Food Research International 44 (2011) 2345–2351

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Nutraceutical properties and toxicity studies of fruits from four Cactaceae species grown in Argentine Northwestern

Iris C. Zampini [a,d], Roxana Ordoñez [a,d,*,1], Norberto P. Giannini [c,d], Pedro G. Blendinger [b,c], María Inés Isla [a,d,1]

[a] INQUINOA (CONICET), (4000) S.M. de Tucumán, Tucumán, Argentina
[b] IER, Universidad Nacional de Tucumán, (4000) S.M. de Tucumán, Tucumán, Argentina
[c] CONICET
[d] Facultad de Ciencias Naturales e IML, Universidad Nacional de Tucumán, Miguel Lillo 205 (4000) S.M. de Tucumán, Tucumán, Argentina

ARTICLE INFO

Article history:
Received 30 November 2010
Accepted 14 March 2011

Keywords:
Phenolic compounds
Betalains
Nutraceutical activities
Argentine Cactaceae fruits

ABSTRACT

Fruits are known as good sources of phytochemicals, essential to prevent degenerative diseases like cancer and cardiovascular diseases. They contain a variety of antioxidants, which are useful to scavenge radical oxygen species (ROS). Considering the importance of natural products as a functional food, comparative studies between the antioxidant activity (AOA) and antimutagenic properties as well as phytochemical profile of Cactaceae fruits (*Lepismium lorentzianum*, *Lepismium lumbricoides, Rhipsalis floccosa, and Pfeiffera ianthothele*) from Argentinean Yungas, were performed. Different assays were applied: ABTS radical scavenging capacity, inhibition of lipid peroxidation using β-carotene-linoleate model system and mutagenicity/antimutagenicity by Ames test. A pigment like betalain was detected in fruits of both *Lepismium* species; *L. lorentzianum* aqueous extracts showed a higher pigment content (60.6 mg BE/100 g FM) than *L. lumbricoides* (9.2 mg BE/100 g FM). Fruit preparations of *L. lorentzianum* (aqueous and ethanolic extractions) showed significantly higher total phenolic compound and pigments content than the other berries. In all preparations, high antioxidant activity was demonstrated. *Lepismium* species were more active than *Rhipsalis* and *Pfeiffera* (SC_{50ABTS} values between 1.3 and 4.5 μg/ml and $IC_{50\ \beta\text{-carotene-linoleate}}$ values between 9 and 45.8 μg/ml). A significant correlation between pigment and phenolic compounds content and AOA was observed. The preparations showed no sign of mutagenicity at tested concentrations (until 50 μg/plate). A significant antimutagenic effect was observed for *L. lumbricoides*, and a weak effect was shown for *P. ianthothele* and *R. floccosa*. Because of the diversity and abundance of bioactive phytochemicals found in these species, food, cosmetic, and pharmaceutical applications could be proposed.

1. Introduction

The role played by dietary factors on health status has long been recognized but it has been only recently that epidemiological and clinical studies have provided a clearer insight on the physiological mechanisms of the effects of bioactive foods on human health (Shahidi, 2009). Plant foods are well known sources of vitamins, such as vitamin C and folic acid, carotenoids, and fiber, and they are naturally free of saturated fat and cholesterol (Brat, George, Bellamy, Du Chauffaut, Scalbert, & Mennen, 2006). In addition, these foods contain significant amounts of polyphenols, a group of phytochemicals recognized as the most abundant antioxidant in our diet that maintain the redox conditions necessary to control cell functions diet (Manach, Scalbert, Morand, Rémésy, & Jimenez, 2004). High consumption of fruits is associated with a lowered incidence of degenerative diseases, including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts (Di Matteo & Esposito, 2003; Kris-Etherton, Hecker, Bonanome, Coval, Binkoski, & Hilpert, 2002; Shahidi, 2004). Exploring the Argentine native edible fruits as sources of physiologically active compounds offers enormous opportunities for the development of novel foods, conservation and propagation of plant species to commercial production (Cardozo, Ordoñez, Zampini, Cuello, Dibenedetto & Isla, 2010). Wild fruits consumed in Tucumán, Salta and Jujuy provinces of Northwestern Argentine have received less attention as functional foods. Hence it was considered pertinent to determine some functional and nutritional properties of fruits obtained from four species of Cactaceae (*Lepismium lorentzianum*, *Lepismium lumbricoides, Rhipsalis floccosa,* and *Pfeiffera ianthothele*), consumed wild by frugivorous vertebrates and children in rural settlements (Schmeda-Hirschmann, Feresin, Tapia, Hilgert, & Theoduloz, 2005), and to determine the relation between their nutritional properties and phytochemical components. Besides its use for food, some of them have been used in

* Corresponding author at: INQUINOA-CONICET, Universidad Nacional de Tucumán, Ayacucho 461, 4000-San Miguel de Tucumán, Argentina. Tel.: +54 381 4107220; fax: +54 381 4248169.
E-mail address: rmordoniez@fbqf.unt.edu.ar (R. Ordoñez).
[1] Both authors have equally contributed.

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doi:10.1016/j.foodres.2011.03.035

traditional medicine in Argentine as cicatrizant and anti-inflammatory for the treatment of buccal and skin diseases, among others (Chifa & Ricciardi, 2002; Hilgert, 2001; Martínez, 2010).

