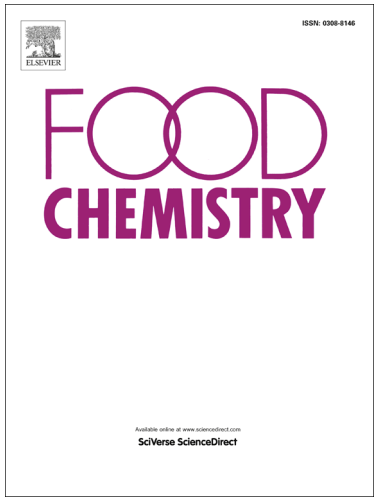

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Polyphenols rich fraction from *Geoffroea decorticans* fruits flour affects key enzymes involved in metabolic syndrome, oxidative stress and inflammatory process

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Running tittle: Polyphenols and biological activity of *chañar* fruits flour

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Abstract

Geoffroea decorticans (chañar), is widely distributed throughout Northwestern Argentina. Its fruit is consumed as flour, arrope or hydroalcoholic beverage. The chañar fruits flour was obtained and 39 phenolic compounds were tentatively identified by HPLC-MS/MS^n. The compounds comprised caffeic acid glycosides, simple phenolics (protocatechuic acid and vanillic acid), a glycoside of vanillic acid, *p*-coumaric acid and its phenethyl ester as well as free and glycosylated flavonoids. The polyphenols enriched extract with and without gastroduodenal digestion inhibited enzymes associated with metabolic syndrome, including α-amylase, α-glucosidase, lipase and hydroxyl methyl glutaryl CoA reductase. The polyphenolic extract exhibited antioxidant activity by different mechanisms and inhibited the pro-inflammatory enzymes (ciclooxygenase, lipooxygenase and phospholipase A_2). The polyphenolic extract did not showed mutagenic effect by Ames test against *Salmonella typhimurium* TA98 and TA100 strains.

These findings add evidence that chañar fruit flour may be considered a functional food with preventive properties against diseases associated with oxidative stress, inflammatory mediators and metabolic syndrome.

Keywords: *Geoffroea decorticans*; chañar flour; polyphenolic compounds; metabolic syndrome; antioxidant activity; anti-inflammatory activity; genotoxicity

1. Introduction

The metabolic syndrome (MetS) is a state of insulin resistance, oxidative stress and chronic inflammation that affect 25% of the world population (Prasad, Ryan, Celzo, & Stapleton, 2012). MetS is characterized by the presence of at least three of the cardiovascular risk factors: obesity, excessive visceral fat storage, dyslipidemia, hypertension, hyperglycemia or type 2 diabetes. Treatments for MetS are mainly based on therapeutic lifestyle changes, often accompanied by pharmacological treatments of MetS-related factors (Prasad et al., 2012). Reduction of postprandial hyperglycemia by inhibition of enzymes involved in carbohydrate metabolism (α-glucosidase and α-amylase), inhibition of lipolytic enzymes including lipase, inhibition of oxidative stress and the delay of inflammatory process are the most common therapeutic approach to treat MetS. Plant-derived foods represent a natural source of phytochemical positively associated with the prevention and regression of MetS clinical manifestations. In particular, plant antioxidants are known to reduce the oxidative stress and inflammatory process associated with obesity and cardiovascular alterations (Abete, Goyenechea, Zulet, & Martinez, 2011). The evidence suggests that a diet enriched in fibers and low in saturated fats, together with increased daily physical exercise, can reduce the incidence of diabetes in people with impaired fasting glucose tolerance by almost 60% (Wagh & Stone, 2004).

The tree *Geoffroea decorticans* (Gill.ex Hook. et Arn.) Burk (Fabaceae), known as chañar, occurs in the native forests of the Gran Chaco region (phytogeographical regions of “Parque chaqueño”, “Monte” and “Espinal”) in Argentina as well as in the Paraguayan and Bolivian Chaco and northern Chile (Scarpa, 2009). It is a xerophytic tree that forms extensive uniform colonies due to reproduction by gemmiferous root. The ripe fruits (drupes) were consumed since pre-colonial times. At present, they are still part of the diets of aboriginal communities like the Wichis, Toba and

Chorotes (Arena & Scarpa, 2007). In summer time, each tree produces between 20 and 30 kg of orange brown colored fruits, which fall from the tree when mature (Figure 1). Under dry conditions, the fruits can be stored for one year before consumption. The fruits and seeds are used raw, roasted, boiled and/or fermented (beverage). The chañar flour was used as ingredient for soups and to make so-called chañar breads. The other popular product made of chañar fruits is a sweet jelly-like syrup called arrope. The arrope is recommended as a sweet for desserts and cocktails as well as the best cough syrup in traditional medicine. Chañar fruits flour and arrope can be used as functional food due to their high level of sugar, fiber and polyphenolic compounds (Costamagna, Ordoñez, Zampini, Sayago & Isla, 2013). The antinociceptive action and antioxidant activity of chañar fruits and arrope was reported (Costamagna et al., 2013; Reynoso, Vera, Aristimuño, Daud & Sánchez Riera, 2013).

Chañar is relevant in the native Argentine forests and is often found associated with algarrobo (*Prosopis*) and mistol (*Zyziphus*) trees. The collection and processing of chañar fruits, that was important in the past, is now disappearing due to the clearance of the native forests for agriculture, mainly to produce transgenic soybean for export. Therefore, studies that add value to this species are important to promote their propagation, conservation and sustainable management in arid areas. The aim of the present study was to assess the polyphenolic profile of chañar fruits flour and to evaluate the efficacy of polyphenolic component against enzymes relevant in hyperglycemia, dyslipidemia, oxidative stress and inflammatory process related with metabolic syndrome.

2. Materials and Methods

2.1. Reagent and standards

All chemicals and reagent used were either analytical reagent or HPLC grade. The chemicals, standard compounds and enzymes purchased from Sigma Aldrich Co., St. Louis, USA were: p-

nitrophenyl α-D- glucopyranoside (pNPG, BCBN2675V); p-nitrophenyl palmitate (pNPP109K5200); hydroxy methylglutaril CoA (HMG-CoA, L 112M4102V); linoleic acid 1393368) ; bovine serum albumin, (BSA,60F-0270); β-carotene (N° 117H2511); thiobarbituric acid (N°034K06055); NADH (D00079245); dinitrophenyl hydrazine (DNPH) (S16313-065); ascorbic acid (A596050-81-7); quercetin (Q4951); ABTS (1392678); lipoxygenase (LOX-1, L2630); secretory phospholipase A2 (sPLA2, 0424023-1); α-glucosidase (N° 117F8205); pepsin (128K7354V) and pancreatin (110M1429V).

COX-2 enzyme immune assay EIA (Kit N° 560131) was obtained from Cayman Chemical, MI, USA.

Lipase pancreatic (N° BCR693); α-amylase (N° 67688), ABTS (N° 11557) and BHT (B1215000) were purchased in Fluka.

2.2. Plant material

Fruits of *Geoffroea decorticans* were collected in January 2011 and January 2012 in Departmento Fernández, Provincia de Santiago del Estero, Argentina. The fruits were lyophilized and the pulp was ground to powder (Helix mill, Metvisa, Argentina). Then, the chañar flour was passed through a 4 mm mesh sieve. The tree growing in the natural habitat and details of the fruits are presented in Figure 1.

