PHYTOTHERAPY RESEARCH
Phytother. Res. (2017)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5897

Stability of an Aqueous Extract of *Larrea divaricata* Cav. during a Simulated Digestion Process

María Rosario Alonso, Ignacio Peralta, Demian Monti, Renzo Martino and Claudia Anesini*

Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

***Larrea divaricata* Cav. (Zygophyllaceae) is a South American plant widely distributed in Argentina that is used in folk medicine to treat inflammatory diseases. The aqueous extract is known to have well-documented biological activities such as antitumour, immunomodulatory, antimicrobial, antiinflammatory and antioxidant. However, its stability in gastrointestinal fluids is unknown. The latter is an important factor to assure the bioavailability of plant extracts intended to be administered via the oral route. The aim of this work was to study the stability of a lyophilized aqueous extract of *L. divaricata* compressed as a pill. To this end, the main polyphenol compound found in the extract, that is, the nordihydroguaiaretic acid, the total polyphenols and flavonoids content and the antioxidant activity such as diphenylpicrylhydrazyl scavenger activity and reducing power were assayed after subjecting the extract to different incubation times in simulated digestive fluids. The HPLC and spectroscopic methods were employed. Although the levels of polyphenols and flavonoids decreased upon incubation in gastric and intestinal fluids, the extract maintained its antioxidant activity related to the presence of nordihydroguaiaretic acid. These results are promising and encourage the potential use of the extract by the oral route as a supplement or phytomedicine with antioxidant activity. Copyright © 2017 John Wiley & Sons, Ltd.**

Keywords: Larrea divaricata; simulated gastrointestinal fluids; DPPH activity; reducing power activity; polyphenols stability.

INTRODUCTION

Medicinal plants are used as therapeutic agents as well as supplements in the food industry; however, little is known about their pharmacokinetics, mainly the stability in gastric and intestinal fluids, which are important factors to assure bioavailability of a drug intended to be administered by the oral route.

Nowadays, the antioxidant properties of plants are being studied exhaustively. This biological activity is known to be related to the scavenging of reactive oxygen species. Reactive oxygen species participate in the oxidation of the cell components affecting the normal physiology and inducing different diseases. The antioxidant activity is related to the presence of polyphenol compounds.

Larrea divaricata Cav. (Zygophyllaceae) is a South American plant widely distributed in Argentina that is used in folk medicine to treat inflammatory diseases. The aqueous extract of its leaves presents nordihydroguaiaretic acid (NDGA) as the major phenolic compound. This extract is known to have well-documented biological activities such as antitumour, immunomodulatory (Anesini *et al*., 1996; Anesini *et al*., 2001), antimicrobial (Anesini and Pérez, 1993; Stege *et al*., 2006), antiinflammatory (Davicino *et al*., 2015) and antioxidant. The antioxidant activity is reflected on the scavenging effect on the diphenylpicrylhydrazyl (DPPH) radical and on the secretion of peroxidase in rat salivary glands (Anesini *et al*., 2004; Alonso *et al*., 2012). It has also been demonstrated that the extract can prevent the oxidation of vitamin C in orange juice in a range of concentrations of 500–1000 μg/mL (Micucci *et al*., 2011). To date, the relationship between the stability in a simulated digestion system and the antioxidant activity of *L. divaricata* extract has not been assessed.

Taken this into account, the present work was aimed at assessing the stability of the active components of the *L. divaricata* aqueous extract and its therapeutic potential as antioxidant to be administered by the oral route.

The stability of the aqueous extract in artificial gastrointestinal fluids was evaluated by the determination of the levels of NDGA by HPLC with diode-array detection and by the quantification of total polyphenols and total flavonoids before and after subjecting the extract, compressed as a pill, to *in vitro* digestion with artificial gastric and pancreatic juices. Furthermore, the antioxidant activities were monitored throughout the digestive process by the measurement of the DPPH radical scavenger activity and the reducing power.

MATERIALS AND METHODS

Plant material and extract. Leaves of *L. divaricata* Cav. were collected in the province of Córdoba, Argentina, and identified by morphological, anatomical and

* Correspondence to: Claudia Anesini, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.
E-mail: canesini@yahoo.com.ar

Contract/grant sponsor: CONICET; contract/grant number: PIP 00067 CO.

Received 21 April 2017
Revised 27 July 2017
Accepted 27 July 2017

histochemical analyses. A voucher specimen (BAFC no. 38) was deposited at the Museum of Pharmacobotany, School of Pharmacy and Biochemistry, University of Buenos Aires. An aqueous extract of the leaves was prepared at 7.5%. To this end, the air-dried leaves were extracted for 10 min with boiling distilled water. The extract was then macerated, filtered and lyophilized. The final yield was 26.6 g% of plant material. The aqueous extract was aliquoted and stored at −20°C until used (Anesini *et al*., 1996).

