Antioxidant Screening of Medicinal Herbal Teas

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Herbal tea consumption is deeply and widely rooted amongst South-American populations. In view of the involvement of oxygen- and nitrogen-reactive species in the ethiogenesis of several diseases, the antioxidant properties of some of the herbal teas most commonly consumed in the southern regions was assessed *in vitro*. Around one-third of the 13 examined herbs, displayed a substantially higher ability to scavenge $ABTS^{+}$ radicals (TEAC assay), and to quench the pro-oxidant species, hypochlorite (HClO) and peroxynitrite ($ONOO^{-}$). Amongst the tested herbs, teas prepared from *Haplopappus baylahuen*, *Rosa moschata* and *Peumus boldus* showed the highest TEAC and HClO-quenching activities. These herbs were around 5- to 7-fold more potent than the least active herbs. Based on the TEAC assay, 150 mL of tea prepared from *H. baylahuen*, *R. moschata* and *P. boldus* would be equivalent to around 200 mg of Trolox®. Teas from *H. baylahuen* and *P. boldus* were also found to be particularly potent in quenching HClO. In the $ONOO^{-}$ assay, *H. baylahuen* and *Buddleia globosa* showed the highest activities. The results obtained suggest that the regular consumption of teas prepared from some of these herbs may be useful potentially to provide the organism with molecules capable of protecting the gastrointestinal tract against certain pathologically relevant oxidant species.

Keywords: herbal teas; antioxidants; free radicals; hypochlorite; peroxynitrite.

INTRODUCTION

The consumption of fruits and vegetables is strongly and inversely associated with the incidence and/or mortality rate of several forms of cancer, cardiovascular, cerebro-vascular and neuro-degenerative diseases (Bazzano *et al*., 2003; Liu, 2003; Riboli and Norat, 2003). Since the occurrence of oxidative stress can either lead to or accompany the progression of the above disorders, the high presence of antioxidants in fruits and vegetables generally has been regarded as an important basis for the health-protecting effects associated with their consumption (Gate *et al.*, 1999; Terry *et al*., 2001).

In addition to fruits and vegetables, herbs of no particular nutritional value can also constitute an important source of antioxidants (Warren, 1999; Ng *et al*., 2000). The term herb refers not only to herbaceous plants, but also to the leaves, bark, roots, seeds, fruits and flowers of shrubs and trees. The leaves from black and green tea (*Camellia sinensis*), long used amongst western and Asian populations, respectively, constitute an important source of potentially health-protecting antioxidants (Higashi-Okai *et al.*, 2001; Wu *et al*., 2003; Higdon and Frei, 2003) and world-wide represent the most popular form of daily herb consumption. More recently, the study of other herbs as a potential source of antioxidants has also stemmed from the need to screen endangered floras, and has been prompted by the increasing assumption that, given their natural origin, plant-derived antioxidants would comprise a group of relatively innocuous compounds.

The consumption of herbs in the form of herbal teas represents a widely established habit and the most popular form of herb consumption amongst people in South America (Jagtenberg and Evans, 2003). Particularly, in Chile, the term 'agüita', refers to an after-meal aqueous solution prepared, at the table, by the simple addition of a small amount of the leaves (and exceptionally bark) from an herb to a cup containing hot (near-boiling) water (Montes and Wilkomirsky, 1985). Herbs consumed in such form correspond mostly to plants to which some mild medicinal or digestive properties have been vernacularly attributed (Evans, 2002). Despite its widespread use, however, in most cases no scientific assessment of these claims has been conducted. Considering the increasing recognition that antioxidant consumption may play a role in preventing several oxidative-stress related diseases, the present study was undertaken to evaluate the antioxidant potential of the main herbal teas consumed in Chile. The study comprised an array of herbs, both endemic as well as

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non-endemic, of which several are also currently consumed world-wide, and implied their antioxidant characterization in terms of their free radical scavenging capacity (e.g. Trolox® equivalent antioxidant capacity) and their ability to quench pro-oxidant species (e.g. hypochlorite and peroxynitrite).

