

## Novel flavones in *Lamium L.* genus

Olívia R. Pereira<sup>a,b</sup>, Maria R.M. Domingues<sup>c</sup>, Artur M. S. Silva<sup>c</sup>, Susana M. Cardoso<sup>a,d\*</sup>

<sup>a</sup>CERNAS - ESA, Instituto Politécnico de Coimbra, Coimbra, Portugal

<sup>b</sup>DTDT, ESSa, Instituto Politécnico de Bragança, Bragança, Portugal

<sup>c</sup>Departamento de Química & QOPNA, Universidade de Aveiro, Aveiro, Portugal

<sup>d</sup>CIMO - ESA, Instituto Politécnico de Bragança, Bragança, Portugal

\*scardoso@esac.pt

**Keywords:** *Lamium L.*, Isoscutellarein derivatives, HPLC-DAD, ESI-MS<sup>n</sup>, RMN

### ABSTRACT

The main aim of this work is to determine the phenolic composition of a *Lamium album L.* purified extract, in order to contribute for the chemical characterization of this plant. Moreover, it also intends to emphasize its antioxidant properties. As established by HPLC-DAD and ESI-MS<sup>n</sup> combined analysis, verbascoside was the major phenolic constituent of *Lamium album L.* purified extract, as previously described by other authors [1]. Still, we could also detect, for the first time in *Lamium* genus, significant amounts of isobervascoside and of isoscutellarein derivatives. As confirmed by NMR analysis, the major isoscutellarein derivative in *Lamium album L.* purified extract was the isoscutellarein-7-*O*-(6-*O*-acetyl- $\beta$ -allosyl)(1 $\rightarrow$ 2)- $\beta$ -glucoside, but isoscutellarein-7-*O*-allosyl(1 $\rightarrow$ 2)glucoside and of *O*-methyl derivatives of these two compounds were also present in considerable amounts. Additionally, the extract showed good antioxidant activities in *in vitro* experiments. Overall, the present study contributes for the chemical and biological characterization of *L. album L.*

### 1. INTRODUCTION

*Lamium L.* (Family: Lamiaceae alt. Labiatae) is a genus that includes about forty species distributed in Europe, Asia and Africa. Many plants of this genus have been used as emergency or famine food during the specific decades of starvation. Currently, they are consumed raw or cooked as a vegetable, and they are a component of several dishes and of food supplements. Additionally, many *Lamium L.* species have been used in traditional and officinal medicine for the treatment of several disorders [2]. Despite the importance of the flavonoid compounds in the beneficial activities of plants, there are few studies focusing this class of second metabolites. In this context, we herein focus the phenolic composition and the antioxidant capacity of *Lamium album L.*, in order to improve the knowledge on the *Lamium L.* genus..

## 2. MATERIALS AND METHODS

### 2.1 Determination of phenolic compounds

The purified ethanolic extracts of *L. album* was obtained by extraction with an 80% ethanolic solution (v/v) and further purification on Strata SPE C18-E cartridges, as previously described [3]. The main flavones in the extract were identified by means of HPLC-DAD and ESI-MS<sup>n</sup> techniques and, the structure of the major flavone was confirmed by NMR spectroscopy [3]. HPLC-DAD was performed with a RP-C18 column 250 mm× 4 mm id, 5µm bead diameter (temperature of 30°C, flow rate of 1 mL/min) and the mobile phase comprised (A) 0.1% formic acid in water and (B) acetonitrile. Phenolic quantification was performed at 340 nm by HPLC-DAD using the external standard method.

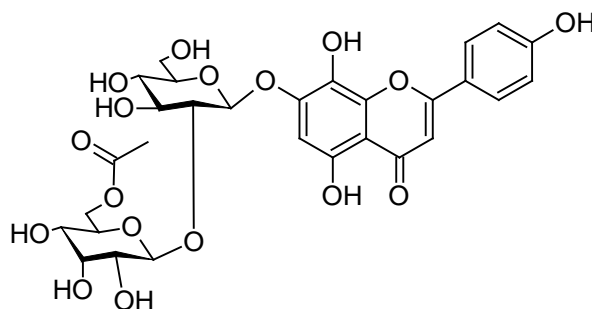
### 2.2 Antioxidant activity

The antioxidant activity of *L. album* purified extract was performed by assessment of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) scavenging potential [4] and reducing power [5]. Additionally, the potential protection of *L. album* purified extract against the generation of reactive oxygen species (ROS) induced by potassium dichromate (25 µM) was conducted in hepatoblastoma HepG2 cells. ROS formation was measured by flow cytometry after a 48 h treatment with *L. album* purified extract at 200 µg/ mL, following a procedure previous described [6].