2.3. Extraction and phenolic content determination

The flour sample (630 g) was extracted four times with methanol:water 70:30 v/v in a 1:2 w/v ratio in an ultrasonic bath for 30 min at 25°C. Then, the combined extract was concentrated under reduced pressure and then lyophilized. The lyophilized extract was suspended in distilled water (acidified with HCl to pH 2) and mixed with Amberlite-XAD 7 resin (500 g) for 1 hour under stirring. The resin was filtered on a Büchner funnel, washed three times with the

119 acidified water and the polyphenols bound to the resin were desorbed with methanol (MeOH). The
120 phenolic-enriched MeOH solution was concentrated under vacuum to afford a crude MeOH extract
121 that was stored at -20°C until use. Total polyphenols were determined by the Folin–Ciocalteau
122 method (Singleton, Orthofer, Lamuela-Raventos, 1999). Results were expressed as mg gallic acid
123 equivalents (g GAE)/100 g dry weight (DW) of soluble principles. The flavonoids content was
124 determined according to Popova, Silici, Kaftanoghu & Bankova, (2005) and expressed as µg
125 quercetin equivalents per mg of dry weight (g QE/ 100 g DW).

126 ***2.4. Enzyme inhibition and antioxidant assays***

127 ***2.4.1. Inhibitory activity of enzymes related to metabolic syndrome***

128 The inhibitory effect on enzyme related to metabolic syndrome was determined using polyphenolic
129 extract with and without gastroduodenal digestion (GD). The polyphenolic extract was submitted to
130 GD into three categories: salivary, gastric and duodenal digestion according to Tenore et al., 2015
131 with minor modifications. Briefly: For the salivary digestion, the extract (4 mg GAE) were mixed
132 with 6 mL of saliva at pH 6.8 (KCl [89.6 g/L], KSCN [20 g/L], NaH_2PO_4 [88.8 g/L], Na_2SO_4 [57.0
133 g/L], NaCl [175.3 g/L], $NaHCO_3$ [84.7 g/L], urea [25.0 g/L] and α-amylase [48.3 mg/mL]). The
134 mixture was incubated for 3 min at 37°C.Then, for the gastric digestion, pepsin (14,800 U)
135 dissolved in HCl 0.1 M was added, pH was adjusted to 2 and the mixture was incubated at 37°C
136 during 2 h. For pancreatic (duodenal) digestion, the pH was adjusted to 6.5 with $NaHCO_3$ (0.5 M).
137 Then, pancreatin (8 mg/mL) and bile salts (50 mg/mL) (1:1, v/v), dissolved in water (20 mL), was
138 added and the mixture was incubated at 37 °C for 2 h. After digestions, polyphenols were extracted
139 with ethyl acetate and the organic phase was taken to dryness and resuspended in DMSO (2 mg
140 GAE/mL).

141 ***2.4.1.1. α-Glucosidase inhibition***

The inhibition of α-glucosidase was determined using *p*-nitrophenyl α-D-glucopyranoside as substrate. The reaction mixture contained 160 µl of 0.1M sodium phosphate buffer (pH 6.9), 5 µL of enzyme (5.46 U/mL) and polyphenolic extract (0.17-1.36 µg/mL). After pre-incubation of the reaction mixture on ice for 5 min, the enzyme reaction was started by adding 5 µL of 25 mM *p*-nitrophenyl α-D-glucopyranoside into this mixture. The reaction was incubated 15 min at 37 °C. Then, 80 µL of 0.2 M sodium carbonate was added. The absorbance was measured at 405 nm in a microplate reader (BiotekELx808). Enzyme inhibition was calculated using the following equation:

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

Where A_0 is the absorbance of the control (blank, without extract) and A_s is the absorbance in presence of the extract. IC_{50} values denote the µg GAE/mL required to inhibit the enzyme by 50%.

2.4.1.2. α-Amylase inhibition

The α-amylase inhibitory activity using starch as substrate was assayed using Amilokit ® (Wiener Lab Group, Rosario, Argentina). The reaction mixture contained 800 µL of 0.01 M sodium phosphate buffer (pH 7.4), 5 µL of enzyme and polyphenolic extract (28-146 µg GAE/mL). After pre-incubation of the reaction mixture on ice for 5 min, the enzyme reaction was started by adding 500 µL of reagent A (substrate) into the reaction mixture. Then, the reaction was incubated at 37 °C for 7 min. After that, 500 µL of reagent B (iodine solution) was added for color development and the reaction mixture was taken to a final volume of 5.3 mL with water. The absorbance was measured at 640 nm in a spectrophotometer (UV2400 PC). Enzyme inhibition was calculated using the following equation:

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

Where A_0 is the absorbance of the control (blank, without extract) and A_s is the absorbance in presence of the extract. IC_{50} values denote the µg GAE/mL required to inhibit the enzyme by 50%.

165 ***2.4.1.3. Lipase inhibition***

166 Lipase activity was assayed by measuring the enzymatic hydrolysis of *p*-nitrophenyl palmitate to *p*-
167 nitrophenol in a microplate reader (BiotekELx808) at 400 nm. Lipase solution (1.0 mg/mL) was
168 mixed with the polyphenolic extract (final concentration between 0.2 and 8.71 µg/mL) and pre-
169 incubated on ice for 5 min. The reaction mixture for standard assay contained 330 µL of sodium
170 phosphate buffer 0.1 M (pH 7) supplemented with 0.6% (w/v) Triton X-100 and 0.15% (w/v) arabic
171 gum, and 20 µL of 10 mM *p*-nitrophenyl palmitate. The enzyme reaction was started by adding 50
172 µL of the lipase/ polyphenolic extract solution into the reaction mixture, and incubated at 37 °C for
173 20 min. Enzyme inhibition was calculated using the following equation:

174 $$\% \text{ inhibition} = (A_0 - A_s)/A_0 \times 100$$

175 Where A_0 is the absorbance of the control (blank, without extract) and A_s is the absorbance in
176 presence of the extract. IC_{50} values denote the µg GAE/mL required to inhibit the enzyme by 50%.

177 ***2.4.1.4. HMG-CoA Reductase inhibition***

178 The HMG-CoA Reductase KIT Assay was used. The reaction mixture contained extract (0.7 to 13
179 µg GAE/mL) or 1µL of parvastatin (as inhibitor), 1x buffer to complete 182 µL, 4 µL of NADPH,
180 12 µL of HMG-CoA and 2 µL of HMG-CoA reductase. The reaction was incubated at 37ºC during
181 10 min. Readings were taken every minute for 10 minutes at 340 nm in a microplate reader. IC_{50}
182 values denote the µg GAE/mL required to inhibit the enzyme by 50%.

183 ***2.4.2. Inhibition of pro-inflammatory enzymes***

184 ***2.4.2.1. Lipoxygenase***

185 Lipoxygenase (LOX) activity was determined using a spectrophotometric method based on the
186 enzymatic oxidation of linoleic acid to the corresponding hydroperoxide. The reaction mixture
187 contained substrate (50 µM linoleic acid in 0.2 M borate buffer pH 9), enzyme (0.9 nM soy LOX-1)

and different concentrations of polyphenolic extract (100-600 µg GAE/mL). The assay to obtain the 100% of LOX activity was performed with DMSO as solvent control. Absorption at 234 nm was recorded as a function of time for 3 min. The polyphenolic concentration causing 50% inhibition of hydroperoxide-release ($IC_{50)}$ was calculated from the concentration–inhibition response curve by regression analysis. The extinction coefficient of 25 mM^{-1} cm^{-1} was used for hydroperoxide quantification. Naproxen was used as a reference anti-inflammatory compound.

2.4.2.2. Cyclooxygenase

The ability of the extracts and/or fractions to inhibit the conversion of arachidonic acid to prostaglandin (PG) by human recombinant COX-2 was determined by enzyme immune assay, EIA. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid to PGH2. PGF2α produced from PGH2 by reduction with stannous chloride was measured by EIA in a microplate reader (BiotekELx 808). The assays were performed in presence of 100-600 µg GAE/mL of polyphenolic extract or commercial anti-inflammatory drugs (nimesulide selective for COX-2). The assay to obtain the 100% of COX-2 activity was performed with and without DMSO as solvent control. Enzyme control was performed with inactivated enzymes by boiling during 3 min. Detection limit was 29 pg of PG/mL. The polyphenolic concentration causing 50% inhibition of enzyme (IC_{50}) was calculated from the concentration–inhibition response curve by regression analysis.