These species are epiphytic Cactaceae growing in rainforest communities of the Southern Yungas, a subtropical montane forest of the eastern slopes of the Andes. The species are mainly distributed in southern South America, and are included in the Appendix II of CITES (i.e., species are trade controlled to avoid use non compatible with their survival).

Our research has focused on their evaluation as a source of health-promoting phytochemicals, including major hydrophilic compounds, antioxidant and antimutagenic capacities as well as their toxicity (genotoxicity).

2. Materials and methods

2.1. Study species

Epiphytic cacti evolved several times in independent lineages (Calvente, 2010; Nyffeler, 2002). The species studied here belong to tribes Hylocereeae with one species in the study area, *P. ianthothele* (Monv.) F.A.C. Weber, and Rhipsalideae with three species, *L. lorentzianum* (Griseb.) Barthlott, *L. lumbricoides* (Lem.) Barthlott, and *R. floccosa* ssp. *tucumanensis* (F.A.C.Weber) Barthlott & N.P. Taylor. The fruits are subespherical berries (*P. ianthothele*: 13.0 mm length, 13.9 mm diameter; *L. lorentzianum*: 6.1×6.9 mm; *L. lumbricoides*: 7.1×6.5 mm; and *R. floccosa*: 7.2×9.5 mm), unarmed except *P. ianthothele*. Ripe fruits are dark violet red in both species of *Lepismium*, and light pink to white in *P. ianthothele* and *R. floccosa* (Fig. 1).

Fruits were collected in natural areas in Tucumán province (Argentine), *R. floccosa* and *L. lumbricoides* were collected in November 2009 in Sierra San Javier Park (26° 47′S, 65° 19′W, 660 masl and in 26° 48′S, 65° 20′W, 723 masl, respectively). *L. lorentzianum* was collected in January 2010 in Aguas Chiquitas Reserve (26° 36′S, 65° 10′W, 624 masl), and *P. ianthothele* was collected in February 2010 in Sierra San Javier Park (26° 47′S, 65° 21′W, 1135 masl).

2.2. Sample preparations and processing

Only ripe fruits without blemishes or damage were selected, cleaned with distilled water, cut into small pieces and the seeds were removed using a sterile clip. The fresh pulp samples were stored at −20 °C until freeze-dried. Afterwards, the samples were grinded, stored at −20 °C until analyzed.

2.2.1. Aqueous (with and without boiling) and ethanolic extractions

10 g of samples was homogenized and extracted twice with 15 ml of distilled water and centrifuged for 20 min at 10,000×*g*. Insoluble fraction (pomace) was consecutively extracted with ethanol at 45 °C for 1 h and centrifuged at 10,000×*g* for 10 min. The residual precipitates were submitted to decoction with 5 ml of distilled water during 10 min at 100 °C. Each extraction was kept at 4 °C prior to biological activity measurement.

2.3. Pigment extraction from fresh fruits and processed fruits

For pigments isolation, all processed fruits (hydroalcoholic and aqueous preparations with and without heating at 100 °C) were chromatographically concentrated by solid phase extraction (SPE) on

Fig. 1. Photograph of *Rhipsalis floccosa* (A), *Lepismium lorentzianum (B), Lepismium lumbricoides* (C), and *Pfeiffera ianthothele* (D).

C_{18} cartridges (Phenomenex) according to the procedure of Stintzing, Schrieber, & Carle (2002). After rinsing with acidified water, the pigment was eluted with acidified methanol (methanol/TFA acidified water at pH 3, 95:5, v/v). *Spectrophotometric Analysis:* the concentration of pigment in the extracts was determined. Briefly: 100 μl of extract aliquots was diluted with 1900 μl of 10 mM of sodium phosphate buffer pH 7.4 and the absorbance was recorded between 350 and 650 nm in a Beckman DU-650 UV/Vis spectrophotometer. The red pigment content, defined as the amount of pigment (mg) in betanin equivalent per 100 g of fresh matter (mg BE/100 g fresh matter), was determined by using the extinction coefficient of betanin at 535 nm (60,000 l/mol × cm). The absorbance at 535 nm for betanin was used to calculate the concentration of the pigments [B] according to the equation.

$$B = [(A \times Df \times MW \times V / m\varepsilon L)]$$

Where

A = the absorption value at the absorption maximum (λ = 535 nm),
Df = the dilution factor,
MW = the molecular weight of betanin (550 g/mol),
V = final volume (l) of each extract,
M = mass of the fruits (g) used to generate the analyzed extract,
ε = the molar extinction coefficient of betanin (60,000 l/mol cm),
L = pathlength of cuvettes (1 cm)

The degradation of pigments was investigated by triplicate measuring betalain concentration of each solution during 3 months.

2.4. Free phenolics content

A derived method of Folin–Ciocalteau (F–C), according to Singleton, Orthofer, & Lamuela-Raventos (1999) was used: The reaction mixture contained 50 μl of each preparation, 2 ml of distilled water, 200 μl of F–C reagent and 800 μl of sodium carbonate (15.9% w/v). The reaction mixture was heated at 50 °C for 5 min in a water bath. Absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalents/100 g fresh matter (mg GAE/100 g FM).

