



Original Research Article

Phytochemical characteristics of *Galeopsis ladanum* subsp. *angustifolia* (Ehrh. ex Hoffm.) Gaudin collected in Abruzzo region (Central Italy) with chemotaxonomic and ethnopharmacological implications

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ABSTRACT

In this work, a phytochemical analysis on the total aerial parts of *Galeopsis ladanum* subsp. *angustifolia* (Ehrh. ex Hoffm.) Gaudin collected in Abruzzo region (Central Italy) has been reported. Nine compounds were identified belonging to five different classes of natural compounds. Additional chemotaxonomic markers were recognized than our previous study, as well. Their presence is perfectly in accordance with the current botanical classification of the species and shows the possibility of a pronounced metabolic variability. The observed phytochemical composition may also suggest the possible use of this species in the ethnopharmacological field just like most *Galeopsis* species since they are endowed with several medicinal properties. Furthermore, no potential toxic component was identified in the studied accession.

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1. Introduction

Galeopsis ladanum subsp. *angustifolia* (Ehrh. ex Hoffm.) Gaudin, [syn. *Galeopsis angustifolia* (Ehrh. ex Hoffm.)], is a small annual herbaceous plant belonging to the Lamiaceae family, subfamily Lamioideae.

The name of the genus derives from the Latin term "galea", meaning "helm", and was coined by the Roman admiral, writer and naturalist Gaius Pliny the Second. In addition, the name of the species derives from Latin but refers to the particular shape of the leaves, lanceolate and narrow.

From the botanical standpoint, the species can reach up to 40 cm height. It is characterized by an ascendant stem with a tetragonal section. The stem is branched only in the upper part and is covered all along with some fuzzy hairs. The leaves are also small, opposite, shortly petiolate, with a full margin. The even small flowers depart from the leaves axillae and

are collected in verticillasters four by four. They are also hermaphrodite and zigomorphic blooming from June to October. The fruit is a schizocarp formed by four glabrous nuculae. The roots are tapped (Fig. 1) (Conti et al., 2005).



Fig. 1. *G. ladanum* (left), particular of the leaves (middle) and of the flowers (right).

G. ladanum subsp. *angustifolia* is typical of the Northern Mediterranean Basin or even Southern Europe.

In Italy, it has a widespread distribution all along

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the national territory with the only exceptions of Apulia, Calabria and Sardinia whereas its presence is uncertain in Sicily (Pignatti, 1982).

The species particularly grows on calcareous rocky cultivated lands and along rivers and railway roads up to 1700 m a.s.l. Its preferred growth area represents the main reason why this species is classified as critically endangered since it is extremely infesting and its population has dramatically decreased during the last years (Pignatti, 1982; Conti et al., 2005) due to the massive use of fertilizers and herbicides to prevent its seeding

In literature, several works deal with the species from the genus *Galeopsis*: from botanical point of view, there are only two studies on the species with morphologic and ecologic aspects (Townsend, 1962; Gibson et al., 2006); as for phytochemistry and chemotaxonomy, many works have focused their attention on specific classes of natural compounds such as diterpenoids (Rodriguez and Savona, 1980; Pérez-Sirvent et al., 1983) or flavonoids (Tomàs-Barberà et al., 1991, 1992). Indeed, only one work explored a combined mix of more classes of natural compounds evidencing the presence of flavonoids and iridoids (Venditti et al., 2013a). However, as far as we know, this is the first study on a specimen collected in Abruzzo region.

On the other hand, few studies are present on the pharmacological properties of the species and of the genus *Galeopsis*. In particular, *G. ladanum* subsp. *angustifolia* is able to exert antioxidant, sedative, neuroprotective and antiacetylcholinesterase activities (Czarnecki et al., 1993; Uriarte-Pueyo and Calvo, 2009) while some *Galeopsis* species are well-known to possess antiinflammatory, astringent, antianemic, expectorant, remineralizing and diuretic effects (Mazza, 2000; Matkowski et al., 2008).