2.4.2.3. Phospholipase A2

Secretory phospholipase A_2 activity was determined using 1,2-diheptanoylthio-glycerophosphocholine (1,2 dHGPC) and Triton X-100 as substrates. The mixture contained 50 µL of buffer Tris–HCl (10 mM, pH 8), 10 µL of 5,5′- dithiobis-2-nitrobenzoic acid (DTNB) (10 mM), 10 µL of PLA2 enzyme (1 mg/mL) and 100-600 µg GAE/mL of the assayed samples dissolved in

DMSO or commercial anti-inflammatory drug (acetylsalicylic acid). The reaction was initiated by the addition of 150 µL of 1,2 dHGPC (1.66 mM) and maintained during 20 min at 25 °C. The absorbance was read at 414 nm during 20 min every 2 min in a microplate reader (BiotekELx808). The polyphenolic concentration causing 50% inhibition of enzyme (IC_{50}) was calculated from the concentration–inhibition response curve by regression analysis.

2.4.3 Antioxidant activity

2.4.3.1. ABTS radical scavenging

The assay was carried out by the improved $ABTS^{\bullet+}$ method as described by Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans (1999). $ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23 ºC) in the dark for 16 h.

2.4.3.1.1.Autographic assay on TLC

An aliquot of each fraction obtained from total polyphenolic extract after fractionation by Sephadex LH 20 was placed on Silica gel F254 plates (4x4 cm). The plates were developed with (toluene: ethyl acetate: formic acid; 4:2:1, v:v:v) as solvent system. After, 3 mL of soft medium (agar 0.9 %) containing 1mL $ABTS^{\bullet+}$ solution was distributed on TLC plate (Zampini, Ordoñez, & Isla, 2010) After solidification, the plate was incubated at room temperature for 1 min in the dark. The antioxidant activity appeared as clear spots against a dark green-blue background.

2.4.3.1.2. Total antioxidant capacity by spectrophotometric assay

$ABTS^{\bullet+}$ solution (1mL; absorbance of 0.7 $\pm$ 0.02 at 734 nm) was added to the sample (1.35 to 12.5µg 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. Results are presented as SC_{50} values in µg GAE/mL required to scavenge 50% ABTS free radicals.

2.4.3.2. Hydroxyl radical scavenging

The reaction mixture contained 50 µL of 10.4 mM 2-deoxy-D-ribose and 100 µL of 50 µM $FeCl_3$ and extract (0.29-2.39 µg GAE/mL). The reaction was carried out with and without 100 µL of 52 µM EDTA. To start the Fenton reaction, 50 µL of 10 mM H_2O_2 and 50 µL of 1.0 mM ascorbic acid were added. The mixture was incubated at 37°C for 60 min. Then, 500 µL of 2-thiobarbituric acid (1%, w/v) dissolved in trichloroacetic acid (3%, w/v) was added. The tubes were heated at 100°C for 20 min. The absorbance was measured at 532 nm. Reaction mixtures without the polyphenolic extract were used as positive control (100% MDA). The negative control contained the full reaction mixture without 2-deoxy-D-ribose. A modification was carried out without the addition of ascorbic acid. The negative control contained the full reaction mixture without 2-deoxy-D-ribose. The positive control was the H_2O_2/Fe^{3+}/ascorbic acid system mixture lacking the extract (100% MDA). Results are presented as SC_{50} values in µg GAE/mL required to inhibit by 50% the degradation of 2-deoxy-D-ribose.

2.4.3.3. Hydrogen peroxide scavenging

A solution of hydrogen peroxide (4 mM) was prepared in phosphate buffer (PBS, pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm using the molar absorptivity 81 $M^{-1}.cm^{-1}$. Samples (7-82.7 µg GAE/mL) were added to the hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing extracts without hydrogen peroxide. Results are presented as SC_{50} values in µg GAE/mL required to inhibit by 50% the degradation of H_2O_2

256 ***2.4.3.4. Protection of protein against oxidative damage***

257 Bovine serum albumin (BSA, 40 mg/mL) in 10 mM sodium phosphate buffer pH 7 was mixed with
258 1 mL of 1.5 mM $FeSO_4$, 1 mL H_2O_2 3 mM and different concentrations of the samples. Then, 400
259 µL of 7 mM DNPH in 2 M HCl was added. The protein was precipitated by adding 500 µL of
260 trichloroacetic acid (4% w/v). The pellet collected by centrifuging for 5 min at 14,000 x *g* was re-
261 dispersed in ethanol/ethyl acetate (1:1, v/v) in order to remove unreacted DNPH. Absorbance at 378
262 nm was read and carbonyl concentration was calculated. Results are reported as PC_{50} values in µg
263 GAE/mL required to inhibit by 50% the degradation of BSA.

264 ***2.4.3.5. Protection of lipid against oxidative damage: β-Carotene bleaching***

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

269 $$AA\% = [(R_{control} - R_{sample})/ R_{control}] \times 100$$

270 Where $R_{control}$ and R_{sample} are the bleaching rates of β–carotene in the reactant mix without
271 antioxidant and in presence of the extracts, respectively. SC_{50} values denote the µg
272 GAE/mLrequired to inhibit β–carotene bleaching by 50%.

273 ***2.5. Mutagenicity***

274 ***2.5.1. Salmonella mutagenicity assay***

275 The mutagenic effect of chañar fruits flour extracts was evaluated on two *S. typhimurium* strains
276 (TA98 and TA100). The plate incorporation assay was performed according to Maron & Ames
277 (1983), by adding 0.1 mL of the overnight bacterial culture, 0.1 mL of chañar polyphenolic extracts
278 at different concentrations (25-100 µg GAE/plate) and 2 mL of top agar on minimal agar. The

plates were then incubated at 37 °C for 48 h. After incubation, his+ revertant colonies were counted and compared to the number of revertant colonies in the controls.

For the experiment with S9 metabolic activation, the S9 mixture was freshly prepared before the assay and kept on ice until needed. The S9 mixture consisted of S9 fraction (Moltox – Molecular Toxicology Inc., USA) containing NADP (Maron & Ames, 1983). The experiment was repeated as above and the only difference was that 500 µL of S9 was added in place of the phosphate. After 72 h of incubation at 37 °C His^+ revertants were counted. The positive controls employed were 4-nitro-*O*-phenylenediamine (4-NPD; Aldrich Chemical Co.), (10 µg/plate) and 2-aminofluorene (2-AF; Merck) (10 µg/ plate). Solvent control was carried out adding 100 µL DMSO/plate. An extract was considered mutagenic when the mean number of revertants was double or greater than two times that of the negative control. Three plates per experiment and two separate experiments were used for each concentration tested and for positive and negative controls.

2.6. Fractionation of the polyphenolic extract

A sample of the polyphenolic extract (1.6 g) obtained from chañar fruits flour was dissolved in MeOH and permeated on Sephadex LH-20. MeOH was used as mobile phase. The eluates with similar TLC profiles (toluene: ethyl acetate: formic acid; 4:2:1, v:v:v), revealed with diphenylboric acid ethanolamine complex, were pooled in four major fractions. All of them were taken to dryness and kept at 4°C for further use. The yields were as follow. F-I: 151 mg; F-II: 1100 mg; F-III: 200 mg; F-IV: 64.7 mg. The fractions F-III and F-IV showed the highest antioxidant activities by autographic assays. The FIII and FIV were analyzed by HPLC-DAD y HPLC-MS/MS^n. Then, FIII and FIV were re-chromatographed on Sephadex LH 20 and eluted with Metanol:H_2O, (8:2, v:v) Nine subfractions (SF-1 to SF-9) were obtained from FIII and eight sub-fractions (SF-1 to SF-8)

were obtained from FIV. All fractions and sub-fractions were dried by evaporation and lyophilized. Dry extracts were kept at 4°C for further experimental use.