HPLC analysis

Nordihydroguaiaretic acid HPLC conditions. The HPLC analysis was performed in a Varian Pro Star instrument equipped with a Rheodyne injection valve (20 μL) and a photodiode array detector set at 280 nm. A reversed-phase column Phenomenex-Kinetex (250 mm × 4.6 mm and 5 μ dp) was used. Samples were eluted with a gradient of A: water and acetic acid (98:2) and B: methanol and acetic acid (98:2) from 70% A to 0% A in 30 min and back to initial conditions. The flow rate of 1.0 mL/min and the separation was conducted at room temperature (18–25°C). Data were analysed with a Varian Star 5.5 programme (Varian, Walnut Creek, CA, USA). Lyophilized aqueous extracts at 10 mg/mL and the pure standard were dissolved in methanol : water (70:30). A calibration curve of NDGA was obtained by injecting 20 μL of a range of 0.8 to 80 μg/mL solutions prepared from a stock solution of 100 μg/mL.

Water employed to prepare working solutions was of ultrapure quality (Milli-Q; Merck Millipore, Billerica, MA, USA). Methanol (HPLC grade; J.T. Baker Ciudad de Mexico) and acetic acid (Merck, Argentina) were used. The Sigma pure standard (Sigma, St Louis, Mo, USA) of NDGA lot 19C-0504 was used for identification and quantification by comparing retention times and by plotting peak areas, respectively (Davicino *et al*., 2011).

Calibration curve and validation method. The NDGA was injected into the column and identified by its retention time. Calibration curves were obtained by plotting the peak area versus the theoretical concentration of analyte. Curves were constructed from two replicate measurements of six concentrations of NDGA over a range of 0.8–80 μg/mL during 3 days. Data were subjected to least-squares regression analysis.

The precision of the analytical method was evaluated on the basis of the coefficients of variation obtained intraday and interday analyses. The accuracy was expressed in terms of relative error of measurement according to the following formula: Accuracy % = (Cex × 100/Ct), where Cex was the experimental concentration and Ct the theoretical concentration (%bias). Limits of detection and quantitation were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than 10, with precision (%CV) and accuracy (%bias) within ±5% (Alonso *et al*., 2006).

Simulated gastrointestinal fluids

Preparation of simulated gastric and intestinal fluids. Simulated gastric fluid was prepared according to the procedure of the United States Pharmacopeia, National Formulary, as follows: 2.0 g NaCl, 3.2 g pepsin and 3.0 mL concentrated HCl and diluted with water to 1 L, pH 1.2–1.8. Simulated intestinal fluid was prepared according to the procedure of the United States Pharmacopeia, National Formulary: 6.8 g monobasic potassium phosphate, 650 mL water, 190 mL of 0.2 mol/L NaOH and pancreatin mix (10 g). In this experiment, the samples subjected to simulated gastric juice were incubated 1 and 2 h at 37°C and churning by a basket stirring element set at 100 rpm. This was conducted to mimic what normally occurs in the stomach where after 1–2 h, the chyme is transported to the small intestine. The simulated intestinal fluid was prepared to mimic the pancreatic fluid; in this case, samples were treated with the fluid for 2–4 h in a vortexing bath at 37°C. The incubation times were selected in accordance to the maximal transit average time in the small intestine.

Incubation of *L. divaricata* aqueous extract in simulated gastrointestinal fluids. The experiment was carried out in triplicate. Briefly, 500 mg of the aqueous extract was compressed as a pill (the amount of the extract used was calculated in relation to the final concentration that exerts antioxidant activities in *in vitro* assays as additive (Micucci *et al*., 2011; Turner *et al*., 2011)) and then incubated with the simulated gastric juice at 37°C for 1 h (step 1). In step 2, the material obtained after incubation with the simulated gastric juice (chyme) was incubated with the intestinal fluid at 37°C, for 2 and 4 h. These steps were carried out to mimic the digestion process discussed previously. Samples obtained in steps 1 and 2 were analysed by HPLC (Goh and Barlow, 2004). In order to test the effect of pH on the extract stability and to determine solubility, controls were done by incubating the extract 1, 2 and 4 h at 37°C at pH 7.0, 1.2 and 6.8, without enzymes.