In Southern Italy, *G. ladanum* subsp. *angustifolia* owns an important use in folk medicine since the infusion, syrup or fluid extract of its leaves and/or flowers are capable to treat respiratory system infections for their high hematopoietic and fluidizing effects in the bronchi (Guarino et al., 2008).

The main reason to establish this work was the lack of literature data on this particular specimen which has never been studied before. In addition, we aimed to check if there was any difference between this specimen and the other studied one (Venditti et al., 2013a) because of environmental conditions.

The other purposes of this work were: i) to perform a general phytochemical analysis on this particular specimen, ii) to verify if the presence of chemosystematic markers is in accordance with the current botanical classification of the species, iii) to provide a phytochemical rationale for the use of also this species in the ethnopharmacological field just like for many *Galeopsis* species even if not all of them such as *G. tetrahit* L. and *G. speciosa* Mill. which are

considered to be extremely toxic causing paralysis (Komarov, 1934).

2. Experimental

2.1. Plant material

A significant exsiccated sample of this species (130.0 g) was collected in the town of Civita di Oricola (Abruzzo region, geographical coordinates: N 42°02'58"; E 13°02'21") at about 600 m a.s.l., during August 2015.

The botanical recognition was performed by one of us (A.V.) using available literature (Pignatti, 1982; Conti et al., 2005).

A representative sample of this collection is stored in our laboratory for further references under the accession number GA12082015.

2.2. Chemicals

Throughout this study, the following reagents and solvents were utilized: ethanol 96% for the extraction procedure of the plant material; *n*-butanol, distilled water, methanol and dichloromethane as pure solvents or in mixtures among them all at different concentrations to be used as eluting systems for the separation procedure via classical column chromatography on 40-63 μ m particle size silica gel; sulfuric acid 2N for the development of TLCs; vanillin/HCl methanolic solution as spray reagent for the detection of iridoids; an aqueous solution of FeCl₃ (3% w/v) as spray reagent for the detection of phenolics; deuterated solvents such as CDCl₃, CD₃OD and D₂O for the identification of the metabolites by NMR Spectroscopy; methanol having RS purity grade for the identification of the metabolites by Mass Spectrometry.

All the natural solvents having high purity grade, if not differently specified, together with the deuterated and the RS purity grade methanol were purchased from Sigma-Aldrich while silica gel was purchased from Fluka Analyticals. Moreover, the pre-coated silica gel TLCs "60 F254" were purchased from Merck.

2.3. Apparatus

The following instrumentation was used: Rotavapor RII by Büchi for the solvent evaporation at reduced pressure; vacuum pump "Jet Standard" by "General Scientific Instrument"; lyophilizer belonging to the "Analitica De Mori" company.

NMR spectra were recorded on a Varian (now Agilent Technologies) Mercury 300 MHz instrument and/or on a Bruker Avance III 400 MHz instrument with the chemical shifts expressed in ppm. The residual internal solvent signal of CD₂HOD (m5, 3.31 ppm) was the reference for spectra in CD₃OD while

the HDO signal (s, 4.79 ppm) was set as reference for spectra in D₂O.

MS spectra were performed on a Q-TOF MICRO spectrometer (Micromass, now Waters, Manchester, UK) equipped with an ESI source operating in the negative and/or positive ion mode. The flow rate of sample infusion was 20 µL/min. with 100 acquisitions per spectrum. Data were analysed by using the MassLynx software developed by Waters.

2.4. Procedures

2.4.1. Extraction of secondary metabolites

The dried plant material (130.0 g) consisting of the total aerial parts was extracted with ethanol 96% until it was completely immersed in the reaction medium (about 500 mL × 48 h). The extraction was repeated three times for an exhaustive extraction procedure. The ethanolic solutions were collected altogether in a same flask and ethanol was evaporated under a reduced pressure by using Rotavapor. Throughout the first concentration of the extract, pH of the solution was checked on normal litmus paper. This preliminary test was necessary in order to verify that pH was between the range of 5.5-8.5 to avoid unwanted secondary reactions in the extract such as the hydrolysis of ester and glycosidic bonds. After ethanol was completely eliminated, a water suspension was obtained. This was frozen and then lyophilized to preserve also temperature-sensitive compounds eventually present. The final dried crude extract having a dark green coloration weighed 2.6 g and is still store inside our laboratory.

