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Metabolic profile and biological activities of *Lavandula pedunculata* subsp. *lusitanica* (Chaytor) Franco: Studies on the essential oil and polar extracts

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ABSTRACT

We investigated the metabolic profile and biological activities of the essential oil and polar extracts of *Lavandula pedunculata* subsp. *lusitanica* (Chaytor) Franco collected in south Portugal. Gas chromatography–mass spectrometry (GC–MS) analysis revealed that oxygen-containing monoterpenes was the principal group of compounds identified in the essential oil. Camphor (40.6%) and fenchone (38.0%) were found as the major constituents. High-performance liquid chromatography with diode array detection (HPLC–DAD) analysis allowed the identification of hydroxycinnamic acids (3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic and rosmarinic acids) and flavones (luteolin and apigenin) in the polar extracts, with rosmarinic acid being the main compound in most of them. The bioactive compounds from *L. pedunculata* polar extracts were the most efficient free-radical scavengers, Fe^{2+} chelators and inhibitors of malondialdehyde production, while the essential oil was the most active against acetylcholinesterase. Our results reveal that the subspecies of *L. pedunculata* studied is a potential source of active metabolites with a positive effect on human health.

1. Introduction

Aromatic plants synthesize a diverse array of organic compounds with important ecological and physiological functions in plant-environment interactions. Essential oils contain secondary metabolites that, together with phenolic compounds, are among the most important ones produced by aromatic plants (Osbourn & Lanzotti, 2009). Monoterpenes and sesquiterpenes are the main constituents of essential oils. They are synthesized through condensations of the universal five-carbon precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which derive from two independent pathways (Dudareva et al., 2005). Phenolic compounds are a chemically heterogeneous group (simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignans and lignins) biochemically synthesized *via* the shikimate and/or acetate pathways (Garcia-Salas, Morales-Soto, Segura-Carretero, & Fernandez-Gutierrez, 2010; Singer, Crowley, & Thompson, 2003).

Nowadays, the demand for plant-derived compounds by food, pharmaceutical and cosmetic industries has increased, because they are accepted by consumers as relatively safe and healthy compared to their synthetic counterparts. *Lavandula* species (Lamiaceae) are some of the most popular aromatic plants and are widely used in food, perfume and pharmaceutical industries (Boelens, 1995; Kim & Lee, 2002). Many *Lavandula* species produce compounds with antimicrobial (Hanamanthagouda et al., 2010; Zuzarte et al., 2009), antioxidant and anti-cholinesterases properties (Costa, Gonçalves, Andrade, Valentão, & Romano, 2011; Costa et al., 2012a; Matos et al., 2009). Natural antioxidants can efficiently protect cells against oxidative stress, because they can act as free radical scavengers, reducing agents, hydrogen donators

Abbreviations: Aβ, amyloid beta; AAPH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; $ABTS^{\cdot+}$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation; ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; ATCI, acetylthiocholine iodide; AUC, area under the curve; BChE, butyrylcholinesterase; BHT, butylated hydroxytoluene; BTCI, butyrylthiocholine chloride; DTNB, 5,5′-dithiobis [2-nitrobenzoic acid]; EDTA, ethylenediaminetetraacetic acid; HD, hydrodistillation; HPLC–DAD, high-performance liquid chromatography–diode array detection; GC–MS, gas chromatography–mass spectrometry; MDA, malondialdehyde; ORAC, oxygen radical absorbance capacity; SDS, sodium dodecylsulphate; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; TE, Trolox equivalents; TEAC, Trolox equivalent antioxidant capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

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and transition metals chelators (Dai & Mumper, 2010). Based on the premise that free radicals are involved in neurodegenerative diseases, such as Alzheimer's disease (AD) (Andersen, 2004), natural antioxidants are of all interest. AD has a complex pathogenesis, being characterised by a deficit in the cholinergic system. The use of cholinesterase inhibitors is an efficient strategy for the treatment of AD, contributing to the enhancement of acetylcholine (ACh) levels in the synaptic cleft (cholinergic hypothesis) (Wilkinson, Francis, Schwam, & Payne-Parrish, 2004). Plant-derived compounds are used in the treatment of AD and this is a positive indicator that natural product discovery is important for AD therapy (Saklani & Kutty, 2008). *Lavandula pedunculata* (Miller) Cav. is an aromatic shrub common in the Iberian Peninsula and traditionally used in Portuguese medicine and as ornamental plant. Franco (1984) considered three subspecies for *L. pedunculata*: subsp. *pedunculata* in northwest Portugal, subsp. *sampaiana* in north and central Portugal, and subsp. *lusitanica* in central and south Portugal. Infusions prepared from flowered aerial parts are traditionally consumed to treat anxiety, insomnia, anorexia, cough and bronchitis (Proença da Cunha, Pereira da Silva, & Roque, 2003; Salgueiro, 2004). The essential oils from *L. pedunculata* have been studied by several authors in the last decades. For instance, the essential oils of *L. pedunculata* harvested in north and central Portugal are characterised by a high amount of oxygenated monoterpenes with 1,8-cineole (2.4–55.5%), fenchone (1.3–59.7%), and camphor (3.6–48.0%) as the most abundant and having an important antifungal activity (Zuzarte et al., 2009). In addition, the anti-acetylcholinesterase activity of the essential oil and extracts of *L. pedunculata* from eastern Portugal has been previously described (Ferreira, Proença, Serralheiro, & Araújo, 2006). In this work, *L. pedunculata* subsp. *lusitanica* (Chaytor) Franco [=*Lavandula* stoechas subsp. *lusitanica* (Chaytor) Rozeira] collected in south Portugal was studied because there are few reports concerning the biological potential of this subspecies. Only Matos et al. (2009) analysed the chemical profile and antioxidant activity of its essential oil. Therefore, we describe the metabolic profile and biological activity of the essential oil and polar extracts from *L. pedunculata* subsp. *lusitanica*. The qualitative and quantitative analysis of the essential oil and extracts was performed by gas chromatography coupled to mass spectrometry (GC–MS) and by high-performance liquid chromatography with diode-array detection (HPLC–DAD), respectively. The antioxidant activity was evaluated by measuring the Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), Fe^{2+}-chelation activity and the inhibition of Fe^{2+}-induced lipid peroxidation in mouse brain homogenates (*in vitro*) and the anti-cholinesterases activity was assessed by the Ellman's method. To our knowledge this is the first report about the phenolic profile and biological potential of extracts from this subspecies and investigations on its essential oil are still scarce.