MATERIALS AND METHODS

Chemicals. 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), hypochlorous acid, β-nicotinamide adenine dinucleotide, reduced form (NADH), 5-amino salicylic acid (5-ASA), 3-morpholinosydnonimine N-ethylcarbamide (SIN-1) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) were all purchased from Sigma-Aldrich (St Louis, MO). 2,2′-azo-bis (2-amidinopropane dihydrochloride; AAPH), was obtained from Waco Pure Chemicals Industries Ltd, Osaka.

All solutions were prepared on the day of the experiment, kept at 4 °C, and protected from the light until use.

Herbal materials. Herbal tea bags were commercial products from two major Chilean producing and commercial companies who claimed and certified that the bag contents were all natural leaves and free of artificial flavoring and preservatives. The following herbs were studied: Anise (*Pimpinella anisum*, L.), Bailahuen (*Haplopappus baylahuen* Remy), Boldo (*Peumus boldus*, Mol), Plantain (*Plantago major*, L.), Chamomile (*Matricaria chamomilla*, L.), Peppermint (*Mentha piperita*, L.), Matico (*Buddleia globosa*, Hope), Mexican-tea (*Chenopodium ambrosioides*, L.), Brier rose (*Rosa moschata* Herrm.), Linden tree (*Tilia* spp.), Lemon Verbena (*Aloysia citriodora*), Lemon Balm (*Melissa officinalis*, L.), Pennyroyal (*Mentha pulegium*, L.). The herbal teas were prepared by adding 150 mL of distilled water (95–100 °C) to the bags (each containing 2 g of herbal material). The infusions were brewed for 5 min, with gentle stirring every 45 s. Upon withdrawing the tea bags, the resulting teas were cooled to 20 °C and used immediately after to assess their antioxidant properties.

Trolox®-equivalent antioxidant capacity (TEAC assay). A solution containing AAPH (2 mM) and ABTS (75 μM), prepared in 20 mM sodium phosphate buffer, pH 7.0, was incubated at 45 °C for 50 min, at which time the optical density at 734 nm increased to near 0.40 Units (in the absence of AAPH, incubation of ABTS generated no color). The resulting solution was afterwards rapidly cooled on ice, nitrogen-bubbled during 3 min, and kept at 4 °C until use. The TEAC assay was carried out at 22 °C. The addition of aliquots of the herbal teas to a cuvette containing the ABTS-AAPH solution led to a decrease in the OD_{734nm} which corresponded to the reduction and disappearance of the $ABTS^{+\cdot}$ radical. The absorbance was recorded continuously (UV-160 Shimadzu spectrophotometer, Kyoto, Japan) and the difference (Δ) in OD_{734nm} seen after 5 min of addition of the teas to the cuvettes was estimated. The TEAC assay was calibrated against Trolox®, a water-soluble vitamin E analogue. On the basis of the ΔOD_{734nm} thus obtained, the antioxidant capacity of the teas was estimated and expressed as Trolox®-equivalents (Campos and Lissi, 1995; Cao and Prior, 1998).

Hypochlorite quenching assay. The reagent 5-ASA (8 μM, dissolved in a 0.1% DMSO aqueous solution) exhibits a peak of maximal fluorescence at a wavelength of 340 nm of excitation and 500 nm of emission. Its fluorescence decreases upon addition of HClO (4 μM). Thus, the hypochlorite-quenching activity of the herbal teas was assayed by measuring the extent of maximal prevention of the loss of fluorescence induced by HClO. The changes in fluorescence were registered at 22 °C, 3 min after the addition of 5-ASA to a mixture of teas plus HClO prepared in sodium phosphate buffer (20 mM), pH 7.0. In the absence of HClO, none of the teas affected the fluorescence displayed by 5-ASA. The hypochlorite-quenching activity of the herbal teas was expressed as 5-ASA-equivalents.