Polyphenols and flavonoids determination

Total polyphenols determination. Polyphenols were determined by spectrophotometry by the Folin–Ciocalteu's method using gallic acid as standard (Makkar *et al*., 1993; Hosseinzadeh *et al*., 2013). The lyophilized extract was weighed and dissolved in distilled water. Briefly, a sample of 1.0 mL of the extract and 1.0 mL of samples incubated in simulated gastrointestinal fluids were transferred to separate tubes containing 7.0 mL of distilled water, 0.5 mL of Folin–Ciocalteu's reagent and 1.5 mL of a 20% anhydrous sodium carbonate solution. Mixtures were then allowed to stand at room temperature for 60 min, and then, the absorbance at 765 nm was measured in an ultraviolet–visible spectrophotometer. The concentration of polyphenols in samples was calculated from a standard curve of gallic acid ranging from 10 to 50 μg/mL (Pearson's correlation coefficient: r^2 = 0.9996). Results were expressed as milligram of gallic acid equivalent per gram extract.

Total flavonoids determination. Flavonoids were determined by spectrophotometry. Briefly, the extract, without being incubated, and samples incubated in simulated gastrointestinal fluids were mixed with aluminium trichloride (10% *w*/*v*) and potassium acetate (1 M) and incubated during 30 min. The absorbance was then measured at 415 nm. Results were expressed as milligram of quercitrin per gram extract and derived from a calibration curve obtained with known concentrations of the flavonoid quercitrin (Sigma) (Dantas Fernandes *et al*., 2012).

Antioxidant activities

Diphenylpicrylhydrazyl scavenging activity. The antioxidant activity of the control extract and samples incubated in gastrointestinal fluids was determined by the assessment of the scavenger activity of the DPPH free radical (Sigma) according to the method described by Wang (Wang *et al*., 2007). The absorbance of DPPH diluted in water was taken as control. The antioxidant capacity to scavenge the DPPH radical was calculated by the following equation: Scavenging effect (%): [(1 – absorbance of sample/absorbance of control) × 100]. Results were expressed as mean ± standard error of the mean of three experiments made in triplicate.

Reducing power activity. The reducing power of the control extract and samples incubated in gastrointestinal fluids was determined using a mixture of phosphate buffer (200 mM, pH 6.6) and potassium ferricyanide (1% *w*/*v*). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10% *w*/*v*) was then added together with distilled water and $FeCl_3$ (0.1% *w*/*v*) and allowed to react for 30 min. The absorbance at 700 nm was measured in a spectrophotometer. The reducing power was directly proportional to the absorbance (Shyu *et al*., 2009).

Statistical analysis. The Student's *t*-test for unpaired measures was used to determine the level of significance. When multiple comparisons were necessary, the Tukey's test was used. Differences between means were considered significant when $p < 0.05$.

