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The phenolic content and antioxidant activity of infusions from Mediterranean medicinal plants

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ABSTRACT

We prepared cold and hot water infusions from ten Mediterranean medicinal plant species and compared their total phenolic content, capacity to scavenge hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, ability to reduce Fe^{3+} and chelate Fe^{2+}, and ability to inhibit Fe^{2+}-induced lipid peroxidation in mouse brain homogenates. In all ten species, the phenolic content of hot infusions (from 147.05 ± 2.32 to 1359.10 ± 33.18 $\mu mol_{GAE}\ g_{dw}^{-1}$) was significantly greater than that of cold infusions (from 106.11 ± 8.10 to 1006.05 ± 54.43 $\mu mol_{GAE}\ g_{dw}^{-1}$) but the difference was greatest in *Lavandula viridis*. All of the extracts contained antioxidants, but infusions from *Myrtus communis*, *Pistacia lentiscus*, *L. viridis* and *Cistus albidus* were the most potent and provided evidence that the free radical scavenging and metal-chelating effects of these extracts might contribute to the prevention of Fe^{2+}-induced lipid peroxidation. We observed a positive correlation between the total phenolic content and the antioxidant activity as measured using all the methods listed above with the exception of hydroxyl radical scavenging. Our results provide a scientific basis for the use of these plant species in folk medicine as well as additional resources for the discovery of novel antioxidants.

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1. Introduction

Herbs and spices have been used since antiquity not only for their culinary properties but also as preservatives and medicines (Dorman et al., 2004). Contemporary dietary programs often recommend certain aromatic and medicinal plants as functional foods, *i.e.* foods that provide additional physiological benefits over and above normal nutritional requirements, such as preventing or delaying the onset of chronic diseases (Nicoli et al., 1999; Fares et al., 2011). The severity of many chronic diseases, including age-related cardiovascular and neurological disorders, is influenced by the abundance of reactive oxygen species (ROS) and other free radicals in the body (Bland, 1995; Tohma and Gulçin, 2010). The progression of such diseases can therefore be delayed by minimizing redox imbalances that generate ROS, and by counteracting their effects (Wu et al., 2011). Antioxidants scavenge ROS and other free radicals by acting as sacrificial substrates for oxidation, thereby terminating the chain reactions that can otherwise cause pathological oxidative damage in cells (Valko et al., 2007).

Many functional foods are rich in antioxidants and can therefore, at least in theory, help to reduce the impact of age-related chronic diseases (Krishnaih et al., 2007). For this reason, there has been a particular interest in the potential health benefits of aromatic and medicinal plants with the greatest capacity for scavenging ROS, and in the identification of the natural antioxidants responsible for these effects. Natural antioxidants with site-specific mechanisms of action would be more potent but less toxic than the natural and synthetic antioxidants currently used in the food processing industry (Liscovitch and Lavie, 2002; Lizcano et al., 2010; Hossain and Rahman, 2011). Plants synthesize a diverse spectrum of antioxidant phenolic compounds as secondary products, which prevent oxidative damage *in planta* but also confer protective effects on humans when the plants are consumed as food (Nićiforović et al., 2010). The antioxidant properties of phenolic compounds reflect a combination of mechanisms, including free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation and acting as a substrate for oxidation (Nićiforović et al., 2010).

The Mediterranean region is characterized by heterogeneous soil and climatic conditions that have produced more than 10,000 medicinal and aromatic plant species with diverse properties worthy of further investigation (Fares et al., 2011; Vanzani et al., 2011). Some of these plants have been used as folk remedies for

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; F–C reagent, Folin–Ciocalteu reagent; GAE, gallic acid equivalents; MDA, malondialdehyde; ROS, reactive oxygen species; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; TE, trolox equivalents; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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generations after preparation in traditional ways such as cooking, infusion or maceration. The systematic investigation of such plants will help to define their precise pharmacological properties and to determine their value as functional foods and as a source of nutraceutical compounds such as novel antioxidants (Miliauskas et al., 2004; Gouthamchandra et al., 2010). Previous studies have shown that the pharmacological properties of folk medicines based on herbal infusions are often connected with the presence of antioxidant phenolic compounds (Dawidowicza et al., 2006). We therefore investigated the relationship between the total phenolic content and antioxidant activity of different Mediterranean plant species traditionally used in Portugal (Table 1) and whether these activities were affected by steeping in hot or cold water. The infusions were subjected to a range of *in vitro* tests to determine antioxidant activity, including the chelation of Fe^{2+}, the reduction of Fe^{3+}, the ability to prevent the decomposition of deoxyribose, the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and protection against Fe^{2+}-induced lipid peroxidation in mouse brain homogenates.

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu's phenol reagent (F–C reagent), gallic acid, Na_2CO_3 and $FeCl_3$ were purchased from VWR (Leuven, Belgium). Phenanthroline and trichloroacetic acid (TCA) were obtained from Panreac (Barcelona, Spain). $K_2S_2O_8$, thiobarbituric acid (TBA), H_2O_2, DPPH and methanol were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium dodecylsulfate (SDS), $K_3[Fe(CN)_6]$, deoxyribose and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (Geel, Germany). $FeSO_4$ and acetic acid were purchased from Merck (Darmstadt, Germany). HCl was obtained from BDH (Poole, England).

