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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.

**Keywords:** *H. lydium, H. orientale, H. confertum,* essential oil, GC-MS, PCA analysis

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## 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 recognised 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* standardisation 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 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 standardisable bioactives (hypericins, hyperphorins and flavonoids), but lacking of the recognised well-established usage in the EU market.

## 2. Experiments

### *2.1 Plant material*

*Morphological description and sampling procedures: 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; Figure 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).

*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; Figure 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).

*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; Figure 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%).

*2.3 Sample preparation*

The aerial parts (air-dried samples, 200 g) were hydrodistilled by Clevenger apparatus for 2 h. The EO samples were diluted in *n*-hexane (HPLC solvent grade, 10%) and injected in GC-FID (injection volume 1 ml, HP-5 and HP-WAX columns) and GC-MS (injection volume 1 µL, DB-5MS column).

*2.4 Gas chromatography-FID*

GC analyses were accomplished by HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m x 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60°C for 10 min, ramp of 5°C $min^{-1}$ up to 220°C; injector and detector temperatures 250°C; carrier gas nitrogen (2mL $min^{-1}$); detector dual FID; split ratio 1:30; injection of 0.5 mL.

*2.5 Gas chromatography–mass spectrometry*

GC/EIMS analyses were performed by a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm, 0.25 µm film thickness) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220°C and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 3°C $min^{-1}$; carrier gas helium at 1mL $min^{-1}$; triplicate injections of 0.1 µL

(10% hexane solution); split ratio 1:30. Identification of EO constituents was based on the comparison with those of authentic standard samples by their linear retention indices (L.R.I.) relative to a series of *n*-hydrocarbons and computer matching by both a homemade library mass spectra as well as two-commercial database (NIST 98 and ADAMS) [28-29]. Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionising gas. The EOs composition is reported as relative percentage composition by internal peak area normalization, not inclusive of solvent peak and all relative response factors being taken as one (Table 1).

*2.6 Statistical analysis*

Principal component analysis (PCA) was carried out using the statistical software package SPSS Version 20.0. The relative percentage composition (%, triplicate samples, table 1) for about 80 constituents previously identified by GC-MS for each EO sample were used to discriminate the three selected plant species. This analysis provides the two-dimensional spatial score plot of investigated exemplars relative to each other. The principal components represent the axes, which are the orthogonal projections for the values representing the highest possible variances, in this experimental case, the first and second principal components. Therefore, a factor analysis was performed, whereby each variable was used to calculate relationships between variable and investigated factors. In addition, a dendogram (cluster) was created to show the relationships among the investigated plant samples and their target volatiles [31].

## 3. Results and discussions

### *3.1 EO fingerprints and volatile markers of H. lydium, H. orientale, and H. confertum*

The three selected Turkish species *H. confertum, H. orientale* and *H. lydium* have been already investigated for their phenolic and hypericins contents [15-21]. The present study carried out for the first time a preliminary screening of their volatile constituents (relative percentage composition, %) with the aim to point out some further target compounds related to their chemotaxonomic classification and collection sites.

In fact, *Hypericum orientale* could be clearly distinguished from the other two studied species by the highest amount of hydrocarbon sesquiterpenes (75.4% ± 4.1, Figure 2). In particular, β-selinene was identified as target volatile for *H. orientale* (37.1% ± 3.2; Table 1). Although hydrocarbon sesquiterpenes (HS) were considered a typical chemical class of this species, Mathis and Ourissons [32, 33] classified French cultivated samples of *H. orientale* L. in the Euhypericum section (subsection Crossophyllum Spach) especially for predominant pinenes and linear hydrocarbons [ α□pinene (43%), undecane (51%)]. Furthermore, Mathis and Ourissons [33] considered predominant the hydrocarbon sesquiterpene α-humulene (always less than 10%) and not β-selinene, which was detected, in huge amounts (37.1% ± 3.2) in our *H. orientale* EO samples. In fact, α-humulene represented only a minor constituent (0.63% ± 0.01) in our samples in comparison with β-selinene and also other more representative HS such as β-caryophyllene (9.68 % ± 0.09), γ-muurolene (4.36% ± 0.87), and cadinene (6.12% ± 0.23). In addition, Mathis and Ourissons (33) found high levels of satured aldehydes (10-40%), which were not detected in our samples. Therefore, the present work pointed out that huge amounts of hydrocarbon sesquiterpenes and absence of

aldehydes could represent two important chemotaxonomic features characterizing the dried flowering aerial parts of North Turkish *Hypericum orientale*.