Peroxynitrite quenching assay. The reagent SIN-1 was employed as a peroxynitrite anion generator. In aqueous solutions, SIN-1 decomposes rapidly into nitric oxide and superoxide anions. These two species react rapidly giving rise to the formation of peroxynitrite (Huie and Padmaja, 1993), which induces the oxidation of NADH (Kirsch and de Groot, 1999). The changes in OD_{340nm} were registered continuously (22 °C) after addition of SIN-1 (75 μM) to a mixture of herbal tea plus NADH (50 μM), prepared in sodium phosphate buffer (20 mM), pH 7.0. In the absence of SIN-1, none of the teas affected the OD_{340nm} displayed by NADH. In the absence of herbal teas, 50% of NADH was regularly oxidized at around 30 min. Thus, the peroxynitrite-scavenging activity of the herbal teas was assayed by measuring the delay (Δtime) in attaining a 50% loss in NADH concentration induced by SIN-1.

Data expression and analysis. Data presented in Tables 1–3 represent the mean values of at least two independent experiments, each conducted in triplicate. The SD of such values are not included as these generally represent less than 10% of the means. Correlation data (r^2) depicted in Fig. 1 (A, B and C) were estimated from linear regression analysis.

RESULTS AND DISCUSSION

Since pre-historical times, herbs have been the basis for nearly all medicinal therapies (Abelson, 1990; Jagtenberg and Evans, 2003). According to the World Health Organization around 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs (Craig, 1999). Most of this therapy involves the use of plant extracts or their active phytochemical components, of which some have been shown to display antioxidant activity (Craig, 1999; Kahkonen *et al.*, 1999). The antioxidant capacity of some herbal teas was screened using three different techniques: TEAC assay, hypochlorite-quenching capacity and peroxynitrite-quenching capacity. The TEAC assay represents a useful experimental approach for assessing the antioxidant potential of samples containing

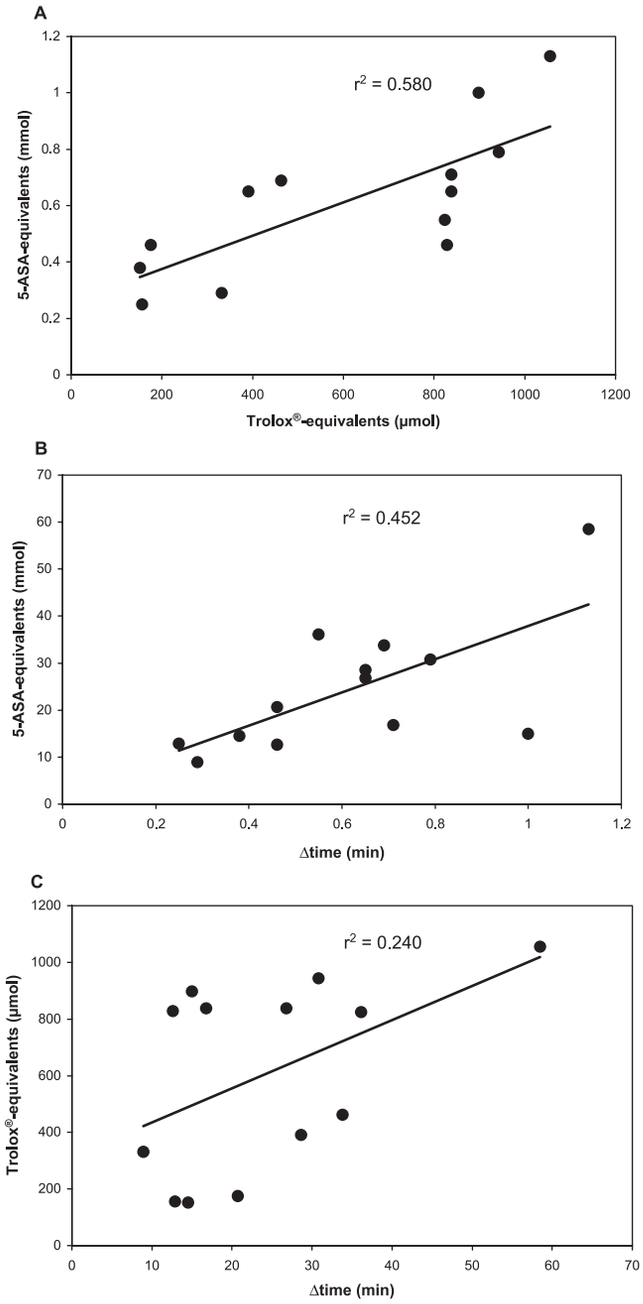

Figure 1. Correlation between TEAC and hypochlorite-quenching activities of herbal teas (A). Correlation between peroxynitrite- and hypochlorite-quenching activities of herbal teas (B). Correlation between TEAC and peroxynitrite-quenching activities of herbal teas (C).

