for colour development (Nieva Moreno et al., 1999). The growth inhibition areas, yellow coloured, were compared with the Rf of the related spots on the TLC plate revealed with UV light and NP (1% methanolic solution of diphenylboric acid aminoethyl ester) reagents.

2.4.3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determination

MIC values of the extracts against the test organisms were determined by two assays: Serial agar macrodilution and broth microdilution methods (CLSI, 2006). The microdilution method was also used to determine MBC values.

2.4.3.1. Serial agar macrodilution method.
The same volume (1 ml) of serial two-fold dilution of each extract was added to 9 ml of MHA (Müeller–Hinton agar) medium. After cooling and drying, the plates were inoculated in spots with 2 μl of each bacterial cell suspension (5×10^4 CFU) and incubated aerobically for 16–20 h at 35 °C. A growth control of each tested strain was included. Controls of ethanol 80° or distilled water were carried out.

MIC_{100} was defined as the lowest concentration of extract at which no colony was observed after incubation.

2.4.3.2. Broth microdilution method.
This test was performed in sterile 96-well microplates. The extracts were transferred to each

microplate well in order to obtain two-fold serial dilutions of the original extract. The inoculum (100 μl) containing 5×10^5 CFU was added to each well. A number of wells were reserved in each plate for sterility control (no inocula added), inocula viability (no extract added), and solvent effect (ethanol 80° or distilled water). Plates were aerobically incubated at 35 °C. After incubation for 16–20 h, bacterial growth was assayed by absorbance measurement at 550 nm. Bacterial growth was also indicated by the presence of turbidity and a pellet on the well bottom.

MIC was defined as the lowest concentration of extract that had restricted growth to a level <0.05 at 550 nm (no macroscopically visible growth).

To confirm MIC and to establish MBC, 10 μl of each culture medium was removed from each well with no visible growth and inoculated in MHA plates. After 16–20 h of aerobic incubation at 35 °C, the number of surviving organisms was determined.

MBC was defined as the lowest extract concentration at which 99.9% of the bacteria have been killed.

MIC values were also determined for different commercial antibiotics. Resistance was defined for each case: levofloxacine (Lvx, MIC ≥ 8 μg/ml), piperacillin/tazobactam (Tzp, MIC ≥ 128 μg/ml), imipenem (Ipm, MIC > 16 μg/ml), meropenem (Mem, MIC > 16 μg/ml), ceftriaxone (Cro, MIC > 128 μg/ml), cefotaxime (Ctx, MIC > 128 μg/ml), ceftacidime (Caz, MIC > 32 μg/ml), cefuroxime (Cxm, MIC ≥ 32 μg/ml), cefepime (Fep, MIC ≥ 32 μg/ml), amikacin (Amk, MIC > 16 μg/ml) and ampicillin/sulbactam (Sam, MIC ≥ 32 μg/ml) for Gram-negative bacteria and oxacillin (Oxa, MIC > 16 μg/ml), streptomycin (Str, MIC ≥ 300 μg/ml), ampicillin (Amp, MIC > 64 μg/ml), methicillin (Met, MIC > 16 μg/ml), gentamycin (Gen, MIC > 100 μg/ml) and vancomycin (Van, MIC > 6 μg/ml) for Gram-positive bacteria. The antimicrobial

agents were supplied by Sigma Chemical Co (USA) and Laboratorio Britania S.A., Argentina.

All experiments were carried out in triplicate.

3. Results and discussion

The selection of plants for this study was based on ethnobotanical data and on their traditional use in the treatment of infectious diseases such as skin infections (bacterial and fungal) (Table 2).

The antibiotic multi-resistant clinical strains assayed in this work were isolated from human infections from a local hospital. Herbal drug preparation methods by traditional healers are mainly carried out by maceration, infusion and decoction. For this reason, we assayed two extraction methods, maceration and decoction.

