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Nutritional and antioxidant properties of *Geoffroea decorticans*, an Argentinean fruit, and derived products (flour, arrope, decoction and hydroalcoholic beverage)

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ABSTRACT

Geoffroea decorticans, popularly known as "Chañar," is widely distributed throughout Northwestern Argentina. Its fruit is consumed in different forms (flour, arrope, and hydroalcoholic beverage) in several Argentinean communities. The aim of this work is to quantify macronutrients and phytochemicals of *G. decorticans* fruit and its derived products as well as to determine its functional properties and toxicity. A high content of fiber and other phytochemicals (carotenoids, ascorbic acid and polyphenols, principally non flavonoids) were detected in Chañar flour. Since physicochemical changes that affect the functional properties of the fruit may occur during processing, different traditional foods were obtained from the fruit and their properties were studied. The phenolic compound content in different arrope types (with and without sugar added) was lower (between 3 and 18 fold) than that of Chañar flour. Condensed tannins were the dominant phenolics in the flour but in arrope without sugar they decreased 21 fold and in arrope with sugar were not detected. The content of ascorbic acid in arrope was similar to that of flour. All preparations obtained from flour and arrope showed hydrogen or electron donor capacity and were active as lipoperoxidation inhibitors. On the other hand, extracts did not show any mutagenic effect. The consumption of Chañar flour, beverage, decoction and arrope could be encouraged for their functional and nutritional properties. The exploitation of this native natural resource could be very important for regional economy.

1. Introduction

In Northwestern Argentina (NOA) there is an extraordinary plant biodiversity with many native fruits which represent an important alternative to production in the regional economy. The Great American Chaco, a vast region having an apparent ecological unit, is immersed in a severe degradation process of its natural resources and biodiversity due to deforestation, desertification and changes in land use (Giménez, Hernández, Gerez, & Rios, 2007). The American Chaco forest is characterized by the presence of "red quebracho" (*Schinopsis quebracho-colorado*), "white quebracho" (*Aspidosperma quebracho-blanco*), *Ziziphus*, *Prosopis*, *Caesalpinia*, *Cercidium*, and *Geoffroea* (Giménez et al., 2007). *Geoffroea decorticans* (Hook & Arn.) Burkart (Fabaceae) (Gallo, 1996), is one of five species of the genus which is distributed in South America (Kileen, García, & Beck, 1993). *G. decorticans*, traditionally known as "Chañar", is a thorny, deciduous, honey plant (10–15 m tall) widely distributed throughout Perú, Bolivia, Paraguay and Argentina. Its fruit is a reddish brown spherical drupe of approximately 10–17 mm in diameter, with a sticky, sweet pulp (Fig. 1A). Many NOA traditional communities utilize its bark, flowers and leaves in the treatment for dysentery (Gallo, 1996). The bark is used to treat respiratory affections (cough, bronchitis, colds). The infusion is used as an expectorant, the syrup is recommended as the best cough syrup, the decoction is used as a remedy for whooping cough, and it is also said to fight flatulence. The crushed bark mixed with sebum facilitates removal of thorns (Demaio, Karlin, & Medina, 2002). Some compounds (5,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone and 7-2′-3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone) were isolated from Chañar stem bark (Vila et al., 1998). The presence of isoflavanones in leaves and twigs and their antifungal activity on phytopathogenic strains were also demonstrated (Quiroga, Sampietro, Sgariglia, Soberón, & Vattuone, 2009).

Fruits of *algarrobo* and Chañar have been a food source for humans and domestic goats in rural communities in the Monte desert since ancient times (Burkart, 1952). Traditionally, Chañar fruits are consumed in *añapa* and *arropes* with and without sugar. Chañar fruit has long been used in folk medicine in many preparations. "Chañar arrope" is administered as an antitussive and against respiratory diseases. The antinociceptive action of Chañar arrope and aqueous extract has been demonstrated (Reynoso, Vera, Aristimuño, Daud, & Sánchez Riera, 2013).

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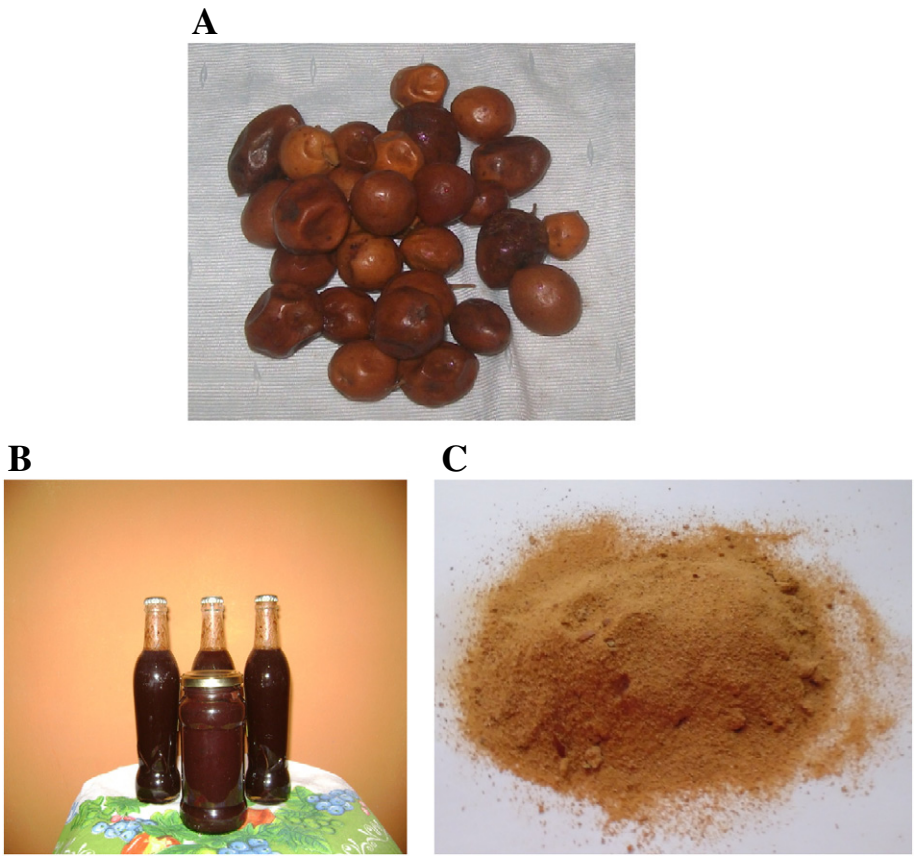

Fig. 1. (A) Ripe Chañar fruits; (B) arrope; (C) Chañar flour.

