Food Research International 48 (2012) 330–335

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Phenolic constituents of *Lamium album*: Focus on isoscutellarein derivatives

Olívia R. Pereira [a,b], Maria R.M. Domingues [c], Artur M.S. Silva [c], Susana M. Cardoso [a,d,*]

[a] CERNAS — Escola Superior Agrária, Instituto Politécnico de Coimbra, Bencanta, 3040-316 Coimbra, Portugal
[b] Departamento de Tecnologias de Diagnóstico e Terapêutica, Escola Superior de Saúde, Instituto Politécnico de Bragança, Av. D. Afonso V, 5300-121 Bragança, Portugal
[c] QOPNA - Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[d] CIMO — Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia — Apartado 1038–5301-854, Bragança, Portugal

ARTICLE INFO

Article history:
Received 9 January 2012
Accepted 30 April 2012

Keywords:
Lamium album
Verbascoside
Isoverbascoside
Isoscutellarein derivatives
High performance liquid chromatography
Electrospray ionization-mass spectrometry

ABSTRACT

Lamium album L. is an edible plant which is consumed raw or cooked, in particular in the Mediterranean and surrounding areas. It is also consumed as tea infusions and as a main component of food supplements, because of its pharmacological effects. Despite being consumed by humans for centuries, the chemical composition of *L. album* L. is far from being understood. In this study, a purified ethanolic extract (PEEL) was prepared and further analyzed by high performance liquid chromatography and electrospray mass spectrometry. Overall, verbascoside accounted for approximately half of the phenolic content of the extract, but this also contained other bioactive phenolic compounds herein detected for the first time in the genus, namely isoscutellarein derivatives. The latter included isoscutellarein-7-*O*-allosyl(1→2)glucoside, its *O*-methyl derivative, three acetyl derivatives of isoscutellarein-*O*-allosyl glucoside and one acetylated form of *O*-methylisoscutellarein-7-*O*-allosyl(1→2)glucoside. From those, the main isoscutellarein derivative was assigned to isoscutellarein-7-*O*-(6-*O*-acetyl-β-allosyl)(1→2)-β-glucoside, as confirmed by NMR. Altogether, isoscutellarein derivatives accounted for almost 30% of PEEL phenolics. Since verbascoside and isoscutellarein derivatives are main components of *L. album* L. ethanolic extract, their possible association to the health benefits of the plant is discussed.

1. Introduction

The genus *Lamium* L. (Family: Lamiaceae alt. Labiatae) comprises about 40 annual or perennial herb species native to the Old World, distributed in Europe, Asia and Africa.

Lamium album L. is a perennial herb commonly known as white dead nettle that has been used as emergency or famine food, particularly during the specific decades of starvation as an alternative nourishment in different countries such as Europe, China and Japan (Baranov, 1967; Luczaj, 2008; Sturtevants, 1919; Turner et al., 2011). In modern times, *L. album* L. is mainly consumed in the Mediterranean and surrounding areas for confection of local dishes (Heinrich, Müller, & Galli, 2006). In fact, the young shoots, leaves and flowers of this plant are edible and consumed raw or cooked as a vegetable. The plant is also commonly used as an ingredient in several dishes including omelets, stews and roasts (Clifford, 2001). Moreover, white dead nettle is the base ingredient for important vegetarian dishes such as the "White Dead Nettle Frittata", "White Dead Nettle, Feta and Watermelon Salad" and the "Deadnettle soup" (Celnet, 2005; Harford, 2007).

L. album L. is also used in teas and in food supplement preparations, the consumption of which is primarily associated to the plant health benefits. In particular, the consumption of food supplements enriched in *L. album* L. extracts are claimed to detoxify the organism, to prevent menstrual disorders, abdominal inflammation and musculoskeletal diseases (Xu, 2008) and to improve fat metabolism (Ninomiya et al., 2006).

Besides the above applications, the flowers of *L. album* L. are attractive to bees and other pollinating insects and hence, are frequently used in honey production (Denisow & Bozek, 2008; Mihaly Cozmuta, Bretan, Mihaly Cozmuta, Nicula, & Peter, 2012).