2. Materials and methods

2.1. Standards and reagents

Apigenin, 5-*O*-caffeoylquinic acid, luteolin and rosmarinic acid were purchased from Extrasynthése (Genay, France). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) tablets, potassium persulphate ($K_2S_2O_8$), thiobarbituric acid (TBA), trizma base (Tris), acetylthiocholine iodide (ATCI), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), acetylcholinesterase (AChE) (Electric-eel, EC 3.1.1.7, Type VI-S), butyrylcholinesterase (BChE) (horse-serum, EC 3.1.1.8), galantamine hydrobromide, malondialdehyde tetrabutylammonium salt (MDA) and butyrylthiocholine chloride (BTCI) were purchased from Sigma–Aldrich (Steinheim, Germany). Formic acid, methanol and iron(II) sulphate ($FeSO_4$) were acquired from Merck (Darmstadt, Germany). Fluorescein, 1,10-phenanthroline and absolute ethanol were obtained from Panreac (Barcelona, Spain). 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), butylated hydroxytoluene (BHT) and sodium dodecylsulphate (SDS) were purchased from Acros Organics (Geel, Germany). Qualitative filter paper was purchased from VWR (Leuven, Belgium). Ethylenediaminetetraacetic acid (EDTA) and sodium chloride (NaCl) were purchased from Fluka (Steinheim, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Plant material and extraction procedure

The aerial parts of *L. pedunculata* subsp. *lusitanica* plants were collected during the flowering period at Campus de Gambelas (Algarve, south Portugal). A voucher specimen was deposited in the herbarium of the University of Algarve under the number ALGU 8080. The plant material was dried at room temperature, ground to powder in a blender to achieve a mean particle size lower than 2 mm and stored at −20 °C until required.

Essential oil was obtained by hydrodistillation (HD) for 3 h, using 50 g of plant material in a Clevenger-type apparatus. Extraction of phenolic compounds was performed using water and ethanol separately and in a 1:1 mixture. The plant material (10 g) was soaked overnight at room temperature in 200 ml of each solvent and the resulting extract was filtered through a 5–13 μm membrane. An infusion was also prepared by homogenizing 1 g of the plant material in 20 ml hot water (90 °C) for 5 min. Finally, the extracts were concentrated to dryness (the water extracts were lyophilized and the ethanol and water:ethanol extracts were dried in a rotary evaporator) and stored at −20 °C. The extraction yields (w/w, in terms of initial dried material) were 1.1% for the essential oil and 22.5%, 22.4%, 19.4% and 19.6% for infusion, water, water:ethanol and ethanol extracts, respectively.

2.3. GC–MS analysis

The essential oil was analysed using an Agilent 6890 Series gas chromatograph equipped with an Agilent 7683 automatic liquid sampler coupled to an Agilent 5973N mass selective detector (Agilent Technologies, Little Falls, DE, USA). A programmed temperature vaporisation injector with a liner filled with glass wool was used operating in the split mode injection (1:100) heated at a constant temperature of 275 °C. The samples were injected using a volume of 1 μl. Helium was used as a carrier gas at a constant pressure mode (13.9 psi). GC analysis was performed on a TRB-5MS (30 m × 0.25 mm ID, 0.25 μm film thickness) capillary column (5% diphenyl, 95% dimethylpolysiloxane; Teknokroma, Spain). The oven temperature was set at 50 °C for 1 min, followed by an increase of 5 °C/min to 240 °C in a 39 min total run. All mass spectra were acquired in electron impact (EI) mode. The transfer line, ion source, and quadrupole analyser temperatures were 280, 230, and 150 °C, respectively, and a solvent delay of 3 min was selected. Fullscan mode electron ionisation mass spectra were recorded, in the range 35–550 Da at 70 eV electron energy, with an ionisation current of 34.6 μA. Data recording and instrument control were performed by the MSD ChemStation software (G1701CA; Version C.00.00; Agilent Technologies). Repeatability was verified by analysing the sample three times and the components were identified by comparison of their retention index, relative to a standard mixture of n-alkanes (Adams, 2001) and by comparison with the Wiley's library spectral data bank (G1035B; Rev D.02.00; Agilent Technologies).