mixtures of free radical-scavenging molecules. As carried out in the present study, the IV_{50} depicted in Table 1 reflects the volume of herbal teas needed to quench or decrease by 50% the OD_{734nm} of a solution containing the long-lived blue-green chromophore $ABTS^{+\cdot}$ radical. Based on the ΔOD_{734nm}-induced by a standard solution of Trolox®, the results were also expressed in terms of Trolox®-equivalents. The results are presented as µmol or mg Trolox® per 150 mL of herbal tea (see Materials and Methods section). As shown, a significant $ABTS^{+\cdot}$-quenching activity was found for all the herbal teas screened, although clear differences were seen amongst them. Thus, the herbal teas presenting the highest antioxidant potential showed activities up to 7-fold higher than those depicted by the least active ones. Accordingly, the herbal teas were classified into three distinguishable groups: the most active one includes the herbs *Haplopappus baylahuen*, *Rosa moschata* and *Peumus boldus.* A moderate TEAC activity was found in herbs such as *Mentha piperita*, *Melissa officinalis* and *Chenopodium ambrosioides*. The least active were *Pimpinella anisum*, *Mentha pulegium* and *Tilia* spp. On the other hand, the capacity of the herbal teas to quench hypochlorite anions is presented in Table 2. Data are expressed as 5-ASA-equivalents that were 'spared' from being consumed by HClO during the *in vitro* assay. The results are presented as mol or mg of 5-ASA per 150 mL of herbal tea. All herbal teas displayed HClO-quenching activity (Table 2). As seen in the TEAC assay, up to five-fold differences in the quenching potency were detected between the highest and lowest herbal tea activities. The most active herbs were *Haplopappus baylahuen*, *Peumus boldus* and *Rosa moschata*, followed by *Plantago major*, *Mentha piperita* and *Melissa officinalis* as medium-potency teas. The least active in quenching HClO were *Tilia* spp., *Chenopodium ambrosioides* and *Mentha pulegium*. As a last experimental approach, the ability of the diverse teas (each assayed at 25 µL) to quench peroxynitrite anions was determined by measuring the time needed to attain a 50% decrease in the concentration of the peroxynitrite-oxidizable substrate NADH. The delay in time (Δtime) needed to attain such a decrease in OD_{340nm} is represented in Table 3. As shown, differences of up to seven-fold were found between the most and least active herbal teas. The highest peroxynitrite-quenching activity was displayed by *Haplopappus baylahuen*, followed by *Buddleia globosa*, *Mentha piperita* and *Rosa moschata*. The lowest activities were observed in *Mentha pulegium*, *Matricaria chamomilla* and *Chenopodium ambrosioides*.

Comparing the three different assays performed, the TEAC and the hypochlorite-quenching activities, and the TEAC and the peroxynitrite-quenching activities of all screened teas were correlated as depicted in Fig. 1A and 1B. The correlation coefficients (r^2) for these comparisons were 0.580 and 0.240, respectively. Figure 1C depicts the correlation between the hypochlorite- and the peroxynitrite-quenching activities of the same teas. The corresponding r^2 value was 0.452.

The results from the present study, which explored the antioxidant potential of 13 herbal teas commonly consumed by the Chilean and other Southern-American populations, indicate that many of the herbs examined display a high ability to scavenge free radicals, and to quench the pro-oxidant species hypochlorite and peroxynitrite. The antioxidant potential of the herbal teas assessed by means of the TEAC assay suggests the presence of water-soluble molecules in these herbs that are able to react directly with ABTS radicals. *Haplopappus baylahuen*, *Rosa moschata* and *Peumus boldus* were particularly TEAC-active amongst the herbs studied. It should be noted, however, that although the TEAC assay constitutes a practical approach for assessing the antioxidant activity of mixtures of phytochemicals (Campos and Lissi, 1995), herbal teas exhibiting a high TEAC should not necessarily be considered to be equally effective in quenching other reactive pro-oxidant species. An example of the latter is *Plantago major*, which despite presenting a high TEAC,