2.4.2. Isolation and identification of secondary metabolites

The isolation of secondary metabolites was conducted by classical chromatography column.

An aliquot of the total dried crude extract (1.8 g) was subjected to a first chromatographic separation using a corresponding amount of silica gel of 80.0 g, ratio 1:40 w/w. In the isolation process, the eluting system was composed by a solution of *n*-butanol and distilled water at a volume ratio of 82:18 (v/v).

During the chromatographic run, the polarity of the eluting solution was raised in order to let the elution of the most polar compounds and so this became a solution of *n*-butanol, methanol and distilled water at concentration 70:10:30 (v/v/v).

From this first chromatographic separation eight compounds were identified by NMR and MS techniques and by comparison with data reported in literature and standards available in our laboratory.

In details, these were: verbascoside (**1**) (Venditti et al., 2016a), 7-[(6-*O*-acetyl-2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-hydroxy-

phenyl)-4*H*-1-benzopyran-4-one (**3**) (Lenherr and Mabry, 1987), 7-[(6-*O*-acetyl-2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-methoxy-phenyl)-4*H*-1-benzopyran-4-one (**4**) (Venditti et al., 2014), 7-[2-*O*-β-D-allopyranosyl-6-*O*-acetyl-β-D-glucopyranosyl)-oxy]-5,8-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-4*H*-1-benzopyran-4-one (**5**) (Venditti et al., 2014) as an only mixture in ratio 1:3:2:1 from the assembly of fractions 26-30 for the total weight of 24.8 mg; harpagide (**6**) and 8-*O*-acetyl-harpagide (**7**) (Venditti et al., 2016b) in mixture in ratio 1:3 from the assembly of fractions 78-82 for the total weight of 76.7 mg; chlorogenic acid (**8**) (Caprioli et al., 2016) and quinic acid (**9**) (Pauli et al., 1988) in mixture in ratio 1:2 from the assembly of fractions 94-178 for the total weight of 39.8 mg.

Since not all compounds could be clearly detected in this chromatographic step, another chromatographic separation was performed on an assembly of fractions deriving from the first one, in particular, 2-19, with a total weight of 981.2 mg.

This time, the correlative amount of silica gel was 40.0 g (ratio 1:40 w/w) and the eluting system consisted of a solution of dichloromethane and methanol at different concentrations.

The first one was 95:5 (v/v) but during the chromatographic run this was changed in order to perform the elution of the most polar compounds passing to 9:1 (v/v), 8:2 (v/v), 7:3 (v/v) and 6:4 (v/v).

From this chromatographic separation, one more compound was identified by the same previous methods. This was martynoside (**2**) (Yalcin et al., 2003) as almost pure compound from the assembly of fractions 54-75 for the total weight of 12.9 mg.

2.5. NMR and MS data of isolated compounds

verbascoside (**1**): ¹H-NMR (300 MHz, CD₃OD): δ 7.66 (1H, d, *J* = 15.9 Hz, H-β(caff.)), 7.22 (1H, br. s, H-2'), 7.12 (1H, br. d, *J* = 8.2 Hz, H-5'), 6.98 (1H, d, *J* = 8.2 Hz, H-4'), 6.92 (1H, br. d, *J* = 8.1 Hz, H-5''), 6.84 (1H, br s, H-2''), 6.73 (1H, d, *J* = 8.1 Hz, H-6''), 6.38 (1H, d, *J* = 15.9 Hz, H-α(caff.)), 5.15 (1H, br s, H-1'''), 4.47 (1H, d, *J* = 8.2 Hz, H-1), 2.81 (2H, overlapped signal, H-β(tyr.)), 1.06 (3H, d, *J* = 5.9 Hz, H-6''').
ESI-MS: *m/z* 647.50 [M+Na]⁺.

