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Effect of extraction conditions on total phenolic content and antioxidant capacity of pretreated wild *Peumus boldus* leaves from Chile

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ABSTRACT

We studied the effect of both heat-drying and freeze-drying on the recovery of total phenolic compounds (TPCs) and antioxidant capacity from leaves of *Peumus boldus* Molina (Boldo), an endemic tree of Chile, using DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging), FRAP (ferric reducing antioxidant power) and ORAC (oxygen radical absorbance capacity) methods. The results indicated that infusions prepared using commercial boldo tea bags had similar or higher TPCs, DPPH, FRAP and ORAC values in comparison with those of the infusions prepared using heat- or freeze-dried leaves. The extraction experiments showed that hydro-alcoholic mixtures are the best solvents to extract antioxidants from boldo leaves, favoring the use of freeze-dried leaves. Considering the alkaloid profile of the extracts of freeze-dried leaves and herbal tea bags, the latter exhibited higher amounts of the alkaloids tested, including boldine, which is well correlated with the results obtained using the ORAC method. These results indicate a great potential to develop commercial boldo extracts and could encourage improved applications of this endemic Chilean plant.

Keywords: Native plant; *Peumus boldus* Molina; Antioxidant; Medicinal plants; Phenolic compounds; ORAC

1. Introduction

A large number of compounds with biological activity that are used in traditional medicine are based on substances derived from plants that are prepared as infusions, extracts or beverages (Gurib-Fakim, 2006). The health benefits associated with herbal infusions are partially attributed to their antioxidant capacity (Higdon and Frei, 2003; Shahidi, 2000). Antioxidants are substances that limit or prevent the oxidation process at a low concentration in comparison to that of the oxidizable substrate (Halliwell, 1996). Molecules with antioxidant activity, such as carotenoids, ascorbic acid, tocopherol and phenolic compounds, have been shown to reduce low-density lipoprotein (LDL) oxidation in vitro, inhibit platelet aggregation, and control blood pressure, in addition to having antibacterial, antiviral, antimutagenic, and antiallergenic activities (Choe and Min, 2009; Halliwell, 1996). In this context, preserving the antioxidants of herbs during processing is fundamental to offering products that maintain the health benefits they promote.

Peumus boldus Molina, commonly known as boldo, is an endemic tree of Chile, which is used to prepare infusions that have several health benefits associated with their essential oils, alkaloids and polyphenols. These infusions are commonly used as digestive stimulants, diuretics, relaxants, and in the treatment of liver and gallbladder disorders. In particular, boldo leaves have been shown to possess more than 30 compounds such as quercetin glycosides, kaempferol derivatives, isorhamnetin glycosides, phenolic acids, and proanthocyanidins, which exert antioxidant and chemopreventive effects (Fernández et al., 2009; O'Brien et al., 2006; Simirgiotis and Schmeda-Hirschmann, 2010). The essential

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oil of the boldo is composed mostly of ascaridole (34.8%), limonene (16.1%) and eucalyptol (11.9%), and has been proven to have anti-inflammatory, carminative, and fungistatic properties (Bittner et al., 2009). Additionally, boldine, the major alkaloid present in the leaves and bark of the boldo, is well known for its antioxidant properties and its trypanocidal activity (O'Brien et al., 2006).

Boldo leaves are available in the market as dried leaves in herbal tea bags; however, the effects that processing has on its antioxidant activities have not been studied. We assessed the effect of heat- and freeze-drying on the recovery of phenolic compounds, antioxidant capacity and the content of alkaloids, such as boldine, from frozen boldo leaves using different solvents and compared them to that obtained from herbal tea bags available in the market.

2. Experimental

2.1. Plant material

Leaves of *P. boldus* were collected from naturally growing trees in the Bio-Bio region of Chile. The samples were frozen and then heat-dried or freeze-dried depending on the experimental design. Additionally, commercially available boldo herbal tea bags ("Té Supremo" brand, Chile) were tested.

2.2. Chemicals

Analytical grade reagents were purchased from Merck S.A. (U.S.A) and Sigma–Aldrich (U.S.A.). Trolox, gallic acid, ascorbic acid and boldine were used as standards to quantify the DPPH, TPC, and FRAP values and the boldine concentration, respectively. The Trolox equivalent was also used to quantify the ORAC value.