Phytochemical analysis by TLC indicated the presence of a large amount of phenolic compounds principally flavonoid in all standardized extracts. Alkaloids, coumarins, terpenoids and saponins were not detected. The tincture was able to extract the largest amount of phenolic compounds. Flavonoids constituted 40–52% of the compounds extracted in *Baccharis boliviensis* and *Chuquiraga atacamensis*. The screening of antimicrobial activity by contact bioautography, used for qualitative antibacterial activity detection, demonstrated that bioactive ethanolic extracts have several phenolic compounds (identified by different chemical reagent) with antibacterial capacity.

Tables 3 and 4 show the antimicrobial activity of the Puna plant tincture against Gram-positive and Gram-negative antibiotic resistant bacteria. The extracts of herbal samples were subjected to determination of the MIC and MBC values. The ethanolic extracts of all assayed plant species were active against all methicillin resistant *Staphylococcus aureus* (MRSA), methicillin sensitive *Staphylococcus aureus* (MSSA), methicillin resistant *Staphylococcus* coagulase negative (MRSCN), methicillin sensitive *Staphylococcus* coagulase negative (MSSCN), and *Enterococcus faecalis* strains. *Baccharis*, *Fabiana*, *Parastrephia* and *Chuquiraga* species showed the best antimicrobial effect on Gram-positive bacteria.

The MIC values for the nine *Staphylococcus aureus* strains studied were: 40–80 µg/ml for *Baccharis*, 20–150 µg/ml for *Fabiana*, 80–600 µg/ml for *Parastrephia* and *Chuquiraga* species. MIC values for the nine *Enterococcus faecalis* strains tested were: 40–80 µg/ml for *Baccharis*, 40–300 µg/ml for *Fabiana*, 80–600 µg/ml for *Parastrephia* and 150–300 µg/ml for *Chuquiraga* species. *Frankenia*, *Tetraglochim* and *Chiliotrichiopsis* extracts were more active on *Staphylococcus aureus* than on *Enterococcus faecalis* (Table 3). The MIC values reported in the present work for Gram-positive bacteria were similar to those obtained for other plant species that grow in arid regions of Argentine (Arias et al., 2004).

Microorganisms *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Morganella morganii* and *Klebsiella pneumoniae* were more resistant to most plant extracts than Gram-positive bacteria (Table 4). The most interesting activity on Gram-negative strains was obtained from *Chuquiraga atacamensis* with MIC values between 300 and 600 µg/ml. *Parastrephia* species were active on *Proteus mirabilis*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* with MIC values between 150 and 1200 µg/ml. *Fabiana* species, *Frankenia triandra*, *Chiliotrichiopsis keideii* and *Tetraglochin cristatum* were the less effective plant extracts against the microorganism assayed. *Frankenia triandra* only showed an important antibacterial effect against *Morganell morganii* and *Pseudomonas aeruginosa* with MIC values of 150 and 300 µg/ml, respectively, *Tetraglochin cristatum* only showed antibacterial effect against *Proteus mirabilis* with MIC values of 600 µg/ml. Other Argentine plant species such as *Larrea cuneifolia*, *Larrea divaricata*, *Zuccagnia punctata* and *Acacia caven* also showed antimicrobial activity against Gram-negative bacteria (Arias et al., 2004; Zampini et al., 2005, 2007).

The lower activity of tested plants against Gram-negative bacteria could be attributed to the presence of an extra outer membrane in their cell wall acting as a barrier for substances including antibiotics (Nikaido and Vaara, 1985). The extracts were bactericidal in most cases. The aqueous extractions were less active than tinctures against Gram-positive and Gram-negative bacteria with MICs values between 600 and 2400 µg/ml.

Table 3
Antimicrobial activity (MIC/MBC) of the Puna plant tincture against Gram-positive sensible and antibiotic resistant bacteria.