The roasted seeds are eaten as a flavoring agent for their almond flavor and high oil content (mainly oleic and linoleic acids) (Maestri, Fortunato, Greppi, & Lamarque, 2001).

Based on their oil content, the seeds may be a potential source of commercial vegetable oil (Maestri et al., 2001). Recent studies indicate that biodiesel obtained from Chañar seeds (recovery of 87% of methyl esters) complies with international standards.

However, there is no nutritional or functional information on this fruit in the databases of food composition in Latin America, the United Kingdom or the United States of America.

Taking these factors into consideration in our continued quest to find new edible plants with functional properties and to promote their reproduction and preservation, we undertook the so far unexplored chemical and biological research of Chañar fruits. The aim of the present work was to determine the nutritional composition, evaluate the antioxidant properties and genotoxicity of flour, decoction, beverage and arrope obtained from Chañar fruits grown in Northwestern Argentina.

2. Materials and methods

2.1. Samples

2.1.1. Plant material

Fruits of *G. decorticans* were collected in January 2011 and 2012 in Departmento Fernández, Santiago del Estero, Argentina.

2.1.2. Products derived from the fruit

Chañar flour: Fruits were air-dried and ground into powder with a particle size of 80 meshes.

Chañar honey or arrope: The Chañar fruits were cooked in a water medium for five hours with sugar to obtain a honeylike syrup known as "arrope". The relation between fruits and sugar content was 1:0.5. They were also cooked in a second preparation without sugar for two hours to obtain an "arrope without sugar".

2.1.2.1. Beverage and decoction of flour and arrope were prepared in order to emulate domestic preparations.

- *Ethanolic Chañar extraction or beverage*: Flour or arrope (150 g) were weighed and extracted with 500 mL 96° ethanol for 20 min at 70 °C. Then, the extracted material was filtered through Whatman No. 4 filter paper. The residues were extracted twofold and all fractions were evaporated. The extracts obtained were named E*Ch*FE (ethanolic extract obtained from Chañar flour) and E*Ch*ArE (ethanolic extract obtained from Chañar arrope).
- *Aqueous Chañar extraction* (*AChE*) *or decoction*: The residues from the preparation of ethanolic extracts were decocted in 500 mL of distilled water for 20 min. The decoction was left to cool at room temperature, filtered through Whatman No. 4 filter paper and the aqueous extracts were lyophilized. The extracts obtained were named A*Ch*FE (aqueous extract obtained from Chañar flour) and A*Ch*ArE (aqueous extract obtained from Chañar arrope).

2.1.2.2. Acetone/water and methanolic extractions of flour were carried out to obtain a preparation with a high content of phenolic compounds or tannin.

– *Acetone–water Chañar flour or arrope extract* (*AWChFE*): Sample (1 g) was extracted with 10 mL acetone: water (70:30, v:v) in an ultrasonic bath for 30 min at room temperature and then centrifuged at 9000 ×*g* for 10 min. The resulting suspension was filtered and the

remaining solids were extracted exhaustively with the same solvent system. All organic extracts were combined and the acetone was evaporated, and the final volume was adjusted to 5 mL. Then, a fraction was subjected to acid hydrolysis by adding sulfuric acid (2 N) to the aqueous fraction. The solution was maintained at 100 °C during 26 h.

– *Methanolic Chañar flour or arrope extract* (*MChFE*): Sample (1 g) was extracted with 12.5 mL methanol:water (70:30, v:v) in an ultrasonic bath for 30 min at room temperature and then centrifuged at 9000 ×*g* for 10 min. The suspension obtained was filtered and the remaining solids were extracted exhaustively with the same solvent system. All organic extracts were combined, evaporated and the final volume adjusted to 5 mL.

2.2. Chemical composition determination

2.2.1. Sugar determination

2.2.1.1. Sugar extraction. Flour or arrope (1 g) were extracted with ethanol 80% (4 mL) at 75 °C for 10 min and then centrifuged at 9000 ×*g* for 5 min (Prado, Gonzalez, Boero, & Sampietro, 1998). The remaining solids were extracted exhaustively with the same solvent system. All organic extracts were combined and then evaporated.

2.2.1.2. Sugar determination. The phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) was used to determine total neutral sugars in aqueous and ethanolic preparations. Reducing sugars were measured using the Somogyi–Nelson method (Nelson, 1944; Somogyi, 1945). Results were expressed as g of glucose/100 g dry weight (DW) of fruits or arrope.

2.2.2. Protein determination

Soluble protein concentration in all preparations was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard. Results were expressed as mg of BSA/100 g dry weight (DW) of fruits or arrope. The total Nitrogen (N) content of lyophilized fruits was determined by Kjeldahl method (AOAC, 1998). Crude protein content was calculated as % N × 6.25.

2.2.3. Mineral analyses

The analysis was carried out by quadrupole inductively coupled plasma mass spectrometry (Q-ICPMS). A Thermo-Elemental X7 series (Thermo Fisher Scientific, Bremen, Germany), equipped with an ASX-100 autosampler model (CETAC Technologies, Omaha, NE), was used (Instituto Superior de Investigación Desarrollo y Servicios en Alimentos, ISIDSA, Córdoba, Argentina).

2.2.4. Total polyphenols

Total polyphenols in flour and arrope extractions were determined by Folin–Ciocalteau's reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999). Results were expressed as mg gallic acid equivalents (mg GAE)/100 g dry weight (DW) of fruits or arrope.

2.2.5. Non-flavonoid polyphenols

Non-flavonoid phenols were measured by the determination of total phenol content remaining after precipitation of the flavonoids with acidic formaldehyde (Zoecklein, Fuelsang, Gump, & Nury, 1990). Results are expressed in mg gallic acid equivalents (mg GAE) per 100 g dry weight of fruits or arrope.

2.2.6. Flavones and flavonols

Two different methods were used to assess the amount of flavonoids present in extractions of flour and arrope.

The total flavonoid content in aqueous preparations was determined with aluminum chloride ($AlCl_3$) according to Zhishen, Mengcheng, and Jianming (1999). 0.3 mL of extracts was added to 0.3 mL distilled water followed by $NaNO_2$ (0.03 mL, 5%). After 5 min at 25 °C, $AlCl_3$ (0.03 mL, 10%) was added. After a further 5 min, the reaction mixture was treated with 0.2 mL, 1 mM NaOH. Finally, the reaction mixture was diluted to 1 mL with water and absorbance was measured at 510 nm.

The $AlCl_3$ method (Lamaison & Carnet, 1990) was used to determine total flavonoid content of the ethanolic, methanolic and acetonic extracts. 0.5 mL of ethanolic 2% $AlCl_3 \cdot 6H_2O$ was added to equal volumes of each extract. The mixture was shaken and the absorbance read at 420 nm after 60 min incubation at room temperature. Flavonoid content was expressed as mg quercetin equivalents (mg QE) per 100 g dry weight (DW) of fruits or arrope.