2.3. Extract preparation

An aliquot of 0.5 g of sample was extracted using 10 mL of deionized water, ethanol, methanol or 50:50 hydro-alcoholic mixtures. The conditions of the extractions were 50 °C for 16 h. In addition, infusions of whole fresh leaves, boldo herbal tea bags, heat-dried leaves, and freeze-dried/milled leaves were prepared. In those cases, 1 g of sample was extracted with 50 mL of water at 90 °C for 5 min. The extracts were filtered and the recovered volume quantified.

2.4. Soluble solids

The content of soluble solids was calculated from the difference in the mass ($mass_{beginning} - mass_{end}$) of a fixed amount of extract ($mass_{beginning}$), which was dried at 100 °C until a constant weight was reached ($mass_{end}$).

2.5. Determination of total phenolic compounds

The TPCs were determined using a modified Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, a mixture of 3.75 mL of deionized water, 0.5 mL of extract, 0.25 mL of Folin-Ciocalteu phenol reagent diluted two-fold in distillated water and 0.5 mL of 10% (w/v) sodium carbonate was prepared. The absorbance at 765 nm was determined after 1 h, and gallic acid was used as the standard (GAE).

2.6. Determination of antioxidant capacity

Three methods were applied to determine the antioxidant power of boldo leaves. (a) The DPPH assay, a free radical scavenging-based method (von Gadow et al., 1997), was performed using 2 mL of DPPH solution (3.6×10^{-5} M methanolic solution) and 50 μL of extract to determine the absorbance at 515 nm. To obtain the calibration curve, the absorbance at 515 nm of a series of concentrations of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), the standard (TS), was measured; (b) The FRAP method, which employs a redox-linked colorimetric assay, was conducted according to Benzie and Strain (1996). Briefly, 3 mL of FRAP solution (10:1:1 of 300 mM acetate buffer pH 3.6, 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ) prepared in 40 mM HCl, and 20 mM $FeCl_3$ $6H_2O$, respectively) was mixed with 0.1 mL of the phenolic extract. The absorbance at 593 nm was recorded after 6 min, and ascorbic acid was used as the standard (AAS); (c) The ORAC was determined according to Garrett et al. (2009), using 200 μL of fluorescein (108 nM in PBS buffer pH 7.4) and 20 μL of phenolic extract. The samples were incubated at 37 °C, and then, 75 μL of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) (79.7 mM in PBS buffer pH 7.4) was added. Fluorescence was followed for 60 min using 485/538 nm excitation/emission wavelengths, and synthetic Trolox was used as the standard (TS).

2.7. Determination of alkaloid concentrations

The method to extract and quantify alkaloids, including boldine, is described in European Pharmacopeia (Cámara et al., 2010). Briefly, 50 mL of hydrochloric acid was added to 1 g of the extract sample and then transferred to a separating funnel, to which was added an organic solvent (a mixture of equal volumes of ethyl acetate and hexane). The alkaloids were obtained in the organic phase. The solvent was evaporated and the extract was dissolved in the mobile phase (16%, v/v, of solution A (0.2%, v/v, of diethylamine and 99.8%, v/v, of acetonitrile) and 84%, v/v, of solution B (0.2%, v/v, of diethylamine and 99.8%, v/v, of acetonitrile adjusted to pH 3.0 with anhydrous formic acid)) for quantification by high pressure liquid chromatography (HPLC) using a UV detector at 304 nm.

2.8. Statistical analysis

Statistical analysis was performed using the GraphPad InStat program, version 3.1. The significance of differences was calculated using a degree of confidence of 95%.

3. Results and discussion

3.1. Pre-selection of boldo infusions

Infusions of freeze-dried, heat-dried, whole frozen boldo leaves and herbal tea bags were tested to determine how the most common preparations of this plant affect the recovery of their antioxidants. In addition, this evaluation guided the choice of the type of conditioning to perform on the boldo leaves, considering the goal of obtaining an extract rich in antioxidants using an extraction process at a lower temperature. To reach this goal, the TPCs and the DPPH, FRAP and ORAC values of different types of boldo infusions were analyzed. As shown in Table 1, the infusion of commercial herbal tea bags presented the highest values of all of the parameters