Bacterial strains	MIC/MBC (µg/ml)											
	1	2	3	4	5	6	7	8	9	10	11	Fenotype of clinical isolate
Staphylococcus aureus												
F1 MRSA	80/80	80/80	80/80	80/300	300/300	20/40	40/150	80/150	600/1200	600/ND	1200/2400	Met^r Oxa^r Gen^r
F7 MRSA	40/80	300/1200	80/150	150/600	600/2400	20/40	40/300	150/600	1200/1200	1200/ND	1200/ND	Met^r Oxa^r Gen^r
F16 MSSA	40/40	80/150	80/300	150/150	300/300	20/40	40/150	80/600	600/600	600/2400	600/2400	Met^s Oxa^s Gen^s Van^s
F19 MSSA	80/150	150/150	80/300	300/2400	300/300	20/40	40/150	150/600	300/600	600/ND	1200/2400	Met^s Oxa^s Gen^s Van^s
F21MSSCN	80/150	600/1200	150/>2400	300/2400	150/1200	20/80	80/300	150/300	600/2400	300/2400	1200/2400	Met^s Oxa^s Gen^s Van^s
F22MRSCN	80/300	150/600	150/>2400	150/1200	300/1200	20/80	80/150	80/150	300/600	600/1200	1200/ND	Met^r Oxa^r Gen^r Van^s
F28 MSSCN	80/80	80/150	80/2400	150/600	150/600	20/80	80/300	80/300	600/1200	600/ND	600/1200	Met^s Oxa^r Gen^r Van^s
F36MSSCN	80/80	150/150	80/2400	300/2400	600/600	20/40	80/150	80/150	2400/2400	300/2400	600/2400	Met^s Oxa^s Gen^s Van^s
ATCC29213	40/40	150/150	80/600	150/600	300/600	20/40	40/150	40/300	300/600	600/ND	600/ND	Control strain
Enterococcus faecalis												
F201	80/150	150/300	80/1200	300/2400	600/2400	40/80	300/1200	300/>2400	R*	1200/ND	2400/ND	Van^s Amp^s Gen^s Str^s
F203	80/300	300/1200	150/600	300/1200	600/2400	40/80	150/600	300/2400	R*	1200/ND	1200/ND	Gen^r Str^r Van^s Amp^s
F205	80/300	300/1200	150/600	600/600	600/2400	150/600	300/1200	300//>2400	R*	1200/ND	1200/ND	Gen^r Str^r Van^s Amp^s
F207	80/150	150/600	80/600	300/300	600/2400	40/80	150/600	300/2400	1200/2400	1200/ND	1200/ND	Van^s Amp^s Gen^s Str^s
F208	80/300	300/1200	80/2400	300/1200	600/2400	40/150	80/300	300/1200	R*	1200/ND	1200/ND	Str^r Van^s Amp^r Gen^r
F209	80/300	150/1200	80/2400	150/2400	300/2400	40/150	80/300	150/1200	1200/2400	1200/ND	2400/ND	Gen^r Van^s Amp^s Str^s
F223	80/300	150/1200	150/2400	300/1200	600/2400	40/150	150/600	300/1200	1200/2400	1200/ND	2400/ND	Gen^r Van^s Amp^s Str^s
F226	80/150	150/1200	150/2400	300/2400	600/2400	40/150	150/600	300/1200	1200/2400	1200/ND	1200/ND	Van^s Amp^s Gen^s Str^s
ATCC29212	80/150	150/600	150/300	150/600	600/1200	40/150	150/600	300/2400	600/2400	600/ND	1200/ND	Control strain

Standardized extracts were used in all cases: (1) *Baccharis boliviensis* (1530 µg of GAE/ml), (2) *Chuquiraga atacamensis* (500 µg of GAE/ml), (3) *Parastrephia lepidophylla* (7060 µg of GAE/ml), (4) *Parastrephia lucida* (2788 µg of GAE/ml), (5) *Parastrephia phyliciformis* (4899 µg of GAE/ml), (6) *Fabiana bryoides* (1608 µg of GAE/ml), (7) *Fabiana densa* (1841 µg of GAE/ml), (8) *Fabiana punensis* (3452 µg of GAE/ml), (9) *Frankenia triandra* (1328 µg of GAE/ml), (10) *Tetraglochim cristatum* (1106 µg of GAE/ml), (11) *Chiliotrichiopsis keidelii* (1616 µg of GAE/ml). R* resistant until 2400 µg of phenolic compound/ml extract. ND: not determined by solubility problems. ^{r}Resistant, ^{s}susceptible; vancomycin (Van), ampicilin (Amp), gentamycin (Gen), streptomycin (Str), methicillin (Met), oxacillin (Oxa).