Hydrocarbon sesquiterpenes (HS) represented the main chemical class of *H. confertum* EO (58.2%± 3.1), too. However, this species showed in parallel the highest level of linear monoterpenes (10.1% ± 1.1) than the other two analysed species (Figure 2). In particular, *H. confertum* was found the only one very rich in the monoterpene α-pinene (7.8% ± 0.34). Principal Component Analysis (PCA) analysis provided further confirmation that *H. confertum* aromatic fingerprint showed the highest variety in terms of monoterpenes, sesquiterpenes and no terpenoid constituents in comparison with the two other species (Figure 2, 3).

Considering *H. lydium*, oxygenated terpenes, both as monoterpenes (OM) and sesquiterpenes (OS), played a crucial role in distinguishing it from *H. orientale* and *H. confertum*. In fact, both OM and OS represented the main *H. lydium* chemical classes (37.4% ± 2.3% OM; 35.7% ± 3.0% OS). In particular, verbenone (22.2% ± 2.04) and cis-linolool oxide (6.83% ± 0.42) were the most abundant oxygenated monoterpenes (OM), while caryophyllene oxide (18.3% ± 0.58), β-selinene (6.28% ± 1.01), and α-eudesmol (11.3% ± 2.01) represented the main oxygenated sesquiterpenes (OS).

The only previous studies on Turkish *H. lydium* EO [15-17] reported a completely different composition as the hydrocarbon monoterpenes were pointed out as the predominant volatiles (76.4% M; α-pinene 71.2%). However, the different collection site (Gümüşhane is separated by a distance of 365 km from Samsun, where our samples were collected) as well as the different sampling procedures (dried flowers and leaves instead of whole dried flowering parts) could justify such a different aromatic fingerprints for the same Turkish species.

Furthermore, GC-MS combined with PCA analysis showed specific volatile chemical classes and singular terpenes related to different *Hypericum* genus sections and groups:

- *H. orientale* Crossophyllum Spach group with hydrocarbon sesquiterpenes (HS) and the predominant β-selinene

- *H. confertum* Taneiocarpium Jaub&Spach group with a mixed composition of sesquiterpenes (large variety of target compounds with singular relative abundance higher than 5%) and hydrocarbon monoterpenes (HM, α-pinene predominant)

- *H. lydium* Drosanthe Spach group with balanced contribution between oxygenated monoterpenes (OM) and sesquiterpene (OS) with two target volatiles (verbenone and caryophyllene oxide; much higher than 10%)

*3.2 H. lydium, H. orientale, and H. confertum vs H. perforatum EO fingerprints*

The present study was performed after the establishment of a GC-MS EOs database of ten wild *H. perforatum* population samples collected in Northen Turkey [34]. Volatile production were monitored also from different plant organs during seven morphogenetic stages [35] in order to compare the suitable balsamic time with that one of *H. perforatum* herbal drug reported in the Eur. Pharmacopeia 8th Ed. [1].

The present paper used this homemade database to compare the EO fingerprints of three further wild Turkish *Hypericum species (H. confertum, H. lydium,* and *H. orientale*) selected among those already characterized in *H. perforatum* standardization bioactives [15-21]. The highest levels of monoterpenes and sesquiterpenes (both HM and OM) simultaneously detected in the EO composition of *H. confertum, H. lydium,* and *H. orientale* were the first discriminating factors which could distinguish them from Turkish *H. perforatum* plant raw material collected in the same region (Samsun) at the

same phenological stage. In addition, α-pinene (8%) in *H. confertum*, verbenone (23%) and β-caryopyllene in *H. lydium*, β-selinene (38%) in *H. orientale* were pointed out as inter-species target volatiles. Due to the fact that spathulenol (OS)/caryophyllene oxide (OS) ratio represented the only common compositional feature among the studied species and *H. perforatum* EOs (34, 35), α-pinene, verbenone/β-caryopyllene, and β-selinene could be regarded as further chemical markers in the multi-targeted detection of *H. perforatum* plant raw material before its inclusion into batch production.