2.5. Total sugar and reducing sugar determinations

Glucose, fructose and sucrose were measured in aqueous (with and without boiling) and ethanolic extractions. Total neutral sugars were also determined by Dubois, Gilles, Hamilton, Rebers, & Smith (1956). Reducing sugars were measured using the Somogyi–Nelson method (Nelson, 1944; Somogyi, 1945). Results were expressed as mg of glucose/100 g fresh matter.

Glucose was determined by the glucose–oxidase method (Jorgensen & Andersen, 1973). Sucrose was estimated by the resorcinol method (Cardini, Leloir, & Chiriboga, 1955). Fructose was measured according to Roe (1934). Results were expressed as mg of glucose/100 g fresh matter.

2.6. Protein determinations

Protein concentration was determined by the method of Lowry, Rosebrough, Farr, & Randall (1951) using bovine serum albumin as the standard.

2.7. Measurement of antioxidant capacity

2.7.1. ABTS free radical scavenging activity

The antioxidant capacity assay was carried out by the improved $ABTS^{\bullet+}$ method as described by Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans (1999). The $ABTS^{\bullet+}$ 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 dilution in ethanol (for alcoholic extracts) or in buffer PBS pH 7.4 (for aqueous extracts) of the stock solution to an absorbance of 0.70 at 734 nm. $ABTS^{\bullet+}$ solution (1 ml) was added to samples (0.625–5 μg GAE or 0.0625–2 μg BE) and mixed thoroughly. Absorbance was recorded at 734 nm, 1 and 6 min after initial mixing.

$$\text{Antioxidant capacity}\,(\%) = A_{ABTS^{\bullet+}} - A_{\text{sample or standard}} / A_{ABTS^{\bullet+}} \times 100$$

The results were calculated based on the calibration curve plotted using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations. Results were expressed in μmol Trolox equivalents per g of fresh matter. SC_{50} values denote the sample concentration required to scavenge 50% ABTS free radicals.

2.7.2. β-carotene-linoleic acid assay

Antioxidant activity of fruits was determined according to Ordoñez, Gomez, Vattuone, & Isla (2006). 1 ml of 0.2 mg/ml β-carotene dissolved in chloroform was added to round-bottom flasks (50 ml) containing 0.02 ml of linoleic acid and 0.2 ml of Tween 20 (polyoxyethylene sorbitan monopalmitate). Chloroform was removed at 40 °C under vacuum. The resulting mixture was diluted with 10 ml of water. To this emulsion, 40 ml of oxygenated water was added. Four milliliter aliquots of the emulsion were added to 0.2 ml of different sample dilutions or synthetic antioxidant (BHT) or solvent control. The mixture was shaken for 2 min and then subjected to thermal oxidation at 50 ° C for 60 min. The absorbance of the solution was monitored at 470 nm by taking measurements at 10 min intervals and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Antioxidant activity (AA) was calculated as percent inhibition relative to control. IC_{50} values denote the sample concentration required (μg GAE/ml) to inhibit 50% β-carotene bleaching.

2.8. Salmonella mutagenicity assay

The mutagenic effects of fruits aqueous extracts were evaluated on two *Salmonella typhimurium* strains (TA98 and TA100). The plate incorporation assay was performed according to Maron & Ames (1983), by adding 0.1 ml of the overnight bacterial culture and 0.1 ml of test compounds at different concentrations (1–50 μg GAE/plate) to the test tubes with 2 ml of top agar and then each tube was plated on minimum medium (Oxoid N°2). In the case of metabolic activation, 0.5 ml of 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.), 40 μg/plate and 2-aminofluorene (2- AF; Merck), 10 μg/plate. Mutagenic effects were assayed with and without metabolic activation (S9 mix fraction). Three plates at three separate experiments were used for each concentration tested and for the positive and negative controls. A sample was considered to be mutagenic when the number of revertant colonies was at least twice the negative control yield and showed a significant response in the analysis of variance.

2.9. Antimutagenicity test

The antimutagenic effects of fruit aqueous extracts were assayed using *Salmonella* assay. The 4-NPD mutagen (40 μg/plate), different concentrations of fruits extracts (1–50 μg GAE/plate) and TA98 or TA100 bacterial culture (0.1 ml) were added to 2 ml of soft agar containing L-hystidin (0.05 mM), D-biotin (0.5 mM) and 0.1 ml of bacterial culture. Then, this mixture was poured onto a plate containing minimum medium. The plates were incubated at 37 °C for 48 h and then, His^+ revertants were counted. The mutagenicity of 4-NPD (positive control in the absence of samples) was defined as 100% mutagenicity. The calculation of percentage remaining mutagenicity was done according to formula

Table 1
Analysis of total phenolic compounds (TPC), total pigments like betalains; total sugars (T); reducing sugar (R); glucose (G); fructose (F); sucrose (S) and proteins (P) of aqueous extracts (AE), etanolic extracts (EE) and aqueous extracts with heating (AE-H) from different fruits.

