

Total antioxidant capacity and polyphenol content of 21 aqueous extracts obtained from native plants of Traslasierra valley (Argentina)

[Capacidad antioxidante y contenido de fenoles totales de 21 extractos acuosos de plantas nativas del Valle de Traslasierra (Argentina)]

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Abstract

The use of medicinal herbs derivatives is an alternative to conventional medicine to treat diseases associated with oxidative stress. In this study we assessed the antioxidant capacity (AC) and the total phenolic (TP) and flavonoid content (FV) of 21 extracts obtained from commercially available supplies of native plants of Traslasierra valley, Córdoba; *Hedeoma multiflorum*, *Mintostachys mollis*, *Lippia turbinata*, *Satureja parvifolia*, *Aloysia triphylla*, *Aloysia gratissima*, *Aloysia polystachya*, *Heterothalamus allienus*, *Xanthium spinosum*, *Gnaphalium gaudichaidianum*, *Flaveria bidentis*, *Hypericum connatum*, *Larrea divaricata*, *Aristolochia macroura*, *Erythraea quitensis*, *Geoffroea decorticans*, *Solanum rutilum*, *Urtica dioica*, *Usnea gracilis*, *Anemia tomentosa* and *Lycopodium saururus*. AC was assessed on the basis of different methods: The scavenging of stable free radicals ABTS and DPPH, the ability to reduce Fe³⁺ in the FRAP assay, and the capacity to inhibit the lipid peroxidation of copper-induced human plasma oxidation. All extracts were able to bleach the radicals in the range of 0.03 - 4.48 μmol of Trolox equivalent/mg dry extract. *S. parvifolia* and *H. connatum* extracts exhibited the highest scavenging activity for both, DPPH radical (1.48 and 1.77 μmol Tx eq/mg dry extract) and ABTS radical (3.20 and 4.48 μmol Tx eq./mg dry extract). *S. parvifolia*, *L. divaricata* and *H. connatum* showed the highest reducing capacity (8.89, 8.44 and 7.72 μmol eq. of ascorbic acid/mg dry extract) following by *H. allienus* and *H. multiflorum*. At 100 μg/mL, *S. parvifolia* and *H. connatum* also showed a very high capacity (> 85%) to inhibit Cu²⁺ - induced human plasma peroxidation. *E. quitensis*, *L. divaricata* and *H. multiflorum* were also potent inhibitors of lipoperoxidation. There was a significant correlation between the TP and FV content and the AC (P<0.001). Taken together, our results suggest that the aqueous extracts of *H. multiflorum*, *H. allienus*, *L. divaricata*, *H. connatum* and *S. parvifolia* may be important sources for the isolation of compounds with potential use as pharmacological or nutritional tools.

Keywords: Antioxidant capacity; Herbal antioxidant; ABTS; DPPH; Oxidative stress; Argentina

Resumen

El empleo de plantas medicinales constituye una alternativa a la medicina convencional para el tratamiento de patologías asociadas con la producción de estrés oxidativo. En este estudio evaluamos la capacidad antioxidante total (AC) y el contenido de fenoles (TP) y flavonoides (FV) de 21 extractos obtenidos de plantas nativas del valle de Traslasierra, Córdoba, Argentina. Las especies estudiadas fueron *Hedeoma multiflorum*, *Mintostachys mollis*, *Lippia turbinata*, *Satureja parvifolia*, *Aloysia triphylla*, *Aloysia gratissima*, *Aloysia polystachya*, *Heterothalamus allienus*, *Xanthium spinosum*, *Gnaphalium gaudichaidianum*, *Flaveria bidentis*, *Hypericum connatum*, *Larrea divaricata*, *Aristolochia macroura*, *Erythraea quitensis*, *Geoffroea decorticans*, *Solanum rutilum*, *Urtica dioica*, *Usnea gracilis*, *Anemia tomentosa* y *Lycopodium saururus*. AC se evaluó utilizando diferentes métodos: La capacidad de captación de los radicales libres estables ABTS y DPPH; la capacidad de reducción del Fe³⁺ mediante el ensayo FRAP y la capacidad de inhibición de la peroxidación lipídica del plasma humano inducida por cobre. Todos los extractos tenían capacidad de captación de ABTS y DPPH en el rango de 0,03- 4,48 μmol de Trolox equivalentes /mg extracto seco. La mayor potencia fue encontrada en *S. parvifolia* y *H. connatum* (1,48 and 1,77 μmol Tx eq. / mg extracto seco para DPPH y 3,20 and 4,48 μmol Tx eq./mg extracto seco para ABTS). *S. parvifolia*, *L. divaricata* y *H. connatum* mostraron la mayor capacidad reductora (8,89, 8,44 and 7,72 μmol eq. de ácido ascórbico/mg extracto seco) seguidos por *H. allienus* y *H. multiflorum*. A la concentración de 100 μg/mL, *S. parvifolia* y *H. connatum*, *E. quitensis*, *L. divaricata* y *H. multiflorum* mostraron también una muy alta capacidad de inhibición de la peroxidación lipídica del plasma inducida por Cu²⁺. Se encontró una significativa correlación entre el contenido de TP y FV y la actividad antioxidante (P<0,001). Tomados en conjunto, nuestros resultados permiten sugerir que los extractos acuosos de *H. multiflorum*, *H. allienus*, *L. divaricata*, *H. connatum* y *S. parvifolia* pueden constituir importantes fuentes para el aislamiento de compuestos con alta actividad antioxidante de uso potencial desde el punto de vista farmacológico y nutricional.