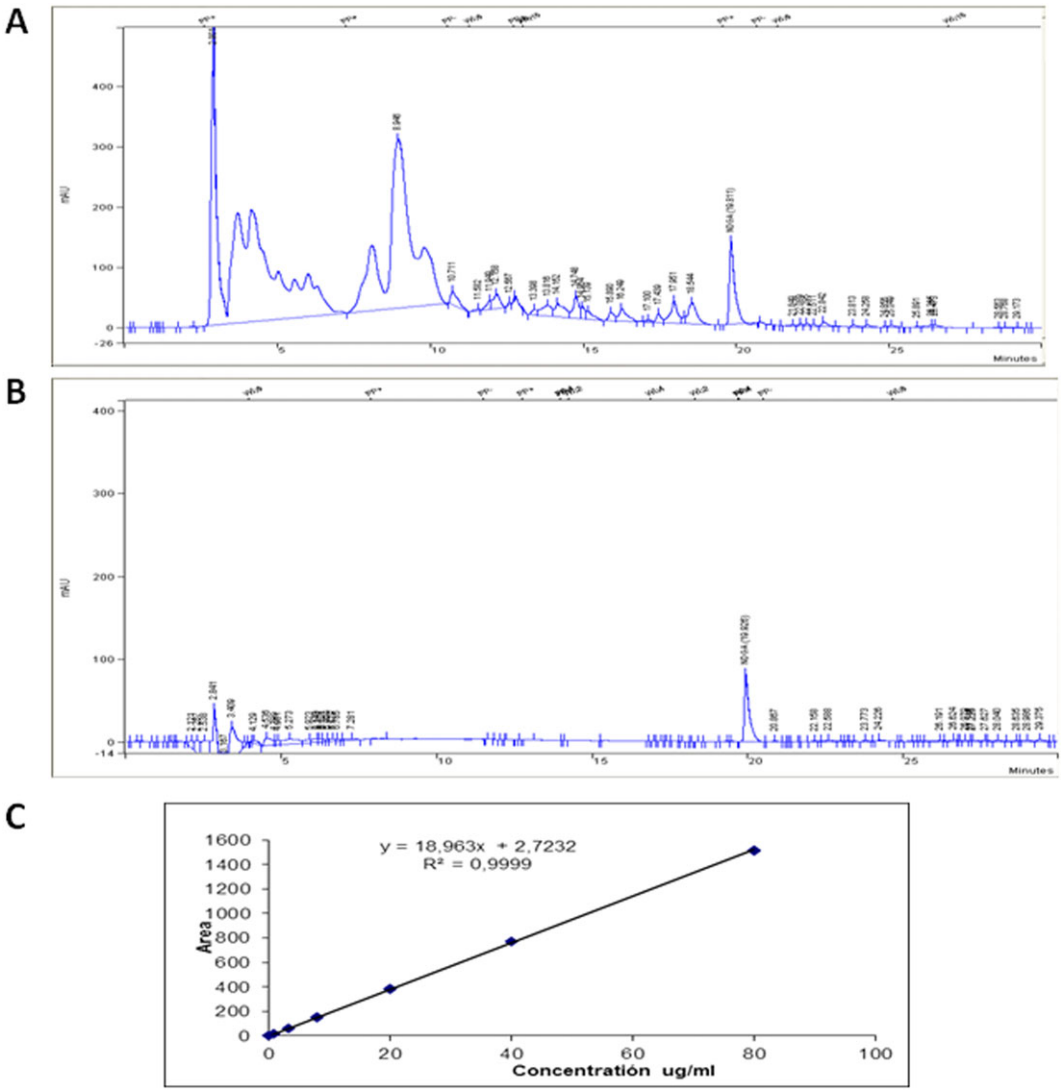

Figure 1. HPLC analysis of AE (A), NDGA (B) and NDGA calibration curve (C). The concentration of NDGA in samples was calculated from a standard curve ranging from 0.8 to 80 μg/mL. A linear response was observed within this concentrations range. AE, aqueous extract; NDGA, nordihydroguaiaretic acid. [Colour figure can be viewed at wileyonlinelibrary.com]

RESULTS AND DISCUSSION

In this work, the effect of intestinal and gastric fluids on the chemical composition and the antioxidant activity of *L. divaricata* extract was studied. Despite some chemical modifications occurring in the gastric and intestinal fluids, the extract retained its antioxidant activity.

The NDGA, the majority polyphenolic compound present in the extract, was identified and quantified by HPLC with the intention to work with a standardized extract (Fig. 1A and B). In order to validate the method, the accuracy and precision (repeatability) were determined. The method accuracy was determined by preparing recovery samples made up of known amounts of NDGA corresponding to three concentration levels (0.8, 8 and 80 μg/mL) in triplicate and within the linearity limits. A good correlation degree ($r^2 = 0.999$) was obtained between concentrations and absorbance of NDGA (Fig. 1C). The accuracy was expressed as the percentage of NDGA recovered in the assay. The method was found to be highly accurate, because the recovery ranged from 98.194% to 100.125%, while the average recovery and Standard Deviation (SD), considering all the concentrations, was 99.418 ± 1.064%, which is largely within the 90–110% range that is considered acceptable (Table 1). Intraday and interday assay precision was ascertained from the peak area ratios obtained by three concentration levels of NDGA (0.8, 8 and 80 μg/mL); the experiments were made in triplicate, intraday and in different days. The mean accuracy of each concentration of NDGA recovered was calculated. The percentage coefficient of variation (CV%) for each concentration of NDGA recovered was less than 3% (Table 1), compared with an acceptance value of 5%.

Taking into account these performance values, it can be concluded that the reverse phase HPLC for the determination of NDGA developed is simple, accurate and specific.

Table 1. Intraday and interday accuracy and precision

	Day 1	Day 2	Day 3	Precision	Accuracy
0.8 μg/mL					
Mean	0.78	0.76	0.81		
SD	0.03	0.02	0.01		
%CV	3.90	2.00	1.01		
%Bias	−2.52	−4.32	1.23		
				2.980	98.194
8 μg/mL					
Mean	8.03	8.09	7.92		
SD	0.07	0.05	0.10		
%CV	0.88	0.61	1.29		
%Bias	0.36	1.08	−0.50		
				0.332	100.125
80 μg/mL					
Mean	80.00	79.99	79.86		
SD	0.38	0.30	0.18		
%CV	0.47	0.37	0.23		
%Bias	−0.005	−0.012	0.005		
				0.121	99.935

CV, coefficient of variation; SD, standard deviation.