	Fruits extracts	Total phenolic compounds (TPC)	Total pigments like betalain	Sugars					Proteins
				T	R	G	F	S	P
		mg GAE/100 g	mg BE/100 g	mg/100 g FM					mg/100 g FM
L. lorentzianum	AE	76.6 ± 8.1^{f}	60.6 ± 10.7^{c}	$66.4 \pm 15.5^{a,b}$	25.2 ± 1.5^{ab}	13.1 ± 1.5^{ab}	14.2 ± 1.5^{a}	5.4 ± 0.5^{b}	1.3 ± 0.2^{bc}
	EE	49.6 ± 5.4^{e}	27.9 ± 2.8^{b}	$58.8 \pm 10.5^{a,b}$	22.3 ± 2.0^{ab}	13.1 ± 1.5^{ab}	15.3 ± 1.2^{a}	2.6 ± 0.3^{a}	2.3 ± 0.5^{d}
	AE-H	28.0 ± 3.8^{d}	8.1 ± 2.8^{a}	140 ± 20^{de}	69.2 ± 10.0^{d}	38.2 ± 5.1^{e}	40.9 ± 8.1^{ef}	15 ± 2.5^{d}	2.2 ± 0.5^{d}
L. lumbricoides	AE	23.8 ± 5.0^{d}	9.2 ± 1.4^{a}	29.7 ± 2.5^{a}	14.0 ± 1.5^{a}	6.8 ± 1.0^{a}	7.2 ± 1.2^{a}	1.4 ± 0.9^{a}	0.5 ± 0.1^{ab}
	EE	$11.3 \pm 2.0^{b,c}$	3.6 ± 1.4^{a}	121 ± 15^{cd}	27.36 ± 3.0^{ab}	13.5 ± 1.5^{ab}	16.5 ± 1.2^{ab}	9.1 ± 0.6^{c}	1.1 ± 0.5^{c}
	AE-H	$10.5 \pm 3.0^{a,b,c}$	1.6 ± 0.4^{a}	162.6 ± 22^{e}	43.7 ± 0.5^{bc}	27.1 ± 5.0^{cde}	20.3 ± 2.0^{abc}	2.1 ± 0.3^{a}	0.8 ± 0.2^{abc}
R. floccosa	AE	18.3 ± 3.0^{c}	ND	74.8 ± 12.5^{bc}	55.5 ± 10.0^{cd}	20.9 ± 3.0^{bcd}	31.5 ± 2.5^{cde}	3.5 ± 0.8^{ab}	0.3 ± 0.1^{a}
	EE	$12.9 \pm 2.0^{b,c}$	ND	99 ± 10^{bcd}	62 ± 10^{d}	31.8 ± 3.5^{de}	32.2 ± 12.5^{de}	2.1 ± 0.5^{a}	0.4 ± 0.1^{a}
	AE-H	$11.1 \pm 2.0^{a,b,c}$	ND	135.1 ± 15^{de}	102.7 ± 12^{e}	55.3 ± 10.0^{f}	56.3 ± 15.0^{f}	5.1 ± 0.8^{b}	0.3 ± 0.2^{a}
P. ianthothele.	AE	$6.5 \pm 1.5^{a,b}$	ND	54.8 ± 10.2^{ab}	49.4 ± 10.9^{cd}	27.6 ± 5.0^{cde}	28.3 ± 6.0^{bcde}	5.9 ± 0.8^{b}	0.9 ± 0.2^{abc}
	EE	4.0 ± 1.3^{a}	ND	62.6 ± 10.5^{ab}	40.5 ± 10.5^{bc}	18.3 ± 1.5^{abc}	20.5 ± 4.0^{abc}	4.2 ± 0.5^{ab}	0.8 ± 0.3^{abc}
	AE-H	$5.2 \pm 2.0^{a,b}$	ND	65.5 ± 10.8^{ab}	$56,1 \pm 10.9^{cd}$	18.8 ± 2.5^{bc}	28.3 ± 5.0^{bcd}	$2,5 \pm 0.5^{a}$	0.9 ± 0.3^{abc}

ND: no detected.
Means ± SD followed by the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$).

given by Ferrer, Sanchez-Lamar, Fuentes, Barbe, & Llagostera (2002); percentage remaining mutagenesis = 100 (His^+ revertants per plate with mutagen and fruit extracts/His^+ revertants per plate with mutagen alone). The antimutagenic effect was considered moderate when the inhibitory effect of fruit extracts was in the range of 25–40% and strong when the inhibitory effect was >40%. An inhibitory effect <25% was considered weak, and it was not recognized as a positive result.

2.10. Statistical analysis

Sampling and analysis were performed in triplicate, and the data are presented as mean ± standard deviation. The correlation between two variants was analyzed using GraphPad Prism 5.0 software 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. Phytochemical analysis

Aqueous extractions with heating (AE-H) had significantly higher total sugar content than alcoholic extract and aqueous extract without heating except to *P. ianthothele*, where the three extracts were not significantly different (Table 1). *L. lumbricoides* presented higher TS content than *R. floccosa* followed by *L. lorentzianum* and *P. ianthothele* in AE-H and EE. Glucose and fructose were significantly more abundant than sucrose in all fruits extractions. The soluble protein content in all preparations of *L. lorentzianum* was significantly higher than the preparations obtained from the other assayed fruits (Table 1). *L. lorentzianum* showed higher TPC content in aqueous extract without heating (76.6 mg GAE/100 g FM) than ethanolic preparation (49.6 mg GAE/100 g FM) and aqueous preparation with heating (28.0 mg GAE/100 g FM). *L. lumbricoides* showed lower content of TPC in all preparations (23.8/11.3/10.5 mg GAE/100 g FM), followed by *R. floccosa* (18.4/12.9/11.1 mg GAE/100 g FM) and *P. ianthothele* (6.5/4.0/5.2 mg GAE/100 g FM). However, the assay is not specific to phenolic compounds and other redox-active molecules interfere in the measurement in an inhibitory, additive or enhancing manner (Prior, Wu, & Schaich, 2005). Among these molecules are reducing sugars present in fruits. Removing sugars from berry extracts with the help of SPE treatment (Bond Elut C_{18} SPE), resulted in the reduction of TPC (F-C method) by 20%.