2.7. Identification of phenolics

2.7.1. HPLC-DAD analysis

The most active fractions of the polyphenolic extract (F-III and F-IV) and subfractions obtained from them were analyzed by HPLC coupled to a diode array detector to set the conditions for HPLC-DAD-MS/MS studies. The HPLC system used for DAD analysis was a Shimadzu (Shimadzu Corporation, Kyoto, Japan) equipment consisting of a LC-20AT pump, a SPD-M20A UV diode array detector, CTO-20AC column oven and a LabSolution software. A MultoHigh 100 RP 18-5µ (250x4.6 mm) column (CS-Chromatographie Service GmbH- Germany) maintained at 35 °C was used. The samples were dissolved in 1 mL MeOH, filtered through a 0.45 µm PTFE filter (Waters) and submitted to HPLC-DAD and HPLC-ESI-MS/MS analysis. The compounds occurring in the mixtures were monitored at 254 and 330 nm, and UV spectra were recorded from 200 to 600 nm for peak characterization. The HPLC analyses were performed using a linear gradient solvent system consisting of 0.1% acetic acid in water (A) and methanol 0.1% acetic acid as follow: 90% A to 43% A over 45 min, followed by 43% A to 0% A from 45 to 60 min, 0% A to 0% A from 60 to 5 min. The flow rate was 0.5 mL/min and the volume injected was 20 µL.

2.7.2. Identification of phenolics by HPLC-ESI-MS/MS

Data were recorded on a HPLC-ESI-MS/MS system which consisted of the HPLC HP1100 (Agilent Technologies Inc, CA-USA) connected through a split to the mass spectrometer Esquire 4000 Ion Trap LC/MS(n) system (Bruker Daltonik GmbH, Germany). Ionization was performed at 3000 V assisted by nitrogen as nebulizing gas at 24 psi and as drying gas at 365ºC and a flow rate of 6

L/min. Negative ions were detected using full scan (m/z 20-2200) and normal resolution (scan speed 10,300 m/z/s; peak with 0.6 FWHM/m/z). The trap parameters were set in ion charge control (ICC) using manufacturer default parameters, and maximum accumulation time of 200 ms. Collision induced dissociation (CID) was performed by collisions with helium background gas present in the trap and automatically controlled through SmartFrag option.

2.8. Statistical analysis

Sampling and analyses were performed in triplicate, and the data are presented as mean ± standard deviation (S.D.). The correlation between the main polyphenolic compounds content of the extracts, and the biological activities was analyzed by the Pearson test correlation coefficients with 95% confidence. Statistical analysis was performed by one way ANOVA followed by Tukey's multiple comparison test ($p<0.05$). All statistical analyses were carried out using the Infostat software.

3. Results and discussion

The macronutrient and phytochemical composition of chañar fruits flour was previously reported (Costamagna et al., 2013). The carbohydrates were the major component (19.75 %) followed by proteins (5%). Furthermore, the chañar fruits flour could be considered a source of dietary fiber (12%), of potassium and polyphenolic compounds (1.5 %) (Costamagna et al., 2013). In the present paper, a polyphenols-enriched hydroalcoholic extract was obtained from chañar fruits flour. The polyphenolic compounds content and flavonoids of the extract were 12.5 ± 1.0 g GAE/100 g dry weight of soluble principle and 2.0 ± 0.2 g QE/100 g dry weight of soluble principle, respectively.

The aim of the present study was to determine the efficacy of polyphenolic component against enzymes involved in hyperglycemia, dyslipidemia, and inflammatory process related with metabolic syndrome and oxidative stress.

3.1. Enzyme inhibition

The metabolic syndrome is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia and disturbances of carbohydrate and fat metabolisms. The activity of the chañar fruits flour polyphenolic extract was assessed towards enzymes associated with metabolic syndrome, including α-glucosidase and α-amylase, pancreatic lipase and HMGCoA reductase, pro-inflammatory enzymes (COX-1, COX-2, LOX, $sPLA_2$) and as antioxidant. The effect of the chañar polyphenols on the different enzymes is summarized in Table 1. Furthermore, the fraction was evaluated for a possible mutagenic effect by the Ames test.

3.1.1. α-Glucosidase and α-amylase inhibition

One therapeutic approach to decrease postprandial hyperglycemia is to suppress the production and/or absorption of glucose from the gastrointestinal tract through inhibition of the enzymes α-amylase or α-glucosidase (Abete et al., 2011). Hypoglycaemic agents used in clinical practice, such as acarbose, competitively inhibit α-glucosidase in the brush border of the small intestine, which consequently delay the hydrolysis of carbohydrates and alleviate postprandial hyperglycemia. However, the continuous administration of these agents may cause several adverse effects, such as diarrhea, abdominal discomfort, flatulence, and hepatotoxicity. Therefore, α-glucosidase novel natural inhibitors are necessary given the therapeutic challenge of type II diabetes mellitus. Phenolic compounds of chañar fruits flour may freely interact with enzymes present in the digestive tract modulating their activity (Abete et al., 2011; Xiao, Chen, & Cao, 2014). The inhibitory activity of polyphenolic extract obtained from chañar flour towards α-amylase was low (IC_{50} values of 25 µg GAE/mL). However, this extract was very active against α-glucosidase (IC_{50} = 0.68 µg GAE/mL) with a relation dose-response with polyphenolic concentration until IC_{50} values (R^2= 0.95), presenting better effect than the reference compound acarbose (IC_{50} = 25 µg/mL), Table 1. These results suggest that chañar fruit flour polyphenols might be able to reduce glucose

uptake/absorption. The selective inhibition of α-glucosidase is the preferred effect for plant extracts to control glucose uptake. Simultaneous inhibition of both enzymes would result in abnormal bacterial fermentation in the colon due to the presence of undigested carbohydrates. Other plants, many of them used traditionally to control diabetes or hyperglycaemia were reported to exert strong inhibition of α-glucosidase and moderate or negligible effect on α-amylase activity (Ranilla, Kwon, Apostolidis, & Shetty, 2010). The activity of polyphenolic extract from chañar fruits flour on α-glucosidase was higher than the activity of hydroalcoholic blueberry extract (Boath, Stewart, McDougall, 2012), pomegranates (Medjakovic & Jungbauer, 2013) and maqui (Rubilar, et al., 2011) fruits. In this sense, chañar fruits flour may offer dietary coadjuvants (therapeutic complements) to control hyperglycemia in diabetic patients. However, further evaluation of their in vivo hypoglycemic activity is necessary to verify these beneficial effects.

3.1.2. Pancreatic lipase and HMGCoA reductase inhibition

The inhibition of pancreatic lipase, which splits triacylglycerols into absorbable monoacylglycerol and fatty acids, is the main prescribed treatment for weight management and obesity in developed countries. Orlistat, one of the two clinically approved drugs for obesity treatment, has been shown to act through inhibition of pancreatic lipase. In order to find alternative natural sources for obesity prevention and treatment, we evaluated the chañar polyphenolic extract on lipase activity. Results are showed in Table 1. The inhibitory activity of chañar polyphenolic extract on lipase (IC_{50}: 4 µg GAE/mL) was higher than that reported for white and green tea polyphenols (Gondoin, Grussu, Stewart, & McDougall, 2010). Polyphenols from common plant foodstuffs such as tea, soybean, mate tea, peanut, or grapevine have been reported as pancreatic lipase inhibitors (Garza, Milagro, Boque, Campion, & Martinez, 2011). A positive correlation between lipase inhibitory activity of

chañar flour and total phenolics (R^2=0.95) was demonstrated as well as in several dietary supplements and fruits (Garza et al., 2011).

