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Effect of different provenances and production conditions on antioxidant properties in ***Buddleja globosa*** leaves

[Efecto de diferentes procedencias y condiciones de cultivo sobre propiedades antioxidantes en hojas de *Buddleja globosa*]

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Abstract

Buddleja globosa Hope (matico) is a medicinal shrub native to Chile whose leaves have been traditionally used for wound and ulcer healing, pathologies associated to oxidative stress. Matico leaves display a high content of polyphenols, compounds with recognized antioxidant capacity, which may contribute to its therapeutic properties. Several factors, however, can modify the polyphenol content of matico leaf extracts, including plant material, production techniques, provenances, leaf age, harvest time, irrigation, and desiccation procedures. Thus, standardized leaf extracts prepared with plants from different provenances and harvest conditions were compared in terms of polyphenol content and their protecting antioxidant effects on rat liver microsomal lipids and thiol groups. All factors tested, but irrigation, changed both polyphenol content and antioxidant properties of matico extracts; water stress only affected their antioxidant properties without changing their polyphenol content. Correlation between polyphenol content and lipid peroxidation inhibition was only significant in the provenance study.

Keywords: *Buddleja globosa* Hope; lipid peroxidation; thiol group oxidation; rat liver microsomes, polyphenol content; harvest time; provenances; irrigation

Resumen

Buddleja globosa Hope (matico) es un arbusto medicinal nativo de Chile cuyas hojas han sido utilizadas en la medicina tradicional como cicatrizante en caso de patologías relacionadas con el estrés oxidativo. Las hojas tienen un alto contenido de polifenoles, compuestos con reconocidos efectos antioxidantes relacionados con la inhibición de la lipoperoxidación. Varios factores pueden afectar a su contenido, entre ellos el origen de la planta, edad de la hoja, momento de cosecha, riego y métodos de secado. En extractos estandarizados preparados de hojas de diferentes tratamientos se compararon el contenido de polifenoles y los efectos antioxidantes protectores de lipidos y grupos tioles microsomales. Todos los ensayos de cultivo y postcosecha mostraron diferencias significativas entre los tratamientos, excepto para la inhibición de lipoperoxidación en el tratamiento de riego. Plantas de diferente origen muestran que el contenido de polifenoles en las hojas es determinado genéticamente y sufre variaciones por efectos ambientales.

Palabras Clave: inhibición de la lipoperoxidación; protección de tioles; contenido de polifenoles; poscosecha; provenencia; riego**.**

List of abbreviations: cb - centibar.

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INTRODUCTION

Buddleja globosa Hope (Buddlejaceae), known in Chile as "Matico", is a shrub whose perennial leaves are traditionally used for healing wounds and ulcers (Mellado *et al*. 1996). They contain phenylpropanoids, iridoids, terpenes and flavonoids (Houghton and Mensah 1999; Mensah *et al*., 2000; Montes and Wilkomirsky, 1987). In recent pharmacological studies different medicinal properties were shown: antioxidant, anti-inflammatory, cicatrizing and analgesic activities (Liao *et al*., 1999; Mensah *et al*., 1998; Mensah *et al*., 2001; Vargas 2001) as well as the regeneration of the mucous membrane in the case of ulcers (Rodríguez *et al*., 2001; Yañez 2001). In general, all the pathologies involve an inflammatory phase in which tissue is dangerously oxidized by reactive oxygen species (ROS). When the production of ROS surpasses the cellular antioxidant capacity, oxidative stress occurs. Polyphenols, including flavonoids, are widely recognized because of their antioxidant properties; they act as free radicals scavenger, especially those derived from molecular oxygen, anion superoxide ($O_2^{\cdot-}$) and hydroxyl radical ($HO^{\cdot}$) (Haliwell, 2007). Polyphenols also act as chelating agents of transition metals such as iron and copper, which generate oxygen free radicals through Haber-Weiss and/or Fenton reactions (Haliwell and Gutteridge, 1990). Moreover, some natural terpenoids are known to inhibit some enzymes belonging to the inflammatory cascade (COX-2 and 5-lipooxigenase) (Liao *et al.*, 1999). The high content of polyphenols and terpenoids in leaves may explain the therapeutic activity of *B. globosa* preparations.

B. globosa is traditionally grown in home gardens for domestic use. Commercial production is based principally on wild collection or on recently established plantations. There are no previous studies on the genetic or environmental variability that would permit the optimization of quality factors such as concentration and activity of compounds through the selection of genetic material or development of management and post harvest techniques.