martynoside (**2**): ¹H-NMR (300 MHz, CD₃OD): δ 7.66 (1H, d, *J* = 15.9 Hz, H-β(caff.)), 7.20 (1H, br. s, H-2'), 7.08 (1H, br. d, *J* = 8.5 Hz, H-5'), 6.94 (1H, d, *J* = 8.5 Hz, H-4'), 6.87 (1H, br. d, *J* = 8.2 Hz, H-5''), 6.84 (1H, br s, H-2''), 6.73 (1H, d, *J* = 8.2 Hz, H-6''), 6.37 (1H, d, *J* = 15.9 Hz, H-α(caff.)), 4.44 (1H, d, *J* = 8.2 Hz, H-1'), 3.88 (3H, s, O-CH₃(caff.)), 3.81 (3H, s, O-CH₃(tyr.)), 2.81 (2H, overlapped signal, H-β(tyr.)), 1.05 (3H, d, *J* = 6.2 Hz, H-6''').
ESI-MS: *m/z* 675.19 [M+Na]⁺.

7-[(6-*O*-acetyl-2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-hydroxy-



-phenyl)-4*H*-1-benzopyran-4-one (**3**): ¹H-NMR (300 MHz, CD₃OD): δ 7.93 (2H, d, *J* = 8.2 Hz, H-2' and H-6'), 7.09 (2H, d, *J* = 8.2 Hz, H-3' and H-5'), 6.79 (1H, s, H-3), 6.63 (1H, s, H-6), 5.07 (1H, d, *J* = 7.9 Hz, H-1'''), 4.95 (overlapped with solvent signal, H-1''), 4.00-3.42 (overlapped signals of carbohydrates), 1.98 (3H, s, CH₃CO).

ESI-MS: *m/z* 675.48 [M+Na]⁺.

7-[(6-*O*-acetyl-2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-methoxy-phenyl)-4*H*-1-benzopyran-4-one (**4**): ¹H-NMR (300 MHz, CD₃OD): δ 8.02 (2H, d, *J* = 8.1 Hz, H-2' and H-6'), 7.12 (2H, d, *J* = 8.1 Hz, H-3' and H-5'), 6.77 (1H, s, H-3), 6.68 (1H, s, H-6), 5.09 (1H, d, *J* = 8.2 Hz, H-1'''), 4.95 (overlapped with solvent signal, H-1''), 3.89 (3H, s, CH₃O), 3.85-3.42 (overlapped signals of carbohydrates), 2.01 (3H, s, CH₃CO).

ESI-MS: *m/z* 689.18 [M+Na]⁺.

7-[2-*O*-β-D-allopyranosyl-6-*O*-acetyl-β-D-glucopyranosyl)-oxy]-5,8dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-4*H*-1-benzopyran-4-one (**5**): ¹H-NMR (300 MHz, CD₃OD): δ 7.58 (1H, br. d, *J* = 8.1 Hz, H-6'), 7.47 (1H, br. s, H-2'), 6.95 (1H, d, *J* = 8.1 Hz, H-5'), 6.74 (1H, s, H-3), 6.64 (1H, s, H-6), 5.08 (1H, d, *J* = 8.2 Hz, H-1'''), 4.95 (overlapped with solvent signal, H-1''), 3.95 (3H, s, CH₃O), 3.94-3.42 (overlapped signals of carbohydrates), 2.15 (3H, s, CH₃CO).

ESI-MS: *m/z* 705.49 [M+Na]⁺.

harpagide (**6**): ¹H-NMR (300 MHz, D₂O): δ 6.36 (1H, d, *J* = 6.1 Hz, H-3), 5.72 (1H, br. s, H-1), 5.05 (1H, br. d, *J* = 6.1 Hz, H-4), 4.77 (overlapped with solvent signal, H-1'), 3.94-3.35 (overlapped signals of carbohydrates), 2.53 (1H, s, H-9), 1.95-1.77 (2H, overlapped signals, Ha-7 and Hb-7), 1.23 (3H, s, H-10).

ESI-MS: *m/z* 387.32 [M+Na]⁺.