Hypercholesterolemia and cardiovascular disease are major health problems. One approach to reduce hypercholesterolemia is to use medicines that inhibit the enzymes essential for cholesterol biosynthesis. HMGCoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis. Inhibition of cholesterol synthesis lowers the hepatocyte cholesterol content and increase expression of low density lipoprotein cholesterol (LDL-c) receptors, responsible for LDL-c uptake via receptor-mediated endocytosis, and consequently LDL-c is rapidly cleared from the bloodstream. Statins (lovastatin, simvasatin, pravastatin, fluvastatin, atorvastatin, pitavastatin) are HMG-CoA reductase inhibitors which are highly effective therapeutic agents for the treatment of hypercholesterolemia. However, statins cause side effects such as new-onset diabetes mellitus (DM). The polyphenolic extract of chañar fruits flour is a natural source of HMG-CoA reductase inhibitors that was able to suppress HMG-CoA reductase activity with a low IC_{50} value of 6 µg GAE/mL, Table 1. Phenolic compounds prevent *de novo* synthesis of cholesterol in the liver via the suppression of HMG-CoA reductase. According to these results, we suggest that chañar polyphenols may help reducing blood cholesterol and triglycerides by inhibition of lipid digestion and absorption as well as by the inhibition of the cholesterol biosynthesis.

3.1.3. Effect of polyphenolic extract after treatment with digestive enzymes

Since a large part of nutrient and non-nutrients are gastro-sensitive, the effect of gastroduodenal digestion on bioactive compounds was studied. The polyphenolic extract after treatment with digestive enzymes is able to inhibit enzymes such as α glucosidase and lipase with similar potency to polyphenolic extract without digestion and consequently decreasing the bioavailability of food nutrients (IC_{50} of 0.80±0.05 and 4.50± 0.20 µg GAE/mL, respectively). Furthermore, the

polyphenolic extract after treatment was active on α-amylase (IC_{50} 58±2 µg GAE/mL) with lower potency than undigested extract (IC_{50} 25±1µg GAE/mL).

3.1.4. Inhibition of pro-inflammatory enzymes

The effect of chañar fruit flour polyphenols extract was measured against the pro-inflammatory enzymes COX, LOX and $sPLA_2$ (Table 1). Products of these enzymes (PGs, leukotrienes and hydroperoxide) are important mediators of inflammation. The extract showed an IC_{50} value of 124 µg GAE/mL against COX-2 while the reference drug nimesulide presented an IC_{50} of 0.39 µg/mL. The IC_{50} value of the extract for LOX was 48 µg/mL with naproxen (selective inhibitor of LOX) presenting an IC_{50} value of 14 µg/mL. The extract was also active on $sPLA_2$ with an IC_{50} value of 225 µg GAE/mL. Therefore, the polyphenolic extract of chañar fruits was able to inhibit the three pro-inflammatory enzymes while other Argentinian fruits such as mistol or algarrobo were selective inhibitors of LOX or COX, respectively (Cardozo et al., 2010; Pérez, et al., 2014). These findings, together with the antioxidant activity observed in the polyphenolic extract of chañar suggest that its consumption may contribute to the reduction of inflammation and could prevent or ameliorate oxidative stress related diseases.

3.2. Effect of polyphenolic extracts on oxidative stress

Four basic mechanisms of antioxidant action have been described and are applicable to polyphenolic compounds: (1) free radical scavenging activity, (2) quenching of singlet oxygen, (3) chelating of transition metals, and (4) inhibition of free radical producing enzymes. The antioxidant capacity of polyphenolic-enriched chañar fruits flour extract was determined using five different experimental models. The polyphenolic extract exhibited effect as ABTS, hydroxyl radical and H_2O_2 scavenger (Table 2), with higher antioxidant potency as $HO^{\bullet}$ scavenger. Furthermore, the extract showed electron or hydrogen donor capacity on ABTS (Table 2). In all experiments, the

polyphenols-enriched extract showed a dose-response relation with antioxidant capacity (R^2 = 0.998, 0.989, 0.977 and 0.964 for ABTS, H_2O_2, $HO^{\bullet}$ and β-carotene assays, respectively (Table 2). Similar antioxidant activity on ABTS radicals was reported for the fruit of *Ziziphus mistol* (SC_{50}= 7.38 μg GAE/mL) and *Prosopis* species (SC_{50} values between 7 and 29 μg GAE/mL), native food plants that grows in northern Argentina (Cardozo, Ordoñez, Alberto, Zampini, & Isla, 2011; Pérez et al, 2014). Chañar extract was 28-folds more active (SC_{50}: 0.3 μg GAE/mL) than mistol as hydroxyl radical scavenger (SC_{50}: 14.13 μg GAE/mL). Mistol ethanolic extract was also able to protect linoleic acid from oxidation in the β-carotene system with IC_{50} value of 10.87 μg GAE/mL, with better activity than chañar.

3.3. Mutagenicity

In light of the potential nutritional and functional applications of chañar fruits flour, it is important to prove the safety of products obtained from this fruit. The current study reports the results of *in vitro* mutagenicity studies. In the Ames test, both on TA98 and TA100 *S. typhymurium* strains, different doses of phenolic-enriched extract did not changed significantly the mutation frequencies when compared to spontaneous ones, either in the presence or absence of metabolic activation (S9 mixture). The results indicate that *G. decorticans* fruit polyphenolic-enriched extract did not contain compounds that could cause base substitution (detected in TA100) and frameshift (detected in TA98) mutations. Furthermore, the extracts did not show the presence of pro-mutagenic compounds. The absence of such an effect by *G. decorticans* fruits against *S. typhimurium* bacterial strains is a positive step towards determining its safe traditional use. Taking together, the promising chemopreventive activity and lack of mutagenic effect of chañar polyphenols in bacterial systems is highly relevant for a possible agroindustrial development of this native fruit.

3.4. Identification of polyphenolics

460 The polyphenolic extract was fractionated by Sephadex LH-20. From the most active antioxidant
461 fractions of *G. decorticans* fruits flour, 39 phenolics were tentatively identified by HPLC-MS/MS^n
462 (Table 3). The assignation was based on comparison with literature, interpretation of the mass
463 spectra and co-chromatography with standards when available. The identity of the sugars and the
464 exact placement of the carbohydrate moieties in the aglycones remain to be established. HPLC
465 traces of the active fractions with the compound number are shown in Figure 2. The structure of the
466 compounds tentatively identified in the chañar flour polyphenolic fractions is presented in Figure 3.
467 Neutral loss scan experiments were carried out to identify the glycosides occurring in the samples.
468 The glycosides were assigned based on the neutral losses of 132, 146, 162 or 176 amu for a pentose,
469 rhamnose, hexose or glucuronic acid moieties from the M-1 ion, leading to the corresponding
470 aglycones (Barros, Dueñas, Ferreira, Carvalho, & Santos-Buelga, 2011; Simirgiotis, Theoduloz,
471 Caligari, & Schmeda-Hirschmann, 2009). The aglycones were identified by the [M-1] ions at 301
472 for quercetin, 315 for isorhamnetin, 285 for kaempferol and luteolin, 287 for the dihydro derivatives
473 eriodictyol and dihydro kaempferol, 269 for apigenin and 271 for naringenin. Further fragmentation
474 of the *m/z* ion of the aglycones yielded diagnostic MS^3 fragments that allowed identification of the
475 genines. For quercetin, the ion at *m/z* 301 led to fragments at 179 and 151 amu, while isorhamnetin
476 yields fragments at m/z 300 and 151 from the [M-1] ion at 315 amu (Schieber, Keller, Streker,
477 Klaiber, & Carle, 2002). The differentiation of the genines kaempferol and luteolin, was based on
478 the MS^3 experiments who allows a clear distinction based on the fragments of the aglycones (Fabre,
479 Rustan, de Hoffmann, & Quetin-Leclercq, 2001). While kaempferol give fragment ions of low
480 intensity, luteolin shows clear ions at *m/z* 241, 199 and 175 amu (Fabre et al., 2001). Flavanones
481 were assigned based on the work of Portet, Fabre, Rozenberg, Habib-Jiwan, Moulis, & Quetin-
482 Leclercq (2008) and Fabre et al. (2001). Eriodictyol fragments to a base ion of *m/z* 151 while