2.2.7. Flavanone and dihydroflavonol

Flavanone and dihydroflavonol content was determined using 2,4-dinitro phenyl hydrazine (DNP) in acid media according to Popova, Silici, Kaftanoglu, and Bankova (2005). An aliquot of each extract sample was diluted with 80% ethanol to a volume of 0.25 mL. Next, 0.5 mL of DNP solution (1 g DNP in 2 mL 96% sulfuric acid, diluted to 100 mL with methanol) was added and heated at 50 °C for 50 min. After cooling to room temperature, 0.3 mL of the previous mixture was diluted to 1 mL with 10% KOH. The resulting solution was centrifuged at 1500 ×*g* for 10 min and 0.25 mL of the supernatants was diluted to 1.5 mL with methanol. Absorbance was measured at 492 nm. Flavanone and dihydroflavonol content was estimated using a naringenin calibration curve with a concentration range of 30–120 μg. Results were expressed as mg naringenin equivalent per 100 g dry weight (mgNGE/100 g DW).

2.2.8. Proanthocyanidin determination

Total proanthocyanidin (PACs) content was determined with 4-dimethylaminocinnamaldehyde (DMAC) according to Prior et al. (2010). 450 μL of the DMAC solution (0.1% in acidified ethanol) was added to 150 μL of 80% ethanol (blank), standard or extracts. Absorbance was read at 640 nm after 20 min at 25 °C. Data were expressed as mg of procyanidin B2 equivalents (mg PB2E) per 100 g dry weight (DW) of fruits or arrope.

2.2.9. Gallotanin determination

Acetonic extract (2 mL) was first hydrolyzed with 4 mL of 2 N H_2SO_4 at 100 °C for 26 h and the gallic acid released was determined with the rhodanine method of Inoue and Hagerman (1988). Twenty microliters of hydrolyzed extract [H(AW*Ch*E)] were made up to 200 μL with distilled water and 200 μL of non-hydrolyzing AW*Ch*E were dried under nitrogen and resuspended in 200 μL of 0.2 N H_2SO_4. Two hundred microliters of 0.2 N H_2SO_4 and 300 μL rhodanine (0.667% methanol) were added to the diluted extracts. After 5 min, 200 μL of 0.5 N potassium hydroxide and 4 mL distilled water were added. The mixtures were left for 10 min at room temperature and absorbance was determined at 520 nm. AW*Ch*E was evaluated in replicates and gallotannin concentrations were expressed as mg gallic acid equivalents per 100 g dry weight of fruits (mg GAE/100 g DW) or arrope.

$$\text{Gallotanins (mg GAE)} = [\text{GH(AW}Ch\text{E)}] - [\text{G(AW}Ch\text{E)}]$$

where [GH(AW*Ch*E)] is the amount of gallic acid present in the hydrolyzed extract and [G(AW*Ch*E)] is the amount of gallic acid present in the non hydrolyzed extract.

2.2.10. Total anthocyanins

Sample (1 g) was extracted with 5 mL 1% HCl in methanol overnight at 5 °C without light. The solution obtained was then filtered through Whatman No. 1 filter paper and the remaining solids were extracted exhaustively with 5 mL of 1% HCl in methanol. All acidic–methanolic extracts obtained were combined and vacuum-concentrated by evaporating

methanol in a rotary evaporator. They were resuspended with 5 mL MILLIQ water to obtain the anthocyanin extract (AE). Total anthocyanins were evaluated by the pH differential method (Lee, Durst, & Wrolstad, 2005). The AE in 25 mM potassium chloride solution (pH 4.5) and 400 mM sodium acetate buffer (pH 1.0) were measured simultaneously at 520 nm and 700 nm, respectively. The content of total anthocyanins was expressed as mg cyanidin-3-glucoside equivalents (mg C3GE) per 100 g of dry weight (DW) of fruits or arrope.

2.2.11. Total alkaloids

Total alkaloids were measured using bromothymol blue (BTB) as the coloring agent (Önal, Kepekçi, & Öztunç, 2005). One milliliter of water, standard or A*Ch*E was added to 2 mL of BTB reagent (4.78×10^{-4} M in phosphate buffer pH 5). After mixing, the ion-pair complex formed was extracted with 5 mL chloroform by shaking vigorously for exactly 1 min. Absorbance was measured at 414 nm against the blank prepared with water. Total alkaloids were calculated as μg of apomorfine chlorhydrate equivalents (μg ACE) per 100 g of dry weight (DW).

2.2.12. Total carotenoids

Samples (1 g) were extracted with 10 mL of hexane:acetone: ethanol (50:25:25, v/v). After centrifugation at 13000 ×*g* for 10 min at 4 °C, the top hexane layer containing the color was recovered and adjusted to 10 mL with hexane. Total carotenoid content was calculated according to Rodríguez-Amaya (1999). The β-carotene absorbance was measured at 450 nm and results were expressed as mg of β-carotene equivalents (mg β-CE) per 100 g of dry weight (DW) of fruits or arrope.

2.2.13. Vitamin C

Fresh samples (0.2 g) were extracted with 0.5 mL of H_3PO_4 2% according to Barros, Heleno, Carvalho, and Ferreira (2010). After centrifuging at 12000 ×*g* for 10 min, the supernatant was reserved to determine the ascorbic acid content using 2,6 dichloroindophenol sodium salt hydrate (IDF). Different dilutions were added to 125 μL of sodium acetate buffer 400 mM, pH 4; 40 μL of IDF and distilled water until 1 mL. After mixing, the absorbance was measured at 515 nm. Vitamin C was calculated and expressed as mg L-ascorbic acid (mg L-AA) per 100 g dry weight (DW) of fruits or arrope.

2.2.14. Determination of fiber content

Fiber content was determined according to Jaafar, Rahman, Mahmod, and Vasudevan (2009). Two grams of samples (flour or arrope) were put into a 250 mL conical flask and 1.25% sulphuric acid solution was added. The sample was heated for about 30 min, filtered using a vacuum filter and washed until traces of acid were undetected using pH paper. A Whatman 5B paper was placed in the Buchner flask. After the acid digestion, the sample was transferred to a 250 mL conical flask and 3.52% NaOH solution was added. The sample was heated again for 30 min. It was filtered using a vacuum filter and washed with water until base was undetected. The whole material was placed in a crucible and dried for 12 h at 120 °C. The crucible was heated in a muffle oven at 550 °C for 12 h and the weight of the crucible was recorded.