Table 1. Trolox®-equivalent antioxidant capacity of herbal teas

Herb	IV_{50} (μL herbal tea)	Trolox®-equiv. (μmol)/150 mL herbal tea	Trolox®-equiv. (mg)/150 mL herbal tea
Tilia spp.	9.9	152	38
Mentha pulegium, L.	9.6	156	39
Pimpinella anisum, L.	8.6	175	44
Chenopodium ambrosioides, L.	4.5	332	83
Melissa officinalis, L.	3.8	391	98
Mentha piperita, L.	3.2	463	116
Buddleia globosa, Hope	1.8	824	206
Matricaria chamomilla, L.	1.8	829	208
Aloysia citriodora, Ort.	1.8	838	210
Plantago major, L.	1.8	838	210
Peumus boldus Mol.	1.7	898	225
Rosa moschata, Herrm.	1.6	943	236
Haplopappus baylahuen, Remy	1.4	1056	264

Herbal teas were prepared and TEAC assayed as described in Material and Methods. Data represent the mean of at least two independent experiments, each conducted in triplicate.

Table 2. Hypochlorite-quenching capacity of herbal teas

Herb	IV_{50} (μL herbal tea)	5-ASA-equiv. (mmol)/150 mL herbal tea	5-ASA-equiv. (mg)/150 mL herbal tea
Mentha pulegium, L.	2.4	0.25	38.3
Chenopodium ambrosioides, L.	2.1	0.29	43.7
Tilia spp.	1.6	0.38	57.4
Pimpinella anisum, L.	1.3	0.46	70.7
Matricaria chamomilla, L.	1.3	0.46	70.7
Buddleia globosa, Hope	1.1	0.55	83.5
Aloysia citriodora, Ort.	0.9	0.65	99.9
Melissa officinalis, L.	0.9	0.65	99.9
Mentha piperita, L.	0.9	0.69	105.6
Plantago major, L.	0.9	0.71	108.1
Rosa moschata, Herrm.	0.8	0.79	120.9
Peumus boldus Mol.	0.6	1.00	153.1
Haplopappus baylahuen, Remy	0.5	1.13	173.3

Herbal teas were prepared and hypochlorite-quenching activity assayed as described in Material and Methods. Data represent the mean of at least two independent experiments, each conducted in triplicate.

Table 3. Peroxynitrite-quenching capacity of herbal teas

Herb	T_{50} (25 μL herbal tea)	Δtime (min)
Chenopodium ambrosioides, L.	36.9	8.9
Matricaria chamomilla, L.	40.6	12.6
Mentha pulegium, L.	40.9	12.9
Tilia spp.	42.5	14.5
Peumus boldus Mol.	43.0	15.0
Plantago major, L.	44.8	16.8
Pimpinella anisum, L.	48.7	20.7
Aloysia citriodora, Ort.	54.8	26.8
Melissa officinalis, L.	56.6	28.6
Rosa moschata, Herrm.	58.8	30.8
Mentha piperita, L.	61.8	33.8
Buddleia globosa, Hope	64.1	36.1
Haplopappus baylahuen, Remy	86.5	58.5

Herbal teas were prepared and peroxynitrite-quenching activity assayed as described in Material and Methods. Data represent the mean of at least two independent experiments, each conducted in triplicate.

exhibited a very low peroxynitrite-quenching activity. Conversely, *Pimpinella anisum* which showed a very low TEAC exhibited moderate but significant hypochlorite- and peroxynitrite-quenching activities. The large differences seen between the most and least active herbs when assayed for their TEAC, HClO- or $ONOO^-$-quenching activities, added to the observation that some herbal teas are particularly active in one but not necessarily in the other assays, suggest that in each herb molecules exist which are able to react selectively with the species $ABTS^{+\cdot}$, HClO or $ONOO^-$. Thus, the latter is reflected by the very low correlation coefficients found in our study between the TEAC and the hypochlorite-quenching activity, and between the TEAC and the peroxynitrite-quenching activity for all the studied herbs.