On the other hand, there is a wide array of commercially available matico preparations, which include crushed plant drug for infusions, leaf extracts prepared through different extraction processes as well as in mixture with other plant species. Due to the distinct physicochemical properties displayed by active principles in a whole extract, including polarity and solubility, different solvents used for extraction will impact the relative content of each principle in the final extract. Thus, the pattern of active principles in these preparations may differ in quality and quantity and, consequently, in their therapeutic properties. Therefore, it is necessary to control the high number of variables leading to obtain standardized preparations which allow demonstration of clinical efficacy and therapeutic safety. The goal of this work is to evaluate the influence of provenance and postharvest procedures on the polyphenol content and antioxidant properties of matico leaf extracts.

To this end, six provenances, three of wild origin and three taken from home garden plants, cultivated in the same environment were studied. In two of the wild provenances, both cultivated plants and wild individuals from the natural populations were compared. The harvest time and distribution of the active compounds within the plant were studied, as well as the activity of well watered and stressed plants. The study of different drying methods even included some treatments not recommended for medicinal plants such as sun light, washing and high temperatures.

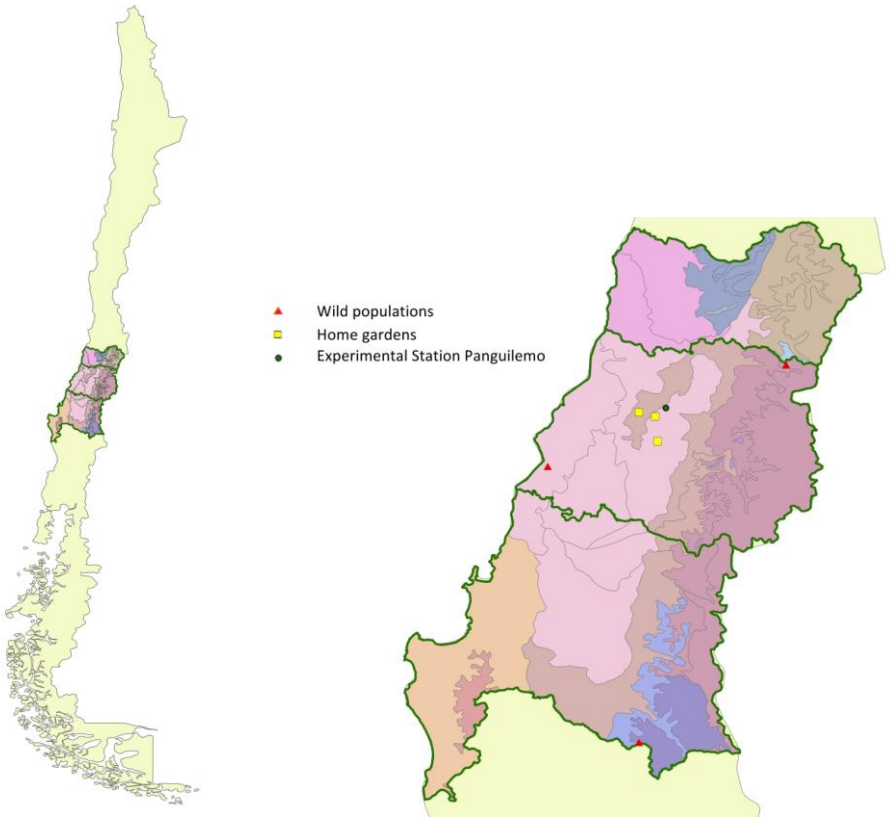
Leaves were selected for the preparation of extracts due to their high polyphenol content, the main responsible of the antioxidant activity of herbal preparations. These features attesting to these active principles as good markers to evaluate the various processes involved to reach an herbal formulation. Thus, standardized hydroalcoholic extracts from *B. globosa* leaves from different provenances were assayed for polyphenol content and the protecting antioxidant effects on lipids and thiol groups occurring in rat liver microsomes.

MATERIAL AND METHODS

Plant material

Six different provenances of *B. globosa* were studied (Figure 1), three of them of wild origin (Los Ruiles, 35°49'S 72°32'W 170 m above sea level [asl] with maritime influenced climate and two mountain sites: Los Queñes, 35°02´S 70°37'W 809 m asl; Tolhuaca, 38°13'S 71°48'W 830 m asl) and three found in home gardens (Pencahue, 35°23'S 71°48'W, 76 m asl, Talca, 35°25'S 71°40'W 92 m asl, San Javier, 35°37'S 71°39'W 140 m asl located in the central valley of Chile with Mediterranean climate).