8-*O*-acetyl-harpagide (**7**): ¹H-NMR (300 MHz, D₂O): δ 6.42 (1H, d, *J* = 6.2 Hz, H-3), 6.06 (1H, br. s, H-1), 4.97 (1H, br. d, *J* = 6.2 Hz, H-4), 4.60 (1H, br. d, *J* = 7.9 Hz, H-1'), 3.94-3.35 (overlapped signals of carbohydrates), 2.83 (1H, s, H-9), 2.24-2.11 (1H, m, Ha-7), 2.03 (3H, s, CH₃CO), 1.99-1.80 (1H, m, Hb-7), 1.41 (3H, s, H-10).

ESI-MS: *m/z* 429.34 [M+Na]⁺.

chlorogenic acid (**8**): ¹H-NMR (300 MHz, D₂O): δ 7.51 (1H, d, *J* = 15.7 Hz, H-10), 7.05 (1H, br. s, H-12), 6.96 (1H, br. d, *J* = 8.3 Hz, H-15), 6.84 (1H, d, *J* = 8.3 Hz, H-16), 6.25 (1H, d, *J* = 15.7 Hz, H-9), 5.37 (1H, m, H-3), 4.41 (1H, m, H-4), 2.27-1.95 (4H, m, Ha-2, Hb-2, Ha-6 and Hb-6).

ESI-MS: *m/z* 377.21 [M+Na]⁺; *m/z* 353.27 [M-H]⁻.

quinic acid (**9**): ¹H-NMR (300MHz, D₂O) δ: 4.12 (1H, m, H-4), 3.99 (1H, overlapped signal with carbohydrates, H-5), 3.52 (1H, overlapped signal with carbohydrates, H-3), 2.11 (4H, m, Ha-2, Hb-2, Ha-6 and Hb-6).

ESI-MS: *m/z* 215.19 [M+Na]⁺; *m/z* 191.09 [M-H]⁻.

3. Results and Discussion

The phytochemical analysis conducted on the ethanolic extract from the total aerial parts of *Galeopsis ladanum* subsp. *angustifolia* evidenced the presence of nine compounds belonging to five different classes of natural compounds: phenyl-ethanoid glycosides, verbascoside (**1**) and martynoside (**2**); flavonoids and, in particular, isoscutellarein derivatives, 7-[(6-*O*-acetyl-2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-hydroxy-phenyl)-4*H*-1-benzopyran-4-one (**3**) and 7-[(6-*O*-acetyl-2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-methoxy-phenyl)-4*H*-1-benzopyran-4-one (**4**) and hypolaetin derivatives, 7-[2-*O*-β-D-allopyranosyl-6-*O*-acetyl-β-D-glucopyranosyl)-oxy]-5,8dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-4*H*-1-benzopyran-4-one (**5**); iridoids, harpagide (**6**) and 8-*O*-acetyl-harpagide (**7**); caffeoyl-quinic acids, chlorogenic acid (**8**); organic acids, quinic acid (**9**) (Fig. 2).

Compared to the previous work (Venditti et al., 2013a) some important differences could be observed during this study.

First of all, only the present study evidenced the occurrence of the phenyl-ethanoid glycosides, verbascoside (**1**) and martynoside (**2**). Moreover, the flavonoid pattern observed in the Abruzzo accession showed no similarities with the sample from Latium even if they belong to the same family of compounds, diglycosidic acetylated isoscutellarein (**3**, **4**) and hypolaetin (**5**) derivatives containing allose. The presence of the hypolaetin derivative containing two glucopyranose units, originally identified in the sample from Latium (Venditti et al., 2013a), was not detected in this accession. On the other hand, the occurrence of 7-[(6-*O*-acetyl-2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**5**), the first derivative of this series of flavonoids with a monoacetylation at C-6 of the glucose moiety which was recognized for the first time in *S. tymphaea* (Venditti et al., 2014), was confirmed in the present study. The occurrence of different acetylated allose-containing derivatives of isoscutellarein/hypolaetin may be due to the different environmental conditions of the sampling areas. In fact, the sample from Latium was collected in a mountain environment at about 1700 m a.s.l. (the maximal survival altitude limit for this species) while the sample from Abruzzo was collected in a plain at about 600 m a.s.l. and so they were subjected to different environmental stresses. In relation to the iridoid pattern, harpagide (**6**) and 8-*O*-acetyl-harpagide (**7**) were evidenced in both studied samples whereas 8-epiloganin, which is considered to be one of the precursors of the decarboxylated carbocyclic iridoids with an 8α-configuration and is commonly found in Lamiales,

