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Sesquiterpene lactones and inositol 4-hydroxyphenylacetic acid derivatives in wild edible leafy vegetables from Central Italy

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ABSTRACT

Many wild species of the Asteraceae family are used as edible leafy vegetables. A distinctive trait characterizing these is the presence of sesquiterpene lactones, responsible of typical bitter taste and putative health-promoting effects against chronic-degenerative diseases. In this study, several edible leafy species of the Asteraceae family have been collected in the wild and characterized for their sesquiterpene lactones and 4-hydroxyphenylacetic acid inositol derivatives content by ultra-high-performance liquid chromatography coupled to Orbitrap high resolution mass spectrometry. The following species were considered: "aspraggine" (*Helminthia echioides*), "barba di becco" (*Tragopogon pratensis*), "caccialepre" (*Reichardia picroides*), "cicoria" (*Cichorium intybus*), "dente di leone" (*Taraxacum officinale*), "erba grassella" (*Hypochaeris radicata*), "grespigno" (*Sonchus asper*, *Sonchus oleraceus*), "ragaggiolo" (*Chondrilla juncea*), "gallina grassa" (*Crepis vesicaria*), "pie di gallina" (*Crepis sancta*) and "radicchiello" (*Crepis leontodontoides*). A database containing retention times and selected *m/z* ions for the identification of fifteen sesquiterpene lactones and six 4-hydroxyphenylacetic acid inositol derivatives was developed. Total sesquiterpene lactone content showed a remarkable variability among species (0.14-140.45 mg kg^{-1} of dry matter). The highest values were observed in *Cichorium* samples (94.29-140.45 mg kg^{-1} dm), that also showed higher lactucine contents. Six 4-hydroxyphenylacetic acid derivatives were identified in *Taraxacum officinale*.

1. Introduction

Sesquiterpene lactones are a large class of over 5000 known plant metabolite compounds, characterizing the subfamily Cichorioideae of the Asteraceae family, but also found in species belonging to the Apiaceae (Umbelliferae) and Magnoliaceae families (Merfort, 2002; Zidorn, 2008).

Sesquiterpene lactones are typically located in laticifers, specialized secretory cells in most of the Asteraceae species, but also present inside the vacuoles of other cell types (Chadwick et al., 2013).

Sesquiterpene lactones are responsible of characteristic bitter taste, which is an important factor for consumer acceptance or rejection. Studies have been carried out to relate bitterness to the presence of these phytochemicals (Price et al., 1990), with special respect to cultivated chicory varieties. D'Antuono et al. (2016), investigated the relations between sensory characters, acceptance and sesquiterpene lactone content of endive and escarole accessions: in this study, bitterness was significantly and inversely related to acceptance, and significantly affected by lactucopicrin and kaempferol malonyl glucoside contents.

Sesquiterpene lactones have also been studied for their putative health effects against chronic-degenerative diseases (Chadwick et al., 2013).

Anti-diabetic properties of some species of the Asteraceae family, such as *Taraxacum officinale*, have been recently attributed to their bioactive compounds, in particular to the classes of phenolic acids and sesquiterpene lactones (Wirngo et al., 2016).

Wild gathered species have been often indicated as potential sources of dietary phytochemicals (Leonti et al., 2006; Sánchez-Mata et al., 2012), among which carotenoids, tocopherols and ascorbic acid are the more frequently reported in leafy vegetables (Morales et al., 2014; Santos et al., 2012; Žnidarčič et al., 2011). Several phenolic compounds, belonging to the classes of hydroxycinnamic acid, flavonoids and anthocyanins were identified in wild gathered species, among which caffeoylquinic acid derivatives resulted the most abundant

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compounds in most of the species analysed (Giambanelli et al., 2017).