The stability of the extract was studied in an *in vitro* digestion model. Despite their limitations (i.e. static model of digestion), *in vitro* gastrointestinal models have been developed for the assessment of bioaccessibility. It has been demonstrated that the evaluation of bioaccessibility in *in vitro* models shows a good correlation with the results obtained in human studies and animal models (Biehler and Bohn, 2010).

The lyophilized extract compressed as a pill was incubated in simulated gastric fluid (1 and 2 h) and in simulated intestinal fluid (2 and 4 h). The percentage of recovery of NDGA was then assessed. The percentage of recovery of NDGA was slightly but significantly lower at shorter incubation times (Table 2). This finding is probably related to the poor water solubility of NDGA. The solubility and recovery could have been increased after longer times and higher incubation temperatures.

In all cases, the percentage of recovery of NDGA was high. Although the NDGA solubility in water was not determined, the high recovery values suggested not only that the pH conditions did not affect the NDGA solubility but also that this compound was not degraded by neither gastric nor intestinal enzymes.

Apart from NDGA, other polyphenols are present in the extract. Flavonoids and phenylpropanoid pathway intermediates such as *p*-coumaric acid, ferulic acid and sinapyl alcohol are found. Flavonoids have previously been described in *L. divaricata*; among them, glycosylated flavonoids such as the *O*-glycosides of quercetin and myricetin and a *C*-glycoside of apigenin have been found (Timmerman *et al.*, 1979). Quercetin-3-methyl ether and kaempferol-3,4′-dimethylether have been described in the aqueous extract of *L. divaricata* (Martino *et al.*, 2014; Martino *et al.*, 2016; Barboza *et al.*, 2006).

Taken these data into account, total polyphenols and total flavonoids were determined in gastric and intestinal fluids. A significant decrease (20%) in the total polyphenols content was observed only in intestinal fluids after 2 and 4 h of incubation. No differences between incubation times were observed (Fig. 2A). Furthermore, a slight decrease, 12% after 1 h and 18% after 2 h of incubation in gastric fluid, in the total flavonoids content was found. On the contrary, incubations in intestinal fluid

Table 2. Percentage of recovery of NDGA after the digestive process

	NDGA			
	Recovery percentage (%)			
	Gastric digestion		Instestinal digestion	
	1 h	2 h	2 h	4 h
	96.04	99.83	83.45	94.09
	91.76	93.75	82.53	95.56
	87.63	103.63	89.95	92.22
	88.81	95.04	84.88	97.38
	88.99	98.89	—	—
	84.34	99.48	—	—
Mean	89.60	98.44**	85.20	94.81**
SD	3.97	3.57	3.31	2.19
%CV	4.43	3.63	3.88	2.31

CV, coefficient of variation; NDGA, nordihydroguaiaretic acid.
**$P < 0.01$: significantly different (Student's *t*-test).

during 2 and 4 h provoked a higher decrease than gastric fluid, reaching values of 45% and 40%, respectively. No differences in the percentages between incubation times were observed (Fig. 2B). It could be suggested that the decrease in the total phenolic concentration at the duodenal phase of the *in vitro* digestion was related to the observed decline in the total flavonoids content.

The observed instability of flavonoids in gastrointestinal fluids was related to neither the temperature of incubation, nor to the pH, as no modifications were observed in controls of the extract incubated during 1, 2 and 4 h in the absence of enzymes and at pH values of 7.0, 1.2 and 6.8 (Fig. 2C and D). The latter conditions can be also regarded as a way of assessing the compounds solubility. As the recovery of total polyphenols and flavonoids was nearly 100%, it can be concluded that the extract was soluble under these experimental conditions.

As compounds were not modified by neither the temperature nor the pH conditions employed, it can be concluded that the only factor responsible for the degradation of such compounds was the presence of intestinal and, to a lesser extent, of gastric enzymes. It is known that polyphenols, mainly flavonoids, are affected under certain pH conditions, the presence of metals and enzymes. Although some authors have demonstrated that flavonoids such as dihydromyricetin, myricetin, myricitrin and quercetin display a pH-dependent instability (Xiang *et al*., 2017), we have demonstrated that, in this extract, total flavonoids were not affected by pH 1.2 and 6.8 despite some of them could be modified.

