



Antimicrobial phenylpropanoids from the Argentinean highland plant *Parastrephia lucida* (Meyen) Cabrera

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ABSTRACT

Ethnopharmacological relevance: The Argentinean highland plant *Parastrephia lucida* (Meyen) Cabrera is used in traditional medicine as an antiseptic and anti-inflammatory crude drug.

Aim of the study: To relate the antimicrobial effect of the crude drug with the constituents of the active fractions and traditional use.

Materials and methods: Assay-guided isolation of the methanol (MeOH) plant extract was carried out using bacteria and yeasts as target organisms. Both ATCC and local strains were included in the study. The antimicrobial fractions and compounds were detected by bioautographic assays. Minimum inhibitory concentrations (MIC) of each extract and fraction were determined and compared with reference antibiotics. Fractions were analyzed by HPLC–DAD, GC–MS, ¹H NMR and ¹³C NMR.

Results: From the MeOH extract of the plant, assay-guided isolation of the antimicrobial constituents led to 12 phenylpropanoids and two simple phenolics. Most of the compounds occurring in the active fractions were *E*-caffeoyl or *E*-cinnamoyl esters including prenyl and phenethyl derivatives. The MIC values of the most active fractions ranged between 12.5 and 200 µg/mL against reference strains and local isolates of *Staphylococcus aureus* and *Enterococcus faecalis*.

Conclusions: The antimicrobial effect found in the crude drug was associated with mixtures of phenylpropanoids, including prenyl and phenethyl esters of caffeic and cinnamic acids. The results support at least in part the traditional use of the plant as local antiseptic.

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1. Introduction

Human beings living in the Andes highlands (Puna) selected the available natural resources for medicine, building, forage, firewood and as cultural elements in spiritual activities (Villagrán et al., 2003; Toursarkissian, 1980). In the traditional medicine of the Andes highlands, *Parastrephia lucida* is used to relieve toothache, by applications of leaves, for bone fractures and bruises and as vulnerary (Villagrán et al., 2003). It is also used as firewood and to feed llamas and alpacas (Ayma et al., 1995). The genus *Parastrephia* (Asteraceae) comprises five species, namely *P. lucida*, *P. lepidophylla*, *P. phyllicaeformis*, *P. quadrangularis* and *P. teretiuscula* (Cabrera, 1978). *P. lucida* Meyen Cabrera, known under the common name *romero*, *tola*, *chachakoa*, *tola de río*, *tola de agua*, is abundant in the Argentinean highlands,

an environment characterized by high altitude (3000–4200 m over sea level), high exposure to ultraviolet radiation, low oxygen concentration, high daily temperature variations and mean annual rainfall of 100–200 mm or even less. Plants from these ecosystems have particular adaptations to environmental stress, including the biosynthesis of secondary metabolites with relevant pharmacological activities (Kleier and Lambrinos, 2005).

The biological activities of alcoholic and aqueous extracts of *P. lucida* reported so far include anti-inflammatory (Alberto et al., 2009), acaricide (Ayma et al., 1995), antioxidant (Zampini et al., 2008), antibacterial (Zampini et al., 2009). However, little is known on the identity of its chemical constituents. Phytochemical studies on other *Parastrephia* species afforded isofraxidin; 4-hydroxy-3-methoxypropionophenone; *p*-hydroxyacetophenone; methoxytremetone and tremetone in *P. lepidophylla* (Bohlmann et al., 1979) while 5,7-dihydroxy-3,8,3',4'-tetramethoxyflavone; umbelliferone, [1-cinnamoyl-oxy-ethyl] benzol; 4-[1'-methoxy-ethyl]-phenol; 1, 1'-bis-[4-ethylphenol]-ether were identified in *P. quadrangularis* (Loyola et al., 1985). Scopoletin and *p*-coumaroyl-oxy

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tremetone occurs in both *Parastrephia* species (Bohlmann et al., 1979; Loyola et al., 1985; Barboza et al., 2009).

Studies on plants with antimicrobial activity and assay-guided isolation can lead to naturally occurring compounds from different structural types and skeletons. The findings can open opportunities to disclose the mechanisms involved in microbial growth inhibition, either separately or associated with synthetic conventional antimicrobials. Phenolics are a family of plant-derived compounds with potentially exploitable effects, including direct antibacterial activity, synergism with antibiotics, and suppression of bacterial virulence (Ríos and Recio, 2005; Cushnie and Lamb, 2011). These compounds can also ameliorate infection by interfering with aspects of bacterial pathogenesis, for example inhibition of quorum sensing, inhibition of enzymes (sortase, urease), neutralization of bacterial toxins, inhibition of virulence factors secretion, etc. (Cushnie and Lamb, 2011).

Some of the most problematic multidrug resistance (MDR) microorganisms currently found in hospitals include *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* bearing extended-spectrum β -lactamases, vancomycin resistant enterococci, methicillin and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA, respectively). Some bacteria like methicillin-resistant *S. aureus* couple MDR with exceptional virulence capabilities (Miller et al., 2005; Alekshun and Levy, 2007). The majority of the mycoses-related deaths were associated with *Candida*, *Aspergillus*, and *Cryptococcus* sp. infection. More than 90% of invasive infections due to *Candida* spp. are attributed to the species: *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* (Pfaller and Diekema, 2007).