2.2.15. Fat measurement

An aliquot of 20 g of Chañar flour or Chañar arrope was used to determine crude fat by extracting with petroleum ether (40–60 °C) in a Soxhlet apparatus during 4 h (AOCS, 1989).

2.3. Measurement of antioxidant capacity

2.3.1. ABTS free radical scavenging activity

The antioxidant capacity assay was carried out by the improved $ABTS^{\bullet+}$ method as described by Re et al. (1999). $ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23 °C) in the dark for 16 h. The $ABTS^{\bullet+}$ solution was obtained by diluting the stock solution to an absorbance of 0.70 at 734 nm in ethanol, acetone or buffer PBS pH 7.4 according to the solvent used to extract preparation. $ABTS^{\bullet+}$ solution (1 mL) was added to the sample (1 to 30 μg GAE/mL) and mixed thoroughly. Absorbance was recorded at 734 nm after 6 min. The percentage of inhibition was measured by the following formula:

$$\% \text{ inhibition} = (A_0 - A_s)/A_0 \times 100;$$

where A_0 is the absorbance of the control (blank, without extract) and A_s is the absorbance in presence of the extract. SC_{50} values denote the μg GAE/mL required to scavenge 50% ABTS free radicals.

2.3.2. DPPH free radical scavenging activity

DPPH radical scavenging activity was measured according to Brand-Williams, Cuvelier, and Berset (1995). DPPH solution (1.5 mL of 300 μM in 96° ethanol) was added to 0.5 mL of different extracts (containing 2–30 μg of GAE/mL) and shaken vigorously. It was kept at room temperature for 20 min and absorbance was measured at 515 nm. The percentage of radical scavenging activity (RSA %) was calculated using the following equation:

$$\text{RSA } \% = [(A_0 - A_s)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_s is the absorbance of the samples at 515 nm. SC_{50} values denote the μg GAE/mL required to scavenge 50% DPPH free radicals.

2.3.3. β-carotene bleaching assay

Antioxidant activity was determined according to the β-Carotene bleaching method following the procedure described by Ordoñez, Gomez, Vattuone, and Isla (2006). The initial absorbance at 470 nm was registered at zero time (t_0) and for 120 min. Antioxidant activity (AA%) was calculated as percent inhibition relative to control using the following equation:

$$AA\% = \left[\left(R_{control} - R_{sample}\right)/R_{control}\right] \times 100$$

where $R_{control}$ and R_{sample} are the bleaching rates of β-carotene in reactant mix without antioxidant and in the presence of the extracts, respectively.

IC_{50} values denote the μg GAE/mL required to inhibit 50% β-carotene bleaching.

2.4. Mutagenicity

Salmonella mutagenicity assay

The mutagenic effects of Chañar extracts were evaluated on two *Salmonella typhimurium* strains (TA98 and TA100). The plate incorporation assay was performed according to Maron and Ames (1983), by adding 0.1 mL of the overnight bacterial culture, 0.1 mL of extracts at different concentrations (25–100 μg GAE/plate) and 2 mL of top agar on minimal agar. In the case of metabolic activation, 0.5 mL S9 mixture was supplemented. His^+ revertants were counted after 72 h of incubation at 37 °C.

The positive controls employed were 4-nitro-o-phenylenediamine (4-NPD; Aldrich Chemical Co.), 20 μg/plate and 2-aminofluorene (2-AF; Merck), 10 μg/plate. As a solvent control 100 μL DMSO/plate was run concurrently with all experiments. Three plates at two separate experiments were used for each concentration tested and for positive and negative controls.

2.5. Statistical analysis

Sampling and analyses were performed in triplicate, and the data are presented as mean ± standard deviation. GraphPad Prism 5.0 software was used to perform analysis of correlation between two variants by Pearson test with the level of significance set at $p < 0.05$ and of variance (ANOVA) with Tukey posttest at a confidence level of 95%.

3. Results and discussion

3.1. Macronutrients and phytochemical compounds in G. decorticans flour

Fruit water content was low and very stable in all samples and there were no statistically significant differences between batches. These characteristics make for easy preservation and microbiological stability of fruits. Flour was obtained from ripe fruits and characterized.

Carbohydrates were the major component of Chañar flour (19.75 ± 0.85%) principally reducing sugar (6.46 ± 0.15%) (Table 1). Comparing the Chañar flour with other exotic flours it can be observed that while its crude protein content was 5.06 ± 0.02 g/100 g DW (Table 1), the flour of edible fruits from other species that grow in the same environments, such as *Ziziphus mistol*, *Prosopis alba* and *Prosopis nigra*, exhibited protein content of 3 and 4.2%, respectively (Cardozo, Ordoñez, Alberto, Zampini, & Isla, 2011; Cardozo et al., 2010). Fat content was 2.5 ± 0.5 g/100 g DW, lower than the one reported by Maestri et al., 2001 for pericarp of Chañar fruits. "Chañar" flour was high in potassium (3100 mg K/100 g) and low in sodium (23 mg Na/100 g). Calcium was the highest component (130 mg Ca/100 g) followed by magnesium (112 mg/100 g), iron (10 mg Fe/100 g) and phosphorus (2.66 mg/100 g).

The total polyphenol content of Chañar flour extracted with 70% aqueous/acetone (1.24 ± 0.03 g GAE/100 g DW) was similar to that of *Z. mistol* flour (1.19 ± 0.068 g GAE/100 g DW) (Cardozo et al., 2011, Table 2). Rufino et al. (2010) classified the fruits according to their dry matter polyphenolic content in the low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g) or high (>500 mg GAE/100 g) categories. Chañar flour would be considered to be in the high category. Consumption of food containing phenolic compounds, principally tannins and flavonoids, has been shown to be associated with possible prevention of chronic illnesses such as cancer, atherosclerosis or cardiovascular disease (Anderson et al., 2004; Joven et al., 2013; Okuda, 2005).

Condensed tannins were the dominant phenolics in the flour (844.40 ± 15.03 mg PB2E/100 g DW) followed by flavones, flavonones and anthocyanins (Table 1). In our working conditions, hydrolizable tannins and alkaloids were not detected in flour. Other compounds such as carotenoids (2.14 ± 0.10 mg/100 g) and ascorbic acid (19.5 ± 0.2 mg/100 g) were detected in low levels. The ascorbic acid daily intake recommended in packaged food is 45 mg. Hence, the consumption of 200 g of Chañar flour may be necessary to cover these requirements in ascorbic acid.

Table 1
Characterization of Chañar flour.