## 4. Conclusion

In the last few years, many Turkish *Hypericum* spp. have been reported to have a *H. perforatum*-like bioactive chemical profile in terms of hypericins, hyperforins, and flavonoids. However, they have not recognized yet in their well-established usage in EU markets. Therefore, they could represent a severe risk of adulteration for valuable plant raw material batches of *Hypericum perforatum*. In this context, this preliminary study defines for the first time the relationship among volatile markers, botanical certification, and plant collection places of *H. confertum, H. lydium,* and *H. orientale* with the aim to provide additional phytochemical discriminating factors to point out potential contamination cases of *H. perforatum* plant raw material.

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Table 1. EO fingerprints of the three analysed *Hypericum* species.

| | | | ***H. lydium*** | | ***H. orientale*** | | ***H. confertum*** | |
|---|---|---|---|---|---|---|---|---|
| ***Compounds*** | ***LRIa°*** | ***LRIb°*** | ***relative percentage composition, %$^{\S}$*** | | | | | |
| 2-E-hexenal | 854 | 856 | | | | | 1.01 | 0.06 |
| 2-E-hexenol | 860 | 865 | | | | | | |
| α–pinene | 941 | 939 | | | 1.32 | 0.09 | 7.82 | 0.34 |
| sabinene | 977 | 976 | | | | | 0.77 | 0.18 |
| β–pinene | 984 | 980 | | | | | 1.09 | 0.09 |
| 6-methyl-5-hepten-2-one | 987 | 985 | | | | | 1.10 | 0.21 |
| myrcene | 991 | 991 | tr$^{\#}$ | | tr | | 0.06 | 0.01 |
| 2-penthylfuran | 993 | 992 | | | | | 0.95 | 0.08 |
| *p*-cimene | 1028 | 1026 | tr | | tr | | 0.34 | 0.09 |
| limonene | 1033 | 1031 | 0.06 | 0.01 | | | | |
| benzene acetaldeide | 1043 | 1043 | 0.05 | 0.01 | tr | | 0.60 | 0.12 |
| acetophenone* | | | | | | | 0.48 | 0.09 |
| cis-linalool oxide | 1076 | 1074 | 6.83 | 0.42 | 1.00 | 0.15 | 0.41 | 0.09 |
| trans-linalool oxide | 1088 | 1088 | 2.05 | 0.31 | 0.69 | 0.19 | | |
| undecane | 1100 | 1100 | | | | | 1.50 | 0.06 |
| linalool | 1101 | 1098 | 0.82 | 0.01 | 0.87 | 0.05 | 0.58 | 0.08 |
| nonanal | 1107 | | 0.79 | 0.11 | | | 0.46 | 0.11 |
| dehydrosabinaketone* | 1126 | | 1.23 | 0.31 | | | | |
| 4-terpineol | 1180 | 1177 | | | | | 0.53 | 0.02 |
| *p*-cymen-8-ol | 1188 | 1190 | 1.85 | 0.06 | | | | |
| α–terpineol | 1190 | 1189 | 1.49 | 0.12 | tr | | 1.33 | 0.11 |
| safranal | 1201 | | | | | | 0.53 | 0.02 |
| verbenone | 1211 | | 22.20 | 2.04 | | | 0.75 | 0.05 |
| trans-carveol | 1223 | | 0.94 | 0.03 | | | | |
| carvacrol | 1223 | | | | | | 0.57 | 0.02 |
| α–cubebene | 13.49 | 1351 | | | 1.38 | 0.09 | 1.33 | 0.12 |
| α–longipinene | 1352 | 1351 | | | 0.73 | 0.09 | | |
| cyclosativene | 1368 | 1371 | | | 0.45 | 0.09 | 1.02 | 0.03 |
| α–copaene | 1377 | 1376 | | | 1.95 | 0.03 | 3.53 | 0.11 |
| β–bourbunene | 1384 | 1388 | tr | | 2.78 | 0.02 | 1.00 | 0.18 |