Palabras Clave: Capacidad antioxidante; Antioxidantes herbales; ABTS; DPPH; Estrés oxidativo; Argentina.

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INTRODUCTION

Medicinal herbs are some of the oldest medicines around the world and their increased use in recent years is an evidence of public interest in having alternatives to conventional medicine.

There is growing evidence that consumption of certain foods, dietary supplements or traditional beverages, guide to a reduction in some parameters of oxidative damage in biological systems (Aruoma et al., 2003; Juan et al., 2006). Oxidative damage is produced by reactive oxygen species (ROS) which are continuously produced during the aerobic life. ROS seems to be involved in the pathogenesis of several human diseases including cancer, diabetes, cardiovascular diseases, ageing, etc. (Gutteridge, 1994). Aerobic organisms are protected from oxygen toxicity by a natural antioxidant defence system constituted by enzymatic and non enzymatic mechanisms (Halliwell, 1996). If this endogenous system is inadequate for the purpose of scavenging the ROS completely, oxidative damage to important macromolecules occurs (Halliwell, 1999). ROS neutralization by natural antioxidants could attenuate the oxidative damage tissue (Delaporte et al., 2002).

The roles of medicinal herbs in the prevention and cure of disease associated with oxidative stress have been attributed, in part, to antioxidant properties of their constituents. Many herbs are rich in compounds with demonstrated antioxidant capacity, particularly the liposoluble and water soluble vitamins (E and C, respectively) and other compounds like the amphipatic polyphenols which can act as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators (Morel et al., 1998; Ulrich-Merzenich et al., 2009).

Frequently used plants in traditional medicine are assumed to be safe, due to their long-term use and natural origin. Many Elgorashi et al., 2002 tific articles have been published on natural products and their diverse effects, but each plant species has several different natural constituents, many of which have not been studied.

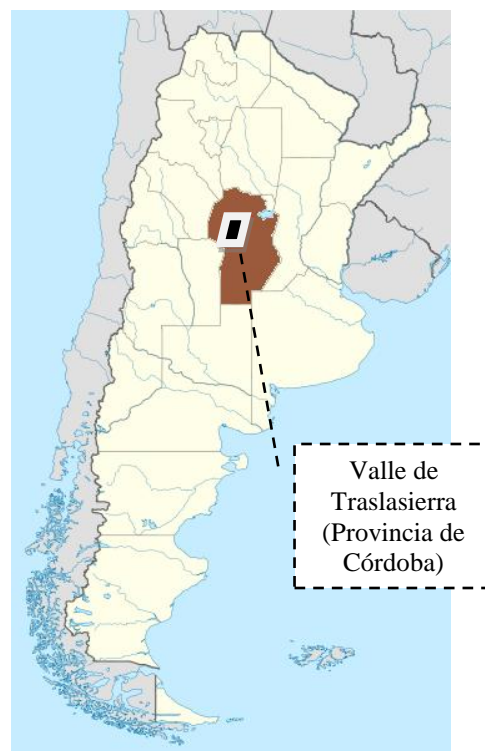
South America countries have a long history of using and producing medicinal and aromatic plants. In the Argentine traditional medicine, this kind of plants is used in the form of infusions or decoctions and this is a common practice among people of rural and also urban communities (Ratera and Ratera, 1980; Toursarkissian, 1980; Alonso, 2004).

Although many pharmacological as well as toxic actions have been reported for Argentinean plants, more information is needed about different properties of those plants widely used as foods, nutritional supplements or in the traditional medicine.

In Argentina, the Cordoba province is remarkable for its diversity and it is a rich source of medicinal plants, many of them used as part of folk remedies (Zuloaga, 1999).

In Cordoba, Traslasierra valley is today, one of main center in the country for medicinal and aromatic herbs production (Fig. 1).

Figure 1. Geographic location of Traslasierra valley, Córdoba, Argentina



Map from Wikimedia Commons.

Located at the foot of the highest peak in Cordoba, the Champaquí hill (2800 m.o.s.l.), the Traslasierra Valley is flanked to the east by the "Sierras Grandes or "Sierra de Comechingones", which drop abruptly downwards, and the "Sierras of Altautina, Pocho and Guasapampa" to the west.