Macronutrients content in flour		Phytochemicals content in flour	
Total soluble sugar g GE/100 g DW	19.75 ± 0.85	Total phenolics mg GAE/100 g DW	1240 ± 30
Reducing sugar g GE/100 g DW	6.46 ± 0.15	Phenolic non flavonoids mg GAE/100gDW	320 ± 10
Total protein g/100 g DW	5.06 ± 0.02	Flavones mg QE/100 g DW	70 ± 0.00
Fat g/100 g DW	2.50 ± 0.5	Flavanones and dyhidroflavanones mg NE/100 g DW	0.02 ± 0.01
Fiber g/100 g DW	12.07 ± 0.20	Condensed tannins mg procyanidin B2/100 g DW	844.40 ± 15.03
		Hydrolizable tannins mg EAG/100 g DW	<DL
		Anthocyanins mg C3GE/100 g DW	1.03 ± 0.01
		Ascorbic acid mg AA/100 g DW	19.50 ± 0.20
		Carotenoids mg Eβ-C/100 g DW	2.14 ± 0.10

<DL: lower of limit of detection method.

3.1.1. Fiber content

Fiber is not a simple and well defined chemical compound, but a combination of chemical substances in composition and structure, such as cellulose, hemicelluloses, lignin, etc. being defined as the "edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine" (Mongeau, 2003). Fiber includes: insoluble fiber (lignin, cellulose and hemicelluloses) and soluble fiber (pectins, β-glucans, galactomanan gums, and a large range of non digestible oligosaccharides including inulin).

Diverse *in vivo* studies have demonstrated that the ingestion of insoluble fiber from fruits and vegetables can produce a significant decrease of the plasmatic concentration of cholesterol, which implies a decrease in the risk of suffering cardiovascular disease, colon cancer, diabetes and obesity. Also, a regulatory activity of the immune system has been attributed to dietary fiber (Brett & Waldron, 1996). For all those reasons, the current fiber intake recommended by the Food and Nutrition Board (Trumbo, Schlicker, Yates, & Poos, 2002) in adults is 21–38 g/day, depending on life stage groups.

The Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006, states that in the EU, a food product can only be declared a source of fiber if it contains 3 g of fiber per 100 g as a minimum. In this sense, the insoluble fiber content of Chañar flour was of 12 g/100 g DW. Thus, it could be considered a "source of fiber", since its contents surpass 3 g/100 g (Table 1). Further research would be desirable to assess the physiological effects of this fiber in the human body, as well as the physico-chemical effects in food products enriched with "Chañar fiber".

3.2. Macronutrients and phytochemicals of Chañar arrope

Fruits are most commonly consumed raw; however, their jams and liquors are also prepared to preserve them for longer periods of time. Physicochemical changes that affect their functional properties may occur during fruit processing. Chañar arrope is an argentinean traditional food obtained by decoction of ripe fruits for 5 or 2 h with and without added sugar, respectively. Macronutrient and phytochemical contents are shown in Table 2. In general, the levels of sugar remained unchanged, but reducing sugar as well as protein, fat and fiber content decreased, probably due to destruction by heating. The phenolic compound content in both arrope types (with and without sugar added) was lower (between 3 and 18 fold) than in Chañar flour.

The total polyphenol and phenolic non flavonoid content of arrope obtained from fruits/water/sugar was lower than the one obtained from arrope without the addition of sugar probably due to the longer decoction time. Condensed tannins were the dominant phenolics in the flour, but in arrope without sugar they decreased 21 fold while in arrope with sugar they and hydrolizable tannin were not detected. Otherwise, carotenoid levels were lower in arrope than in flour (Table 2). Ascorbic acid was stable in the arrope containing sugar. The results indicate that some components such as tannin are more heat labile than others and that adding sugar protects some components from destruction.

Table 2
Characterization of Chañar arrope.

Macronutrients content	Arrope with sugar	Arrope without sugar	Phytochemicals content	Arrope with sugar	Arrope without sugar
Total soluble sugar g GE/100 g arrope	37.25 ± 3.50	18.70 ± 2.10	Total phenolics mg GAE/100 g arrope	153 ± 4	220 ± 5
Reducing sugar g GE/100 g arrope	21.40 ± 1.10	2.40 ± 0.20	Phenolic non flavonoids mg GAE/100 g arrope	38 ± 1	80 ± 2
Soluble Protein g/100 g arrope	0.06 ± 0.01	0.18 ± 0.02	Flavones mg QE/100 g arrope	4 ± 1	3 ± 1
Fat g/100 g arrope	0.04 ± 0.01	0.18 ± 0.02	Flavanones and dyhidroflavanones mg NE/100 g DW	<DL	<DL
Fiber g/100 g arrope	0.15 ± 0.01	1.20 ± 0.5	Condensed tannins mg procyanidin B2/100 g arrope	<DL	31 ± 4
			Hydrolizable tannins mg EAG/100 g arrope	<DL	<DL
			Anthocyanins mg C3GE/100 g arrope	0.11 ± 0.03	0.17 ± 0.01
			Ascorbic acid mg AA/100 g arrope	10.48 ± 0.10	3.30 ± 0.50
			Carotenoids mg Eβ-C/100 g arrope	0.25 ± 0.03	1.10 ± 0.50

Yield of arrope with sugar: 1680 g of arrope was obtained from 1000 g of fruits without seeds with 500 g of sugar and 500 mL of water.
Yield of arrope without sugar: 1280 g of arrope was obtained from 1000 g of fruits without seeds with 500 mL of water.
<DL: lower of limit of detection method.

3.3. Macronutrients and phytochemicals of two food preparations obtained with Chañar flour

Two preparations simulating domestic processing were obtained from flour, an aqueous preparation with heating for 20 min (decoction) and an ethanolic extract (beverage) without heating. Sugar contents were significantly higher in the beverage (12.32 ± 0.7 g GE/100 g) than in the decoction (1.92 ± 0.7 g GE/100 g) (Table 3). Soluble proteins for ethanolic and aqueous preparations were 47.8 ± 4 and 19.7 ± 2 mg BSA/100 g DW, respectively.

The aqueous and ethanolic preparations exhibited lower free total phenolic amounts (193 and 277 mg GAE/100 g DW, respectively) than Chañar flour (1240 mg GAE/100 g DW). The aqueous extracts contained a higher level of phenolic non flavonoid than ethanolic preparations (Table 3). The ascorbic acid content in the ethanolic preparation (24.5 mg/100 g) was similar to that obtained from Chañar flour (19.5 mg/100 g) indicating that it could be consumed as an alcoholic beverage. Ascorbic acid and carotenoids were not detected in the aqueous preparation, but it contained polyphenolic compounds.