2.2. Plant material and preparation of aqueous extracts

Samples from 10 species (Table 1) were collected in spring from plants growing wild in the Algarve region (South Portugal). The plant material was dried at 40 °C and ground to powder in a blender. The aqueous extracts were prepared by homogenizing 1 g of the plant material in 20 ml cold water for 2 h (room temperature) or hot water (90 °C) for 5 min with agitation (1:20, w/v). The cold and hot infusions were then centrifuged at 1100 × *g* for 10 min and the supernatant was filtered for use in the subsequent assays.

2.3. DPPH free radical scavenging assay

DPPH free radical scavenging was measured as described by Brand-Williams et al. (1995). We mixed 10 μl of diluted plant infusion (0.1–10 mg ml^{-1}) with 190 μl DPPH solution (100 μM in methanol) in a clear 96-well microplate (NUNC, Rochester, NY, USA) and measured the absorbance at 515 nm after 30 min at room temperature using an Infinite 200 microplate reader (Tekan, Grödig, Austria). The DPPH radical stock solution was prepared daily to minimize the loss of free radical activity during sample preparation (Blois, 1958). A calibration curve was prepared using Trolox in the concentration range 0.8–4 mM. The results were expressed as mmol Trolox equivalents (TE) per gram dry weight ($mmol_{TE}\ g_{dw}{}^{-1}$).

2.4. Measurement of reducing activity

The reducing properties of the extracts were determined using $FeCl_3$ as described by Pulido et al. (2000) with modifications. Briefly, 250 μl of each extract (0.4, 0.8, 1.6 or 2 mg ml^{-1}) was mixed with 250 μl sodium phosphate buffer (200 mM, pH 6.6) and 250 μl 1% $K_3[Fe(CN)_6]$ and was incubated at 50 °C for 20 min. We then added 250 μl 10% TCA and centrifuged the mixture at 650 rpm for 10 min. We also mixed 50 μl of the supernatant with 200 μl of water and 10 μl 0.1% $FeCl_3$ in a 96-well microplate. Reducing activity was measured by determining the absorbance at 700 nm.

2.5. Inhibition of lipid peroxidation in mouse brain homogenates using thiobarbituric acid reactive substances (TBARS)

C57BL/6 mice (male, 25–30 g, 3–4 months old) were acquired from the Animal House of the Centre for Molecular and Structural Biomedicine, University of Algarve, Portugal. The mice were housed in polyacrylic cages in a temperature-controlled room (20–23 °C), 40–55% relative humidity, with *ad libitum* access to food. Animal experiments were conducted in accordance with ethical guidelines for clinical research and EU guidelines for animal research. The mice were killed by CO_2 asphyxiation and the cerebral tissue (whole brain) was rapidly dissected, placed on ice and weighed

Table 1
Botanical names, families, local names, medicinal properties and plant parts of the 10 plants included in this investigation.

Botanical name	Family	Local name	Reported medicinal properties/indications	Plant part tested
Cistus albidus L.	*Cistaceae*	Roselha grande	Anti-inflammatory and antiviral	Aerial part
Centaurium erythraea Rafn.	*Gentianaceae*	Fel-da-terra	Asthma, eczema, jaundice, intestinal parasitic infestation, rheumatism, wounds and sores, to reduce blood pressure, diabetes, gastrointestinal smooth muscle spasm, edema and digestive disorders, liver and gall bladder stimulant	Aerial part
Lavandula viridis L'Hér	*Lamiaceae*	Rosmaninho-verde	Antiseptic, anti-inflammatory and antimicrobial	Aerial part
Myrtus communis L.	*Myrtaceae*	Murta	Stomachic, hypoglycemic, cough and oral diseases, antimicrobial, for constipation, appetizing, anti-hemorrhagic and externally for wound healing	Leaf
Olea europaea L.	*Oleaceae*	Oliveira	Abdominal colic, baldness, paralysis, rheumatic pains and hypertension	Leaf
Paronychia argentea Lam.	*Caryophyllaceae*	Erva-prata	Gastric analgesic, bladder and prostate ailments, abdominal ailments, stomach ulcers, kidney stones and hypoglycemia	Aerial part
Pistacia lentiscus L.	*Anacardiaceae*	Lentisco	In the treatment of coughs, sore throats, eczema, stomach aches, kidney stones and jaundice	Leaf
Pterospartum tridentatum (L.) Willk.	*Fabaceae*	Carqueja	Bechic and emollient, against liver, bladder, kidney and rheumatism problems and in the treatment of diabetes	Aerial part
Ruscus aculeatus L.	*Liliaceae*	Gilbarbeira	Diuretic and mildly laxative, venous insufficiency, vasoconstrictor and anti-inflammatory	Aerial part
Thymus lotocephalus G. López & R. Morales	*Lamiaceae*	Tomilho-cabeçudo	Antiseptic, antimicrobial and antitussive	Aerial part

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