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

M.P.A. Carabajal [b], M.I. Isla [a,b], I.C. Zampini [a,b,*]

[a] Facultad de Ciencias Naturales e IML, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina
[b] INQUINOA (CONICET), T4000INI San Miguel de Tucumán, Tucumán, 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.

* Corresponding author at: INQUINOA (CONICET), T4000INI San Miguel de Tucumán, Tucumán, Argentina.
E-mail address: zampini@csnat.unt.edu.ar (I.C. Zampini).

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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*

2.6. ABTS radical cation decolorization assay

2,2′-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS solution (7 mM) with ammonium persulfate (2.45 mM) and the mixture was allowed to stand in dark at room temperature for 12–16 h before use (Re et al., 1999).

Freeze-dried infusions were dissolved in dimethylsulfoxide (DMSO) to evaluate antioxidant capacity. $ABTS^{\bullet+}$ solution (absorbance of 0.7 ± 0.02 at 734 nm) was added to different concentrations of each sample and mixed thoroughly. Ascorbic acid (0–6.5 μg/ml) and butylhydroxytoluene (0–40 μg/ml) were used as reference antioxidant compounds, while DMSO was used as negative control. The reactive mixture was allowed to stand at room temperature and absorbance was recorded at 734 nm, 1 min and 6 min after initial mixing. The assay was carried out in triplicate. Dose–response curves were constructed and results were expressed in terms of concentration (μg of freeze-dried infusion/ml) that scavenged 50% of free radicals (SC_{50}).

2.7. The Ames test

In order to determine if compounds present in the medicinal plant infusions affect the viability of *Salmonella typhimurium* strains (TA98 and TA100) a microscopic examination (40×) was carried out (Mortelmans and Zeiger, 2000) on each sample dose evaluated. When a sample shows an antimicrobial effect, there may be "thinning" or complete absence of the background lawn compared to the negative or solvent control (Mortelmans and Zeiger, 2000).

The plate incorporation assay was performed using *S. typhimurium* strains, TA98 and TA100, with and without metabolic activation (S9 mix fraction) (Maron and Ames, 1983). From freeze-dried infusions dissolved in DMSO, serial dilutions were made and a concentration range between 125 and 500 μg GAE/plate was tested for the mutagenic effect. One hundred microliters of an overnight culture of bacteria (cultivated for 16 h at 37 °C) and one hundred microliters of different concentrations of the test sample were added to 2 ml of top agar, containing L-histidine (0.05 mM), D-biotin (0.5 mM), and then each tube was plated on minimum medium (Oxoid No. 2). The assay was performed in duplicate 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, 10 μg/plate; Aldrich Chemical Co.) and 2-aminofluorene (2-AF, 10 μg/plate; Merck). Plates were incubated at 37 °C for 48 h. The influence of metabolic activation was tested by adding 500 μl of S9 mixture prepared with S9 fraction obtained from the liver of Sprague–Dawley rats pretreated with a polychlorinated biphenyl mixture (Aroclor 1254) as previously described in detail by (Maron and Ames, 1983). In this assay, the plates were incubated at 37 °C for 72 h. The revertant colonies of each plate were counted manually and the mutagenicity relation (His^+ revertant per plate/His^+ spontaneous revertant) was calculated. 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) or the mutagenicity relation ≥2 (Maron and Ames, 1983).

The antimutagenic effect of plant infusions were evaluated using a variation of the Ames test by the plate incorporation assay (Maron and Ames, 1983). The same strains used in mutagenic test were employed in the antimutagenic assay against a direct mutagen. Different concentrations of samples (125–250–500 μg GAE/plate), the mutagen 4-NPD (10 μg/plate), and bacterial cultures (0.1 ml) were added to 2 ml of molten top agar. The mutagen and all samples were dissolved in DMSO. The combined solutions were vortexed and poured onto minimal medium. Plates were incubated at 37 °C for 48 h, and colonies were counted. Samples were tested in duplicate for two independent experiments. The mutagenicity of 4-NPD (in the absence of samples) was defined as 100% mutagenicity. Antimutagenic activity was calculated as inhibition percentage (IP) of mutagenic activity, $IP = [1 - (a/b)]*100$, where a = revertant colonies in the presence of the sample, b = revertant colonies in the absence of test sample. Green tea (*Camellia sinensis* leaves) purchased in a local supermarket, was included as positive control (concentration range from 125 to 500 μg GAE/plate) and DMSO was used as the negative control (100 μl/plate). The antimutagenic effect was considered moderate when the inhibitory effect was between 25% and 40% and strong when the inhibitory effect was >40%. An inhibitory effect <25% was considered weak, and it was not recognized as a positive result (Ikken et al., 1999).