483 dihydrokaempferol shows the loss of water. The compounds tentatively identified comprised caffeic
484 acid glycosides, simple phenolics (protocatechuic acid and vanillic acid), a glycoside of vanillic
485 acid, *p*-coumaric acid and its phenetyl ester and several flavonoids. The flavonoids included free or
486 glycosylated flavonols (kaempferol, kaempferol methyl ether, quercetin, isorhamnetin), flavones
487 (luteolin and apigenin), dihydroflavones or flavanone (naringenin and eriodictyol) and
488 dihydroflavonols (dihydrokaempferol and taxifolin). Monoglycosides, diglycosides and
489 triglycosides occurs in the extract.

490 The main compounds were protocatechuic acid **4**, vanillic acid **6** and *p*-coumaric acid **9** in the
491 Fraction III, while Fraction IV afforded luteolin **26**, kaempferol rhamnoside hexoside **27**, apigenin
492 **35** and dihydroxy methoxy flavone **36** as main constituents. The sub-fractions were also analyzed
493 and all compounds identified in F-III y F-IV were confirmed in each sub-fraction. The sub-fraction
494 7 of F-IV yielded a mixture that was not found previously in F-IV : isorhamnetin **30**, isorhamnetin
495 rhamnoside hexoside **31**, dihydroxy methoxy flavone isopentyl and isoprenyl esters **38** and **39** as
496 accompanying compounds. The tentative identification and structure of the compounds is presented
497 in Table 3 and Figure 3, respectively. Four quercetin glycosides were identified, including a
498 hexoside, a pentosylhexoside, a rhamnosylhexoside and a dihexosiderhamnoside.
499 Isorhamnetinhexoside, hexoside pentoside, hexoside rhamnoside and rhamnosylhexoside occurs in
500 the complex mixtures. Kaempferol hexoside, rhamnosyl hexoside and hexosyl rhamnoside are
501 constituents of the extract as well as the rhamnosyl glucuronate of kaempferol methyl ether. The
502 flavone luteolin occurs as hexoside, hexosyl rhamnoside and rhamnosyl dihexoside while the
503 dihydro derivatives naringenin, eriodictyol and dihydrokaempferol were identified as
504 monohexosides. In the fruits, apigenin, luteolin, kaempferol, quercetin, isorhamnetin, and
505 dihydroxymethoxyflavone also occurs as aglycones.

From the flowers of chañar, Silva, López de Ruiz, & Ruiz, (2004) described the isolation and identification of several flavonoid aglycones, including 3,3´,4´-trihydroxyflavone, kaempferol, quercetin, rhamnetin, isorhamnetin, morin, penduletin, jacein, jaceidin, patuletin, artemetin, myricetin, naringenin, tricin, diosmetin, zapotin, 5,7-dihydroxy-2´-methoxyflavone, apigenin, baicalin, quercetagetin hexamethyl ether, gossypetin, quercetin 3',4',5,7-tetramethyl ether and 5,7-dihydroxy-4´-methoxyflavone. From the stem bark of chañar collected in the Bolivian Chaco, Vila, Balderrama, Bravo, Almanza, Codina, Bastida, Connolly (1998) reported the isolation of several isoflavanones, including (3R)-5,7,2',3'-tetrahydroxy-4'-methoxy-5'-prenylisoflavanone; (3R)-7,2',3'-trihydroxy-4'-methoxy-5'-prenylisoflavanone and (3S)-3,7,2',3'-tetrahydroxy-4'-methoxy-5'-prenylisoflavanone. Our finding shows a complex mixture of phenolic acids and flavonoids differing in the oxidation patterns, number and placement of the sugar moieties. Further work should be undertaken to fully characterize the compounds and to establish the chemical variability in different populations of this valuable food resource of arid environments.

The content of major component of F- III such as protocatechuic acid (PCA), vanillic acid (VA) and p-coumaric acid could be responsible for the effect of chañar flour extract on enzymes involved in metabolic syndrome, mainly α-glucosidase and α-amilase (D'Archivio, Scazzocchio, Giovannini, & Masella, 2014, Xiao, Chen, & Cao, 2014). It has been shown that the beneficial effects of PCA and VA are mostly associated with its antioxidant as well as antihyperglycemic activities (D'Archivio et al., 2014). PCA and VA have a key role in counteracting inflammation, due to its ability to inhibit the synthesis and/or activity of most inflammatory mediators and regulatory pathways involved in inflammation (D'Archivio et al., 2014). Furthermore, both acids seems to have chemopreventive potential because they inhibits in vitro chemical carcinogenesis and exerts

528 pro-apoptotic or antiproliferative effects in different tissues (Tanaka, Tanaka, & Tanaka, 2011;
529 D'Archivio et al., 2014).
530 In relation to flavonoids of chañar flour, flavones from F-IV such as luteolin and apigenin and its
531 derivatives exhibits antioxidant and anti-inflammatory activities mainly as potential inhibitors of
532 COX-2 (González-Castejón & Rodriguez-Casado, 2011; Ya-Di et al., 2011). Luteolin inhibited α-
533 glucosidase in a noncompetitive mechanism and the luteolin-enzyme binding was driven mainly by
534 hydrophobic interactions with conformational changes of the enzyme. The luteolin had a high
535 affinity close to the active site pocket of α-glucosidase and indirectly inhibited the catalytic activity
536 of the enzyme. Recently it was also reported that the glycosylation of flavonoids lowered the
537 inhibition against α-glucosidase depending on the conjugation position and the class of sugar
538 moieties. The decreased inhibitory effect against α-glucosidase after glycosylation may be due to
539 the increasing molecular size and polarity, and the non-planar structure. When a hydroxyl moiety is
540 substituted by a glycoside, the steric hindrance may happen, which weakens the binding interaction
541 between flavonoids and α-glucosidase (Xiao et al., 2014).
542 Flavonols such as kaempferol and quercetin and its derivates were found to be especially effective
543 lipoxygenase inhibitors while quercetin was found to be an effective inhibitor of PLA2 in human
544 leukocytes (Kim et al., 2014).
545 The hypocholesterolemic activity of polyphenolic extract of chañar flour could be related to the
546 presence of flavanone such as naringenin and eryodictiol. In previous report was demonstrated that
547 the statin-like flavanones extracted from bergamot peel, exert a similar behavior respect to
548 commercial simvastatin on a model of hypercholesterolaemic rats (Di Donna et al., 2014). Other
549 studies showed eriodictyol as an inhibitor of rat platelet 5- and 12-lipoxygenases which are
550 involved in the biosynthesis of several bioregulators that are closely related to the pathogenesis of

diseases such as allergy, atherosclerosis and cancer (González-Molina, Domínguez-Perles, Moreno, & García-Viguera, 2010). Flavanones show strong antioxidant and radical scavenging activity, antiviral, antimicrobial activities, beneficial effects on capillary fragility, and an ability to inhibit human platelet aggregation as well as anti-ulcer properties (Tomás-Navarro, Vallejo, & Tomás-Barberán, 2014).