As stated previously, polyphenols are known to exert antioxidant activities. For example, the consumption of polyphenol-rich plant extracts results in an increase of the antioxidant capacity and in a reduction of lipid peroxidation in the plasma (Garcia-Alonso *et al*., 2006; Jensen *et al*., 2008), thus preventing the development of several oxidative diseases. In order to determine if there was a correlation between the level of compounds and the antioxidant activity, the DPPH scavenger activity and the reducing power were studied in simulated fluids. As shown in Fig. 3A, the DPPH scavenger activity decreased in intestinal fluids together with the decrease of flavonoids levels in the same fluid. Nevertheless, the percentage of decrease of the antioxidant activity was near 12% in comparison with the decrease of flavonoids levels, which reached a value of 45%. These results suggest that other polyphenols, such as NDGA, would compensate the decrease in the flavonoids levels. The same phenomenon was observed when the reducing power was assessed. Such activity decreased in the intestinal fluid but only at 2 h of incubation. After this time, the activity decreased only by 14%, suggesting that other polyphenols would compensate the decrease in the flavonoids levels in that fluid (Fig. 3B). The NDGA exerts well-known antioxidant activities. It has previously been demonstrated that NDGA presents DPPH scavenger activity and prevents linoleic acid peroxidation (Turner *et al*., 2011). Therefore, if the levels of NDGA did not change in gastric and intestinal fluids, this compound would preserve

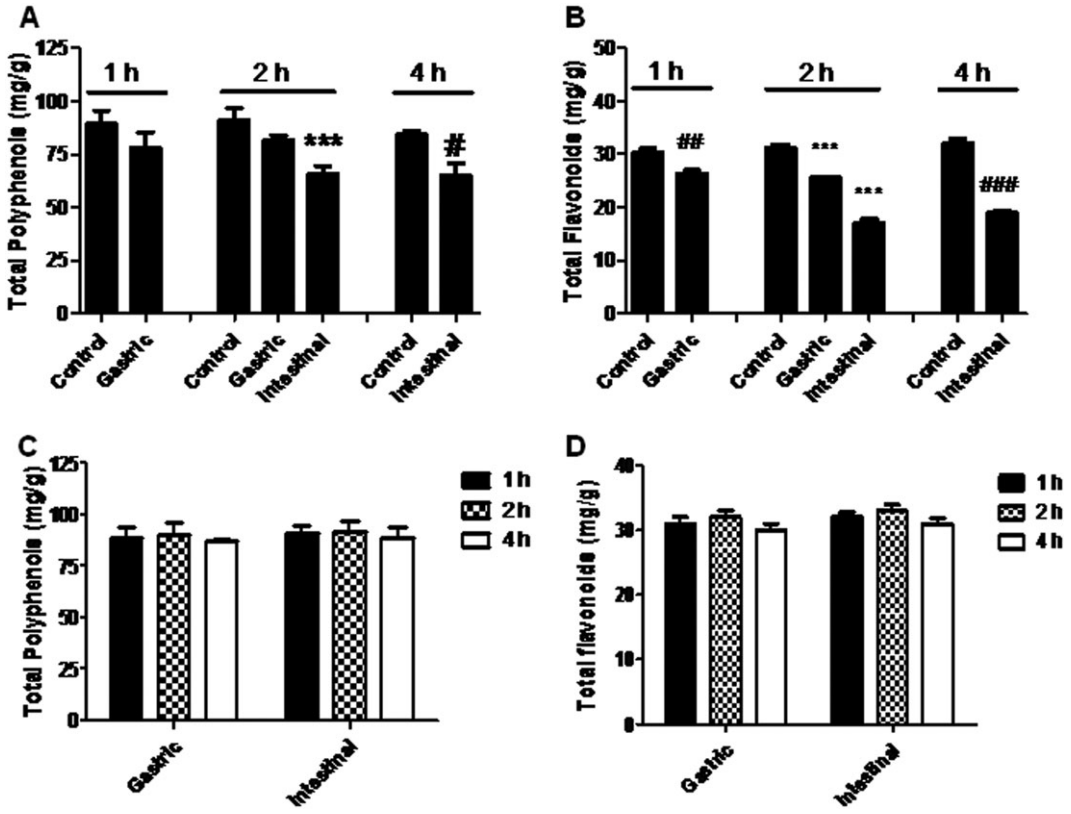

Figure 2. Total polyphenols and flavonoids during simulated digestive processes. Polyphenols and flavonoids were determined by spectrophotometry in extracts subjected to gastric and intestinal simulated digestion. (A,B) Polyphenols were expressed as milligram of gallic acid equivalent per gram extract; flavonoids were expressed as milligram of quercitrin per gram extract. Control: aqueous extract incubated at different temperatures without gastrointestinal fluids. (C,D) Controls extract done in gastric and intestinal fluids at pH 1.2 and 6.8 without enzymes. Results represent means ± standard error of the mean of three determinations. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$: significantly different with respect to control (Student's *t*-test). $^{***}p < 0.001$: significantly different with respect to control (Tukey's multiple comparison test).