Goleniowski et al. (2006) reported that medicinal native plants from Sierras Grandes make up approximately 30% of the total Argentina medicinal

native flora. More recently, 41 plants of Córdoba province were tested for their phenol content and antioxidant capacity using a model food system (Borneo et al., 2009)

Apart from these 41 plants, numerous other natives plants of Traslasierra valley are used in Argentina traditional medicine but they have not been yet investigated for their antioxidant activity. The aim of the present study was to examine the total antioxidant potential and the total phenolic and flavonoid content of 21 aqueous extracts obtained from native plants of Traslasierra valley (Table 1).

MATERIAL AND METHODS

Plant material and preparation of extracts

Commercially available supplies of dried plant materials used throughout the study were bought in bulk from the Almacén Natural Prama, Villa de las Rosas, Córdoba, Argentina.

Herbal samples were authenticated by Dr. E. Spegazzini (Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata) and they were free of preservative and artificial flavoring. Herbal were maintained preserved from light and humid and processed before 3 month of the purchased. Extracts were obtained from infusions or decoctions prepared in common way in which teas are consumed for human population.

Table 1. Botanical species and their popular use

Plant	Family	Popular name	Popular use
<i>Hedeoma multiflorum</i> (Benth)	Lamiaceae	Tomillo serrano	Digestive tract diseases, Flavoring
<i>Minthostachys mollis</i> (Kunt, Griseb)	Lamiaceae	Peperina	Anti-inflammatory, Digestive tract diseases, spasmolytic.
<i>Lippia turbinata</i> (Griseb)	Lamiaceae	Poleo	Flavouring, Digestive tract diseases, sedative
<i>Satureja parvifolia</i> (Philippi) Epling	Lamiaceae	Muña muña	Anti- rheumatic, spasmolytic, aphrodisiac
<i>Aloysia triphilla</i> (L'Hérit.) Britt.	Verbenaceae	Cedrón	Spasmolytic, sedative
<i>Aloysia gratissima</i> (Gill. et Hook)	Verbenaceae	Palo amarillo	Cholagogue, gastric and duodenal ulcer
<i>Aloysia polystachya</i> (Griseb et Moldenke)	Verbenaceae	Te de burro	Digestive tract diseases, gastric disorders
<i>Heterothalamus allienus</i> (Spreng.) O.Kuntze	Asteraceae	Romerillo	Antifungal, ornamental
<i>Xanthium spinosum</i> (Lam.)	Asteraceae	Cepa caballo	Diuretic
<i>Gnaphalium gaudichaidianum</i> DC	Asteraceae	Vira Vira	Antipyretic, antitussive, fatigue
<i>Flaveria bidentis</i> (L.) O. Kuntze	Asteraceae	Contrayerba	Antiseptic, vermifuge, diuretic
<i>Hypericum connatum</i> (Lam.)	Clusiaceae	Cabotoril	Cardiotonic
<i>Larrea divaricata</i> (Cav.)	Zygophyllaceae	Jarilla	Anti-rheumatic, antigout, bruises
<i>Aristolocchia macroura</i> (Gomez)	Aristolochiaceae	Mil hombres	Anti-rheumatic, diuretic
<i>Erythraea quitensis</i> (HBK)	Gentianaceae	Canchalagua	Anti-rheumatic, digestive tract diseases
<i>Geoffroea decorticans</i> (Gill. ex Hook. & Arn.) Burkart)	Fabaceae	Chañar	Antitussive, expectorant
<i>Solanum rutilum</i> (Gr.)	Solanaceae	Espina colorada	Diuretic, antigout
<i>Urtica dioica</i> (L.)	Urticaceae	Ortiga	Depurative, antihypertensive, diabetes
<i>Usnea gracilis</i> (Stirt)	Parmeliaceae	Barba de la piedra	Antimicrobial
<i>Anemia tomentosa</i> (Sav.) Swartz.	Esquiceaceae	Doradilla	Diuretic, antihypertensive, antitussive
<i>Lycopodium saururus</i> (Lam.)	Licopodeaceae	Cola de quirquincho	Aphrodisiac

Briefly, herbal samples (10 g) were weighed into 250 mL Erlenmeyer flasks and 200 mL of boiling distilled water were added and left to cool down to 40 °C. Decoctions were prepared by boiling 10 g of herbal samples in 250 mL of distilled water for 20 min. After cooling, the extractives were filtered, lyophilized and the dry matter was maintained at -20 °C until use it.

For bioassay analyses, samples of each extract were dissolved in dimethyl sulfoxide (DMSO, 10 mg/mL). Stock solutions were serially diluted with the solvent to obtain different concentrations (1-250 µg/mL).