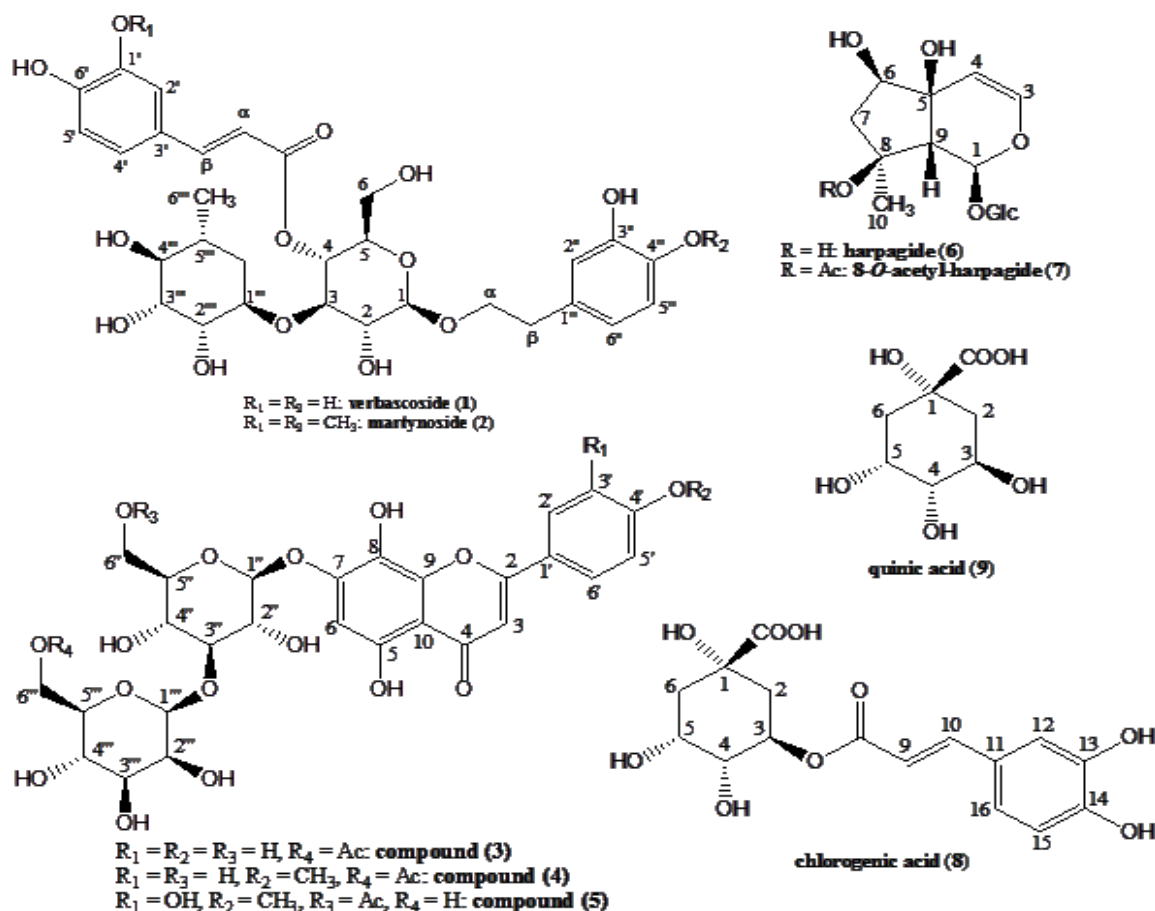


Fig. 2. Structures of isolated compounds in *G. ladanum*.

was detected only in the specimen collected in Latium. Lastly, about the most polar components, only the sample collected in Abruzzo region, evidenced a modest amount of two organic acids, chlorogenic acid (8) and quinic acid (9).

