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Evaluation of antioxidant and antimutagenic activity of herbal teas from native plants used in traditional medicine in Argentina

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ABSTRACT

The Jarilla species are commonly used in Argentinean local communities to heal different ailments and are frequently used as infusions or decoctions. Herbal mixture infusions composed of *Zuccagnia punctata* Cav. (Zp), *Larrea cuneifolia* Cav. (Lc), *Larrea divaricata* Cav. (Ld), also incorporating an exotic plant species *Hovenia dulcis* Thunb. (H), were developed and the total phenolic and flavonoid content, antioxidant activity and mutagenic/antimutagenic capacity were analyzed and compared with the single-plant teas. The phenolic contents ranged from 29.5 mg GAE/l to 1139 mg GAE/l, and the flavonoid content was between 20.1 and 62 mg EQ/l. The mixture with higher content of *H. dulcis* (H mix) infusion showed the highest preference score in a sensory evaluation. Free radical scavenging capacity was determined by the ABTS assay and the most active was the mixture with equal quantities of each plant species (1/4 mix infusion). None of the plant extracts showed mutagenic effects against *Salmonella typhimurium* tester strains TA98 and TA100 with and without metabolic activation. The antimutagenic properties against a direct mutagen, 4-nitro-*o*-phenylendiamine (4-NPD), of three herbal mixtures showed about 30% of inhibition of mutagenicity. Four phenolic compounds were identified in the infusions prepared with the plant mixtures. The developed beverages in this work could be important dietary sources of antioxidant and antimutagenic compounds for prevention of chronic diseases.

1. Introduction

The popular knowledge about the medicinal use of plants is part of the cultural heritage of a village. This traditional knowledge is transmitted from generation to generation without leaving a written record, in most cases (Ratera and Ratera, 1980). Despite the great cultural, social, political and environmental changes that people were subjected to, oral tradition and knowledge of the plant world are still in force. Therefore, many rural communities still consider traditional medicine a fundamental axis of their health systems.

Plants and derived products have many beneficial properties, which are associated with the presence of secondary metabolites, especially phenolic compounds (El Gharras, 2009; Yáñez et al., 2012). Research has indicated that plants have non-nutritive components, most of which are known as chemopreventive agents, which may provide protection against a variety of illnesses, including cancer and coronary heart diseases (Ferguson, 1994; Kaliora et al., 2014). The amount and type of each secondary metabolite present in a plant depends mostly on environmental factors, so those plants that grow in extreme ecosystems, such as in the northwest region of Argentina, are of particular interest (Ncube et al., 2012).

In Argentina, medicinal plants have been used since ancient times for the treatment of a range of diseases. Among the Argentine flora, about 602 plant species are known to possess therapeutic properties (Ratera and Ratera, 1980; Alonso, 2004; Goleniowski et al., 2006). The "Jarilla" species inhabit an arid ecosystem, with low temperature, temperature fluctuations, low absolute humidity and high solar radiation (Alonso and Desmarchelier, 2005). They are widely used by the local communities for the treatment of different ailments, like rheumatism, inflammation of respiratory and intestinal tract, gastric disturbance and venereal disease. They are also used as emetic, antimicrobial and antifungal agents, among others (Cabrera, 1965; Soraru and Bandoni, 1978; Ratera and Ratera, 1980; Kiesling, 1994; Del Vitto et al., 1997; Quiroga et al., 2001; Davicino et al., 2011). People commonly use these plants in infusions or decoctions, and frequently combine "Jarilla" species.

Among the species selected in this work, *Zuccagnia punctata* Cav. has been studied for its biological properties. Alcoholic extracts from this

Abbreviations: Zp, *Zuccagnia punctata*; Lc, *Larrea cuneifolia*; Ld, *Larrea divaricata*; H, *Hovenia dulcis*; Zp mix, mixture containing higher proportion of *Z. punctata*; Lc mix, mixture containing higher proportion of *L. cuneifolia*; Ld mix, mixture containing higher proportion of *L. divaricata*; H mix, mixture containing higher proportion of *H. dulcis*; 1/4 mix, mixture containing equal amounts of each plant species.

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plant have demonstrated antibacterial and antifungal activities (Quiroga et al., 2001; Svetaz et al., 2004; Zampini et al., 2005; Aguero et al., 2010; Svetaz et al., 2010; Zampini et al., 2012), antioxidant capacity (Ávila et al., 2001; Morán Vieyra et al., 2009) and a potent cytoprotective effect (De La Rocha et al., 2003). *Larrea cuneifolia* Cav. methanol and chloroform extracts have exhibited larvicidal activity against *Culex quinquefasciatus* larvae (Batallán et al., 2013). Moreover, its ethanol extract has antibacterial activity against Gram-positive (Amani et al., 1998; Quiroga et al., 2001) and Gram-negative bacteria (Zampini et al., 2007). *Larrea divaricata* Cav. aqueous extract demonstrated tumoristatic capacity (Anesini et al., 1997). Methanol and dichloromethane extracts from this plant have a cytotoxic effect *in vitro* (Bongiovanni et al., 2007). In addition, this plant species has demonstrated antimicrobial, anti-inflammatory and anti-ulcerogenic activity (Gisvold and Thaker, 1974; Anesini and Perez, 1993; Amani et al., 1998; Quiroga et al., 2001, 2004; Pedernera et al., 2006; Davicino et al., 2007).

