SHORT COMMUNICATION

Unusual molecular pattern in Ajugoideae subfamily: the case of *Ajuga genevensis* L. from Dolomites

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**ABSTRACT**

We analysed the ethanolic extract from *Ajuga genevensis* L. (Lamiaceae) growing in Dolomites, part of Italian Alps. Three new compounds for this species were identified: rosmarinic acid (1), oleanolic acid (2) and maslinic acid (3), representative of two different classes of chemical compounds (phenylpropanoids and pentacyclic triterpenes). *A. genevensis* resulted to be a valuable source of these compounds endowed with interesting biological activities (i.e. antioxidant, neuroprotective, anti-inflammatory, antiproliferative). The recognition of compounds (1), (2) and (3) may also confirm the ethnomedicinal uses of this plant. From a chemotaxonomical point of view, it is worth noting that iridoids were not evidenced in this accession. Iridoids are considered chemotaxonomic marker in Lamiales, and, in contrast with a previous study on this species, the presence of aucubin was not confirmed. In addition, the presence of large amounts of rosmarinic acid (1) was unexpected for a species that does not belong to subfamily Nepetoideae.

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1. Introduction

Ajuga genevensis L. is an herbaceous perennial plant included in the Ajugoideae subsection of the Lamiaceae family (Tutin et al. 1972) presenting purplish-blue flowers in verticillaster. This species is typical of Europe and Asia, while in Italy it is present only in the central and northern regions. A. genevensis may be distinguished from the congener A. reptans L. for the absence of stolons and also for the pubescence which covers all the surface of the stems (Pignatti 1982; Conti et al. 2005). Its favourite habitat is represented by meadows and bushes of hills and mountains surviving till 1800 m of altitude above sea level.

Although the genus Ajuga has been widely studied, there is very little literature about A. genevensis, reporting on the presence of neo-clerodane diterpenoids (Malakov et al. 1991, 1992). Among the iridoid compounds, which are considered chemotaxonomical markers for species belonging to Lamiaceae family, only aucubin was previously evidenced in A. genevensis (Kostecka-Madalska & Rymkiewicz 1971).

2. Results and discussion

The polar fraction of A. genevensis L. analysed in this study was principally composed by rosmarinic acid (1) and, in minor amount, oleanolic acid (2) and maslinic acid (3) (Figure 1). These compounds belong to two different classes that are phenylpropanoids for compound (1) and pentacyclic triterpenes for compounds (2) and (3).

Rosmarinic acid (1) was already evidenced in this genus, but only in cell cultures of roots and callus from A. reptans (Murakami et al. 1997), while it was identified for the first time during the present study in a living organism of an Ajugoideae: A. genevensis.

Compound (1) was already isolated from several species of Lamiaceae family as Rosmarinus officinalis (Bai et al. 2010), Salvia officinalis (Lu & Foo 1999), Salvia limbata (Gohari et al. 2010), Salvia miltiorrhiza (Dai et al. 2010), Orthosiphon stamineus Benth (= Orthosiphon aristatus var. aristatus) (Ho et al. 2010) and many others belonging to Nepetoideae subfamily (Pedersen 2000). Rosmarinic acid (1) was contained in a considerable amount in A. genevensis, being the main secondary metabolite and accounting to 3.86% w/w of the crude extract (0.26% on the dry plant material), thus this species may be a valuable source of this compound which showed a very strong antioxidant and neuroprotective activities (Popov et al. 2013; Coelho et al. 2015; Hasanein & Mahtaj 2015).

Also compounds (2) and (3) are endowed with interesting biological activities e.g. anti-proliferative (Leal et al. 2013) and anti-inflammatory (Galipalli et al. 2014; Martin et al. 2014), they are also present in considerable amount (1.4% w/w from the crude extract; 0.08% on the dry plant material) in this plant. Oleanolic acid (2) was yet evidenced in a species belonging to this genus i.e. A. relicta P.H. Davis (Kokdil et al. 2002), on the contrary maslinic acid (3) (0.23% w/w from the crude extract; 0.016% on the dry plant material) was recognised here for the first time in Ajuga.

A. genevensis is one of the many indigenous species used in Austrian territory as a traditional herbal drug mainly against respiratory tract diseases (Vogl et al. 2013) and the isolated compounds may be responsible of the curative properties, confirming so, on phytochemical basis, the ethnopharmacological use of this plant. In fact in literature, it is reported the anti-inflammatory action of both oleanolic (2) and maslinic (3) acids and their derivatives (Hyun Woo 1971; Nataraju et al. 2009; Huang et al. 2011). In addition, a recent study demonstrates
that maslinic acid (3) has also antinociceptive and antiallodynic effects (Nieto et al. 2013). All the substances 1–3 may contribute with a synergistic action (as a phytocomplex) to the curative properties of A. genevensis.

Moreover, from a chemotaxonomical point of view, it is worth noting that iridoids were not isolated from this accession (although aucubin was detected in a previous study by Kostecka-Madalska & Rymkiewicz 1971), whereas large quantities of rosmarinic acid were obtained. The presence of iridoids and the absence of rosmarinic acid are considered chemotaxonomic markers for five subfamilies of the Lamiaceae including the Ajugoideae to which A. genevensis belongs, whereas the presence of large quantities of rosmarinic acid and the absence of iridoids is a feature of subfamily Nepetoideae. Rosmarinic acid has never been isolated in large quantities from the other five subfamilies. Therefore, the chemical content of A. genevensis is chemotaxonomically very unusual for a member of the Lamiaceae not belonging to the Nepetoideae.

Variability in plants is a constant, as the result of efficient adaption to the environment. Therefore, even constituents already reported and considered as markers and active constituents must be confirmed and determined by the phytochemical analysis, starting from the raw material used in herbal remedies, including the marketed ones.

3. Experimental

Experimental details are available as supplementary materials.

4. Conclusion

The content of the polar fraction of A. genevensis L. thus consisted of rosmarinic acid (1) as the main compound followed by oleanolic acid (2) and maslinic acid (3). The amount of these active principles in the plant was relatively high, so A. genevensis may be considered a good source of these compounds. These findings might give a rationale for the use of A. genevensis in folk medicine also by a phytochemical point of view. Nevertheless, it was extremely interesting to not find iridoids in this species, ajugol in particular which is considered a chemotaxonomic marker of the genus. However, there are many species of Lamiales that lack iridoids, but especially the presence of large amounts of rosmarinic acid was unexpected for a species that does not belong to subfamily Nepetoideae of the Lamiaceae.
Disclosure statement

No potential conflict of interest was reported by the authors.

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