4. Conclusion

The chañar fruits have been a relevant food source for the South American amerindians since pre-hispanic times. Different ethnic groups of Argentina consider the chañar tree as a gift from God and are cared for by the Pachamama (mother earth). On the other hand, are considered as health- and energy-giving trees. At present, the sweet and pleasant tasting fruits are still consumed raw or processed into several products used in the local cuisine. The ripe fruits flour contains a complex mixture of polyphenols (phenolic acids and flavonoids) that present relevant functional properties. These findings further support the idea that a diet including chañar fruits flour or chañar fruits, may be preventive against diseases that are associated with oxidative stress, inflammatory mediators and metabolic syndrome. Our results on the functional properties of chañar fruits flour encourages further clinical studies to determine optimal dietary regimens to achieve the desired beneficial health effects. In addition, components of chañar fruits flour are attractive targets for the scientific community to develop novel food products for treatment/prevention of these life-threatening diseases. The nutritional and functional properties that were demonstrated for the chañar fruits flour could stimulate the preservation of chañar forests including sustainable development and management for commercial purposes, contributing to regional development in arid zones.

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Figures

Figure 1: *Geoffroea decorticans* tree growing in the Provincia de Tucumán, Argentina. A: Detail of the tree trunk and bark; B: Tree in the "parque chaqueño" formation; C: Ripe fruits in the tree; D: Ripe fruits are ready to be collected when they fall from the tree.

Figure 2: HPLC chromatograms of polyphenolic extract from *Geoffroea decorticans* fruits Ultraviolet (UV) detection at 254 nm. A) Fraction III; B) Fraction IV; C) Sub fraction -7.

Figure 3: Compounds tentatively identified in chañar fruits. *Identified by comparison with standards.

Figure 1:

A

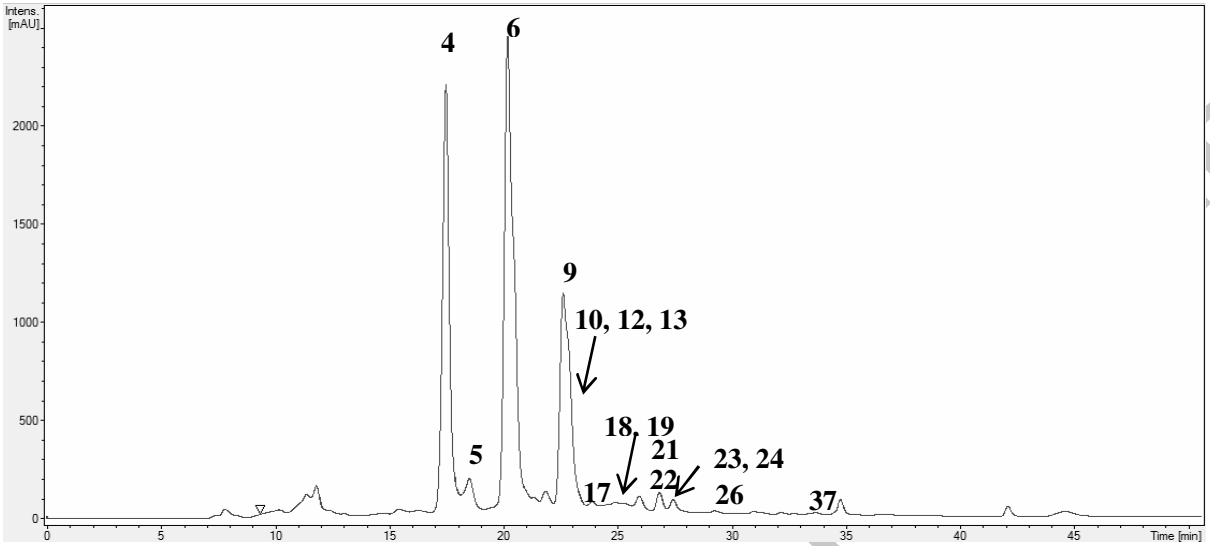
B

C

D

Figure 2:

A



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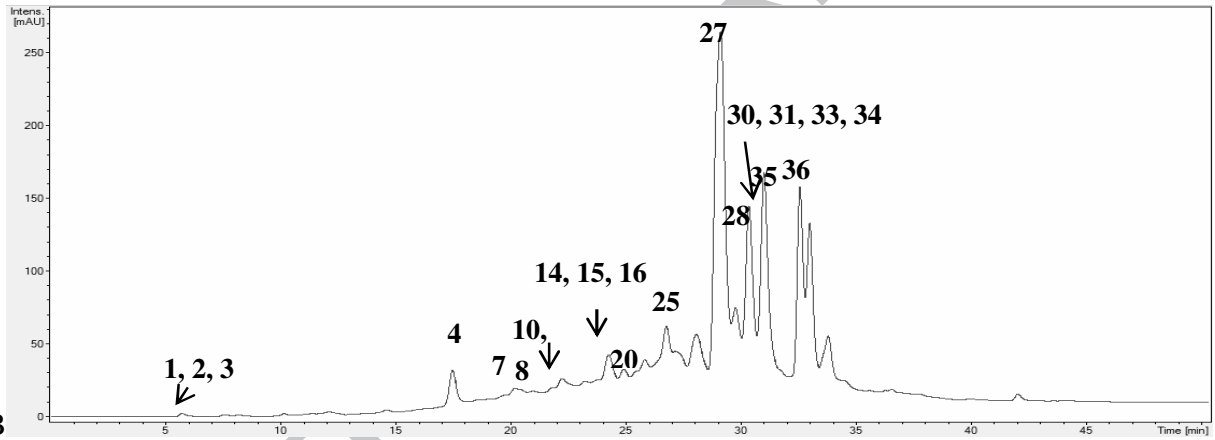

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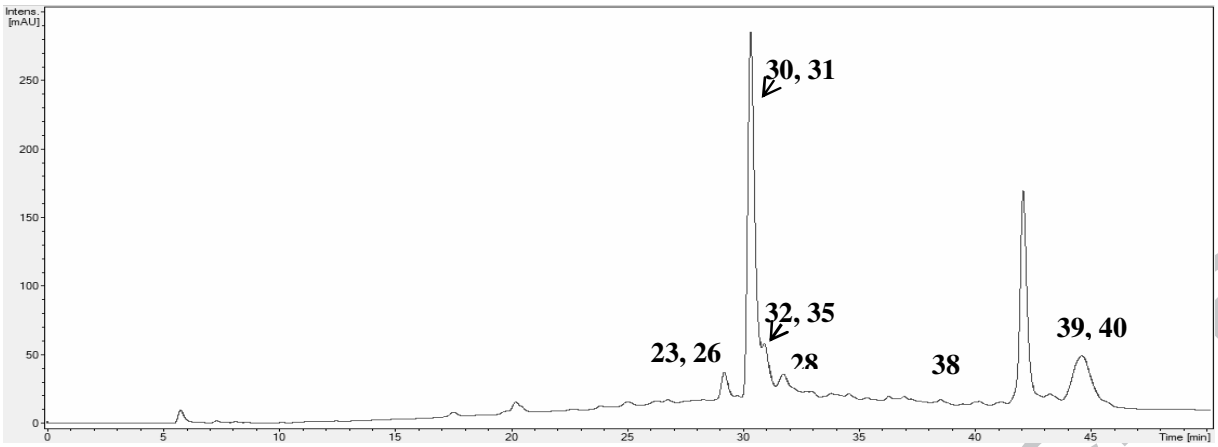
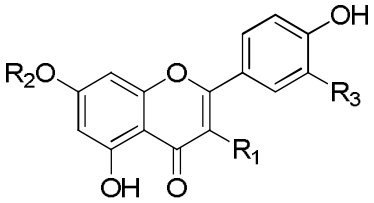
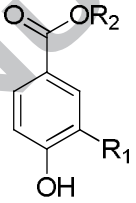


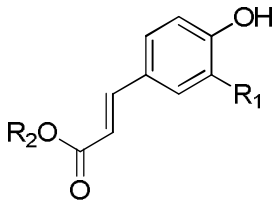
Figure 3;