On the other hand, *Hovenia dulcis* Thunb. belongs to a small genus of Rhamnaceae that is indigenous to East Asia. It is invasive in South American rainforests and was introduced in the early 19th century. The fresh fleshy peduncles of *H. dulcis* contain high levels of sugar and tastes like a combination of raisins, clove, cinnamon and sugar (Hyun et al., 2010). They have a long history as a food supplement and traditional herbal medicine for the treatment of liver diseases and alcoholic poisoning in China (Wang et al., 2012).

Because of their considerable benefits, plants with medicinal potential could be used in human nutrition as an infusion or tea to improve health (Farzaneh and Carvalho, 2015). The aim of this work was to formulate teas with mixtures from "Jarilla" species (*Z. punctata* Cav., *L. divaricata* Cav. and *L. cuneifolia* Cav.) with the addition of peduncles of an exotic fruit (*H. dulcis* Thunb.), to improve the taste, and evaluate its antioxidant, mutagenic and antimutagenic effects using *in vitro* systems.

2. Materials and methods

2.1. Chemical substances

All chemicals and reagents were of analytical grade and were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA), Merck (Darmstadt, Germany), Cicarelli (Santa Fe, Argentina) and Anedra (Buenos Aires, Argentina).

2.2. Plant material

Aerial parts (leaves and stem) of *Z. punctata* Cav., *L. cuneifolia* Cav. and *L. divaricata* Cav. were harvested from Amaicha del Valle, Tucumán, Argentina at 2000 meters above sea level, in September 2013. Peduncles of *H. dulcis* Thunb. were collected from Horco Molle, Tucumán, Argentina in April 2013. The plants were identified by Dra. Soledad Cuello, Laboratory of Investigation in Natural Products (LIPRON-INQUINOA-CONICET) and voucher specimens (*Z. punctata*: LIL 612170; *L. cuneifolia*: LIL 614829; *L. divaricata*: LIL 614299; *H. dulcis*: LIL 614300) were deposited at the Herbarium of Fundación Miguel Lillo (Tucumán, Argentina).

The samples were dried at room temperature in a dark place.

2.3. Infusion preparation and standardization

The infusions of aerial parts from each plant species and peduncles of *H. dulcis* were prepared (Zp, Lc, Ld and H infusions) by the usual domestic preparation technique; 2 g of ground air-dried plant material were added to 200 ml of boiling distilled water. Infusions were maintained at room temperature for 10 min and filtered with Whatman No. 4 paper. In addition, five herbal mixture teas using four plant species were prepared and compared with the single-plant teas. Four mixtures were prepared with 50% of a plant and the other 50% is comprised of equal parts of the other remaining plants (Zp mix, Lc mix, Ld mix and H mix infusions), and the fifth mixture was prepared with equal parts of each plant species (1/4 mix). Table 1 shows the plant composition of each herbal infusion. The infusions were lyophilized to determine the yield of extracted metabolites (dried weight) in each of them. The results were expressed as g freeze-dried infusion/l tea. The samples (freeze-dried infusions) were stored at −16 °C prior to their use in the biological assays.

In order to standardize the teas, their total phenolic (Singleton et al., 1999) and flavonoid contents (Woisky and Salatino, 1998) were analyzed. The results were expressed as milligrams of gallic acid equivalents per liter of infusion (mg GAE/l) and milligrams of quercetin equivalents per liter of infusion (mg QE/l), respectively.

2.4. High-performance liquid chromatography (HPLC)

The HPLC fingerprints of all herbal teas were obtained with a 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). An XBridge™ C18 column (4.6 mm × 100 mm, 5 μm; Waters Corporation, Milford, MA) with a two-gradient solvent system was used.

The system 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 45 min and 57%–100% B from 45 to 65 min) were used for separation of components from each sample. The flow rate was set at 0.5 ml/min. From freeze-dried infusions, solutions of 2 mg/ml were injected, except for *H. dulcis*, of which a solution of 30 mg/ml was used. Data collection was carried out with Empower™ 2 software. The compounds occurring in the mixture were monitored at 275 and 330 nm, and UV spectra were recorded from 200 to 600 nm for peak characterization.

2.5. Acceptability test

The sensory evaluation of teas was carried out by asking an untrained panel to score the acceptability with respect to appearance, taste and odor using a 7-point verbal hedonic scale, which varied from dislike extremely or like extremely (Watts et al., 1992). The panelists ($n = 50$) were students and staff members of Facultad de Ciencias Naturales e IML (UNT, Tucumán, Argentina) who had no previous experience in the assessment of herbal teas. All samples were evaluated under white light illumination at room temperature. For the evaluation, a minimum of 20 ml of sample per evaluator was served in identical containers named with letters from A to I. Warm water was provided for rinsing between samples.

Table 1
Plant composition of each herbal infusion.

Infusion	Composition
Zp	2 g *Zuccagnia punctata*
Lc	2 g *Larrea cuneifolia*
Ld	2 g *Larrea divaricata*
H	2 g *Hovenia dulcis*
Zp mix	1 g *Z. punctata* + 0.33 g *L. cuneifolia* + 0.33 g *L. divaricata* + 0.33 g *H. dulcis*
Lc mix	0.33 g *Z. punctata* + 1 g *L. cuneifolia* + 0.33 g *L. divaricata* + 0.33 g *H. dulcis*
Ld mix	0.33 g *Z. punctata* + 0.33 g *L. cuneifolia* + 1 g *L. divaricata* + 0.33 g *H. dulcis*
H mix	0.33 g *Z. punctata* + 0.33 g *L. cuneifolia* + 0.33 g *L. divaricata* + 1 g *H. dulcis*
1/4 mix	0.5 g *Z. punctata* + 0.5 g *L. cuneifolia* + 0.5 g *L. divaricata* + 0.5 g *H. dulcis*

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