3.2. Pigments like betalain in cacti fruits

Extraction and quantification of pigments of each hydroalcoholic and aqueous processed fruits preparations with and without heating are shown in Table 1. The visible spectra absorption maxima (λmax) for the betalain pigments for both *Lepismium* species in pH 7.4 solution were between 535 and 538 nm, quite similar to previously

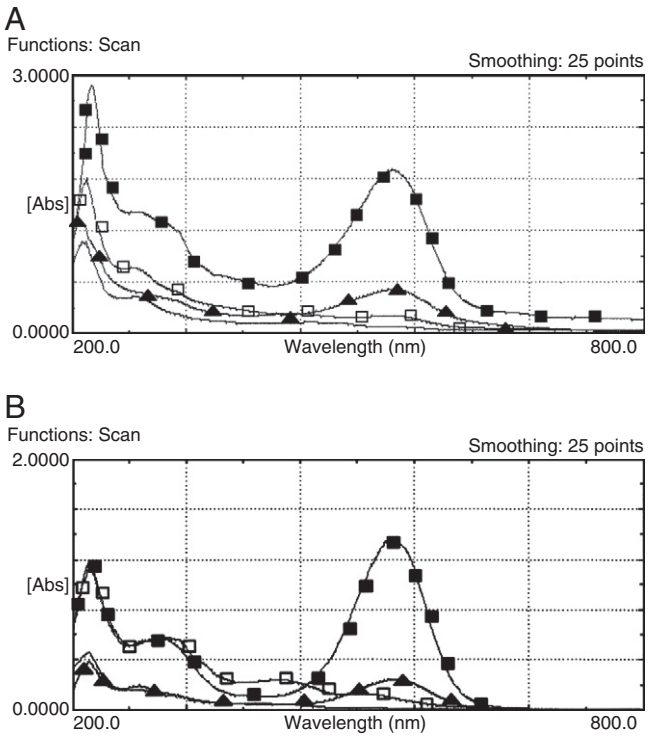

Fig. 2. UV–Visible spectra of *Lepismium* fruit aqueous extract. A) *L. lorentzianum* extract obtained with (-□-) and without (-■-) heating, and *L. lumbricoides* extract obtained with (-▲-) and without (—) heating. B) Pigment extracted from aqueous extract of *L. lorentzianum* (-■-), *L. lorentzianum* pigment after heating, (-□-) pigment extracted from aqueous extract of *L. lumbricoides* (—) and *L. lumbricoides* pigment after heating (-▲-).

Table 2
Scavenging activity of ABTS radical cation (SC_{50}) of different preparation obtained with four types of cacti fruits collected from NWA and expressed as phenolic compounds and pigment like betalains.

	AE	AE-H	EE	AE	AE-H
	SC_{50} (μg GAE/ml extract)			SC_{50} (μg BE/ml extract)	
L. lorentzianum	1.90 ± 0.20^a	1.80 ± 0.15^a	1.30 ± 0.10^a	1.52 ± 0.12^a	0.52 ± 0.03^a
L. lumbricoides	2.10 ± 0.23^a	2.00 ± 0.17^a	1.40 ± 0.10^a	0.82 ± 0.05^b	0.28 ± 0.01^b
R. floccosa	3.40 ± 0.28^b	3.00 ± 0.25^b	2.40 ± 0.22^b		
P. ianthothele	4.50 ± 0.41^b	4.10 ± 0.35^c	3.20 ± 0.25^c		

Aqueous extracts (AE), ethanolic extracts (EE) and aqueous extracts with heating (AE-H).
Means ± SD followed by the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$).

reported data for betacyanins (Kanner, Harel, & Granit, 2001; Slawomir & Mizrahi, 2002) (Fig. 2). *L. lorentzianum* extracts showed higher pigment content expressed as betanin equivalents (60.6; 27.9 and 8.1 mg BE/100 g FM, respectively) than *L. lumbricoides* (9.2; 3.6 and 1.6 mg BE/100 g FM, respectively). Pigment was not detected in *R. floccosa* and *P. ianthothele*. The pigment content in both analyzed *Lepismium* species was higher than *Hylocereus* fruits, other very well-known cacti edible specie (Tang & Norziah, 2007; Wybraniec & Mizrahi, 2002). The red-violet color of all *Lepismium* preparations decreased during processing at 100 °C for 10 min. The color declined more slowly than when the pigment isolated was subjected to heating (Table 1, Fig. 2).

The pigment in the aqueous extracts may be more stable because of protective effects conferred by other components such as phenolic compounds. The pigment was stable at room temperature and 4 °C during 3 months. Our results are the first reports of the detection of betalain in *Lepismium* species. The *Lepismium* pigments are easily obtainable at low cost because they can be removed by water extraction. These characteristics of *Lepismium* pigments give them considerable potential for use in food.