2.8. Statistical analysis

Results were expressed as means ± standard deviation (SD) of three measurements. Statistical analyses were performed using the Infostat program v.2008.e, and analysis of variance (ANOVA) was employed to determine whether the means obtained for the herbal infusions differ significantly from each other. The significance was established using the Tukey test. The probability level of $p \leq 0.05$ was considered significant.

3. Results and discussion

As shown in Table 2, the yield as extracted principles from different plant sources varied from 1.1 to 2.7 g/l, and Lc had the highest content. Total phenolic concentrations varied largely between the medicinal plant infusions analyzed. The values ranged from 29.5 mg GAE/l to 1139 mg GAE/l. The lowest total phenolic compounds content correspond to *H. dulcis* peduncles (H infusion). Zp and Lc infusions exhibit the highest concentrations (1139 ± 78.3 and 1042 ± 17 mg GAE/l, respectively). All the mixtures have lower total phenolic content when compared to the single-plant infusions, being the richest Ld mix. The other mixtures showed a similar phenolic content. This decrease of the phenolic compound content in the infusions prepared from the herb mixture could be attributed to the addition of *H. dulcis* species, which display the lowest content of polyphenol compounds.

The content of phenolic compounds obtained for the herbal infusions analyzed in this study are consistent with those reported for other plant infusions, such as green, white and red tea (*C. sinensis*, 1387, 613 and 318 mg GAE/l, respectively), or peppermint infusions (*Mentha piperita*, 501 mg GAE/l), among others (Atoui et al., 2005; Moraes-de-Souza et al., 2008; Komes et al., 2010; Fu et al., 2011; Jimenez-Zamora et al., 2016).

The flavonoid content in the infusions is also shown in Table 2. Zp infusion exhibits the highest flavonoid content and the *Larrea* infusions (Lc and Ld) showed similar amounts of these metabolites ($p \leq 0.05$). The flavonoid content in H infusion cannot be determined by the methodology used; another author reported the presence of some flavonoids by HPLC method in *H. dulcis* fruit-stalks in low concentrations (Park

Table 2
Soluble principles, phenolic and flavonoids content of each herbal infusion.

Sample	Soluble principles[1] (g/l)	Phenolic compounds (mg GAE/l)	Flavonoids (mg QE/l)
Zp	2.1 ± 0.1^{b}	1139.0 ± 78.3^{a}	62.0 ± 0.6^{a}
Lc	2.7 ± 0.1^{a}	1042.0 ± 17^{a}	34.3 ± 0.9^{b}
Ld	1.5 ± 0.1^{cde}	781.0 ± 18^{b}	34.2 ± 0.1^{b}
H	1.4 ± 0.1^{de}	29.5 ± 1.3^{e}	<LDM
Zp mix	1.9 ± 0.1^{bcd}	528.0 ± 16^{cd}	26.5 ± 1.1^{c}
Lc mix	1.9 ± 0.3^{bc}	502.0 ± 3.2^{d}	20.1 ± 2.3^{d}
Ld mix	1.8 ± 0.4^{bcd}	791.0 ± 56^{b}	25.6 ± 2.9^{c}
H mix	2.0 ± 0.1^{bc}	580.0 ± 5.3^{cd}	23.3 ± 1.8^{cd}
1/4 mix	1.1 ± 0.1^{e}	628.0 ± 3.2^{c}	23.0 ± 0.2^{cd}

Note: <LDM: Below the limit of detection of the method. Values (mean ± SD) followed by the same letter are not significantly different ($p \leq 0.05$).
[1] Grams of freeze-dried infusion obtained from one liter of infusion.