During the last decades food health attributes have become an important issue of concern for consumers, clearly influencing their choices. In parallel, the search for food constituents related to health properties has incredibly raised. This provides the base knowledge to understand the beneficial properties of a particular food product and further stimulate consumers' interest in it. In the particular case of *L. album* L., the phenolic compounds have been closely associated with the antioxidant properties of the plant (Matkowski & Piotrowska, 2006; Valyova, Dimitrova, Ganeva, Mihova Kapchina-Toteva, & Petkova Yordanova, 2011), as well as to its remaining health benefits (Paduch et al., 2008; Paduch, Wójciak-Kosior, & Matysik, 2007).

In this way, several *L. album* L. phenolic compounds have already been detected, which include the flavonoids quercetin, quercetin-3-*O*-glucoside, rutin, isoquercitrin, kaempferol-3-*O*-glucoside and tiliroside,

* Corresponding author at: CERNAS — Escola Superior Agrária, Instituto Politécnico de Coimbra, Bencanta, 3040-316 Coimbra, Portugal. Tel.: +351 239 802940; fax: +351 273 239 802979.
E-mail address: scardoso@esac.pt (S.M. Cardoso).

0963-9969/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2012.04.009

the phenolic acids protocatechuic, chlorogenic, vanillic and caffeic and the phenylpropanoid glycoside ester derivatives lamalboside, acteoside and isoacteoside (Budzianowski & Skrzypczak, 1995; Paduch et al., 2007; Yalcin & Kaya, 2006). However despite that information, a detailed knowledge of the *L. album* L. phenolic constituents, as well as their content in the plant, is still missing. Hence, these two topics will be herein described in detail.

2. Experimental

2.1. Chemicals

The phenolic standards verbascoside, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside and naringenin-7-*O*-glucoside were obtained from Extrasynthese (Genay Cedex, France). Gallic acid was obtained from Sigma Chemical Co (St Louis, MO, USA), while Folin–Ciocalteu reagent, Na_2CO_3, formic acid and ethanol were purchased from Panreac (Barcelona, Spain). *n*-Hexane, methanol and acetonitrile with HPLC purity were purchased from Lab-Scan (Lisbon, Portugal). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA). DMSO-d_6 containing 0.03% of TMS was obtained from CortecNet (Paris, France).

2.2. Plant material

The *L. album* were purchased as a mixture of flowers, leaves and stems from *O SEGREDO DA PLANTA — Produtos Naturais e Biológicos, Lda.* (Seixal, Portugal). The plants have been cultivated under an organic regime and after collection, its aerial parts (flowers, leaves and stems) were dried in a ventilated incubator at 20 to 35 °C for 3 to 5 days.

2.3. Extraction of phenolic compounds

The aerial parts (flowers, leaves and stems) of *L. album* (5 g) were ground together and defatted three times with 150 mL of *n*-hexane. The residue was extracted with 150 mL of an 80% ethanol solution (*v*/*v*) at room temperature, for 1 h and the resulting mixture was filtered. The residue was similarly re-extracted five times and the filtrated solutions were combined, concentrated, frozen at −20 °C and freeze-dried. The dried extract (ethanolic extract) of *L. album* was stored under vacuum, in a desiccator in dark, for subsequent use (Pereira, Silva, Domingues, & Cardoso, 2012). This procedure was performed in triplicate.

2.4. Purification of phenolic compounds

The ethanolic extracts were further purified for phenolic enrichment. For that, approximately 0.4 g of each ethanolic extract was dissolved in 15 mL of water and eluted in three Strata SPE C18-E cartridges (2 g, Waters, Milford, MA, USA). The cartridges were then washed three times with 30 mL of water, and the phenolic compounds were recovered by elution with 20 mL of methanol. The residue was concentrated, frozen at −20 °C and freeze-dried to give the purified ethanolic extract (PEEL) (Pereira et al., 2012).

2.5. Quantification of total phenolic compounds

Total concentration of phenolic compounds was determined according to the adapted Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965). A mixture of 250 μL of Folin–Ciocalteu reagent and 0.5 mL plant extract solution (0.4 mg/mL) was prepared. After 3 min, 1 mL of Na_2CO_3 (200 g/L) and 3.25 mL of milliQ water were added. The mixture was homogenized and incubated for 10 min at 70 °C, and then kept at room temperature for 30 min. The absorbance was measured at 700 nm and the amount of total phenolic compounds was expressed as gallic acid equivalent (mg GAE)/g dried weight of plant material using a calibration curve of gallic acid as standard (5 to 37.5 μg/mL). This procedure was performed at least in duplicate for the three PEEL samples.