3.3. Antioxidant activity

The free radical scavenger potential of preparations obtained from four cacti fruits were tested by the $ABTS^{\bullet+}$ assays. In general, all preparations were scavengers with SC_{50} values between 1.3 and 4.5 μg GAE/ml (Table 2). *Lepismium* preparations were more active on $ABTS^{\bullet+}$ than *R. floccosa* and *P. ianthothele*. All ethanolic extracts were more active than aqueous extracts.

A positive correlation was observed between the total phenolic compounds content and $ABTS^{\bullet+}$ scavenging activity in all preparations ($R^2 = 0.822$; Pearson r = 0.90, Fig. 3).

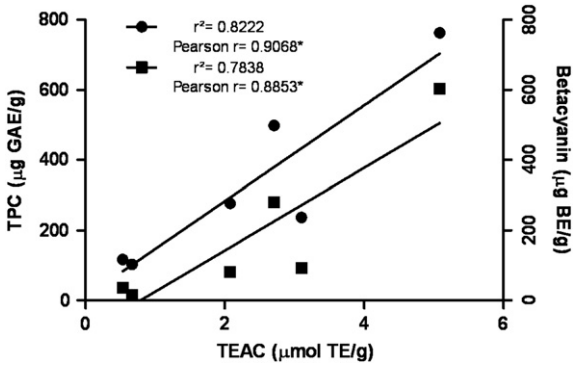

Fig. 3. Linear regression plots and Pearson's correlation coefficients of ABTS-scavenging activity (Trolox equivalent antioxidant capacity, TEAC) with respect to total phenols (TPC) and betalain content of two *Lepismium* species fruits. The correlations were significant at the 0.05 level (two-tailed) for values marked with an asterisk.

The SC_{50} values for $ABTS^{\bullet+}$ scavenging activity in betanin equivalents were 1.52 and 0.82 μg BE/ml by *L. lorentzianum* and *L. lumbricoides*, respectively. The antioxidant capacity of different *Lepismium* preparations was stable after processing at 100 °C during 10 min (Table 2). A correlation between pigment content and antioxidant capacity of all *Lepismium* preparations was observed ($R^2 = 0.78$; Pearson r = 0.88).

Percentage inhibition of lipid peroxidation by different concentrations of cacti extracts was calculated (Fig. 4). Once more, the antioxidant potential was significantly higher in ethanolic extract (IC_{50} values between 9 and 20.10 μg GAE/ml extracts) than aqueous extracts (IC_{50} values between 25.00 and 45.80 μg GAE/ml). *L. lorentzianum* and *L. lumbricoides* were the cacti fruit extracts that show the best effect protective on lipid peroxidation.

3.4. Mutagenic and antimutagenic activity

In order to assess the mutagenic and antimutagenic effects of different preparations of Cactaceae fruits, induction or suppression of revertant colonies was examined in *S. typhimurium* strains (TA98 and TA100). The number of spontaneous revertant for both strains with or without S9 metabolism was determined in each set of experiment and indicated as untreated sample in Table 3. The results of mutagenicity assay of fruit extracts are presented in Table 3. Different concentrations of the fruits aqueous extracts did not show any mutagenic effect on TA98 and TA100 strains in the presence or absence of S9 microsomal fraction. The possible antimutagenic potential of Cactaceae fruit aqueous extracts was examined against 4-NPD in the same tester strains using plate incorporation assay. The results of

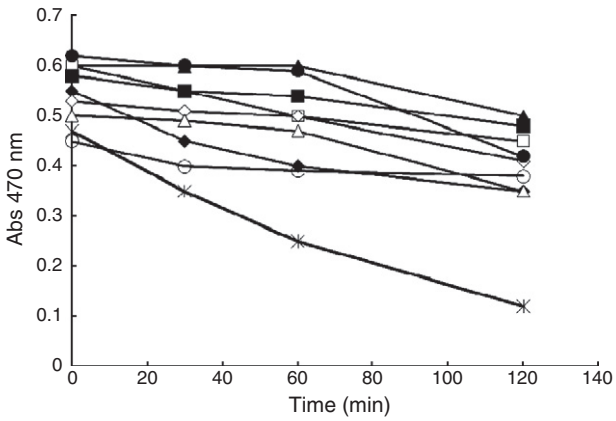

Fig. 4. Percentage of antioxidant activity (AA%) relative to control using the β-carotene bleaching assay: control (-*-); *L. lorentzianum*: alcoholic extracts (-●- $IC_{50} = 12.80$ μg GAE/ml extracts); aqueous extracts (-○-; $IC_{50} = 25.01$ μg GAE/ml extracts); (B) *L. lumbricoides*: alcoholic extracts (-◆-; $IC_{50} = 9.10$ μg GAE/ml extracts); aqueous extracts (-◇- $IC_{50} = 36.21$ μg GAE/ml extracts); (C) *R. floccosa*: alcoholic extracts (-■- $IC_{50} = 17.90$ μg GAE/ml extracts); aqueous extracts (-□- $IC_{50} = 35.80$ μg GAE/ml extracts); (D) *P. ianthothele*: alcoholic extracts (-▲- $IC_{50} = 20.1$ μg GAE/ml extracts); aqueous extracts (-Δ- $IC_{50} = 45.80$ μg GAE/ml extracts). Data are expressed as means ± standard deviations.