Figure 1. Location of the sampled plantations and wild populations of *Buddleja globosa*



Leaf samples of five plants for each provenance were taken in November when the cloned plants cultivated in a random design in the Experimental Station Panguilemo (35°21'S 71°35'W 117 m asl, Mediterranean climate) were five years old. To study the effect of wild versus cultivated conditions, leaves of the corresponding cultivated plants and wild populations of Los Ruiles and Los Queñes were gathered at the same time. All treatments were analyzed in four replicates.

Leaf ages

Young, mature and senescent leaves were harvested in January from five randomly chosen plants cultivated in the Experimental Station Panguilemo (Mediterranean climate), and analyzed in four replicates. The age of leaves was assigned according to their upper, central and lower position within the branches, respectively.

Irrigation studies

The effect of irrigation was studied in plants cultivated in a split plot design with two irrigation levels where the different provenances were arranged randomly within each plot. Two rows of border plants separated the treatments. One of the plots was watered when the soil humidity reached 20 cb (well watered plants) and the other at 40 cb (stressed plants). The soil humidity was determined by three tensiometers per treatment at a 20 cm depth. Leaf samples of both treatments were taken at the end of the season, in March, from five randomly chosen plants and analyzed in four replicates.

Seasonal effect

To assess the seasonal effect five randomly chosen plants were harvested monthly during the growing season starting in November (mid-spring in the southern hemisphere) when the plants were forming new leaves, until March (end of summer) when the lower leaves had become senescent. All samples were analyzed in four replicates.

All leaf samples of the former experiments were immediately taken to the laboratory and dried under environmental conditions protected from direct sun light.

Postharvest procedures

At the end of December until the first days of January, when the environmental temperatures were highest, leaves of the post-harvest experiment were dried under the following conditions: shade (inside the laboratory, at environmental temperature of about 25°C), exposed to full sun light (outside), washed and successive drying in oven at 40°C, oven drying at 40°C, 60°C, and 80°C.

Extraction

Dry leaves were extracted by Laboratorios Ximena Polanco. All extracts were prepared at the concentration 1:5 (g of vegetable drug: mL hydroalcoholic solution) and the final alcoholic degree of all of them was 50%.

Animals for antioxidant experiments

Adult male Sprague Dawley rats (200-250 g), derived from a stock maintained at the Universidad de Chile, were used. They were allowed free access to pelleted food, maintained with controlled temperature (22ºC) and photoperiod (lights on from 07:00 to 19:00 h). Protocols approved by the Institutional Ethical Committee of Chemical and Pharmaceutical Sciences School, Universidad de Chile were used for all animal procedures.

Microsomal preparation for antioxidant experiments

Animals were fasted for 15 h with water *ad libitum* and sacrificed by decapitation. Livers were perfused *in situ* with 4 volumes of 25 ml 0.9% w/v NaCl, excised, and placed on ice. All homogenization and fractionation procedures were performed at 4ºC and all centrifugations were performed using either a Suprafuge 22 Heraeus centrifuge or an XL-90 Beckmann ultracentrifuge. Liver tissue (9 -11 g wet weight), devoid of connective and vascular tissue, was homogenized with five volumes of 0.154 M KCl, with eight strokes in a Dounce Wheaton B homogenizer. Homogenates were centrifuged at 9,000xg for 15 min; sediments were discarded and supernatants were centrifuged at 105,000xg for 60 min. Sediments (microsomes, enriched in endoplasmic reticulum) were stored at –80ºC until use. Microsomal protein was determined according to Lowry et al. (1951).

Determination of polyphenols

The principal antioxidant property of polyphenols is based in the capacity to scavenge free radicals, a phenomenon which is evaluated in this study. Thus, the total polyphenol concentration of *B. globosa* extracts was determined essentially by the method described by Price et al. (1989). The mixture reaction contained in a final volume of 5 mL: herbal extract 50 µL, Folin Ciocalteau reagent 250 µL, 20% w/v sodium carbonate 750 µL and distilled water 3,950 µL. Blanks contained all the reagents except the herbal extract. Then, reaction mixtures were incubated for 2 hours in darkness. At the end of this period, the absorbance was determined at 760 nm in a UV3 Unicam UV-VIS spectrophotometer, using their respective blanks as reference. Catechin ([Catechin(+)-cyanidol-3-(2R,3S)-2-4,4-dihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyrane-3,5,7-triol-(+)-trans-3,3´,4´,5,7-pentahydroxy-flavone]) was used as reference standard; this compound is widely recognized as an antioxidant agent.