In Western countries, lettuce and chicory (*Lactuca sativa* L. and *Cichorium intybus* L.) represent the main dietary sources of sesquiterpene lactones. Lactucin, 8-deoxylactucin, lactucopicrin and their saturated 11β,13-dihydroderivatives are the most abundant sesquiterpene lactones of these leafy vegetables, found in both the free form or as glycosides (Ferioli et al., 2015; Graziani et al., 2015). Total sesquiterpene lactones were quantified in the range of 128–2045 and 383-2497 mg kg^{-1} of dry matter for endive and chicory, respectively, by Ferioli et al. (2015). Significant varietal differences were also found by Graziani et al. (2015) who, taking into account free sesquiterpene lactones, determined the highest amount of this class of compounds in the radicchio "Treviso Precoce", with 189.7 mg kg^{-1}, and the lowest in "Treviso Tardivo", with 45.8 mg kg^{-1}.

Traditional diets of Mediterranean area rural populations also made ample use of wild edible leafy greens, among which species of the Asteraceae family played a prominent role. After a steady declined following industrialization and urbanization, the interest of consumers for wild plant uses and biodiversity is presently following an upwards trend, because of an association to health promotion and diversification (La Vecchia, 2009; Salvatore et al., 2005). In Italy the food uses of several wild leafy vegetables of the Asteraceae family have been well-documented (Guarrera and Savo, 2016, 2013; Sansanelli and Tassoni, 2014). A detailed list of wild taxa traditionally used in vegetable mixtures, indicating also their phytochemical and nutritional profile, was reported by Guarrera and Savo (2016). These species are typically gathered in the wild, before flowering. Wild species collected at the beginning of spring time are mostly consumed raw in salads. Later in summer, leaves become of less pleasant texture and taste, so they are consumed cooked, traditionally accompanying a cereal-based main course or, more recently as a side dish. These species could therefore play a role to integrate local economies (Leonti et al., 2006), and also to supply dietary phytochemicals, like pigments, ascorbic acid, phenolics, and sesquiterpene lactones (Sánchez-Mata et al., 2012).

On the basis of this background, some edible wild species of the Asteraceae family were considered for this study: "aspraggine" (*Helminthia echioides* (L.) Gaert.), "barba di becco" (*Tragopogon pratensis* L.), "caccialepre" (*Reichardia picroides* (L.) Roth.), "cicoria" (*Cichorium intybus* L.), "dente di leone" (*Taraxacum officinale* Weber), "erba grassella" (*Hypochaeris radicata* L.), "grespigno" (*Sonchus asper* (L.) Hill, *Sonchus oleraceus* L.), "ragaggiolo" (*Chondrilla juncea* L.), "radicchiello" (*Crepis leontodontoides* All.), "pie di gallina" (*Crepis sancta* (L.) Babc.) and "gallina grassa" (*Crepis vesicaria* L.). The main aim of the study was the characterization of sesquiterpene lactones and 4-hydroxyphenylacetic acid inositol derivatives, using a ultra-high-performance liquid chromatography (UHPLC) coupled to Orbitrap-MS. Wild species were compared to a *Cichorium intybus* commercial type.

2. Materials and methods

2.1. Chemicals and reagents

For the analyses performed by UHPLC coupled to high resolution mass spectrometry: methanol (MeOH) LC–MS grade was purchased from Sigma-Aldrich; water and formic acid, both of LC–MS grade, were obtained by Scharlau (Barcelon, Spain); ammonium acetate (purity 97%) was supplied by Panreac (Barcelona, Spain). A mixture of acetic acid, caffeine, Met-Arg-Phe-Ala-acetate salt and Ultramark 1621 (proteoMass LTQ/FT-hybrid ESI positive), and a mixture of acetic acid, sodium dodecyl sulphate, taurocholic acid sodium salt hydrate and Ultramark 1621 (fluorinated phosphazines) (ProteoMass LTQ/FT-Hybrid ESI negative) from Thermo Fisher Scientific (Waltham, MA, USA) were used in the Orbitrap analyser for calibration. Silica cartridges (Si-1 cartridges, 3 mL reservoir, 500 mg sorbent mass), for SPE purification, were bought from Phenomenex (Torrance, CA). Santonin was purchased by Sigma-Aldrich.

Table 1
List of edible wild leafy vegetable species of the Asteraceae family included in the experiment.