Chemicals

2, 2-Diphenyl - 1-picryl hydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 2, 2'- azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), and quercetin-3-rutinoside (rutin), were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemical used were of the highest analytical grade.

Determination of total phenol and total flavonoid content

Total phenol concentration in selected medicinal plant extractives were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965), using caffeic acid as the standard and expressing the results as µmol equivalents of caffeic acid (CAE) per mg of dry extract.

Total flavonoids were estimated in the plant extract using a colorimetric method, based on the formation of a complex flavonoid-aluminium (Sakanaka et al., 2005). Briefly, aliquot of extracts or standard solutions (rutin) were mixed with 400 µl of distilled water and 30 µL of a 5% sodium nitrite solution. After 6 minutes, a 10% aluminium chloride sodium (30µl) was added and the mixture was allowed to stand for 5 minutes. After that, 200 µL of 1M NaOH solution and 240 µl of distilled water were added and the absorbance was measured at 510 nm. All determinations were done in triplicate and values were calculated from a calibration curve obtained with rutin. Final results were expressed as µmol of rutin equivalents (RE) per mg dry extract.

DPPH•radical scavenging activity

Reduction of this radical was determined according to Cavin (1998) with some modifications. For the assay, 10 µL of the plant extract was added to 990 µL of a 0.04 mg/mL DPPH solution in methanol. A series of concentrations ranging from 1 to 100 µg dry extract/mL were tested. The mixtures were shaken vigorously and incubated in the dark for 20 min after which the reduction of DPPH absorption was measured at 517 nm. A calibration curve was constructed by measuring the reduction in absorbance of the DPPH solution in the presence of different concentrations of Trolox (0-400 µM). Results were expressed as µmol of Trolox equivalents (TE)/ mg dry extract. All determinations were performed in triplicate. In the scavenging assays, DMSO was used as negative control.

ABTS⁺ radical scavenging activity

Other assay used to determine the antioxidant capacity of extracts was the TEAC assay (scavenging of the radical 2,2'-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid, ABTS) (Re et al., 1999) with some modifications. ABTS⁺ radical was generated by reacting 7 mM ABTS⁺ solution in water with 2.45 mM potassium persulfate in the dark for 12-16 h. Absorbance of the reactant was later adjusted to 0.700 ± 0.02 with PBS at a wavelength of 734 nm. Radical scavenging reaction was started by addition of 10µl of appropriately diluted extracts to 990 µl the ABTS⁺ solution. The absorbance of the mixture was recorded at 734 nm 25 min after addition of the sample. A Trolox calibration curve (0-200 µM) was constructed. Results were expressed as µmol equivalent of Trolox (TE)/mg dry extract. All determinations were performed in triplicate.

Ferric reducing activity (FRAP assay)

The ferric reducing activity of the plant extracts was estimated based on the FRAP assay (Benzie, 1996). The solutions for this assay consisted of 300 mmol/L acetate buffer, 10 mmol/L TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mmol/L of HCl and 20 mmol/L FeCl₃ · 6H₂O. Reagent for this assay was prepared on the day of assay by mixing 25 mL acetate buffer with 2.5 mL TPTZ solution and 2.5 mL FeCl₃ · 6H₂O.

The assay was performed as followed: 990 µL of the FRAP reagent were added to 10 µL of appropriately diluted extracts or buffer. Absorbance readings at 593 nm were recorded 20 min after the

start of the reaction. The change in absorbance was related to the absorbance changes of a standard solution of ascorbic acid tested in parallel. Results were expressed as μmol of ascorbic acid equivalents (AAE) / mg of dry weight of the extract.

Lipid peroxidation assays

Human plasma was oxidatively modified by a non-enzymic method. 100 μL of heparin plasma ($200 \pm 20 \mu\text{g}$ total cholesterol) was diluted with 350 μL PBS and the oxidation was started by adding 50 μL CuSO_4 10 mM. After 180 min of incubation at 37 °C the reaction was stopped by adding EDTA. These incubations and relevant controls were performed in the presence of the different extracts (100 $\mu\text{g}/\text{mL}$). Only one time point at 3 h for incubation was selected. This was in accordance with previous studies which indicated that this was the optimal period for oxidative modification of human plasma to occur. (Schinella et al., 2007). Thiobarbituric acid reactive substances (TBARS) production were used as an indicator of lipid peroxidation (Pompella et al., 1987). Results are expressed as percentage of inhibition related to controls without the extract. Butylated hydroxytoluene (BHT) was used as a positive control.

Statistics

Data were expressed as means \pm SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparisons test. Differences were considered significant at $p < 0.05$. The inhibitory concentration 50% (IC_{50}) was calculated from concentration/effect regression line.