The presence of all these compounds is important from a chemotaxonomic point of view. Verbascoside (1) is a very common phenyl-ethanoid glycoside in nature and in particular in Lamiaceae. In fact, its presence has been reported several times before in this family such as in *Ajuga*, *Sideritis* and *Stachys* genera (Venditti et al., 2013b, 2013c; Frezza et al., 2017). Nevertheless, its presence in *Galeopsis* genus was reported for the first time during this study.

On the other hand, martynoside (2) represents a new compound for the species. In the *Galeopsis* genus, its presence has been already documented, in particular for *G. pubescens* Besser (Calis et al., 1984) whereas, in the Lamiaceae family, its presence seems to be more limited. In fact, it has been isolated only from *Stachys affinis* Bunge (Venditti et al., 2017a), which belongs to the same sub-family Lamiaceae, anyway.

From a mere chemotaxonomic standpoint, these phenyl-ethanoid glycosides gain here much more importance because they were found together with iridoids thus raising to the rank of similar

chemotaxonomic marker (Jensen, 1992). Also, the three flavonoids strictly represent new phytochemicals for the genus. Actually, similar compounds have been isolated from *G. ladanum* even if without the same structure (Uriarte-Pueyo and Calvo, 2010). Meanwhile, the same compounds have been also identified in *Sideritis* and *Stachys* species (Venditti et al., 2016c, 2016d, 2017b). Indeed, acetylated flavonoids containing allose with an isoscutellarein/hypolaetin base structure are considered to be chemosystematic markers because their distribution is restricted to several genera in the Lamiaceae subfamily and more specifically to *Galeopsis*, *Pogostemon*, *Stachys* and *Sideritis* (Tomàs-Barberà et al., 1992). Considering also the differences observed between the samples from Latium and Abruzzo localities, it seems that the biosynthesis of flavonoidic metabolites in this species may be deeply influenced by environmental conditions. Conversely, harpagide (6) and 8-O-acetylharpagide (7) are not new phytochemicals even for the species (Venditti et al., 2013a). As a matter of fact, these two compounds, are the chemotaxonomic markers for excellence of the Lamiaceae family and their occurrence has been widely reported both in the *Galeopsis* genus (Sticher et al., 1975; Sticher and Weisflog, 1975) and in the Lamiaceae family (Venditti et al., 2013c, 2014, 2016b, 2016d, 2016e, 2016f; Frezza



et al., 2017). In addition, chlorogenic acid (**8**) is a new compound for the genus even if it represents the most common caffeoyl-quinic derivative present in nature. In the Lamiaceae family, it has been reported in *Stachys* and *Hyssopus* genera (Venditti et al., 2014, 2015a). From the chemosystematic standpoint it has a relatively low importance since it is widespread in several other families e.g. Compositae (Venditti et al., 2015b; Venditti et al., 2016d), Apiaceae (Venditti et al., 2016g) and Hypericaceae (Esposito et al., 2013; Mandrone et al., 2015) but it is endowed with interesting biological activities as underlined in the next section.

Lastly, quinic acid (**9**) is another new phytochemical for the genus found during this study even if it also is a very spread organic acid.