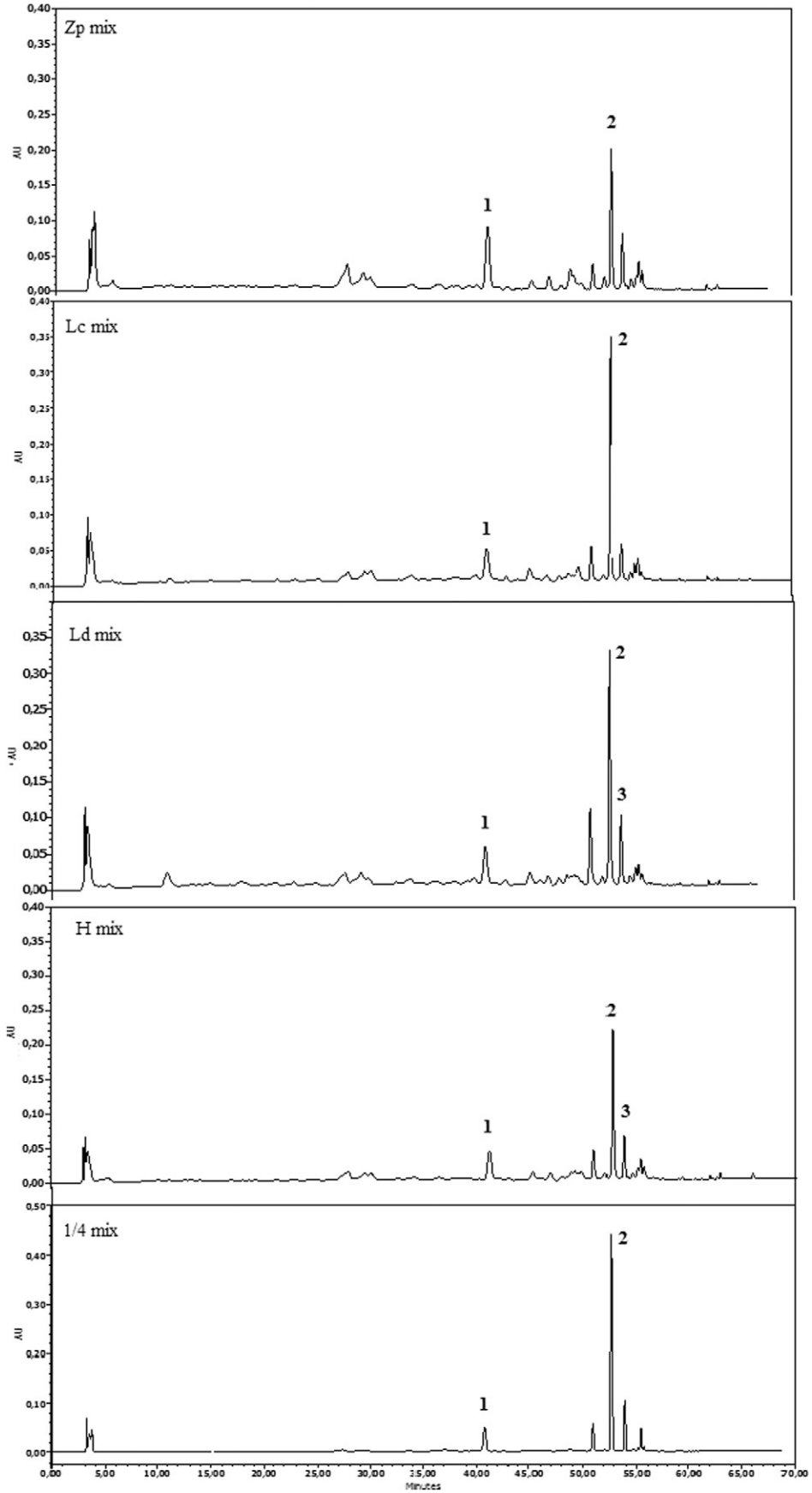

Fig. 1. HPLC profiles of herbal mixture infusions at 275 nm. Zp mix: mixture tea with higher content of *Z. punctata*; Lc mix: mixture tea with higher content of *L. cuneifolia*; Ld mix: mixture tea with higher content of *L. divaricata*; H mix: mixture tea with higher content of *H. dulcis* and 1/4 mix: mixture tea with equal quantities of each plant species. **1**: cinnamic acid; **2**: nordihydroguaiaretic acid; **3**: 2′, 4′-dihydroxychalcone.

et al., 2016). Lc mix as well as H mix exhibit the lowest flavonoid content, while the remaining herbal mixture infusions have similar flavonoid contents (Table 2).

The chromatographic patterns by HPLC-DAD of each infusion were obtained. HPLC fingerprints of the five herbal mixtures showed similar profiles (Fig. 1) with difference in absorption intensity of peaks. Four compounds was identified by the retention time and UV–VIS spectra absorption **1**, cinnamic acid, **2**, nordihydroguaiaretic acid (Fig. 1), **3**, 2′,4′-dihydroxychalcone and **4**, 2′,4′-dihydroxy-3-metoxychalcone (spectra at 330 nm; data not shown). Compounds **1**, **3** and **4** were also identified in Zp sample (data not shown) and compound **2** was found in Lc and Ld samples. The presence of these compounds in each plant species were previously reported (Valesi, 1972; Ávila et al., 2001; De La Rocha et al., 2003; Davicino et al., 2011; Moreno et al., 2015). Cinnamic acids are known by its antioxidant and antitumoral properties (Liu et al., 1995; Foti et al., 1996, 2004; Cinkilic et al., 2014), antibacterial (Liu et al., 2010; Yang et al., 2016) and antifungal activities (Sadeghi et al., 2013). 