## 3. RESULTS AND DISCUSSION

### 3.1 Polyphenols of the *L. album* extract

The phenolic compounds of *L. album* purified extract accounted for 500.7±50.0 mg/g of extract (Table 1). Approximately 30% of these phenolics were isoscutellarein derivatives, showing a typical UV spectra with maxima at 278, 302 and 333 nm. Note that the presence of isoscutellarein derivatives in the *Lamium* genus counteracts previous chemotaxonomic studies [7]. The main isoscutellarein derivative in the purified extract accounted for 37.4 ± 4.4 mg/g of extract and it was assigned to isoscutellarein-7-*O*-(6-*O*-acetyl-β-allosyl)(1→2)-β-glucoside (Fig. 1), on the basis of its characteristic mass spectrometry fragmentation pattern ( $m/z$  651 → 609 → 285 → 241, 243, 199, 175) and NMR analysis (data not shown). The remaining isoscutellarein derivatives detected in the enriched phenolic extract included two structural isomers of the latter compound, namely the 7-*O*-allosyl(1→2)glucoside (MW 610 Da) and the 4'-*O*-methyl-7-*O*-allosyl(1→2)glucoside (MW 624 Da), as well as the 4'-*O*-methyl-7-*O*-(6-*O*-acetylallosyl)(1→2)glucoside (MW666).

**Figure 1.** Structure of isoscutellarein-7-*O*-(6-*O*-acetyl- $\beta$ -allosyl)(1 $\rightarrow$ 2)- $\beta$ -glucoside.

### 3.2 Antioxidant capacity

The EC<sub>50</sub> value in DPPH and reducing power assays ( $11.2 \pm 0.9$  and  $67.9 \pm 8.7$   $\mu\text{g/mL}$ , respectively) for *L. album* purified extract were good, comparing to the standards used ( $2.5 \pm 0.4$  and  $27.1 \pm 1.2$   $\mu\text{g/mL}$  for the ascorbic acid and BHA, respectively). More, the exposure of HepG2 cells to the non-toxic concentration of *L. album* purified extract (200  $\mu\text{g/mL}$ ) resulted in a decrease rate of ROS production under oxidative stress conditions of approximately 27%. Overall, the extract showed good antioxidant capacity.

**Table 1.** Total phenolics and antioxidant activities of *L. album* purified extract.

Total Phenolics (mg/g extract)	EC <sub>50</sub> Radical Scavenging <sup>(1)</sup> ( $\mu\text{g/mL}$ )	EC <sub>50</sub> Reducing Power <sup>(2)</sup> ( $\mu\text{g/mL}$ )	Protection of ROS Production <sup>(3)</sup> (% of control)
500.7 $\pm$ 50.0	11.2 $\pm$ 0.9	67.9 $\pm$ 8.7	26.7 $\pm$ 8.1

Mean values  $\pm$  standard deviations of three independent assays; <sup>(1)</sup> Amount of extract able to reduce 50% of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH $\cdot$ ) at a concentration of 60  $\mu\text{M}$ ; <sup>(2)</sup> Amount of extract required to provide an absorbance of 0.5 by reducing 3.5  $\mu\text{M}$  Fe<sup>3+</sup> (3.5  $\mu\text{M}$ ) to Fe<sup>2+</sup>; <sup>(3)</sup> ROS increment was induced by potassium dichromate at 25  $\mu\text{M}$  for 48 h. Protective effect was measured in the presence of *L. album* extract (200  $\mu\text{g/mL}$ )

## 4 CONCLUSIONS

Since isoscutellarein derivatives are present in considerable amounts in *L. album* purified extract, it is possible that they are associated to the health benefits of this plant, including the antioxidant properties of the extract herein demonstrated. Further studies focusing these issues should now be addressed.

### **Acknowledgements**

The authors acknowledge the financial support provided by the FCT to CERNAS (project PEst-OE/AGR/UI0681/2011) and CIMO and of the FCT as well as FSE (III Quadro Comunitário de Apoio) to QOPNA (project PEst-C/QUI/UI0062/2011), REDE/1504/REM/2005 (that concerns the Portuguese Mass Spectrometry Network). Olívia R Pereira was supported by a PhD grant (SFRH/PROTEC/49600/2009).

### **References**

- [1] J Budzianowski, L Skrzypczak, *Phytochemistry*, 1995, 38, 997-1001
- [2] A Matkowski & M Piotrowska, *Fitoterapia*, 2006, 77, 5, 346-353
- [3] OR Pereira, MRM Domingues, AMS Silva, SM Cardoso, *Food Res Int*, 2012, 48, 330-335
- [4] A Ferreira, C Proenca, MLM Serralheiro, MEM Araujo, *J Ethnopharm*, 2006,108, 31-37
- [5] M Oyaizu M, *Jpn J Nutr*, 1986, 44, 307-15
- [6] MJ Perez, B Castaño, S Jimenez, MA Serrano, JM Gonzalez-Buitrago, JGG Marin, *Toxicol Appl Pharm*, 2008, 232, 327-336
- [7] F A Tomás-Barberán et al, *Phytochemistry*, 1988, 27, 8, 2631-2645