Microsomal lipid peroxidation assay

The extent of microsomal lipid peroxidation following Cu^{2+}/ascorbate preincubation was estimated by determining TBARS according to Letelier *et al.* (2005). Mixtures (1 mL final volume) contained 1 mg/mL microsomal protein, 25 ηM $CuSO_4$, 1 mM sodium ascorbate, 50 mM phosphate buffer, pH 7.4. Blanks contained all the reagents but microsomal protein. Blanks and samples were incubated for 20 min at 37ºC with constant agitation. Afterwards, 250 µL of TCA 0.24 M (4ºC) were added and all mixtures were centrifuged at 10,000 x g for 10 min at 4ºC using a Suprafuge 22 Heraeus. Then, mixtures of 500 µL of the supernatants and 500 µL of 35 mM TBA were incubated at 50ºC for 1 hour. At the end of this period, the absorbance of the sample was measured at 532 nm in a UV3 Unicam UV-VIS spectrophotometer, using their respective blanks as reference. Results are expressed in ηmols of TBARS conjugated/min/mg of microsomal protein using the extinction coefficient 156 mM^{-1} x cm^{-1} of malondialdehyde as reference. Reaction rates were determined at conditions where product formation was linearly dependent to time and protein concentration.

Values for inhibition of lipid peroxidation were obtained with 0.16µL of each extract, corresponding to the IC_{50} of the Pencahue provenance; a 20-fold higher volume (3µL) of each extract was used for protection of thiol groups.

Microsomal thiol content

Microsomal thiols were titrated with DTNB. Microsomes (1 mg/mL total protein) were incubated with 25 ηM Cu^{2+}/1 mM ascorbate for 30 min at 37ºC. Afterwards, microsomal thiol content was titrated with DTNB according to Letelier et al. (2005). Thiol concentration was estimated by the equimolar apparition of 5-thio-2-nitrobenzoic acid (ε_{410} = 13,600 M^{-1} cm^{-1}). Reaction rates were determined at conditions where product formation were linearly dependent to time and protein concentration.

Statistical Analyses

Data of at least four independent determinations were analysed by ANOVA and means separated by LSD, Tukey or Kruskal-Wallis ($p \leq 0.05$), according to the data conditions given in each assay.

Correlation analysis was performed using Pearson regression and r^2 coefficients were considered statistically significant when $p<0.05$.

RESULTS

Provenances

The cultivated plants of the six assessed provenances showed significant differences in polyphenol content, antilipoperoxidant effects and protection in the protein thiol groups. Polyphenol content ranged from 47.8 to 58.8 nmol μl^{-1}, being lowest in the "Pencahue" plants followed by the "Tolhuaca" accession, the first being a home garden and the second a wild provenance. Both provenances showed, at the same time, the highest protection of microsomal thiol groups but one of the lowest inhibition effects of lipid peroxidation. On the other hand, the "Talca" provenance had both the highest polyphenol content and percentages of lipid peroxidation inhibition (Table 1). In this regard the polyphenol content and the inhibition of lipid peroxidation are significantly correlated ($r = 0.83$; $p = 0.04$, Pearson).

Table 1. Polyphenol content, inhibition of lipid peroxidation and protection of microsomal thiol groups of different provenances cultivated under the same environmental conditions.

Provenance	Polyphenols (nmol catechin μl^{-1})	Lipid peroxidation inhibition (%)	Protection on thiol-groups (%)
Pencahue	47.8 c	50.0 c	40.7 a
Talca	58.8 a	71.1 a	32.7 bc
San Javier	55.8 ab	64.6 b	25.6 c
Los Ruiles*	57.7 ab	62.9 bc	37.9 ab
Tolhuaca*	54.5 b	57.6 d	41.9 a
Los Queñes*	57.9 ab	60.8 cd	27.5 c
Statistical Test	Tukey	Kruskal-Wallis	Tukey

* Wild provenances

Values in columns followed by different letters indicate significant difference ($p \leq 0.05$)

When comparing the wild and cultivated plants from the same origin the assessed provenances, "Los Ruiles" and "Los Queñes", responded differently: The cultivated plants of "Los Ruiles" did not differ significantly from plants of their wild population, except for the thiol group protection, where cultivated plants showed better effects, whereas in the "Los Queñes" provenance wild plants had significantly higher antilipoperoxidant and thiol group protective effects, but lower polyphenol content (Table 2).