2′,4′-dihydroxychalcone and 2′,4′-dihydroxy-3-metoxychalcone has been found to possess antigenotoxic, antioxidant and cytoprotective effects (Ávila et al., 2001; De La Rocha et al., 2003; Zampini et al., 2008; Morán Vieyra et al., 2009), to modulate the ABCB1 multidrug transporter P-gp (Chieli et al., 2012), and antibacterial activity (Zampini et al., 2012). Moreover, the lignan nordihydroguaiaretic acid possesses several beneficial properties. It had been reported to be implicated in cancer prevention (Hwu et al., 2011; Mundhe et al., 2015), to have antimutagenic and antitumorigenic activities (Wang et al., 1991), as well as antioxidant properties by different mechanisms (Lee et al., 2003; Anesini et al., 2004; Guzmán-Beltrán et al., 2008). In addition, these compounds can serve as chemical markers for quality control of the herbal mixtures teas.

Sensory evaluation has been demonstrated to be an effective instrument to study acceptability of a product. Whereas infusions prepared with "Jarilla" species have a strong bitter taste and the typical odor of herbs, and *H. dulcis* tastes like a combination of raisin, clove, cinnamon and sugar, the peduncles of the last plant species were mixed with "Jarilla" species in order to obtain palatable teas to consumers. According to the acceptability test, H infusion was more pleasant than the other single-plant infusions, with a mean score of 6.40 (Fig. 2). The H mix infusion was the most accepted among the five herbal mixtures prepared. This acceptability may be due to the sweetness proportioned by the fruit-stalks, present in greater amounts in H mix (mean score = 5.25). The scores of the other mixture infusions are not significantly different among themselves according to ANOVA analysis ($p \leq 0.05$), with mean score between 2.45 and 3.90. The infusion prepared with *L. divaricata* was the least accepted from the panelists, with a mean score of 1.30 (Fig. 2).