	R_1	R_2	
4	OH	H	Protocatechuic acid*
5	OCH_3	Hexose	
6	OCH_3	H	Vanillic acid*

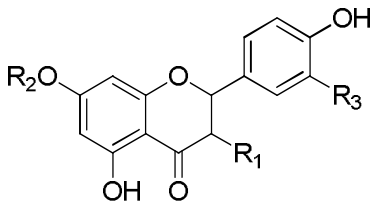
	R_1	R_2	R_3	
35	H	H	H	Apigenin*
32	H	Dirhamnoside	H	
26	H	H	OH	Luteolin
22	H	Hexose	OH	
23	H	Hexose-rhamnose	OH	
17	H	Rhamnose-dihexose	OH	



	R_1	R_2	R_3	
28	OH	H	H	Kaempferol*
20		Hexoside	H	
19		Hexoside-rhamnoside	H	
18		Hexoside-pentoside	H	
27		Rhamnose-hexose	H	
29	OH	CH_3	H	
25		Rhamnose-glucuronic acid		
33		Dirhamnoside		
34		Rhamnose-glucuronic acid		

	R_1	R_2	
	OH	H	Caffeic acid
1	OH	Hexose-rhamnose	
2	OH	Hexose	
3	OH	Dihexose	
9	H	H	*p*-Coumaric acid*
37	H	Phenethyl	

	R_1	R_2	R_3	
	OH	H	OH	Quercetin*
15	Hexose		OH	
10	Hexose-pentose		OH	
14	Rhamnose-hexose		OH	
12	Dihexose-rhamnose		OH	
30	OH	H	OCH_3	Isorhamnetin
11	Hexose		OCH_3	
13	Hexose-pentose		OCH_3	
21	Hexose-rhamnose		OCH_3	
24	Prenyl, hexose		OCH_3	
31	Rhamnose-hexose		OCH_3	



	R_1	R_2	R_3	
	H	H	H	Naringenin
8	H	Hexose	H	
	H	H	OH	Eriodictyol
16	H	Hexose	OH	
	OH	H	H	Dihydrokaempferol
7	OHexose		H	H

Table 1: Effect of polyphenols enriched extract of Chañar flour and reference compounds on Enzymes related to carbohydrate metabolism, fat metabolism and inflammatory processes. Results are reported as IC_{50} values in µg GAE/mL.

Enzyme	Polyphenols enriched extract IC_{50} values (µg GAE/mL)	Reference compound	Reference compounds IC_{50} values (µg/mL)
Enzymes related to carbohydrate metabolism			
α amylase	25.00±1.00[b]	Acarbose	1.25±0.10
α-glucosidase	0.68±0.05[a]	Acarbose	25.00 ± 1.00
Enzymes related to fat metabolism			
Lipase	4.00±0.20[a]	Orlistat	0.08±0.01
Hydroxymethyl glutaril CoA reductase	6.00±1.00[a]	Pravastatin (SIGMA I5909)	1µl inhibition 18%
Enzymes related to inflammatory processes			
COX-2	124±5[b]	Nimesulide	0.39±0.10
LOX	48±2[a]	Naproxen	14.00 ±1.00
PLA2	225±5[c]	Acetylsalicylic acid	65.00± 1.00

Different letters (a, b, c) in the same column in each biological assay show significant differences among effect of polyphenols on enzyme activity according to Tukey's test ($p \leq 0.05$).

Table 2: Antioxidant activity of polyphenols enriched extract of Chañar flour. Results are presented as SC_{50} or IC_{50} in µg GAE/mL

Assay	**Polyphenols enriched extract**	Reference compound	Reference compounds IC_{50} values (µg/mL)
$ABTS^{\bullet+}$ (SC_{50})	2.8 ± 0.2^{b}	Quercetin	6.7±0.3
		BHT	7.7±0.4
H_2O_2 (SC_{50})	23.0 ± 1.0^{c}	Quercetin	12.0±1.0
$HO^{\bullet}$ (SC_{50})	0.30 ± 0.05^{a}	Quercetin	30.0±2.0
% Lipid protection β-Carotene (IC_{50})	22.0 ± 1.0^{c}	Quercetin	9.8 ±0.9
		BHT	3.9 ±0.2
BSA protection (50% protection)	18.0 ± 1.0^{c}	Quercetin	15.0±1.0

Different letters (a, b, c) in the same column show significant differences among antioxidant effect of polyphenols by different mechanisms according to Tukey's test ($p \leq 0.05$).

Table 3. Tentative identification of phenolic compounds in the most active antioxidant fractions of chañar fruit

	Rt (min)	$[M-H]^-$	MS/MS	Tentative identification
1	6.2	487	341, 179, 161	Caffeic acid hexosiderhamnoside
2	6.3	683	341 (2M-1)	Caffeic acid hexoside
3	6.6	503	341	Caffeic acid dihexoside
4	16.7-18.0	153	109	Protocatechuic acid
5	18.0-19.1	329	167	Vanillic acid hexoside
6	19.1-21.3	167	152, 123, 107	Vanillic acid
7	20.3	449	287	Dihydrokaempferolhexoside
8	20.6	433	271, 151	Naringeninhexoside
9	22.0-23.9	163	119	*p*-coumaric acid
10	22.7-23.0	595	301, 179, 151	Q-hexosidepentoside
11	22.9	477	315	Isorhamnetin/Rh hexoside
12	23.1-23.2	771	301, 179, 151	Q-dihexosiderhamnoside
13	23.2	609	477, 315, 300	Isorhamnetin/Rh hexosidepentoside
14	23.5	609	301, 179, 151	Q-rhamnosidehexoside
15	23.5	463	301, 179, 151	Q hexoside
16	23.6-23.9	449	287, 151	Eriodictyolhexoside
17	23.8	755	609, 285	Luteolinrhamnosidedihexoside
18	24.0	579	447, 285	Kaempferolhexosidepentoside
19	24.7	593	447, 285	Kaempferolhexosiderhamnoside
20	24.8	447	285	K hexoside
21	24.9-25.0	623	477, 315, 207	Isorhamnetin/Rh hexosiderhamnoside
22	26.3	447	285	Luteolinhexoside
23	26.8-29.5	593	285	Luteolin hexose rhamnose
24	27.6	545	477, 315, 300	Prenylisorhamnetinhexoside
25	28.3	621	299, 284	K methyl ether rhamnosideglucuronate
26	28.6-28.7	285	267, 241, 217, 199, 175	Luteolin
27	28.7	593	285	K rhamnosidehexoside
28	29.7-31.7	285	257, 239, 199, 119	Kaempferol
29	29.7	299	284, 151	Kaempferol methyl ether
30	29.7	315	300	Isorhamnetin/Rhamnetin
31	29.9	623	315, 299, 285, 236	Isorhamnetin/Rhrhamnosidehexoside
32	30.4	561	269	Apigenindirhamnoside
33	30.4	591	299, 269	K methyl ether dirhamnoside
34	30.4	621	299	K methyl ether rhamnosideglucuronate
35	30.2-30.4	269	225, 177, 149, 133	Apigenin
36	32.4	283	268	Dihydroxymethoxy flavoneGenkwanin
37	32.9	267	163, 119	*p*-coumaric acid phenethyl ester
38	38.5	367	283, 176	Dihydroxymethoxyflavoneisopentyl ester

39	43.0-44.1	365	283	Dihydroxymethoxyflavoneisoprenyl ester

The *Geoffroea decorticans* fruits (chañar) are consumed in Argentina as flour, arrope or beverage

39 phenolic compounds were tentatively identified in the polyphenolic extract of chañar flour

Polyphenols from *chañar* fruits flour affects enzymes involved in metabolic syndrome

Polyphenols from *chañar* fruits flour exhibited antioxidant and anti-inflammatory activities

Chañar flour could be used as functional food alone or combined with other flours