Table 2. Polyphenol content, inhibition of lipid peroxidation and protection of microsomal thiol groups of wild and cultivated plants of the same provenances

Provenance	Origin of samples	Polyphenols (nmol catechin μl^{-1})	Inhibition of lipid peroxidation (%)	Protection on thiol-groups (%)
Los Ruiles	Wild	54.9 a	68.4 a	31.3 b
	Cultivated	57.7 a	64.6 a	37.9 a
Los Queñes	Wild	42.0 b	67.7 a	43.1 a
	Cultivated	57.9 a	57.6 b	27.5 b

Values in columns followed by different letters indicate significant difference ($p \leq 0.05$)

Age of the leaves

Green leaves, both young and mature, had significantly higher polyphenol content than senescent leaves, but inhibition of lipid peroxidation was quite similar. Extracts from young leaves also protected microsomal thiol groups best (Table 3).

Table 3: Polyphenol content, inhibition of lipid peroxidation and protection of microsomal thiol groups of leaves harvested in different positions within the plant

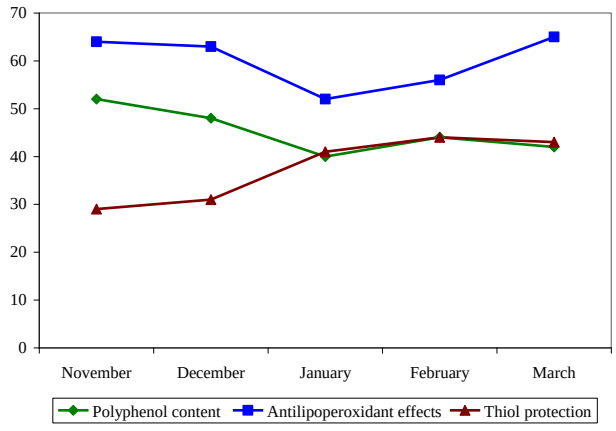
Age of the leaf	Position within the plant	Polyphenols (nmol catechin µl^{-1})	Inhibition of lipid peroxidation (%)	Protection on thiol-groups (%)
Young	upper	45.0 a	65.6 b	39.6 a
Middle-aged	central	43.5 a	66.7 ab	33.7 b
Senescent	lower	36.2 b	67.7 a	29.8 b

Values in columns followed by different letters indicate significant difference by LSD ($p \leq 0.05$)

Moment of leaf harvest

Figure 2 shows that polyphenol content in the leaves is highest early in the growing season and decreases during the growing season, then remains steady from January to March. Inhibition of lipid peroxidation is significantly higher in November and March and lower during the summer months. Protective effects of microsomal thiol groups increase until January and then stabilize until the end of the season.

Figure 2. Fluctuations in polyphenol content (nmol/µl), inhibition of lipid peroxidation (%) and protection of microsomal thiol groups (%) in leaves harvested at different moments.



Water supply

The extracts of leaves taken from stressed plants showed significantly higher polyphenol contents and thiol protection than those of the well watered plants, whereas no significant differences could be observed in the antilipoperoxidant effects among different irrigation treatments (Table 4).

Table 4. Effect of water supply on polyphenol content, inhibition of lipid peroxidation and protection of microsomal thiol groups.

Irrigation level	Polyphenols (nmol catechin µl^{-1})	Inhibition of lipid peroxidation (%)	Protection on thiol-groups (%)
Abundant (20 cb)	41.8 b	62.1 a	37.5 b
Stressed (40 cb)	45.2 a	62.2 a	47.9 a

Values in columns followed by different letters indicate significant difference by LSD ($p \leq 0.05$). cb: centibar

Drying method

Highest polyphenol contents in extracts were obtained from leaves dried under environmental conditions protected from direct sun light, whereas leaves dried at high temperatures (60°C and 80°C) produced extracts low in polyphenols.

Even so, the inhibition of lipid peroxidation of the 80°C treatment was highest together with the sun dried and washed and oven dried leaves.