Tag	Species	Common name	Locality	Voucher[a] ID N.
CJb	*Chondrilla juncea* L.	Ragaggiolo	Bertinoro	32685
CIb	*Cichorium intybus* L.	Cicoria	Bertinoro	11130
CIm	*Cichorium intybus* L.	Cicoria	Monterchi	11130
CLm	*Crepis leontodontoides* All.	Radicchiello	Monterchi	32104
CSb	*Crepis sancta* (L.) Babc.	Pie di gallina	Bertinoro	50753
CVb	*Crepis vesicaria* L.	Gallina grassa	Bertinoro	32025
HEb	*Helminthia echioides* (L.) Gaert.	Aspraggine	Bertinoro	67875
HEm	*Helminthia echioides* (L.) Gaert.	Aspraggine	Monterchi	67875
HRm	*Hypochaeris radicata* L.	Erba grassella	Monterchi	68416
RPb	*Reichardia picroides* (L.) Roth.	Caccialepre	Bertinoro	12125
SAb	*Sonchus asper* (L.) Hill.	Grespigno	Bertinoro	11999
SOb	*Sonchus oleraceus* L.	Grespigno	Bertinoro	60627
TOb	*Taraxacum officinale* Weber	Dente di leone	Bertinoro	69024
TPb	*Tragopogon pratensis* L.	Barba di becco	Bertinoro	33317
CIc	*Cichorium intybus* L.	Cicoria	Commercial	–

[a] In: PAL – Herbarium Mediterraneum Panormitanum (www.herbmedit.org/Home.html).

2.2. Plant material

Some wild leafy species were selected and collected during spring 2015, on the basis of previous documentation of their traditional food uses in two areas of Central Italy.

Collection sites were Bertinoro, (province of Forlì-Cesena, Emilia-Romagna; lat. 44°.15′, long. 12°.13′, alt. 254 m), and Monterchi, (province of Arezzo, Tuscany; lat. 43°.49′, long. 12°.11′, alt. 310 m). Collection was carried out at the end of March (early spring) to evaluate their bioactive compound content at a stage when they are usually consumed as raw salads.

The list of samples is reported in Table 1. The identification was based on Flora Europea (Tutin et al., 1964-1980; Tutin et al., 1964) and confirmed by the on-line vouchers of the Herbarium Mediterraneum Panormitanum, (2017) (www.herbmedit.org).

Thirteen wild species, all belonging to the Asteraceae family, were included in the experiment. Ten wild samples were gathered in Bertinoro and four in Monterchi, as also specified in Table 1. *Cichorium intybus* and *Helminthia echioides* were collected in both localities. Different species belonging to *Crepis* and *Sonchus* genera, were included to highlight within-genus similarities or differences. Moreover, a commercial variety of *Cichorium intybus* (Catalogna chicory), purchased in a local supermarket, was used as control.

After harvest the samples were immediately brought to the laboratory, washed and cleaned off decayed parts. The material was then frozen overnight and subsequently freeze-dried for 4 days. Freeze dried samples were stored at −18 °C until further analyses.

2.3. Sesquiterpene lactones extraction, identification and quantification

2.3.1. Sesquiterpene lactones extraction and purification

The method described by Ferioli and D'Antuono (2012) was adopted, with some modifications. About 300 mg of freeze-dried sample was weighed and 85 μL of santonin (c = 1.2 mg mL^{-1}) were added as internal standard. The mixture was sonicated with 3 mL of methanol:H_2O (80:20, *v*/*v*), at pH 4 (acidified with formic acid) for 10 min. The mixture was then centrifuged at 4136 *g* for 10 min and the supernatant fraction collected and purified from other interfering compounds by SPE. Before SPE purification, the cartridges were conditioned with 6 mL of dichloromethane/*i*-propanol 1/1 (*v*/*v*) and equilibrated with 6 mL of dichloromethane. At the end of this procedure the samples were loaded and eluted with 6 mL of dichloromethane/ethyl acetate 3/2 (*v*/*v*). Loading and elution fractions after collection were dried under reduced pressure at 35 °C, recovered with 1 mL of methanol water 1/1 (*v*/