3.4. Macronutrients and phytochemicals of food preparations obtained from Chañar arrope

Ethanolic and aqueous extractions from *arrope* (EC*h*ArE and AC*h*ArE, respectively) were carried out to determine their macronutrient and bioactive phytochemical composition (Table 3). Sugar and soluble protein content were significantly lower than that of arrope (Table 3). In general, the level of phenolic compounds in both preparation types (aqueous and ethanolic) was lower than in Chañar arrope. The aqueous preparations showed a higher phenolic compound content than ethanolic ones. Condensed tannins were the dominant phenolics in arrope without sugar, but in preparations obtained from both arrope types they and hydrolizable tannin were not detected. Otherwise, carotenoid levels in ethanolic preparations were similar to those obtained in arrope while in aqueous preparations they were not detected (Table 3). Ascorbic acid content was lower in ethanolic preparations than that arrope. The results indicate that the ethanolic preparation obtained from Chañar arrope showed a higher content of some phytochemicals (carotenoids, ascorbic acid) while the aqueous preparation showed principally phenolic components.

3.5. Antioxidant activity of food preparations obtained from Chañar flour and extracts enriched with phenolic compounds and tannins

Oxidative stress, caused by reactive oxygen species (ROS), is known to cause the oxidation of biomolecules leading to cellular damage. In the human body, the toxic effects of ROS are regularly opposed by a number of endogenous defense and protective mechanisms. These self-defense systems may also be supported by antioxidant compounds taken as foods, and medicinal plants.

Table 3
Macronutrient and Phytochemical content of food preparations of "Chañar".

Chañar products	Total sugar	Reducing sugar	Soluble protein	Phenolic compounds	Phenolic non flavonoids	Flavones	Anthocyanins	Carotenoids	Ascorbic acid
	g GE/100 g	g GE/100 g	mg AE/100 g	mg GAE/100 g	mg GAE/100 g	mg QE/100 g	mg C3GE/100 g	mg Eβ-C/100 g	mg AA/100 g
Flour									
AC*h*FE	1.92 ± 0.70	0.70 ± 0.10	82.70 ± 4.00	193 ± 8	90 ± 1	80 ± 0	0.12 ± 0.02	<DL	<DL
EC*h*FE	12.32 ± 0.70	8.21 ± 0.10	47.80 ± 4.00	277 ± 3	20 ± 1	90 ± 1	0.35 ± 0.02	0.12 ± 0.04	24.50 ± 0.05
Arrope									
AC*h*ArE without sugar	0.63 ± 1.00	0.07 ± 0.20	31.56 ± 0.12	60 ± 4	8 ± 0	<DL	0.01 ± 0.00	<DL	<DL
EC*h*ArE without sugar	5.78 ± 1.00	2.18 ± 0.20	35.50 ± 3.00	17 ± 1	4 ± 1	<DL	0.12 ± 0.01	1.16 ± 0.02	1.95 ± 0.02
AC*h*ArE with sugar	25.29 ± 2.00	8.73 ± 0.20	36.70 ± 0.60	142 ± 8	30 ± 1	<DL	0.09 ± 0.01	<DL	<DL
EC*h*ArE with sugar	10.32 ± 2.00	5.40 ± 0.20	29.80 ± 0.50	41 ± 1	7 ± 0	<DL	ND	0.21 ± 0.01	2.45 ± 0.02

The results in ethanolic Chañar flour extract (EC*h*FE) and aqueous Chañar flour extract (AC*h*FE), are expressed per 100 g DW flour. The results in aqueous arrope extract (AC*h*ArE) and ethanolic arrope extract (EC*h*ArE) are expressed per 100 g arrope; <DL: lower of limit of detection method.

Table 4
SC_{50} and IC_{50} of food preparations with flour of Chañar and extracts enriched with phenolic compounds and tannins.

Extractions	DPPH•	ABTS•+	β-carotene
	SC_{50} μg EAG/ml		IC_{50} μg GAE/ml
E*Ch*FE	310 ± 10	9 ± 2	23 ± 3
A*Ch*FE	50 ± 10	6 ± 2	10 ± 2
AW*Ch*FE	350 ± 15	4 ± 1	45 ± 4
M*Ch*FE	390 ± 10	13 ± 2	43 ± 3
A*Ch*ArE without sugar	65 ± 5	18 ± 2	18 ± 2
E*Ch*ArE without sugar	18 ± 2	3 ± 1	15 ± 1
AAr*Ch*E with sugar	150 ± 10	13.5 ± 0.5	55 ± 5
EAr*Ch*E with sugar	45 ± 5	2.5 ± 0.5	57 ± 5

Ethanolic Chañar flour extract (E*Ch*FE); aqueous Chañar flour extract (A*Ch*FE); acetonic Chañar flour extract (AW*Ch*FE), methanolic Chañar flour extract (M*Ch*FE), aqueous arrope extract (A*Ch*ArE); ethanolic arrope extract (E*Ch*ArE).

Natural foods (fruits, vegetables) and food derived antioxidants such as carotenoids, vitamins and phenolic phytochemicals, have lately received growing attention because they are known to function as chemopreventive agents against oxidative damage.

The antioxidant activity of food preparations obtained from Chañar flour and Chañar arrope and the organic extracts enriched with phytochemicals were analyzed in the present study. All preparations exhibited DPPH and ABTS reducing capacity (Table 4, Figs. 2 and 3A and B) with SC_{50} values between 18 and 390 μg GAE/mL for DPPH and 2.5 to 18 μg GAE/mL for ABTS. All extracts were able to protect lipids from oxidation with IC_{50} values of 10 to 57 μg GAE/mL (Figs. 2C and 3C). In all cases, polyphenols showed a dose–response relationship ($R^2 > 0.75$) with antioxidant capacity in the decoction and the alcoholic beverage as well as in the extract enriched with polyphenolic compounds, principally tannins. In fact, polyphenols are considered to be the most active antioxidant derivatives in plants. However, it has been shown in other studies that phenolic content does not necessarily correspond to antioxidant activity. The latter is the result of the combined activity of a wide range of compounds, including phenolics, carotenoids, ascorbic acid and other components. Vitamin C can act by scavenging the oxygen radicals present in the medium by way of chemical reactions, consequently making them unavailable as auto-oxidation propagators. In addition, it has a high vitamin E regenerating capacity (Hamilton, Gilmore, Benzie, Mulholland, & Strain, 2000).

Phenolic compounds act by neutralizing and scavenging free radicals and also chelating transition metals. The intermediate compounds formed by the action of the phenolic antioxidants are relatively stable due to charge distribution throughout the aromatic ring system. The antioxidant capacity of these compounds is attributed to the reducing power of the aromatic hydroxyl group, which reduces the reactive free radicals (Saito, Kohno, Yoshizaki, & Niwano, 2008).