Table 5. Effect of different drying methods of *Buddleja globosa*-leaves on polyphenol content, inhibition of lipid peroxidation and protection of microsomal thiol groups

Drying method	Drying period	Polyphenols (nmol catechin µl^{-1})	Inhibition of lipid peroxidation (%)	Protection on thiol-groups (%)
Under shade	9 days	43.1 a	54.4 b	47.9 a
At full sun light	32 h	40.5 b	63.9 a	46.7 ab
Washing + drying in oven at 40° C	48 h	38.4 c	63.9 a	41.5 abc
Oven at 40° C	48 h	40.4 bc	51.7 b	37.2 c
Oven at 60° C	8 h	25.1 e	41.3 c	40.0 bc
Oven at 80° C	6 h	32.8 d	62.2 a	43.8 abc
Statistical Test		Kruskal-Wallis	Tukey	Tukey

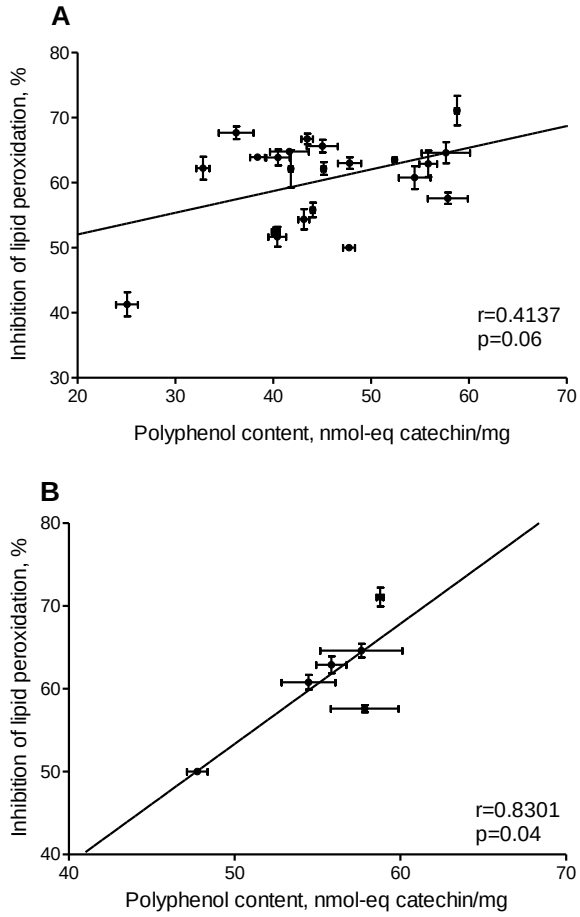
Values in columns followed by different letters indicate significant difference ($p \leq 0.05$)

For the thiol-group protection significant differences could only be observed between the leaves dried under shade and the oven dried at 40°C and 60°C (Table 5).

Relationship between polyphenol content and protective effects

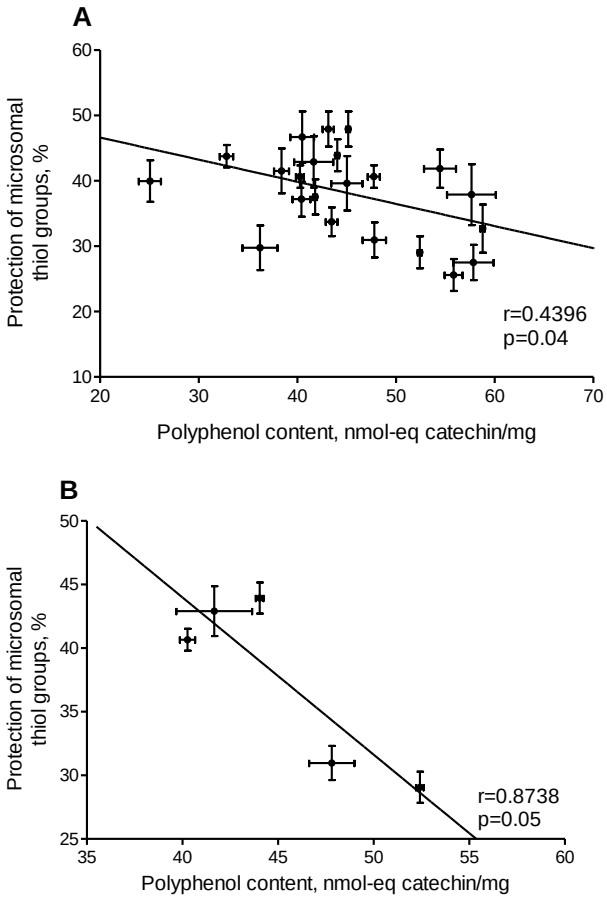
A series of correlation studies were performed between polyphenol content and the antioxidant protective properties of matico extracts, which are shown in Figures 3 and 4.