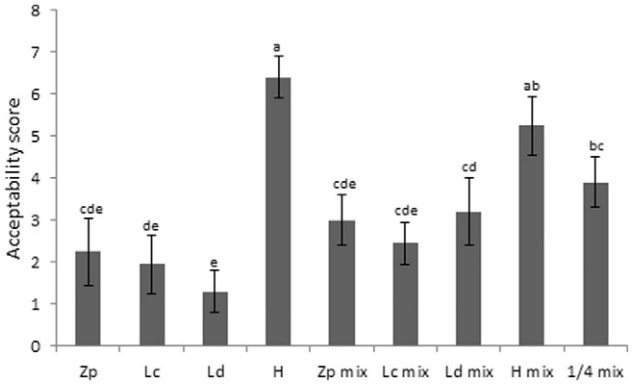

Fig. 2. Sensory evaluation of the nine medicinal herbal infusions. Note: Values (mean ± SD) followed by the same letter are not significantly different ($p \leq 0.05$). Error bars represent the standard error of the mean.

Table 3
Free radical scavenging activity of single-plant and mixture infusions.

12	Sample	SC_{50} (μg/ml)
Positive controls	Ascorbic acid	4.5 ± 0.5^{a}
	BHT	7.7 ± 0.4^{a}
Single-plant infusions	Zp	17.1 ± 0.1^{ab}
	Lc	71.2 ± 3.3^{c}
	Ld	19.7 ± 0.2^{ab}
	H	236.2 ± 18.3^{d}
Mixture herbal infusions	Zp mix	69.9 ± 2.6^{c}
	Lc mix	64.6 ± 5.3^{c}
	Ld mix	17.1 ± 1.2^{ab}
	H mix	27.9 ± 1.4^{b}
	1/4 mix	15.4 ± 1.7^{ab}

Values (mean ± SD) followed by the same letter are not significantly different ($p \leq 0.05$). SC_{50}: concentration (μg of freeze-dried infusion/ml) that scavenged 50% of free radicals.

Plants have a wide variety of free radical scavenging molecules, from which the majority are phenolic compounds. Such compounds may be useful in preventing cancer and other mutation-related diseases, by fortifying physiological defense mechanisms, or by favoring the intake of protective factors (Lewandowska et al., 2016). The free radical scavenging potential of herbal preparations was obtained by the $ABTS^{\bullet+}$ assay. As shown in Table 3, the SC_{50} value range from 17.1 to 236.2 μg/ml.

Table 4
Results obtained in the mutagenic activity evaluation assay against *S. typhimurium* TA98 and TA100 strain.

