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Hypericum spp. volatile profiling and the potential significance in the quality control of new valuable raw material

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ABSTRACT

The genus *Hypericum* (Guttiferae) is one of the most representative species in temperate zones and Turkey is one of the most important Mediterranean sites. Due to the increasing commercial value of Hyperici herba (*Hypericum perforatum*), many wild Turkish *Hypericum* species have received currently a considerable renewed interest as potential substitutes of the well-established *H. perforatum* crops for their similar content in the standardization bioactives (hypericins, hyperforins, and flavonoids). The present paper reported the volatile fingerprints of three selected wild Turkish *Hypericum* species recently characterized as *H. perforatum* bioactive-like profiles but lacking of the requested well-established usage in the EU market. In this context, the volatile constituents of the three-selected *Hypericum* spp. were investigated as additional discriminating markers to enhance the likelihood that this adulterating plant raw material will be detected before it is incorporated into finished *H. perforatum* products.

1. Introduction

H. perforatum is one of the most valuable herbal drugs in European Pharmacopoeia [1a–2]. Nowadays, *Hypericum perforatum* plant raw material is sold as dried whole, cut, or powdered forms in the EU market not only for herbal drug production but also for dietary supplements. The Mediterranean basin has been recognized as a hot spot for *Hypericum perforatum* provisions as it displays considerable morphological and phytochemical diversity associated with numerous endemic *Hypericum* species [3–8]. In particular, Turkey is one of the most important sites for the genus *Hypericum* as there are a total of 96 *Hypericum* species in the flora of Turkey from 19 sections, 46 of which are endemic [3–6]. However, Turkish *H. perforatum* does not represent the main source as it has not been domesticated yet and it is generally collected from its natural habitat [9–10]. However, there is a recently renewed research interest in several wild Turkish *Hypericum* spp. as alternative sources of the well-known *H. perforatum* bioactives. Due to the lacking of recognized well-established usage in EU markets for these species, they may represent a risk of *H. perforatum* adulteration. Considering the studies on *H. perforatum* standardization bioactive constituents, wild Turkish *Hypericum* species have been deeply investigated in the last years [11–21]. The Turkish traditional medicine focuses on *Hypericum* species especially for the treatments of skin damages, diarrhea and ulcers [22–26]. In the present study, three wild Turkish *Hypericum* species (*Hypericum lydium*, *H. orientale*, and *H. confertum*) were selected among those already investigated on their content in the official standardization bioactive constituents described for *Hypericum perforatum* [15–17,19,21].

Hypericum lydium is an herbaceous perennial plant, which is limited to Turkey and Northern Iraq. This plant grows in sparse populations on rocky slopes and *Pinus* woodland. Its seeds have exhibited physical dormancy concerning the presence of hard seed coat [3,5]. There are only few reports on *H. lydium* reporting the presence of hypericin and the evaluation of the essential oil antioxidant activity [15–17].

The perennial herbaceous *Hypericum orientale* L. is widely distributed in Northern Turkey and Georgia and grows naturally in igneous stony slopes or woodland [3,5]. Although there are much information about the content of its hypericin and flavonoid content [18–19], there are no published data on the essential oil composition of *H. orientale*.

Regarding *Hypericum confertum* Choisy, it is herbaceous perennial growing in abies woods and rocky igneous slopes of Turkey. There are some data on its content of hyperforin, hypericin, and flavonoids [20, 21]. More recently, the *H. confertum* essential oil has been evaluated for its anti-angiogenic activity [27].

In the present paper, GC–MS analysis of hydrodistilled essential oils (EOs) was performed to screen plant volatile constituents as it represents the first choice technique in the official quality control of aromatic plant material [1b]. Specific commercial and home-made databases on linear retention indeces (L.R.I) and mass spectra of essential oil

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constituents were comparing with the GC–MS fingerprints of the three Turkish *Hypericum* species to complete the phytochemical investigation of still poorly studied wild plant species. Moreover, specific target aromatic compounds were pointed out as further potential quality parameters related to chemotaxonomic classification and plant collection sites. Due to the increased industrial interest in *Hypericum perforatum* for the production of herbal drugs as well as dietary supplements in the EU market, this study provides for the first time additional multi-targeted phytochemical screenings based on inter plant-habitat discriminating volatiles potentially useful to avoid the contamination of *H. perforatum* with other wild *Hypericum* spp. which show similar composition in the well-known standardizable bioactives (hypericins, hyperphorins and flavonoids), but lacking of the recognized well-established usage in the EU market.

2. Experiments

2.1. Plant material

2.1.1. Morphological description and sampling procedures

2.1.1.1. Hypericum lydium Boiss. Stems 10–75 cm, erect, glabrous, with numerous prominent dark and amber glands. Leaves on main stem 9–35 mm, linear or narrowly oblong-lanceolate, often revolute, rounded, glabrous or rarely undulate-papillose. Inflorescence cylindrical or narrowly pyramidal to subspicate, 10-many-flowered. Sepals equal, united at the base, lanceolate to oblong, acute or subacute with sessile glands all round. Petals 6–12 mm, rarely red veined. Capsule 6–8 mm, ovoid and gradually acuminate to subglobose and rostrate [5]. The aerial parts of *H. lydium* plants were collected from Havza district of Samsun province localized in Northern part of Turkey (40° 55′N Lat. 35° 37′E Long. and 580 m elevation; Fig. 1) in June at full flowering stage. The plant material was air-dried under shade and powdered by using a laboratory mill. Plant material was identified by Dr. Hasan Korkmaz, Department of Biology, University of 19 Mayıs, Samsun, Turkey. Voucher specimen was deposited in the herbarium of Ondokuz Mayis University Agricultural Faculty (OMUZF # 109_2).