The mechanism by which carotenoids protect biological systems from free radicals depends on the transfer of energy from the excited oxygen to the carotenoid molecule. They react mainly with the peroxide radical and molecular oxygen (Tapiero, Townsend, & Tew, 2004).

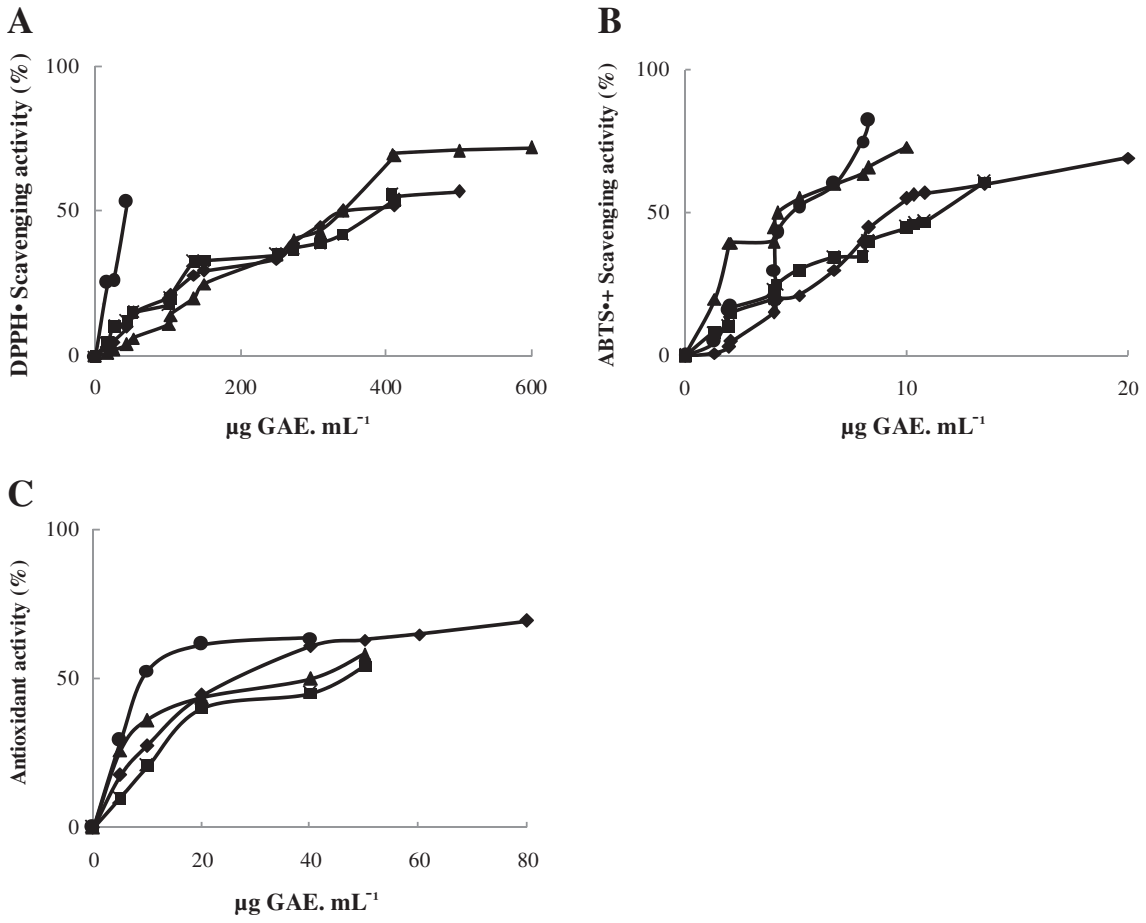

Fig. 2. Antioxidant activity of food preparations with Chañar flour and extracts enriched with phenolic compounds and tannins. Ethanolic Chañar flour extract (E*Ch*FE, -◆-); aqueous Chañar flour extract (A*Ch*FE, -—-); acetonic Chañar flour extract (AW*Ch*FE, -▲-); methanolic Chañar flour extract (M*Ch*FE, -■-). (A) DPPH free radical scavenging activity (B) ABTS•+ free radical scavenging activity (C) β-carotene bleaching assay.