2.6. HPLC apparatus and chromatographic conditions

The HPLC analysis was performed on a Varian 9010 separation module equipped with a PDA Varian Prostar detector and data acquisition and remote control of the HPLC system were done by Varian Star chromatography Workstation® (Lake Forest, CA, USA) software. The column used was a 250 mm × 4 mm id, 5 μm bead diameter, end-capped Nucleosil C18 (Macherey-Nagel) and its temperature was maintained at 30 °C.

Gradient elution was carried out with a mixture of 0.1% (*v*/*v*) of formic acid in water (solvent A) and acetonitrile (solvent B), which were degassed and filtered before use. The solvent gradient consisted of a series of linear gradients, starting from 10 to 20% of solvent B over 6 min, 20 to 25% of solvent B over 6 min, 25 to 40% over 30 min, increasing to 45% at 50 min and to 100% of solvent B over 5 min decreasing to 10% of solvent B after 5 min followed by the return to the initial conditions. The flow rate used was 1 mL/min. For the HPLC analysis, the samples (10 mg) were dissolved in 2 mL of methanol, filtered through a 0.2 μm Nylon membrane (Whatman) and 10 μL of each solution was injected. The UV–vis spectra were recorded between 220 and 500 nm and the chromatographic profiles were recorded at 340 nm.

2.7. Identification and quantification of the phenolic compounds

Identification of the compounds was performed by HPLC–DAD and ESI-MS analysis. The compounds were firstly identified according to the retention time and UV–vis spectra of the HPLC eluting peaks. After three manual collections, further characterization of the eluted compounds was accomplished by electrospray ionization mass spectrometry (ESI-MS and ESI-MS^n) using a Linear Ion trap LXQ mass spectrometer (ThermoFinnigan, San Jose, CA, USA), following the general procedure previously described (Pereira et al., 2012). Moreover, the most abundant isoscutellarein derivative (fraction 9) was further analyzed by NMR spectroscopy. To accomplish that, approximately 3 mg of freeze-dried material of this HPLC fraction was dissolved in DMSO-d_6 and the ^1H and ^{13}C NMR spectra were recorded at 298 K on a Bruker Avance 500 spectrometer operating at 500.13 MHz and 125.77 MHz, respectively. The phase sensitive ^1H-detected (^1H,^{13}C) *g*HSQC (heteronuclear single quantum coherence, using gradient pulses for selection) spectrum was recorded with 216 transients over 256 increments (zero-filled to 512) and 2 K data points with spectral widths of 4500 Hz in F_2 and 20 kHz in F_1. The repetition time was 1.9 s. A cosine multiplication was applied in both dimensions. The delays were adjusted according to a coupling constant ^1J(CH) of 147 Hz. The *g*HMBC (heteronuclear multiple quantum coherence, using gradient pulses for selection) spectrum was recorded with 240 transients over 256 increments (zero-filled to 1 K) and 2 K data points with spectral widths of 4500 Hz in F_2 and 25 kHz in F_1. The repetition time was 1.9 s. A sine multiplication was applied in both dimensions. The low-pass *J*-filter of the experiment was adjusted for an average coupling constant ^1J(CH) of 147 Hz and the long-range delay utilized to excite the heteronuclear multiple quantum coherence was optimized for 7 Hz.

Taking into account the nature of the phenolic compounds (phenylethanoids and flavones), their quantification was performed at 340 nm (Galvez, Martin-Cordero, Houghton, & Ayuso, 2005) by the external standard method. The detection and quantification limits (LOD and LOQ, respectively) were determined from the parameters of the calibration curves represented in Table 1, being defined as 3.3 and 10 times the value of the regression error divided by the slope, respectively (Ermer & Miller, 2005; Snyder, Kirkland, & Dolan, 2010).

Fractions 2 and 3 (verbascoside, isoverbascoside) were quantified using verbascoside as a reference compound. Apigenin-7-*O*-glucoside was used to quantify fractions 4 [isoscutellarein-7-*O*-allosyl(1→2)