RESULTS AND DISCUSSION

Scavenging of free radicals

The antioxidant capacities of plant extracts largely depend on their composition and conditions of the test system and they are influenced by many factors, which cannot be fully described with one single method. Therefore, it is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of the antioxidant action. The antioxidant activity of plant extract was evaluated on the basis of different methods: the scavenging effects of the stable ABTS and DPPH free radicals, the ability to reduce ferric(III) iron to ferrous (II) iron in the FRAP

reagent, and the capacity to inhibit the lipid peroxidation using as biological system the copper-induced human plasma oxidation.

Because of their high reactivity, most free radicals react rapidly with oxidizable substrates. Methods used for evaluation of radical-trapping properties often utilize stable model free radicals as indicators for radical-scavenging abilities, among which 1,1-diphenyl-2-picrylhydrazyl radical (DPPH \cdot) and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS $^{\cdot+}$), have gained the highest popularity.

Plants extracts may contain a wide variety of radical scavenging molecules, such as phenolic and nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites (Cai et al., 2004) which act by mechanisms that may involve hydrogen atom transfer or electron transfer (Litwinienko and Ingold, 2007; Musalik et al., 2009).

The results on the free radical scavenging activity of the different extracts are shown in Table 2. The data presented in this study demonstrate that all the reported species possess free radical scavenging activity although in a wide range.

The preparations were able to reduce the stable free radical DPPH to the yellow-coloured 1,1-diphenyl-2-picrylhydrazyl. The highest DPPH scavenging activity was observed with *H. conatum* and *S. parvifolia* extracts. (1.77 and 1.48 μmol TE/mg dry extract respectively). Lower but important scavenging activities were observed with *L. divaricata* and *H. multiflorum*. The lowest radical scavenging activity was exhibited by *U. dioica*, *L. saururus* and *U. gracilis* ($< 0.1 \mu\text{mol}$ TE/mg dry extract)

ABTS radical cation decolorization assay is another excellent tool for determining the antioxidant capacity of different compounds or herbal extracts.

In this assay, again *H. conatum* and *S. parvifolia* extracts showed the highest scavenging capacity (4.48 and 3.20 μmol TE /mg dry extract respectively). Lowest activity were observed with *U. dioica*, *L. saururus* and *U. gracilis* ($< 0.2 \mu\text{mol}$ TE/mg dry extract).

Reducing activity

Antioxidant activity of plant extracts was also tested using the FRAP assay, which is a simple assay that gives fast, reproducible results. Benzie and Strain (1996) FRAP is versatile and can be readily applied to different kind of materials like teas, wines, plant

extracts and biological fluids. In this assay, the antioxidant activity is determined on the basis of the ability of an extract to reduce ferric (III) to ferrous (II).

Table 2 show a wide range of differences in the reducing capacity of the studied extracts. The FRAP values varied from 0.28 to 8.89 μmol of AAE / mg dry extract. *S. parvifolia*, *L. divaricata* and *H. connatum* were found to have the highest antioxidant

activity (8.89, 8.44 and 7.72 μmol AAE/mg dry extract respectively) followed by *H. allienus* (5.20 μmol AAE/mg), *H. multiflorum* (3.66 μmol AAE/mg) and *A. macroua* (3.16 μmol AAE/mg). Lowest FRAP values were founded in *U. dioica*, *L. saururus* and *U. gracilis* extracts (< 0.5 μmol AAE/mg dry extract).