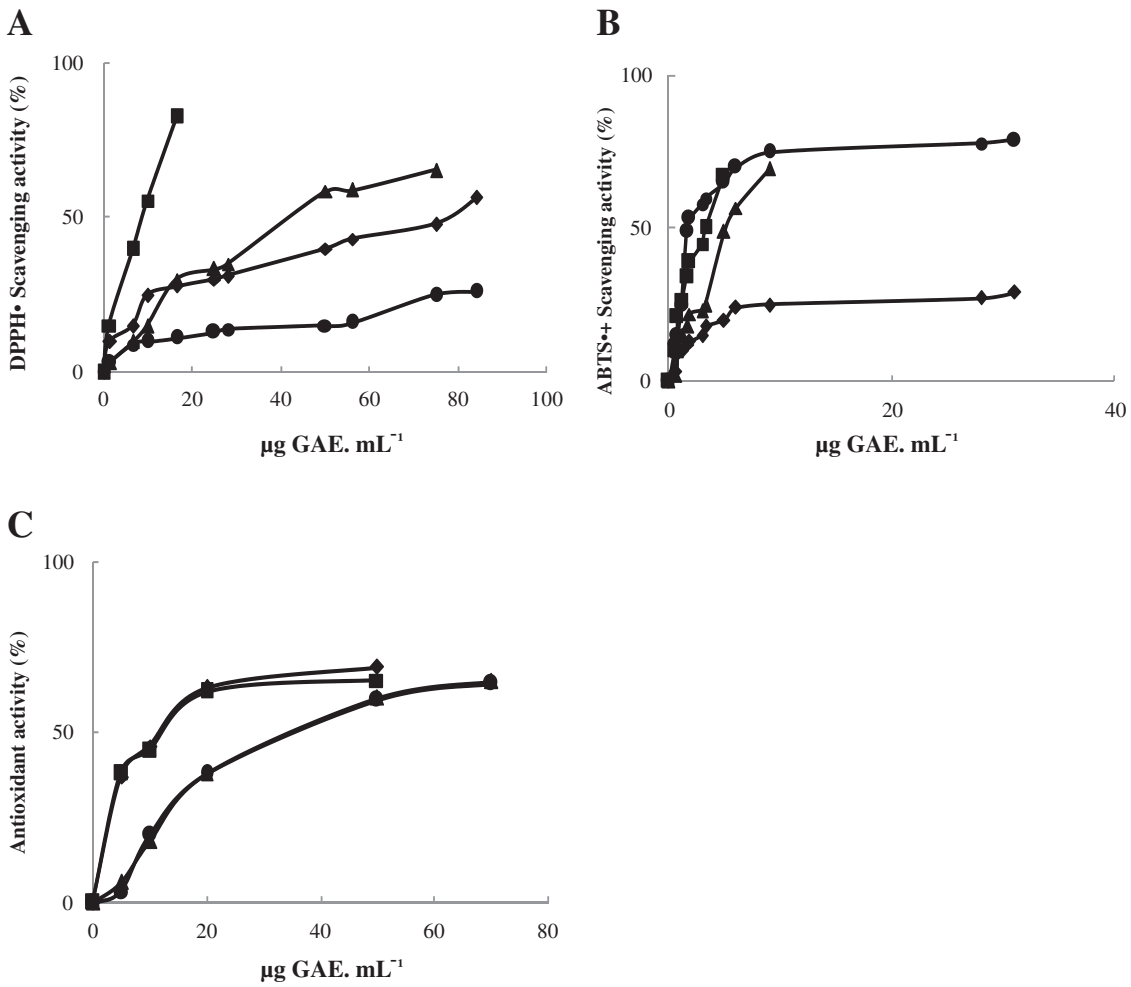

Fig. 3. Antioxidant activity of extractions obtained from arrope. Aqueous arrope extract without sugar (A*Ch*ArE, -■-); ethanolic arrope extract without sugar (E*Ch*ArE, -◆-); aqueous arrope extract with sugar (A*Ch*ArE, -●-); ethanolic arrope extract with sugar (E*Ch*ArE, -▲-).(A) DPPH free radical scavenging activity (B) ABTS$^{\bullet+}$ free radical scavenging activity (C) β-carotene bleaching assay.

According to our results the extracts have higher antioxidant potency in one assay system than in the others (Table 4). All extracts exhibited a significantly higher antioxidant activity in the ABTS, and β-carotene system than in the DPPH system.

3.6. Mutagenic activity

In light of the potential nutritional and functional applications of Chañar flour, it is important to show that the products obtained from it are safe to consume. The current study reports the results of a toxicology evaluation including *in vitro* mutagenicity studies (Table 5). In the Ames test, it was shown that in the presence of different doses of food preparations and extracts enriched with phenolic compounds, the mutation frequencies did not change significantly when compared to spontaneous ones, both on TA98 and TA100 strains, either in the presence or absence of metabolic activation (S9 mixture). These results indicate the inexistence of compounds in *G. decorticans* that cause base substitution (detected in TA100) and frameshift (detected in TA98) mutations and neither do extracts present pro-mutagenic compounds. The absence of such an effect by *G. decorticans* against *S. typhimurium* bacterial strains is a positive step towards determining its safe use in traditional consumption. Considering the popular use of this fruit and its promising chemopreventive activity, a lack of mutagenic effect in bacterial systems is highly relevant.

4. Conclusion

The present study indicates a high content of fiber and other phytochemicals (carotenoids, ascorbic acid and polyphenols, principally non flavonoids) which are potent scavengers of free radicals and inhibitors of lipid peroxidation in Chañar flour. Chañar arrope, a traditional food obtained from Chañar fruits showed a lower content of some phytochemicals than Chañar flour probably due to modifications caused by cooking, but the antioxidant properties were conserved and absence of mutagenicity was shown. Our data highlights the good antioxidant properties of Chañar extracts and flour that make them of interest in preparing food supplements or additives with antioxidant and preservative properties.

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Table 5
Mutagenicity of food preparations with flour of Chañar and extracts enriched with phenolic compounds and tannins on *Salmonella* microsome assay (TA98 and TA100 strains).

Samples	Concentration (μg GAE/plate)	TA98		TA100	
		−S9	+S9	−S9	+S9
Negative control[1]		33 ± 2[a]	25 ± 3[a]	135 ± 5[b]	148 ± 5[b]
Positive control[2]		3065 ± 184	830 ± 65	2498 ± 208	1210 ± 87
AC*h*FE	25	35 ± 1[a]	25 ± 4[a]	124 ± 18[b]	139 ± 15[b]
	50	27 ± 2[a]	23 ± 5[a]	142 ± 20[b]	120 ± 13[b]
	100	28 ± 1[a]	35 ± 3[a]	123 ± 23[b]	140 ± 13[b]
EC*h*FE	25	24 ± 3[a]	29 ± 2[a]	143 ± 4[b]	130 ± 11[b]
	50	27 ± 1[a]	34 ± 2[a]	131 ± 18[b]	116 ± 5[b]
	100	21 ± 4[a]	22 ± 5[a]	120 ± 4[b]	159 ± 2[b]
AWC*h*FE	25	29 ± 1[a]	25 ± 1[a]	142 ± 10[b]	137 ± 18[b]
	50	25 ± 2[a]	23 ± 2[a]	149 ± 1[b]	135 ± 15[b]
	100	33 ± 4[a]	17 ± 5[a]	150 ± 2[b]	161 ± 1[b]
MC*h*FE	25	31 ± 3[a]	27 ± 3[a]	111 ± 5[b]	128 ± 9[b]
	50	26 ± 5[a]	25 ± 1[a]	129 ± 3[b]	123 ± 5[b]
	100	31 ± 2[a]	20 ± 3[a]	124 ± 8[b]	156 ± 3[b]
AC*h*ArE without sugar	25	22 ± 5[a]	22 ± 3[a]	121 ± 5[b]	118 ± 2[b]
	50	34 ± 1[a]	22 ± 3[a]	138 ± 1[b]	128 ± 2[b]
	100	31 ± 1[a]	28 ± 1[a]	113 ± 9[b]	176 ± 4[b]
AC*h*ArE with sugar	25	33 ± 4[a]	20 ± 2[a]	129 ± 2[b]	145 ± 2[b]
	50	40 ± 2[a]	24 ± 5[a]	140 ± 8[b]	155 ± 2[b]
	100	46 ± 9[a]	20 ± 1[a]	136 ± 9[b]	140 ± 1[b]

Ethanolic Chañar flour extract (EC*h*FE); aqueous Chañar flour extract (AC*h*FE); acetonic Chañar flour extract (AWC*h*FE), methanolic Chañar flour extract (MC*h*FE), aqueous arrope extract (AC*h*ArE).

[1] The number of spontaneous revertants was determined with the corresponding solvents.

[2] Positive controls employed were 4-nitro-o-phenylendiamine (4-NPD) at 20 μg/plate and 2-aminofluorene (2-AF) at 10 μg/plate, without and with S9 mix, respectively. With metabolic activation (+S9), without metabolic activation (−S9). Data are means ± SD of three plates at two separate experiments. Values followed by the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$).

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