		TA98		TA100	
Infusions	Treatment (μg GAE/plate)	−S9	+S9	−S9	+S9
Zp	125	27 ± 5^{a}	30 ± 2^{abc}	113 ± 2^{ab}	126 ± 5^{a}
	250	28 ± 5^{a}	28 ± 3^{abc}	115 ± 6^{ab}	137 ± 12^{ab}
	500	24 ± 3^{a}	27 ± 5^{abc}	135 ± 7^{ab}	145 ± 8^{ab}
Lc	125	21 ± 5^{a}	29 ± 3^{abc}	116 ± 11^{ab}	160 ± 3^{ab}
	250	22 ± 1^{a}	17 ± 2^{a}	99 ± 13^{ab}	130 ± 7^{ab}
	500	27 ± 1^{a}	ND	98 ± 7^{ab}	165 ± 1^{ab}
Ld	125	25 ± 1^{a}	45 ± 1^{c}	109 ± 22^{ab}	130 ± 3^{ab}
	250	24 ± 2^{a}	33 ± 2^{abc}	153 ± 23^{b}	145 ± 6^{ab}
	500	27 ± 3^{a}	40 ± 1^{bc}	124 ± 15^{ab}	119 ± 16^{a}
H	125	30 ± 5^{a}	29 ± 3^{abc}	124 ± 15^{ab}	165 ± 7^{ab}
	250	30 ± 7^{a}	30 ± 3^{abc}	92 ± 7^{a}	132 ± 6^{ab}
	500	22 ± 3^{a}	20 ± 4^{ab}	117 ± 13^{ab}	126 ± 16^{a}
Zp Mix	125	27 ± 5^{a}	23 ± 4^{ab}	128 ± 9^{ab}	150 ± 5^{ab}
	250	21 ± 1^{a}	31 ± 2^{abc}	113 ± 27^{ab}	133 ± 5^{ab}
	500	27 ± 2^{a}	34 ± 5^{abc}	105 ± 22^{ab}	142 ± 3^{ab}
Lc Mix	125	ND	ND	ND	ND
	250	ND	ND	ND	ND
	500	ND	ND	ND	ND
Ld Mix	125	35 ± 3^{a}	24 ± 2^{abc}	115 ± 27^{ab}	156 ± 2^{ab}
	250	20 ± 1^{a}	38 ± 3^{abc}	119 ± 15^{ab}	130 ± 3^{ab}
	500	27 ± 6^{a}	39 ± 1^{bc}	122 ± 11^{ab}	114 ± 13^{a}
H Mix	125	32 ± 4^{a}	31 ± 3^{abc}	140 ± 27^{ab}	149 ± 5^{ab}
	250	30 ± 3^{a}	26 ± 2^{abc}	124 ± 25^{ab}	151 ± 7^{ab}
	500	29 ± 2^{a}	30 ± 4^{abc}	111 ± 13^{ab}	164 ± 10^{ab}
1/4 Mix	125	33 ± 7^{a}	35 ± 3^{abc}	134 ± 4^{ab}	165 ± 14^{ab}
	250	29 ± 1^{a}	28 ± 2^{abc}	109 ± 15^{ab}	129 ± 5^{ab}
	500	29 ± 1^{a}	30 ± 1^{abc}	105 ± 8^{ab}	189 ± 10^{b}
Negative control[1]		25 ± 2^{a}	33 ± 4^{abc}	106 ± 18^{ab}	152 ± 25^{ab}
Positive control[2]		1222 ± 109^{b}	890 ± 31^{d}	800 ± 51^{c}	810 ± 87^{c}

Note: (−S9) without and (+S9) with metabolic activation. A sample was considered mutagenic when the number of revertant colonies was at least twice the negative control yield and showed a significant response in the analyses of variance.
ND: Not determined. These concentrations affect the viability of the *Salmonella* strains.
[1] The number of spontaneous revertant colonies (means ± SD) determined without the addition of the samples, only with the vehicle, DMSO.
[2] Mean number of revertants induced by 4-nitro-*o*-phenylendiamine (10 μg/plate—in the assay without metabolic activation) and 2-aminofluorene (10 μg/plate—in the assay with metabolic activation). Values in the same column (mean ± SD) followed by the same letter are not significantly different ($p \leq 0.05$).

Among the single plant infusions, the most active was Zp (SC_{50} = 17.1 μg/ml equivalent to 9.5 μg GAE/ml), followed by Ld (SC_{50} = 19.7 μg/ml equivalent to 10.2 μg GAE/ml). It is known that *Z. punctata* has flavonoids with an excellent singlet oxygen quenching and free radical scavenging ability (Ávila et al., 2001; Morán Vieyra et al., 2009), that could be the responsible of scavenging activity observed in this work. Another author also studied the antioxidant potential of *L. divaricata* aqueous extracts, displaying a significant superoxide and catalase activity and DPPH radical scavenging capacity (Turner et al., 2011). Despite the low activity shown by *H. dulcis* freeze-dried infusions (SC_{50} = 236.2 μg/ml equivalent to 5 μg GAE/ml), polysaccharides present in the peduncles were studied for antioxidant capacity demonstrating superoxide radical scavenging activity, strong inhibition effect on lipid peroxidation and ion-chelating activity (Wang et al., 2012). This is the first report on antioxidant properties of *L. cuneifolia* aqueous extract. Regarding the mixtures prepared, the 1/4 mix which contained equal quantities of each plant species was the most active followed by Ld mix and H mix (Table 3).

In the evaluation of freeze-dried plant infusions against *Salmonella* strains, some concentrations of Lc and Lc mix showed inhibition of the *Salmonella typhimurium* growth, so these sample concentrations were omitted for the mutagenic and antimutagenic assessment in the Ames test.