Table 2. Antioxidant activity of plant extracts

Plant	DPPH ^a	ABTS ^a	FRAP ^b	lipid peroxidation (% inhibition) ^c
<i>Hedeoma multiflorum</i>	0.70 \pm 0.12	1.02 \pm 0.04	3.66 \pm 0.40	89.4 \pm 3.2
<i>Minthostachys mollis</i>	0.27 \pm 0.04	0.70 \pm 0.02	2.32 \pm 0.34	23.1 \pm 9.9
<i>Lippia turbinata</i>	0.18 \pm 0.02	0.64 \pm 0.02	1.50 \pm 0.16	23.9 \pm 2.6
<i>Satureja parvifolia</i>	1.48 \pm 0.10	3.20 \pm 0.15	8.89 \pm 0.90	90.1 \pm 1.2
<i>Aloysia triphylla</i>	0.35 \pm 0.06	0.52 \pm 0.05	2.92 \pm 0.25	49.4 \pm 11.6
<i>Aloysia gratissima</i>	0.34 \pm 0.05	0.49 \pm 0.01	2.59 \pm 0.21	27.0 \pm 7.1
<i>Aloysia polystachya</i>	0.51 \pm 0.06	0.75 \pm 0.01	2.55 \pm 0.28	42.4 \pm 3.6
<i>Heterothalamus allienus</i>	0.59 \pm 0.05	2.41 \pm 0.04	5.20 \pm 0.32	91.2 \pm 2.0
<i>Xanthium spinosum</i>	0.12 \pm 0.02	0.53 \pm 0.02	0.88 \pm 0.08	ND
<i>Gnaphalium gaudichaidianum</i>	0.42 \pm 0.02	2.32 \pm 0.07	2.89 \pm 0.72	41.7 \pm 4.3
<i>Flaveria bidentis</i>	0.23 \pm 0.01	0.79 \pm 0.02	2.14 \pm 0.16	36.1 \pm 1.9
<i>Hypericum connatum</i>	1.77 \pm 0.09	4.48 \pm 0.07	7.72 \pm 0.67	86.5 \pm 3.9
<i>Larrea divaricata</i>	0.76 \pm 0.06	0.70 \pm 0.01	8.44 \pm 0.64	80.1 \pm 2.6
<i>Aristolocchia macroua</i>	0.10 \pm 0.01	0.49 \pm 0.03	3.16 \pm 0.39	60.4 \pm 4.3
<i>Erythraea quitensis</i>	0.39 \pm 0.04	0.76 \pm 0.02	2.43 \pm 0.14	83.7 \pm 2.0
<i>Geoffroea decorticans</i>	0.16 \pm 0.03	0.92 \pm 0.01	1.44 \pm 0.17	27.4 \pm 1.7
<i>Solanum rutilum</i>	0.20 \pm 0.03	0.74 \pm 0.02	1.11 \pm 0.10	38.1 \pm 1.3
<i>Urtica dioica</i>	0.03 \pm 0.01	0.19 \pm 0.02	0.49 \pm 0.04	28.0 \pm 2.6
<i>Usnea gracilis</i>	0.03 \pm 0.01	0.16 \pm 0.01	0.28 \pm 0.02	ND
<i>Anemia tomentosa</i>	0.43 \pm 0.03	1.28 \pm 0.08	2.42 \pm 0.54	20.6 \pm 1.0
<i>Lycopodium saururus</i>	0.04 \pm 0.02	0.16 \pm 0.01	0.48 \pm 0.02	13.5 \pm 2.3

^a Results are expressed as μmol equivalents of Trolox/ mg dry extract

^b Results are expressed as μmol equivalents of ascorbic acid/mg dry extract

^c Extracts were assessed at a final concentration of 100 $\mu\text{g}/\text{ml}$

ND: not determined

Inhibition of lipid peroxidation

Proteins, nucleic acids and lipids are significant targets of cellular injuries. Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids components of different cellular structures. (Janero, 1990)

Metal ions, at micromolar concentrations, plays a role in human plasma lipids oxidation (Gaut and Heinecke, 2001). It has been suggested that low density lipoprotein (LDL) oxidation induced in vitro in whole plasma is expected to reflect the oxidation in vivo more adequately than *in vitro* oxidation of the isolated lipoprotein (Spranger et al., 1998). For that reason we assessed the antioxidant capacity of extracts for lipoperoxidation protection using whole human plasma. All extracts were tested at a final concentration of 100 µg/mL and the inhibition of human plasma lipids peroxidation was assayed by the TBARS test.

Almost all tested extracts inhibited peroxidation and among those, *S. parvifolia*, *L. divaricata*, *H. connatum*, *E. quitensis*, *H. multiflorum* and *H. allienus* were the most active extracts with values of percentage of inhibition greater than 80% while *A. tomentosa* and *L. saururus* showed the lowest activity (< 20% of inhibition).

Total phenolics and flavonoids content

Table 3 reports the results of percentage of yield, the total phenolics and total flavonoids content of each plant extract. The amounts of total phenolics varied widely in the different analysed extracts and ranged from 0.16 to 2.07 µmol equivalents of caffeic acid/mg of dry extract. This variation can be expected for plant extracts due to the presence of other constituents and/or the presence of different types of phenols. Among plant extracts, *H. connatum*, *S. parvifolia* and *A. triphylla* contained the highest amount of phenolics (2.07, 1.90 and 1.70 µmol CAE/mg dry extract respectively). The lowest level of phenolic content was observed in *U. dioica*, *U. gracilis* and *L. saururus*. (0.21, 0.19 and 0.16 CAE/mg dry extract, respectively).

Among plant extracts, *H. connatum* and *S. parvifolia* contained the highest amount of flavonoids (0.94 and 0.91 µmol RE/ mg dry extract) whereas the lowest level was found in *U. gracilis*, *L. saururus*, *U. dioica* and *A. macroura* (0.05 to 0.08 RE/mg dry extract).

Several studies have evaluated the relationships between antioxidant activities of plant products and their phenolic contents (Shahidi, 2003). In our case, comparison of total phenolics and flavonoid contents with antioxidant activities show significant correlations (Fig. 2).

In our work, *L. divaricata* and *A. macromoura* extracts showed a high ability to reduce the FRAP reagent and to inhibit the lipoperoxidation with relatively low concentrations of total phenols and flavonoids.

This relatively high antioxidant activity of extracts with low phenolic content suggests that the type of phenolics could be a determinant of these activities rather than their amounts.