Table 4
Antimicrobial activity (MIC/MBC) of the Puna plant tincture against Gram-negative antibiotic resistant bacteria.

Bacterial strains	MIC/MBC (µg/ml)											Fenotype of clinical isolate
	1	2	3	4	5	6	7	8	9	10	11	
Escherichia coli F301	2400/2400	600/2400	2400/2400	1200/2400	2400/2400	1200/1200	1200/2400	1200/1200	R*	1200/2400	1200/2400	Lvx^r Cro^r Ctx^r Cxm^r Sam^r Mem^r
Escherichia coli F331	1200/2400	600/2400	2400/>2400	600/2400	1200/1200	1200/2400	2400/2400	1200/>2400	R*	1200/2400	1200/ND	Lvx^r Tzp^r Cro^r Ctx^r Caz^r Cxm^r Fep^r Sam^r Mem^r
Enterobacter cloacae F302	2400/2400	600/2400	150/2400	1200/2400	300/2400	1200/2400	2400/2400	1200/>2400	R*	1200/ND	600/ND	Lvx^r Tzp^r Cro^r Ctx^r Cxm^r Sam^r Mem^r
Klebsiella pneumoniae F364	2400/2400	600/2400	2400/2400	2400/>2400	R*	1200/2400	2400/2400	2400/2400	R*	R*	2400/ND	Cro^r Ctx^r Cxm^r Fep^r Mem^r Sam^r
Proteus mirabilis F304	1200/2400	300/2400	300/2400	600/2400	1200/2400	1200/1200	1200/1200	1200/2400	1200/2400	600/1200	1200/ND	Cxm^r Sam^r Mem^r
Morganella morganii F339	1200/2400	300/2400	2400/2400	1200/1200	R*	1200/2400	1200/2400	2400/2400	150/2400	1200/ND	2400/ND	Sensitive
Pseudomonas aeruginosa F305	1200/2400	300/2400	600/2400	300/2400	1200/2400	1200/1200	2400/2400	2400/2400	300/1200	R*	2400/ND	Lvx^r Tzp^r Cro^r Ctx^r Caz^r Ipm^r Amk^r Mem^r
ATCC 35218 *Escherichia coli*	1200/2400	600/>2400	2400/2400	1200/2400	2400/>2400	1200/2400	1200/2400	1200/1200	R*	2400/ND	1200/ND	Control strain
ATCC 700603 *Klebsiella pneumoniae*	2400/>2400	600/>2400	2400/2400	1200/>2400	R*	1200/2400	2400/2400	2400/2400	R*	2400/2400	1200/ND	Control strain

(1) *Baccharis boliviensis*, (2) *Chuquiraga atacamensis*, (3) *Parastrephia lepidophylla*, (4) *Parastrephia lepidophylla*, (5) *Parastrephia lucida*, (6) *Fabiana bryoides*, (7) *Fabiana phyliciformis*, (8) *Fabiana densa*, (9) *Frankenia triandra*, (10) *Tetraglochin cristatum*, (11) *Chiliotrichiopsis keidelii*. ^{r} Resistant, ^{s} susceptible, R* resistant until 2400 µg of phenolic compound/ml de extract. levofloxacine (Lvx), piperacillin/tazobactam (Tzp), imipenem (Ipm), ceftriaxone (Cro), cefotaxime (Ctx), ceftacidime (Caz), cefuroxime (Cxm) cefepime (Fep), amikacine (Amk), ampicillin/sulbactam (Sam) and meropenem (Mem). ND: not determined by solubility problems.

4. Conclusions

The antimicrobial activity of 11 extracts obtained from Argentine endemic plant species was demonstrated. According to the *in vitro* bioassay results, ethanol–water was probably the best solvent for the extraction of bioactive compounds; however, both tinctures and decoctions showed antimicrobial activity against sensitive as well as multi-resistant clinical isolates. These preliminary studies are highly interesting as they open new avenues for further studies which would allow the validation of the traditional use of these plants in the treatment of infections.

Acknowledgements

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