Figure 3. Relation of the polyphenol content in *B. globosa* leaves and inhibition of lipid peroxidation of (A) all extracts and (B) those obtained from different provenances.



There was no significant correlation between polyphenol content and anti-lipid peroxidant activities of all extracts, regardless of the provenance and postharvest procedures (Figure 3A, r = 0.4137, p = 0.06). Notably, when considering extracts from different provenances but obtained through the same postharvest procedure, this correlation increases (Figure 3B, r = 0.8301, p = 0.04). When considering drying method or harvest time, a significant correlation between polyphenol content and the anti-lipid peroxidant effect was not observed (data not shown).

Figure 4. Relation of the polyphenol content in *B. globosa* leaves and protection of microsomal thiol groups of (A) all extracts and (B) those obtained from leaves harvested at different moments.



A low but significant correlation between polyphenol content and the protection of microsomal thiol content was observed, regardless of the provenance and postharvest procedures (Figure 4A, r = 0.4396, p = 0.04). Noteworthy, the slope of this correlation is negative, showing that the higher the polyphenol content, the lower the protection of microsomal thiol content. This correlation increases when only considering extracts from plants harvested at different seasons (Figure 4B, r = 0.8738, p = 0.05). When considering provenance or drying methods, a significant correlation between polyphenol content

and the protection of microsomal thiol groups was not observed (data not shown).

Finally, there was no correlation found between anti-lipid peroxidant effects and protection of microsomal thiol content, regardless of analyzing all extracts or considering each provenance or postharvest procedure (data not shown).

DISCUSSION

Polyphenols and antioxidant effects

Polyphenol content is considered to be a good indicator for the antioxidant protective effects of *B. globosa* in virtue to their high concentration in plant leaves and their widely recognized antioxidant properties.

Antioxidant properties displayed *in vitro* are mainly due to polyphenol capacity to scavenge free radicals and to chelate transition metal ions, which generate oxygen free radicals through Haber-Weiss and/or Fenton reactions (Haliwell and Gutteridge, 1990). Numerous authors evaluate the scavenging properties of herbal preparations by measuring the bleaching of synthetic free radicals, such as DPPH, a stable nitrogen-centered free radical. In biological systems, however, the main free radicals are oxygen-centered, which are quite unstable. As a way to evaluate the antioxidant protecting properties of matico extracts used in this study, we chose to measure the protection of biomolecules from the oxidative damage elicited by Cu^{2+}/ascorbate, an oxygen free radical generating system.

Our data show that there was no significant correlation of polyphenol content versus inhibition of lipid peroxidation or protection of microsomal thiol groups, without considering plant provenance, drying method, age of leaves, or harvesting time. When grouping data in terms of the variables measured, we did find a low but significant correlation solely between plant provenance of the extracts and their antilipoperoxidante activity. This finding suggests that when all other variables (drying method, harvesting time, etc.) are kept constant it may be possible to confirm that polyphenol content is a good indicator for the antioxidant properties of a *B. globosa*-leaf extract. It is possible that a higher sampling number will improve the correlation between both parameters. In addition, the lack of statistical significance for these parameters without consideration of the variables tested suggests that the drying method, age of leaves and harvesting time may differentially alter the relative composition of polyphenols of the extract. Physiological antioxidants which protect membranes are lipophilic compounds, such as β-carotene and vitamin E, while the main soluble antioxidant is glutathione. Therefore, changing the relative composition of polyphenols, *e.g.* water soluble, partially soluble and insoluble, may alter the antioxidant activity of the extract in terms of protection of microsomal components, such as lipids and proteins. This potential difference between the antioxidant mechanisms involved in the protection of lipids and proteins may underlie the lack of significance in the correlation between polyphenol content and protection of thiol groups and between inhibition of lipid peroxidation and protection of thiol groups.

Notably, we found that polyphenol content and the protection of microsomal thiol content were inversely correlated, especially when considering the seasonal effect for the harvest of matico plants. As a corollary, the active principles involved in protecting lipids and thiol groups in proteins occurring in the microsomal preparation are likely to be different. Stress, whether by irrigation or by sunlight, may elicit selective structural changes of the active principles involved in thiol group protection, explaining the decrease of microsomal thiol group protection with the increase in polyphenol content elicited by the stress conditions mentioned.