Conversely, *A. triphylla* is relatively rich in total phenolics, but its activity as scavenger of free radicals is low.

Our results agree with those of Shahidi (2003) who reported that differences in antioxidant activities of plant extracts could be due to different qualitative and quantitative composition of their phenolic constituents.

The data presented in this study demonstrate that almost all the reported species possess antioxidant activity. Indeed, their aqueous extracts scavenged the free radicals DPPH and ABTS, reduced the Fe⁺³ of FRAP reagent and inhibited the lipid peroxidation of human plasma.

Among the assessed extracts, *Satureja parvifolia* (muña muña) and *Hypericum connatum* (cabotoril) showed the highest activity to scavenge DPPH and ABTS free radicals, to reduce the Fe(III) to Fe (II) in FRAP reagent and a very high capacity to inhibit lipid peroxidation of human plasma. Also *Heterothalamus allienus* demonstrated high capacity to scavenge ABTS radical, reducing activity and inhibition of lipid peroxidation.

Oxidative damage is considered a likely cause of arsenic-induced tissue damage, particularly those with high oxygen consumption rate. The high antioxidant activity of *H. allienus* could explain the demonstrated protective activity of this plant against the arsenite-induced renal injury (Soria et al., 2008).

On the other hand, some biological and pharmacological actions have been reported for *S. parvifolia* and *H. connatum*, the two plants with the highest antioxidant capacity in the all studied models.

S. parvifolia showed antiprotozoal and antiplasmodial activity (Van Baren et al., 2006) and it was very active against different microorganisms and

was suggested that this might be due to high concentration of flavonoids (Hernandez et al., 2000)

H. connatum also demonstrated to have other pharmacological actions.

Previous investigations of extracts of this plant showed them to be active against the feline immunodeficiency virus (FIV), which shares biological and pathogenic features with the human immunodeficiency virus (HIV) (Schmitt et al., 2001). In Argentina, the plant is widely used as cardiogenic

and recently antiherpetic and antifungal activities were also demonstrated. (Fusco et al., 2007; Fritz et al., 2007).

Nevertheless, to our best knowledge, the potent antioxidant properties of these two plants have not been yet reported. Further investigations are necessary to determine which components of muña muña (*Satureja parvifolia*) and cabotoril (*Hypericum connatum*) extracts are responsible for such antioxidant capacity.

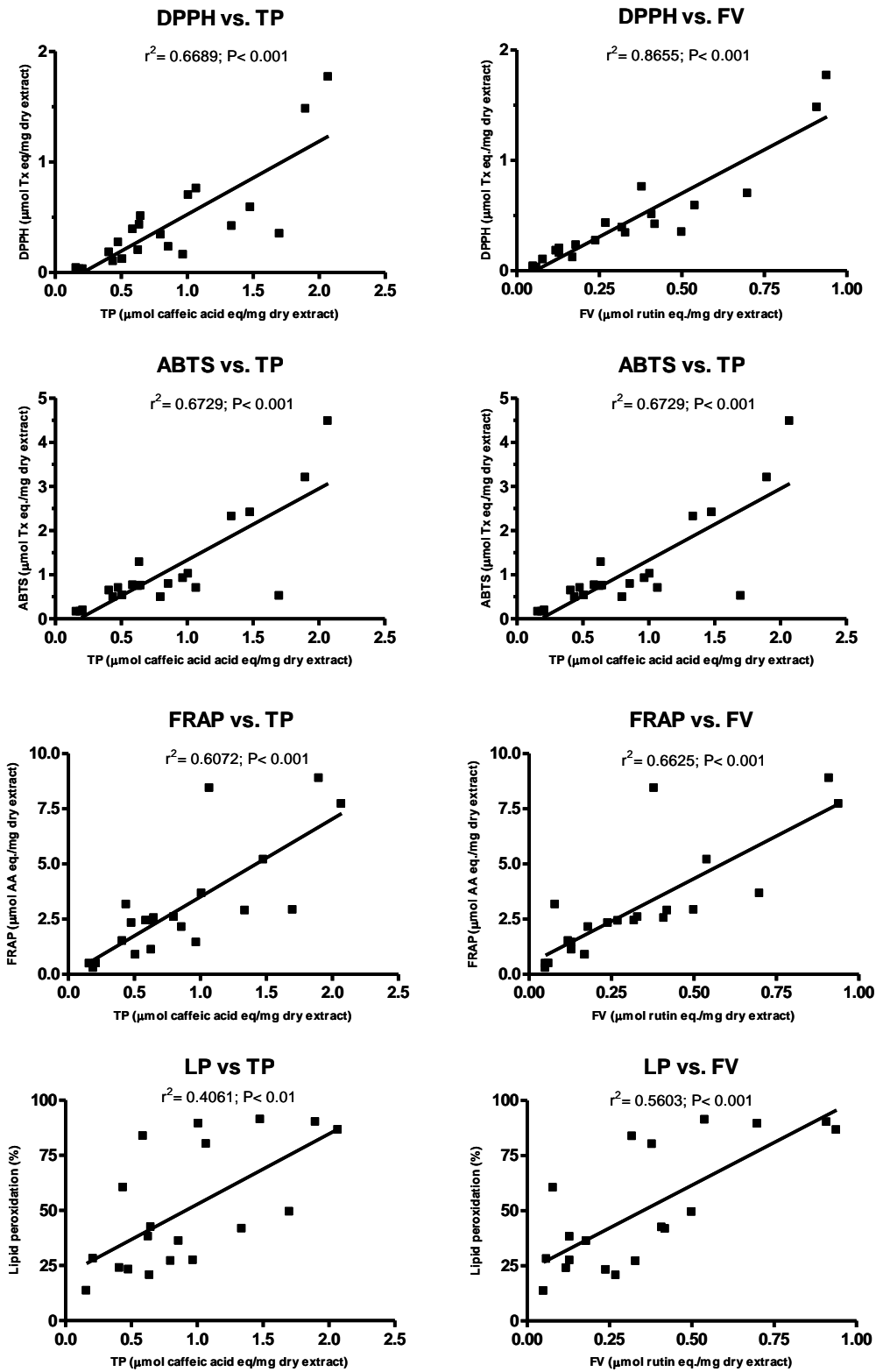
Table 3. Extracts yield and total content of phenolics and flavonoids