2.1.1.2. Hypericum orientale L.. Stems 7–45 cm, erect or decumbent. Leaves 10–40 mm, oblong or elliptic-oblong to oblanceolate or linear. Sepals unequal, narrowly oblong and ovate, obtuse to round, with margin amber-glandular-denticulate. Petals 10–20 mm, entire without black glands. Capsule 7–14 mm, ovoid to ovoid-cylindrical [5]. The aerial parts of *H. orientale* plants were collected from Tavşandağı mountain of Amasya province localized in Northern part of Turkey (40° 51′N Lat. 35° 29′E Long., 2300 m elevation above sea level; Fig. 1) in June at full flowering stage. The plant material was air-dried under shade and powdered by using a laboratory mill. Dr. Hasan Korkmaz, Department of Biology, University of 19 Mayıs, Samsun, Turkey identified plant material. Voucher specimen was deposited in the herbarium of Ondokuz Mayis University Agricultural Faculty (OMUZF # 131).

2.1.1.3. Hypericum confertum Choisy. Stems 10–35 cm, erect or ascending from a rooting and branching base, glabrous to pubescent. Leaves 7–15 mm, lanceolate to oblong-linear, pruinose to pubescent. Inflorescence narrowly pyramidal to cylindrical, 3–20 flowered. Sepals lanceolate to oblong or ovate, acute to round, 3–5 ribbed, black-glandular-ciliate. Petals 7–16 mm. Capsules 6–9 mm, narrowly ovoid [5]. Aerial parts of *H. confertum* plants were collected from Uludağ mountain of Bursa province localized in Northern part of Turkey (39° 53′N Lat. 36° 28′E Long. 2000 m elevation above sea level; Fig. 1) in June at full flowering stage. The plant material was air-dried under shade and powdered by using a laboratory mill. Dr. Hasan Korkmaz, Department of Biology, University of 19 Mayıs, Samsun, Turkey identified plant material. Voucher specimen was deposited in the herbarium of Ondokuz Mayis University Agricultural Faculty (OMUZF # 132).

2.2. Chemicals

Commercial compounds (5–10 mg, Sigma, Aldrich, Extrasynthese, Fluka) and isolated compounds were part of a homemade database (Department of Pharmacy- University of Pisa), where each compound was used as a reference material only after GC–MS grade purity determination (98–99%).

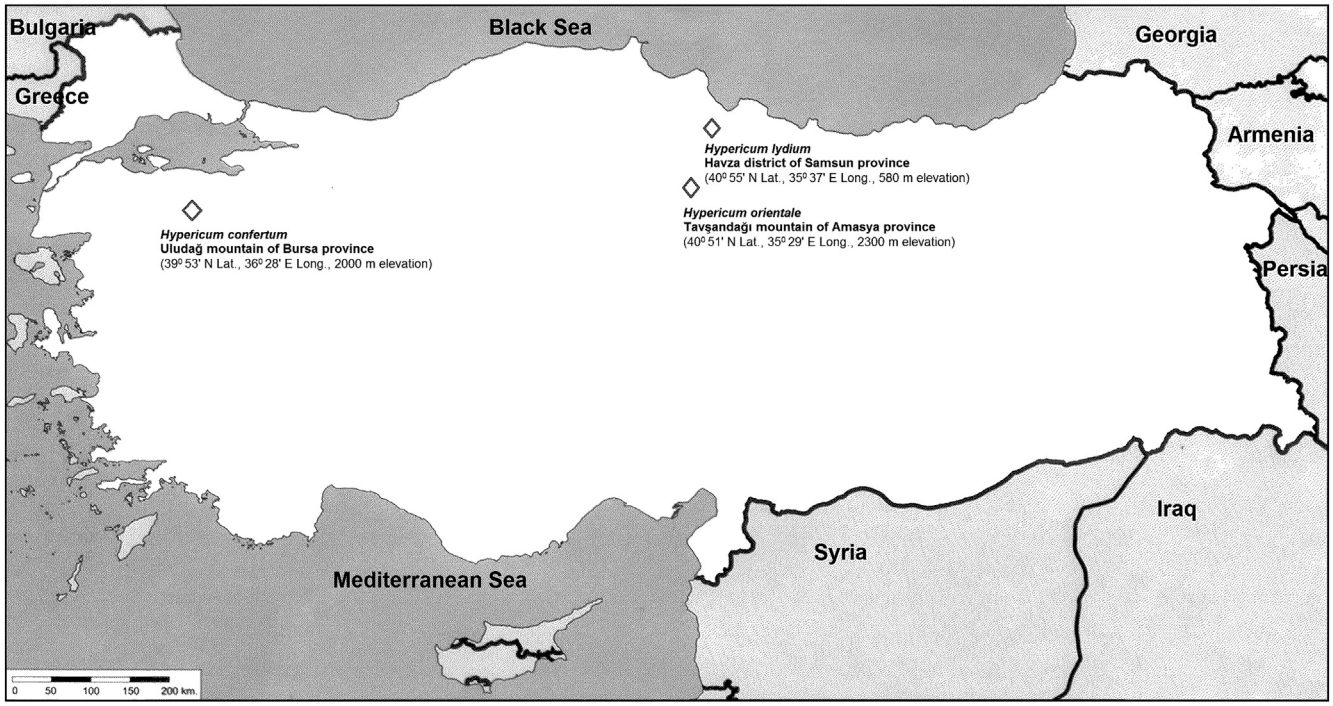

Fig. 1. The collection sites of the three analysed species *H. orientale*, *H. confertum*, and *H. lydium*.

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