Effects of different plant material

The present results showed no clear tendency for wild or home garden provenances to have better effects. The leaf extract from the provenance "Talca" inhibited best the lipid peroxidation, whereas the thiol-group protection was highest in leaf extracts from "Pencahue" and "Tolhuaca". The assessed provenances differed significantly in their polyphenol content, although in former studies neither the flavonoid nor the tannin content varied significantly among the same accessions, both groups of compounds being polyphenols (Vogel *et al*., 2004).

While the leaf extracts of the "Los Queñes"-provenance showed higher inhibition of lipid peroxidation and microsomal thiol-group protection in samples taken from wild plants, in the "Los Ruiles"-provenance leaves from cultivated plants had higher thiol-group protection than the plants growing in their natural habitat. The different responses of wild and cultivated plants of the same provenance could be due to the fact that the "Los Ruiles"-

population is located near the sea side at a similar altitude as the experimental crop, while "Los Queñes" is a mountain habitat.

The fluctuation of the polyphenol content during the summer months showed highest values at the beginning of the growing season when young leaves were growing actively. The fact that young and mature leaves have the highest polyphenol concentrations explains that the harvest in November (mid-spring), when leaves just are starting their development, shows maximum contents. A similar effect was observed in former studies with highest flavonoid and tannin contents in spring time (Vogel *et al*., 2002).

On the other hand, senescent leaves do not reduce their antilipoperoxidant properties. So plants can be harvested until the end of the season without losing complete effectiveness. In the case of the microsomal thiol-group protection the best effects are observed from January to March (summer months) in spite of the decreasing protection with the age of the leaves observed in this study.

B. globosa grows in its natural habitat near water sources and develops significantly better in sites with sufficient water supply (Vogel *et al*., 2005). The major polyphenol content in plants exposed to drier conditions may be a natural reaction to stress that enhances the quality of the plant material. Water stress, however, may not be a good strategy to increase the polyphenol content due to the expected lower leaf yield (Vogel *et al*., 2005).

To obtain high polyphenol content in leaf-extracts the best drying method is at environmental temperatures. Polyphenol concentration decreases with oven drying at high temperatures and washing. This result agrees with that of Leiva (2001) who found that drying temperatures above 45°C negatively affected the flavonoid content. Drying at environmental temperature also gave the best results for the thiol-group protecting effects. On the other hand, it is surprising that the best inhibition of lipid peroxidation was obtained by drying leaves by exposing them to full sun, by washing and successive drying, and oven drying at 80°C, being conditions thought to decrease quality of medicinal plant material. But, as explained above, these stress conditions do not affect the different phenols in the same way because of their different physical and chemical characteristics. Considering environmental drying conditions, under shade and exposed to full sun, the factor sun light negatively affected only the polyphenol content. For the protection of lipids and microsomal thiol groups drying in full sun may be proposed as a fast and cheap dehydration method. When comparing the washed and unwashed leaves, both treatments oven-dried at 40°C, it was shown that washing did not decrease any of the assessed characteristics. That means that dusty *B. globosa* leaves may be washed before drying without loosing quality. Oven-drying at increasing temperatures affected only the polyphenol content, but the best antioxidant protection effect was obtained even at the highest temperature (80°C). Probably, the polyphenols which are not affected by oven-drying are those involved in the antioxidant effect on membrane components which may be the most stable lipophilic polyphenols. Considering these results, this method would permit a fast dehydration process.

The present results give an answer as to which is the best treatment for any of the characteristics assessed. Because of the complex relationship between polyphenol content and antioxidant properties, it is difficult to select an outstanding genotype or to determine the best cultivating, harvesting or drying conditions based on the simultaneous evaluation of the three characteristics studied in the present project. Further studies to assess pharmacological effects of the extracts obtained by different plant material may elucidate this problem.

CONCLUSIONS

Difference of polyphenol content among cultivated provenances, indicate that this character is determined genetically and suffers variation due to environmental effects. Polyphenol content was related to the inhibition of lipid peroxidation.

Leaves from cultivated plants had the same or even higher polyphenol content than those from wild plants.

Age of the leaves affected the polyphenol content, being lowest in senescent leaves, whereas their inhibition of lipid peroxidation was highest. Extracts from young leaves showed the best protection on thiol-groups.

Less watered plants showed higher polyphenol contents and protection on thiol groups, whereas the inhibition of lipid peroxidation was not affected by irrigation.

Shade drying resulted in leaves with highest polyphenol content and best protective effects on thiol groups, followed by extracts from leaves dried

under sun. Inhibition of lipid peroxidation was surprisingly highest in the case of leaves dried at full sun, at 80°C or even washed before drying.

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