The presence of all these compounds is also important from the ethno-pharmacological standpoint. In fact, all the characterized compounds in the ethanolic extract from the aerial parts of *G. ladanum* subsp. *angustifolia* are well known to exert outstanding pharmacological properties. In particular, verbascoside (**1**) exhibits strong antioxidant, antimicrobial, antiinflammatory, antihypertensive and anticancer properties (Ahmad et al., 1995; Avila et al., 1999; Speranza et al., 2010). On the other hand, martynoside (**2**) presents interesting antiestrogenic and cytotoxic activities, instead (Saracoglu et al., 1995; Papoutsi et al., 2006). The three characterized flavonoids (**3-5**) possess antioxidant, anticholinesterase and neuroprotective effects (Uriarte-Pueyo and Calvo, 2010). Harpagide (**6**) and 8-O-acetyl-harpagide (**7**) are endowed with several medicinal effects i.e. antitumor, antibacterial, antiinflammatory, antiviral, analgesic, antiarthritis (Konoshima et al., 2000; Xie et al., 2005; Binyu et al., 2013; Chung et al., 2016). Chlorogenic acid (**8**) exerts antioxidant, radical scavenging, chemopreventive, anticarcinogenesis, antiobesity and anticardiovascular properties (Nardini et al., 1995; Xiang and Ning, 2008; Cho et al., 2010; Sato et al., 2011). Finally, quinic acid (**9**) is a strong antioxidant and astringent compound (Pero et al., 2009).

4. Concluding remarks

The phytochemical analysis performed on an ethanolic extract of the total aerial parts *Galeopsis ladanum* subsp. *angustifolia* (Ehrh. ex Hoffm.) Gaudin collected in Abruzzo, showed the presence of verbascoside (**1**), martynoside (**2**), 7-[(6-O-acetyl-2-O-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-hydroxy-phenyl)-4H-1-benzopyran-4-one (**3**), 7-[(6-O-acetyl-2-O-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-methoxy-phenyl)-4H-1-benzopyran-4-one (**4**), 7-[2-O-β-D-allopyranosyl-6-O-acetyl-β-D-glucopyranosyl)-oxy]-5,8dihydroxy-2-

(3-hydroxy-4-methoxy-phenyl)-4H-1-benzopyran-4-one (**5**), harpagide (**6**), 8-O-acetyl-harpagide (**7**), chlorogenic acid (**8**) and quinic acid (**9**).

Comparing these results with those observed for the same species collected in Latium region, several qualitative differences were observed regarding the occurrence of verbascoside (**1**), martynoside (**2**), 7-[(6-O-acetyl-2-O-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-hydroxy-phenyl)-4H-1-benzopyran-4-one (**3**) and 7-[(6-O-acetyl-2-O-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5,8-dihydroxy-2-(4-methoxy-phenyl)-4H-1-benzopyran-4-one (**4**), chlorogenic acid (**8**) and quinic acid (**9**) in only the present studied accession.

These could likely be related to the different environmental conditions of the two places of growth. In fact, the sample from Latium was collected in a mountain environment at about 1700 m (a.s.l.) altitude survival limit of the species while the sample from Abruzzo was collected in a plain at about 600 m a.s.l. and so they were subjected to different environmental stresses.

The presence of all these compounds is, actually, in accordance with the current botanical classification of this species as a *taxon* in the *Galeopsis* genus since the chemotaxonomic markers of the family (harpagide and 8-O-acetyl-harpagide) and of the sub-family (the acetylated allose-containing flavonoids derivatives with isoscutellarein/hypolaetin skeleton), were identified.

The further presence of phenylethanoid glycosides was well-documented in the present study, suggesting them as additional chemosystematic markers.

The occurrence of all these compounds in the studied species also suggests their possible use in ethnopharmacology since all of them are endowed with important and interesting medicinal properties. In fact, verbascoside (**1**) exhibits strong antioxidant, antimicrobial, antiinflammatory, antihypertensive and anticancer activities. Martynoside (**2**) presents interesting antiestrogenic and cytotoxic activities. The three characterized flavonoids (**3-5**) possess antioxidant, anticholinesterase and neuroprotective effects. Harpagide (**6**) and 8-O-acetyl-harpagide (**7**) exerts antitumor, antibacterial, antiinflammatory, antiviral, analgesic and antiarthritis properties. Chlorogenic acid (**8**) shows antioxidant, radical scavenging, chemopreventive, anticarcinogenesis, antiobesity and anticardiovascular effects. Quinic acid (**9**) is a strong antioxidant and astringent compound.

Finally, no potential toxic compound was identified during this study and this inserts *G. ladanum* subsp. *angustifolia* among the *Galeopsis* species with pharmacological interests.

Conflict of interest

The authors declare that there is no conflict of interest.

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