The results of mutagenic assay, with and without metabolic activation, are shown in Table 4. In the presence of different doses of the samples, the mutation frequencies did not change significantly when compared to the spontaneous one indicating the absence of compounds in the extracts that neither cause base substitution (detected in TA100) and frameshift (detected in TA98) mutations nor promutagenic effect. To our knowledge, this is the first report on genotoxicity studies of *L. cuneifolia*, *L. divaricata* and *H. dulcis* aqueous extracts. Hydroalcoholic extracts of *Z. punctata* have been studied previously for genotoxicity on human hepatoma HepG2 cells and DNA damage was not observed (Zampini et al., 2008).

Antimutagenic activity of the freeze-dried plant infusions was also assayed, against a direct-acting mutagen (4-NPD), displaying a positive response in at least three herbal mixtures. As shown in Table 5, among the single-plant infusions, the only one that exhibits a weak effect was Ld with a percentage mutagenicity inhibition of 13% to TA98 and 11% to TA100 at the highest doses. The aqueous extract of *L. divaricata* has been tested for antitumoral activity, with *in vitro* and *in vivo* assays demonstrating a positive response (Anesini et al., 1995, 1997, 1998; Davicino et al., 2011). Previously, it was demonstrated that the hydroalcoholic extract of *Z. punctata* has an antigenotoxic effect (Zampini et al., 2008); however, no antimutagenic activity was found for Zp on the Ames test. Zp mix has a minor effect on TA98 strain only, at the highest concentration (19%). The most interesting results were obtained from Ld mix (percentage inhibition for TA98 = 30.3% and for TA100 = 27%, respectively, at 500 μg GAE/plate = 1.1 mg/plate), H mix (percentage inhibition for TA98 = 29.8% and for TA100 = 27.8% respectively, at 500 μg GAE/plate = 1.8 mg/plate) and 1/4 mix (percentage inhibition for TA98 = 30.8% and for TA100 = 29.5% respectively, at 500 μg GAE/plate = 0.9 mg/plate), as shown in Table 5. Similar values were obtained for green tea extract, a plant species with well-known antimutagenic/antigenotoxic properties (Kuroda and Hara, 1999; Bhattacharya and Giri, 2013) used as positive control. Green tea showed a percentage inhibition of 33.9% and 28.2% to TA98 and TA100, respectively, against 4-NPD mutagen, at 250 μg GAE/plate (0.39 mg of soluble principles/plate). This is the first study that reported antimutagenic activity for infusions prepared with mixture of herbaceous plants native from the northwest of Argentina.

Table 5
Effect of medicinal plant infusions against the mutagenicity of 4-NPD to *S. typhimurium* TA98 and TA100.