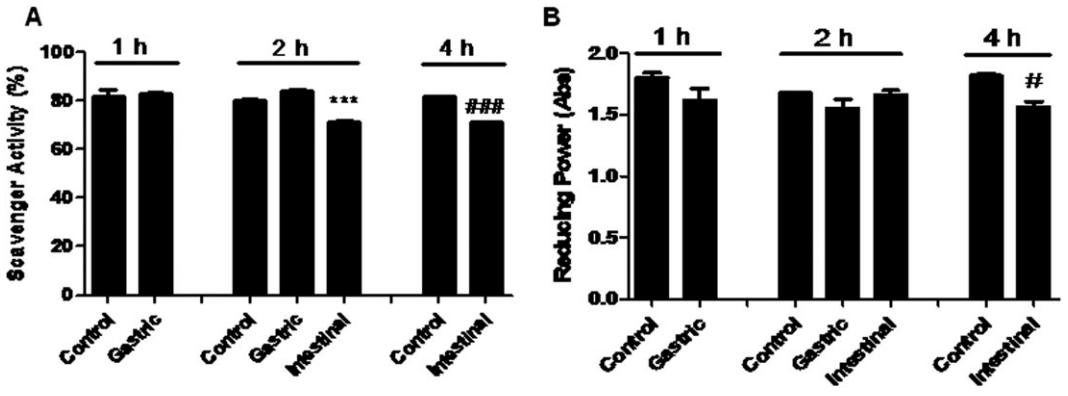

Figure 3. Antioxidant activities during simulated digestive process. Diphenylpicrylhydrazyl (A) and reducing power activities (B) were determined in extracts subjected to gastric and intestinal simulated digestions. Control: aqueous extract incubated at different temperatures without gastrointestinal fluids. Results represent means ± standard error of the mean of three determinations. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$: significantly different with respect to control (Student's *t*-test). $^{***}p < 0.001$: significantly different with respect to control (Tukey's multiple comparison test).

the antioxidant properties in the extract, despite the decrease in flavonoids levels.

It is noteworthy that the DPPH scavenging and the reducing activities are related to the presence of polyphenols, which are capable of donating hydrogen atoms and electrons. Not only NDGA but also other polyphenols could compensate the decrease of flavonoids in intestinal fluids, thus allowing the extract to retain its maximum antioxidant activity.

CONCLUSIONS

These results are promising and encourage the study of other pharmacokinetics parameters related to gastric and intestinal absorption and metabolism, which could support the use of the extract as a supplement or phytomedicine with antioxidant activity to be used by the oral route.

Acknowledgements

This work was supported by CONICET (grant number PIP 00067 CO). Authors thank Miss Rita Lemos for her technical assistance.

Conflict of Interest

There are no conflicts of interest.

REFERENCES

Alonso MR, García M, García Bonelli C, Ferraro G, Rubio M. 2006. Validated HPLC determination of cynarin in biological samples. *Acta Farm Bonaer* **25**(2): 67–70.

Alonso MR, Peralta IN, Lemos R, Davicino R, Anesini C. 2012. Influence of environment conditions on the chemical composition and antioxidant activity of *Larrea divaricata* aqueous extract. *Med Aromat Plant Sci Biotechnol* **6**(1): 28–32.

Anesini C, Pérez C. 1993. Screening of plants used in Argentine folk medicine for antimicrobial activity. *J Ethnopharmacol* **39**(2): 119–128.

Anesini C, Genaro A, Cremaschi G, Sterin Borda L, Cazaux C, Borda E. 1996. Immunomodulatory activity of *Larrea divaricata*. *Fitoterapia* **67**: 329–334.

Anesini C, Ferraro G, Lopéz P, Borda E. 2001. Different intracellular signals coupled to the antiproliferative action of aqueous extract from *Larrea divaricata* Cav and nor-dihydroguaiaretic acid on a lymphoma cell line. *Phytomedicine* **81**(1): 1–7.

Anesini C, Turner S, Borda E, Ferraro G, Coussio J. 2004. Effect of *Larrea divaricata* Cav extract and nordihydroguaiaretic acid upon peroxidase secretion in rat submandibulary glands. *Pharmacol Res* **49**: 441–448.

Barboza GE, Cantero JJ, Núñez CO, Ariza Espinar L. 2006. Flora Medicinal de la Provincia de Córdoba (Argentina). Museo de Botánica, UNC: Córdoba, Argentina.