The observation that teas obtained from *Haplopappus baylahuen*, *Peumus boldus* and *Rosa moschata* exhibited the highest HClO-quenching activities is of particular interest in view of the role that such species play in the ethiogenesis of inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease (Kruidenier *et al.*, 2003). As known, hypochlorite is a potent oxidant generated by neutrophiles in a reaction catalysed by myeloperoxidase through the hydrogen peroxide-dependent oxidation of chloride (Winterbourn, 2002). Mesalamine (5-ASA), a salicylate with antioxidant activity (Simmonds *et al.*, 1999), is widely used in the treatment of IBD (Prakash and Markham, 1999; Reinacher-Schick *et al.*, 2000). According to our estimates, in terms of sparing 5-ASA from HClO-mediated oxidation, 150 mL of *Haplopappus baylahuen* tea would be equivalent to 173 mg of 5-ASA. Since mesalamine is often given to IBD patients at a dose of 1 g per day, the possibility should be considered that regular ingestion of this herbal tea could be of some use in IBD patients receiving 5-ASA treatment. The latter would follow to the extent to which the HClO-quenching activity of *Haplopappus baylahuen* seen *in vitro* also occurred *in vivo* after its ingestion. On the other hand, since the antiinflammatory activity of 5-ASA contributes to its therapeutic effect on IBD, it remains to be established whether those teas possessing HClO-quenching activity may also exert some antiinflammatory action.

In contrast to the very limited knowledge on the chemistry of *Haplopappus baylahuen*, teas from *Peumus boldus*, which in our study were particularly active in quenching HClO, have been reasonably described in terms of their antioxidant composition (Speisky and Cassels, 1994). Flavonoids and aporphine-like alkaloids are believed to account for most of the antioxidant-related phytochemicals occurring in boldo (Speisky and Cassels, 1994; Cassels *et al.*, 1995; Schmeda-Hirschmann *et al.*, 2003). Boldine, the single most important alkaloid present in this herb, has been extensively characterized in terms of its antioxidant (Speisky *et al.*, 1991), cytoprotective (Bannach *et al.*, 1996; Jiménez *et al.*, 2000) and antiinflammatory (Backhouse *et al.*, 1994) activities. It is likely that all these actions contribute to the protection afforded by boldine against the colonic damage associated with neutrophil-infiltration seen in a rodent model of experimental colitis (Gotteland *et al.*, 1997). Recently, however, Schmeda-Hirschmann *et al.* (2003) reported that in aqueous solutions of boldo, the mean catechin-to-total alkaloids ratio approaches 37:1, suggesting that rather than boldine, the former molecules would account for most of the antioxidant activity displayed by boldo teas. Since catechins are able to quench HClO (Scott *et al.*, 1993; Sakagami *et al.*, 1995), their contribution to the removal of hypochlorite by boldo teas may be thus appreciable.

Finally, our interest in assessing the peroxynitrite-quenching activity of herbal teas stems from the apparent involvement of peroxynitrite anions in the ethiogenesis of pathologies associated with ischemia-reperfusion injury (Nonami, 1997), and in the oxidation of LDL (Carr *et al.*, 2000), a key atherogenic event. Results from the present study indicate that teas from *Haplopappus baylahuen*, *Buddleia globosa* and *Mentha piperita* showed the highest peroxynitrite-quenching activities. The activity of the latter two herbs was, however, only half of that displayed by *H. baylahuen*. Since neither *Buddleia globosa* nor *Mentha piperita* were particularly active in the TEAC and HClO assays, in order to gain a broader action against free radicals and reactive species, the use of these herbs should be considered in combination with others displaying potent TEAC and hypochlorite-quenching activities.

In conclusion, based on the *in vitro* data presented here, it is suggested that the regular ingestion of teas prepared from herbs such as *H. baylahuen*, *R. moschata* or *P. boldus* may be useful to provide the gastrointestinal tract with molecules capable of protecting against certain pathologically relevant oxidants species. At this point we ignore whether the molecules which *in vitro* account for the high free-radical scavenging and oxidant-quenching activity of some of these herbs are actually absorbed under *in vivo* conditions. Thus, the actual extent to which the practice of consuming herbal teas might contribute systemically to the overall antioxidant capacity of the organism remains to be assessed.

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