Table 3
Mutagenicity of different Cactaceae fruits on TA98 and TA100 strains.

	Treatment (μg GAE/plate)	TA98		TA100	
		−S9	+S9	−S9	+S9
L. lorentzianum[1]	1	32 ± 4^{a}	35 ± 6^{a}	147 ± 10^{a}	92 ± 8^{a}
	10	35 ± 3^{ab}	45 ± 2^{abc}	138 ± 13^{a}	145 ± 8^{c}
	50	41 ± 6^{abc}	46 ± 3^{abc}	124 ± 9^{a}	112 ± 6^{ab}
L. lumbricoides[1]	1	27 ± 5^{a}	42 ± 4^{abc}	123 ± 9^{a}	152 ± 2^{bc}
	10	32 ± 8^{a}	40 ± 7^{ab}	135 ± 10^{a}	153 ± 11^{bc}
	50	29 ± 3^{a}	51 ± 2^{bc}	127 ± 8^{a}	130 ± 7^{bc}
P. ianthothele[1]	1	30 ± 5^{a}	50 ± 2^{bc}	132 ± 15^{a}	90 ± 12^{a}
	10	31 ± 5^{a}	45 ± 5^{abc}	144 ± 11^{a}	130 ± 10^{bc}
	50	52 ± 3^{c}	40 ± 3^{ab}	129 ± 12^{a}	100 ± 4^{a}
R. floccosa[1]	1	32 ± 6^{a}	50 ± 4^{bc}	143 ± 14^{a}	90 ± 9^{a}
	10	34 ± 9^{ab}	53 ± 3^{cd}	124 ± 10^{a}	100 ± 12^{a}
	50	48 ± 2^{bc}	64 ± 2^{cd}	215 ± 18^{b}	147 ± 13^{bc}
Untreated[2]		42 ± 3^{abc}	45 ± 3^{abc}	130 ± 12^{a}	103 ± 2^{a}
Positive control (4-NPD)[3]		2861 ± 114	880 ± 15	1998 ± 38	1450 ± 28

(−S9) without and (+S9) with metabolic activation.
A sample was considered to be mutagenic when the number of revertant colonies was at least twice the negative control yield and showed a significant response in the analysis of variance.
Means ± SD followed by the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$).
[1] Mean number of revertants induced by Cactaceae fruit extracts [Mean and ± S.D. of three plates].
[2] The number of spontaneous revertants was determined in a assays without a sample,
[3] Mean number of revertants induced by reference mutagens [2-AF, 2-aminofluorene (10 μg/plate), positive control for +S9; 4-NPD, 4-nitro-o-phenylenediamine (40 μg/plate) positive control for −S9].

Table 4
Effect of Cactaceae fruit extracts against the mutagenicity of NPD to *S. typhimurium* TA98 and TA100.

Extracts	Treatment (μg GAE/plate)	TA98	TA100
L. lorentzianum[1]	1	2870 ± 72^{bcd}	1880 ± 42^{ef}
	10	2545 ± 114^{a}	1869 ± 33^{ef}
	50	2630 ± 158^{ab}	1908 ± 20^{fg}
L. lumbricoides[1]	1	3142 ± 89^{d}	1882 ± 35^{ef}
	10	2620 ± 75^{ab}	1679 ± 21^{bc}
	50	3129 ± 74^{d}	1501 ± 50^{a}
P. ianthothele[1]	1	2915 ± 92^{bcd}	1984 ± 32^{gh}
	10	3009 ± 150^{cd}	2030 ± 20^{h}
	50	2620 ± 58^{ab}	1691 ± 19^{bcd}
R. floccosa[1]	1	3090 ± 96^{d}	1786 ± 36^{de}
	10	2680 ± 103^{ab}	1742 ± 42^{cd}
	50	2747 ± 52^{abc}	1639 ± 19^{b}
Untreated[2]		42 ± 3	130 ± 12
Positive control (NPD)[3]		2861 ± 114^{bcd}	1998 ± 38^{gh}

Means ± SD followed by the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$).
[1] Mean number of revertants in presence of NPD and different concentration of Cactaceae fruit extracts,
[2] The number of spontaneous revertants was determined in assays without a sample,
[3] Mean number of revertants induced by 4-NPD, 4-nitro-o-phenylenediamine (40 μg/plate).

antimutagenic assay of the fruits extracts are presented in Table 4. A significant inhibitory effect against the mutagenicity of 4-NPD in the absence of metabolic activation was observed for *L. lumbricoides* and *R. floccosa* for TA100. The linear regression analysis between extract dose and antimutagenic response against test mutagen showed positive correlation with respect to dose dependent response in TA100 ($R^2 = 0.86$; Pearson $r = 0.92$) for *L. lumbricoides*.

4. Conclusions

In this manuscript we report for the first time the presence of pigment like betalain in two species of *Lepismium* and nutritional content, antioxidant and antimutagenic activity of processed fruits of four wild cactaceas obtained from Argentinean Yungas. *Lepismium* fruit is a potential source of pigments which can be used as a natural red purple food colorant. Otherwise, the use of the extract instead of individual antioxidants allows taking advantage of additive and synergistic effects of different phenolic compounds, reducing sugars and pigments present in the samples. Further studies are needed to isolate the active principles present in fruits, as well as to elucidate the role of various interacting phytocompounds in influencing therapeutic potential and efficacy *in vivo*.