Biehler E, Bohn T. 2010. Methods for assessing aspects of carotenoid bioavailability. *Curr Nutr Food Sci* **6**: 44–69.

Dantas Fernandes AJ, Ferreira MRA, Perrelli Randau K, Pereira de Souza T, Lira Soares LAL. 2012. Total flavonoids content in the raw material and aqueous extractives from *Bauhinia monandra* Kurz (Caesalpiniaceae). *Scientific World J* 923462 7. https://doi.org/10.1100/2012/923462.

Davicino R, Alonso R, Anesini C. 2011. "In vivo" and "in vitro"activity of *Larrea divaricata* Cav. on EL-4 cells. *Hum Exp Toxicol* **30**: 965–971.

Davicino R, Peralta I, Martino R, Alonso R, Anesini C. 2015. Preventive anti-inflammatory activity of an aqueous extract of *Larrea divaricata* Cav. and digestive and hematological toxicity. *Int J Pharm Sci Res* **6**(2): 1000–1010.

Garcia-Alonso J, Rosa G, Vidal-Guevera ML, Periago MJ. 2006. Acute intake of phenolic rich juice improves antioxidant status in healthy subjects. *Nutr Res* **26**: 330–339.

Goh LML, Barlow PJ. 2004. Flavonoid recovery and stability from *Ginkgo biloba* subjected to a simulated digestion process. *Food Chem* **86**: 195–202.

Hosseinzadeh R, Khorsandi K, Hemmaty S. 2013. Study of the effect of surfactants on extraction and determination of polyphenolic compounds and antioxidant capacity of fruits extracts. *PLoS One* **8**(3): e57353. https://doi.org/10.1371/journal.pone.0057353.

Jensen GS, Wu X, Patterson KM, *et al*. 2008. *In vitro* and *in vivo* antioxidant and antiinflammatory capacities of an antioxidant rich fruit and berry juice blend. Results of a pilot and randomized double blind, placebo controlled, crossover study. *J Agric Food Chem* **56**: 8326–8333.

Makkar HPS, Bluemmel M, Borowy NK, Becker K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J Sci Food Agric* **61**: 161–165.

Martino R, Canale F, Sülsen V, *et al*. 2014. A fraction containing kaempferol-3,4′-dimethylether from *Larrea divaricata* Cav. induces macrophages activation on *Candida albicans* infected mice. *Phytother Res* **28**: 917–924. https://doi.org/10.1002/ptr.5086.

Martino R, Barreiro Arcos ML, Alonso R, Sülsen S, Cremaschi G, Anesini C. 2016. Polyphenol rich fraction from *Larrea divaricata* and its main flavonoid quercetin-3-methyl ether induce apoptosis in lymphoma cells through nitrosative stress. *Phytother Res* **30**(7): 1128–1136. https://doi.org/10.1002/ptr.5615.

Micucci P, Alonso MR, Turner S, Davicino R, Anesini C. 2011. Antioxidant and antimicrobial activities of *Larrea divaricata* Cav. aqueous extract on vitamin C from natural orange juice. *Food Nutr Sci* **2**(1): 35–46.

Shyu YS, Lin JT, Chang YT, Chiang CJ, Yang DJ. 2009. Evaluation of antioxidant ability of ethanolic extract from dill (*Anethum graveolens* L.) flower. *Food Chem* **115**(2): 515–521.

Stege P, Davicino R, Vega A, Casali Y, Correa S, Micalizzi B. 2006. Antimicrobial activity of aqueous extracts of *Larrea divaricata* Cav. (jarilla) against *helicobacter pylori*. *Phytomedicine* **13**: 724–727.

Timmerman B, Valesi A, Mabry T. 1979. Flavonoids from *Larrea nitida*, *divaricata* and *cuneifolia*. *Rev Lat Quím* **10**: 81–83.

Turner S, Davicino R, Alonso R, Ferraro G, Filip R, Anesini C. 2011. Potential use of low-NDGA *Larrea divaricata* extracts as antioxidant in foods. *Rev Peru Biol* **18**(2): 159–164.

Wang KJ, Yang CR, Zhang YJ. 2007. Phenolic antioxidants from Chinese toon fresh young leaves and shoots of *Toona sinensis*. *Food Chem* **101**: 365–371.

Xiang D, Wang CG, Wang WQ, *et al*. 2017. Gastrointestinal stability of dihydromyricetin, myricetin, and myricitrin: an *in vitro* investigation. *Int J Food Sci Nutr* 1–11.