The aim of this work was to identify the antimicrobial agents from the aerial parts of *P. lucida*. A bio-guided isolation and identification was carried out using human multi-resistant Gram positive and Gram negative pathogenic bacteria and *Candida* species. In this study, the effect of the extracts and fractions was assessed on American Type Culture Collection (ATCC) strains and clinical isolates of a local hospital.

2. Materials and methods

2.1. Plant material

The aerial parts of *P. lucida* (Meyen) Cabrera were collected from January to February 2006 at 3600 m.o.s.l in Antofagasta de la Sierra, Provincia de Catamarca, Puna de Atacama. A voucher specimen no. 607923/LIL, was deposited in the Herbarium of "Fundación Miguel Lillo", Tucumán, Argentina and the plant was authenticated by Lic. Soledad Cuello. The samples were dried at 40 °C. The parts used were leaves and stems (aerial parts), according to the traditional use.

2.2. Reagents

Gallic acid (GA), 3-(4, 5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT), diphenylboric acid- β -ethylamino ester (NP), KOH were supplied by Sigma-Aldrich (Missouri, USA). Analytical grade MeOH was from Merck Argentina (Buenos Aires, Argentina). HPLC grade organic solvents were from Sintorgan Argentina (Buenos Aires, Argentina) and water was Milli Q Plus quality. For column chromatography, silica gel 60 (0.040–0.063 mm, 230–400 mesh, Merck, Darmstadt, Germany) was used. Analytical TLC was carried out using pre-coated plates (Kieselgel 60 F254, 0.2 mm, Merck, Germany).

2.3. Extraction and isolation of bioactive fractions

Dried and powdered plant material was macerated in methanol (MeOH) (250 g of dry plant material/L) for 7 days with gentle shaking or stirring (40 cycles/min) at room temperature. The methanol extract (MeOH) was filtered through Whatman no. 4 filter paper and concentrated under reduced pressure at 40 °C to obtain 75 g of a crude MeOH extract, which was stored at 4 °C until use. A liquid–liquid partition of the crude MeOH extract was performed with dichloromethane (DCM) and water to obtain two phases. The aqueous phase (AQ) was lyophilized. The DCM phase (33 g) was taken to dryness and permeated in a chromatographic column (80 cm length $\times$ 4 cm internal diameter) using Sephadex LH-20 gel as support and eluting with methanol (Henke, 1998). Some 85 drops/tube were collected and combined into seven fractions A to G, based on TLC profiles revealed with NP reagent. Fraction C (82.5 mg/g DCM fraction) was chromatographed on silica gel (column length 30 cm, internal diameter 2.5 cm) eluting with the following gradient of increasing polarity: CHCl_3 , CHCl_3 :EtOAc, EtOAc and EtOAc:MeOH. Fractions of 10 ml each were collected and combined into 19 pools (C I–C XIX based on TLC analysis (SiO_2 , CHCl_3 :MeOH 7:3 v/v). The fractionation is summarized in Fig. 1.

2.4. Phytochemical screening

2.4.1. Thin layer chromatography

The crude MeOH, AQ, DCM extracts and DCM fractions from the Sephadex column were analyzed by TLC. The solvent mixtures used were EtOAc:acetic acid:water (10:0.5:0.5 v/v/v) and

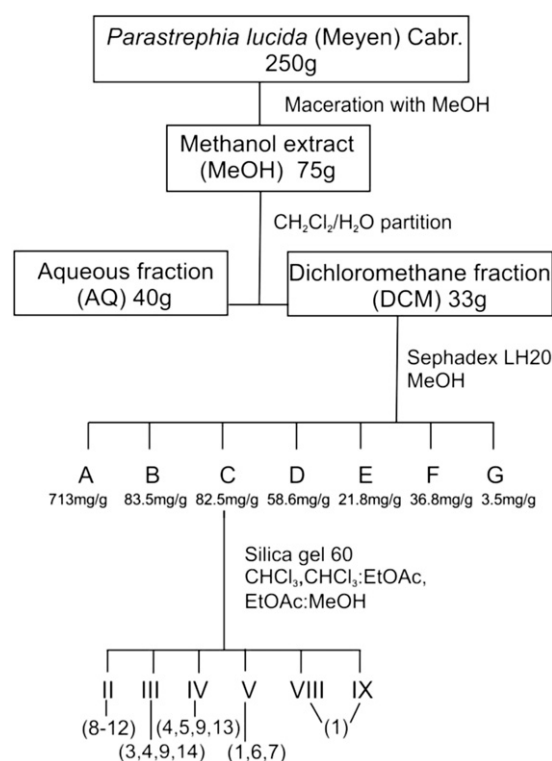


Fig. 1. Bioguided fractionation of antimicrobial compounds from *Parastrephia lucida* aerial parts. Compounds: Cinnamic acid (1), caffeic acid methyl ester (3), ferulic acid methyl ester (4), ferulic acid ethyl ester (5), 3-(4-hydroxy-phenyl)-propionic acid methyl ester (6), 3-(3,4-dihydroxy-phenyl)-propionic acid methyl ester (7), isoferulic acid prenyl ester (8), ferulic acid prenyl ester (9), methyl ferulic acid prenyl ester (10), ferulic acid 2-phenethyl alcohol (11), dimethylcaffeic acid 2-phenethyl ester (12), vanillin (13) and 4-hydroxy-3-methoxy benzoic acid methyl ester (14).

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