Infusions	Treatment (μg GAE/plate)	TA98 No. Rev./plate	Inhibition percentage (%)	TA100 No. Rev./plate	Inhibition percentage (%)
Zp	125	933 ± 151^{abc}	0	776 ± 72^{ab}	0
	250	865 ± 194^{abcd}	5.8 ± 2.0^{f}	732 ± 81^{ab}	4.6 ± 0.8^{ghi}
	500	916 ± 140^{abcd}	0	735 ± 86^{ab}	4.2 ± 0.4^{ghi}
Lc	125	1030 ± 62^{a}	0	744 ± 126^{ab}	2.9 ± 0.2^{hi}
	250	906 ± 196^{abcd}	0	671 ± 50^{ab}	12.5 ± 1.5^{cd}
	500	874 ± 52^{abcd}	4.8 ± 1.3^{f}	770 ± 135^{ab}	0
Ld	125	890 ± 126^{abcd}	3.1 ± 0.8	770 ± 116^{ab}	0
	250	844 ± 27^{abcd}	8.1 ± 1.0^{ef}	725 ± 18^{ab}	5.5 ± 0.9^{ghi}
	500	797 ± 84^{abcd}	13.2 ± 1.9^{de}	677 ± 165^{ab}	11.7 ± 2.0^{de}
H	125	983 ± 164^{a}	0	744 ± 99^{ab}	2.9 ± 1.2^{hi}
	250	937 ± 118^{abc}	0	718 ± 135^{ab}	6.4 ± 0.4^{fgh}
	500	916 ± 147^{abcd}	0	755 ± 10^{ab}	1.6 ± 0.3^{i}
Zp Mix	125	989 ± 186^{a}	0	827 ± 216^{ab}	0
	250	954 ± 73^{ab}	0	922 ± 81^{a}	0
	500	744 ± 59^{abc}	18.9 ± 1.6^{cd}	732 ± 10^{ab}	4.6 ± 0.1^{ghi}
Lc Mix	125	ND	ND	ND	ND
	250	ND	ND	ND	ND
	500	ND	ND	ND	ND
Ld Mix	125	755 ± 35^{abc}	17.8 ± 2.6^{cd}	709 ± 23^{ab}	7.6 ± 0.4^{efg}
	250	776 ± 50^{abcd}	15.5 ± 1.5^{cd}	687 ± 72^{ab}	10.4 ± 1.1^{def}
	500	640 ± 85^{bcd}	30.3 ± 3.2^{b}	560 ± 36^{bc}	26.9 ± 3.0^{b}
H Mix	125	882 ± 45^{abcd}	3.9 ± 0.7^{f}	728 ± 10^{ab}	5.1 ± 0.1^{ghi}
	250	731 ± 27^{abc}	20.4 ± 3.0^{c}	566 ± 86^{bc}	26.2 ± 2.1^{b}
	500	645 ± 48^{bcd}	29.7 ± 2.0^{b}	554 ± 10^{bc}	27.8 ± 1.3^{b}
1/4 Mix	125	770 ± 45^{abcd}	16.1 ± 1.9^{cd}	639 ± 40^{abc}	16.7 ± 2.0^{c}
	250	649 ± 28^{bcd}	29.3 ± 3.0^{b}	677 ± 87^{ab}	11.7 ± 2.3^{de}
	500	636 ± 35^{cd}	30.7 ± 3.8^{b}	541 ± 66^{bc}	29.4 ± 2.0^{b}
Green tea	125	762 ± 24^{abc}	17.3 ± 1.0^{cd}	658 ± 44^{ab}	14.2 ± 1.5^{cd}
	250	609 ± 8^{de}	33.9 ± 0.9^{b}	551 ± 27^{bc}	28.2 ± 1.2^{b}
	500	305 ± 13^{ef}	65.1 ± 1.4^{a}	332 ± 1^{cd}	56.7 ± 1.4^{a}
Negative control[1]		31 ± 6^{f}		113 ± 28^{d}	
100% mutagenicity[2]		918 ± 90^{abcd}		767 ± 56^{ab}	

ND: Not determined. These concentrations affect the viability of the *Salmonella* strains.
[1] The number of spontaneous revertant colonies (mean ± SD) determined without the addition of the samples, only with the vehicle, DMSO.
[2] Mean numbers of revertants induced by 4-nitro-*o*-phenylendiamine, 4-NPD (10 μg/plate). Values in the same column (mean ± SD) followed by the same letter are not significantly different ($p \leq 0.05$).

The presence of compounds with antioxidant activity demonstrated in the plant species considered could be contributing to the antimutagenic effect observed in the mixtures infusions prepared from them.

4. Conclusion

In this study, antioxidant innovative infusions have been developed from mixtures of native plant species used traditionally as medicines in northwest Argentina, with the addition of sweet peduncles of *H. dulcis* used in traditional medicine in China.

The antimutagenic activity of infusions prepared with plant mixtures was demonstrated, while infusions prepared from single plants had no effect. The mixture containing equal amounts of each plant species (1/4 mix) showed the highest antimutagenic activity, followed by Ld mix and H mix. These mixture infusions also exhibit radical scavenging capacity, so this activity could be contributing to the antimutagenic effect. These results indicate that some of developed medicinal infusions could be a natural source in the research of compounds with preventive activity in mutation related diseases.

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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