Acknowledgments

This research was partially supported by grants from Consejo de Investigación de la Universidad Nacional de Tucumán (26D-430, CIUNT, Tucumán, Argentina) and Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 704/PIP 1025, CONICET; Buenos Aires, Argentina).

References

Brat, P., George, S., Bellamy, A., Du Chauffaut, L., Scalbert, A., & Mennen, L. (2006). Daily polyphenol intake in France from fruit and vegetables. *The Journal of Nutrition*, *136*, 2368−2373.
Calvente, A. M. (2010). Filogenia molecular, evolução e sistemática de *Rhipsalis* (Cactaceae). Doctoral Dissertation, Universidade de São Paulo.
Cardini, C. E., Leloir, L. F., & Chiriboga, J. (1955). The biosynthesis of sucrose. *The Journal of Biological Chemistry*, *214*, 149−155.
Cardozo, M. L., Ordoñez, R. M., Zampini, I. C., Cuello, A. S., Dibenedetto, G., & Isla, M. I. (2010). Evaluation of antioxidant capacity, genotoxicity and polyphenol content of non conventional foods: Prosopis flour. *Food Research International*, *43*, 1505−1510.
Chifa, C., & Ricciardi, A. (2002). Cactáceas medicinales en la flora chaqueña de Argentina usadas por las comunidades aborígenes Toba y Wichi. : Comunicaciones Científicas y Tecnológicas.
Di Matteo, V., & Esposito, E. (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Current Drug Targets—CNS and Neurological Disorder*, *2*, 95−107.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350−356.
Ferrer, M., Sanchez-Lamar, A., Fuentes, J. L., Barbe, J., & Llagostera, M. (2002). Antimutagenic mechanisms of *Phyllanthus orbicularis* when hydrogen peroxide is tested using *Salmonella* assay. *Mutation Research*, *517*, 251−254.
Hilgert, N. I. (2001). Plants used in home medicine in the Zenta River basin, Northwest Argentina. *Journal of Ethnopharmacology*, *76*, 11−34.
Jorgensen, O. S., & Andersen, B. (1973). An improved glucose–oxidase–peroxidase coupled assay for the β-fructofuranosidase activity. *Analytical Biochemistry*, *53*, 141−145.
Kanner, J., Harel, S., & Granit, R. (2001). Betalains—A new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry*, *49*, 5178−5185.
Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., & Hilpert, K. F. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, *113*(9B), 71−88.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, *193*, 265−275.
Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jimenez, L. (2004). Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, *79*, 727−747.
Maron, D. M., & Ames, B. N. (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutation Research*, *113*, 173−215.
Martínez, G. J. (2010). Los remedios naturales en la prevención y cuidado de la salud oral de los tobas del Chaco Central (Argentina). *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas*, *9*, 109−122.
Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *The Journal of Biological Chemistry*, *153*, 375−380.
Nyffeler, R. (2002). Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from trnK/matK and trnL–trnF sequences. *American Journal of Botany*, *89*, 312−326.
Ordoñez, A. A., Gomez, D., Vattuone, M. A., & Isla, M. I. (2006). Antioxidant activity of *Sechium edule* (Jacq) Swartz. *Food Chemistry*, *97*(3), 452−458.
Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*, 4290−4303.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*, 1231−1237.
Roe, J. H. (1934). A colorimetric method for the determination of fructose in blood and urine. *The Journal of Biological Chemistry*, *107*, 15−22.
Schmeda-Hirschmann, G., Feresin, G., Tapia, A., Hilgert, N., & Theoduloz, C. (2005). Proximate composition and free radical scavenging activity of edible fruits from the Argentinian Yungas. *Journal of the Science of Food and Agriculture*, *85*, 1357−1364.

Shahidi, F. (2004). Functional foods: Their role in health promotion and disease prevention. *Journal of Food Science*, *69*, 146−149.
Shahidi, F. (2009). Nutraceuticals and functional foods: Whole versus processed foods. *Trends in Food Science and Technology*, *20*, 376−387.
Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. *Method in Enzymology*, *299*, 152−178.
Slawomir, W., & Mizrahi, Y. (2002). Fruit flesh betacyanin pigments in *Hylocerus* Cacti. *Journal of Agricultural and Food Chemistry*, *50*, 6086−6089.
Somogyi, M. (1945). A new reagent for the determination of sugar. *The Journal of Biological Chemistry*, *160*, 61−68.
Stintzing, F. C., Schrieber, A., & Carle, R. (2002). Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia R. ficusindica* (L.) Mill.] by high-performance liquid chromatography electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry*, *50*, 2302−2307.
Tang, C., & Norziah, M. H. (2007). Stability of betacyanin pigments from red purple pitaya fruit (*Hylocereus polyrhizus*), influence of pH, temperature, metal ions and ascorbic acid. *Indo Journal Chemistry*, *7*(3), 327−331.
Wybraniec, S., & Mizrahi, Y. (2002). Fruit flesh betacyanin pigments in *Hylocereus cacti*. *Journal of Agricultural and Food Chemistry*, *50*, 6086−6089.