Plant	Extract	Yield (%)	Total polyphenols*	Total flavonoids**
<i>Hedeoma multiflorum</i>	infusion	12.9	1.01 ± 0.04	0.70 ± 0.07
<i>Minthostachys mollis</i>	infusion	20.8	0.48 ± 0.01	0.24 ± 0.01
<i>Lippia turbinata</i>	infusion	16.8	0.41 ± 0.04	0.12 ± 0.01
<i>Satureja parvifolia</i>	infusion	14.9	1.90 ± 0.05	0.91 ± 0.02
<i>Aloysia triphylla</i>	infusion	14.9	1.70 ± 0.19	0.50 ± 0.04
<i>Aloysia gratissima</i>	infusion	17.6	0.80 ± 0.03	0.33 ± 0.03
<i>Aloysia polystachya</i>	infusion	14.4	0.65 ± 0.04	0.41 ± 0.01
<i>Heterothalamus allienus</i>	infusion	15.9	1.48 ± 0.02	0.54 ± 0.03
<i>Xanthium spinosum</i>	decoction	23.1	0.51 ± 0.02	0.17 ± 0.02
<i>Gnaphalium gaudichaidianum</i>	infusion	5.9	1.34 ± 0.04	0.42 ± 0.02
<i>Flaveria bidentis</i>	decoction	12.8	0.86 ± 0.01	0.18 ± 0.02
<i>Hypericum connatum</i>	infusion	16.1	2.07 ± 0.14	0.94 ± 0.02
<i>Larrea divaricata</i>	infusion	17.1	1.07 ± 0.02	0.38 ± 0.03
<i>Aristolocchia macroura</i>	decoction	12.1	0.44 ± 0.05	0.08 ± 0.01
<i>Erythraea quitensis</i>	infusion	18.5	0.59 ± 0.04	0.32 ± 0.02
<i>Geoffroea decorticans</i>	decoction	7.1	0.97 ± 0.01	0.13 ± 0.01
<i>Solanum rutilum</i>	decoction	17.8	0.63 ± 0.03	0.13 ± 0.02
<i>Urtica dioica</i>	infusion	18.5	0.21 ± 0.02	0.06 ± 0.02
<i>Usnea gracilis</i>	decoction	9.9	0.19 ± 0.02	0.05 ± 0.01
<i>Anemia tomentosa</i>	infusion	14.9	0.64 ± 0.01	0.27 ± 0.03
<i>Lycopodium saururus</i>	infusion	5.2	0.16 ± 0.01	0.05 ± 0.02

* Results are expressed as μmol of caffeic acid equivalents / mg dry matter.

** Results are expressed as μmol of rutin equivalents /mg dry matter.

Figure 2. Correlations between total phenolics and flavonoids content and antioxidant activity.



CONCLUSION

Extracts of vegetables rich in phenolics are increasingly of interest in several fields. In the food industry because they could retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. From the medical point of view, supplementation with antioxidants would be useful for diseases associated with oxidative stress.

In terms of *in vitro* antioxidant activities and phenolic content, *Hedeoma multiflorum*, *Heterothalamus allienus*, *Larrea divaricata*, and particularly *Hypericum connatum* and *Satureja parvifolia*, could be excellent sources of antioxidant products with potential use as pharmacological or nutritional tools.

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