| β–elemene | 1390 | 1391 | | | 0.60 | 0.06 | 0.40 | 0.03 |
| α–gurjunene | 14.07 | | | | | | 1.10 | 0.09 |
| β–caryophyllene | 1420 | 1419 | | | 9.68 | 0.09 | 4.74 | 0.12 |
| β–gurjunene | 1432 | 1434 | tr | | 1.06 | 0.16 | 1.78 | 0.14 |
| aromadendrene | 1440 | 1441 | | | 0.59 | 0.08 | 0.90 | 0.14 |
| E- β–farnesene | 1455 | 1455 | | | | | 1.26 | 0.17 |
| α–humulene | 1457 | 1457 | | | 0.63 | 0.01 | 0.86 | 0.11 |
| alloaromadendrene | 1461 | 1461 | tr | | 0.58 | 0.02 | 2.21 | 0.08 |
| drima-7,9(11)-diene | 1467 | | | | 0.45 | 0.08 | 0.67 | 0.07 |
| cis-muurola-4-(14),5-diene* | 1469 | 1470 | | | | | 0.67 | 0.04 |
| γ–muurolene | 1479 | 1480 | 1.28 | 0.44 | 4.36 | 0.87 | 7.22 | 0.25 |
| α–amorphene | 1485 | 1485 | | | 0.45 | 0.08 | | |
| germacrene D | 1482 | 1485 | | | | | 3.51 | 0.24 |
| β–selinene | 1486 | 1490 | 6.28 | 1.01 | 37.07 | 3.23 | 5.69 | 0.87 |
| valencene | 1493 | | | | 0.55 | 0.05 | | |
| trans-muurola-4-(14),5-diene* | 1493 | 1494 | | | | | 2.34 | 0.14 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| α–selinene | 1496 | 1498 | 2.25 | 0.39 | 2.81 | 0.75 | 5.25 | 0.63 |
| α–muurolene | 1499 | 1500 | | | 0.85 | 0.01 | 1.02 | 0.09 |
| cis-γ–cadinene | 1511 | 1513 | 2.13 | 0.22 | | | 2.98 | 0.32 |
| δ–cadinene | 1519 | 1523 | 2.12 | 0.07 | 6.12 | 0.23 | 6.55 | 0.10 |
| trans- calamenene | 1521 | 1523 | | | 1.26 | 0.09 | 0.39 | 0.11 |
| trans-cadina-1(2), 4-diene* | 1531 | | | | | | 0.58 | 0.01 |
| α–cadinene | 1540 | 1539 | | | 0.57 | 0.04 | 0.72 | 0.03 |
| α–calacorene | 1546 | 1546 | tr | | 0.52 | 0.06 | 0.50 | 0.06 |
| trans-nerolidol | 1563 | 1564 | | | | | 0.53 | 0.06 |
| spathulenol | 1577 | 1578 | 3.17 | 0.26 | 1.10 | 0.12 | 1.69 | 0.68 |
| caryophyllene oxide | 1582 | 1583 | 18.34 | 0.58 | 4.81 | 0.11 | 4.48 | 0.50 |
| globulol | 1587 | 1585 | | | 1.04 | 0.06 | 0.28 | 0.01 |
| b -oplopenone* | 1604 | 1606 | | | 0.69 | 0.09 | | |
| humulene epoxide II | 1609 | 1608 | tr | | 1.78 | 0.12 | 1.33 | 0.09 |
| 1,10-diepi-cubenol* | 1617 | 1619 | | | | | 0.76 | 0.35 |
| 1-epicubenol* | 1629 | 1630 | | | | | 0.30 | 0.09 |
| γ–eudesmol | 1634 | 1654 | 2.11 | 0.06 | tr | | | |
| cis- cadin-4-en-7-ol* | 1637 | | | | | | 0.51 | 0.07 |
| τ–cadinol (epi-a) | 1645 | | | | 0.46 | 0.06 | 1.00 | 0.03 |
| τ–muurolol (epi-a) | 1646 | | | | | | 0.54 | 0.07 |
| α–muurolol | 1650 | | | | | | 0.66 | 0.04 |
| caryophyla-4(14,) 8(15)-dien-5-ol | 1641 | | 0.79 | 0.08 | | | | |
| himachalol | 1654 | | | | | | | |
| α–cadinol | 1659 | | | | 0.59 | 0.01 | 1.63 | 0.07 |
| α–eudesmol* | 1658 | | 11.33 | 2.01 | | | | |
| selina-11-en-6-a-ol | 1661 | | | | 0.81 | 0.09 | | |
| santalol-7-a | 1687 | | | 3.88 | 0.43 | | | |
| n- heptadecane | 1700 | | | | | | 0.62 | 0.07 |
| 6,10,14-trimethylpentadecanone* | 1843 | 1843 | | | 0.63 | 0.03 | 0.73 | 0.05 |
| n- nonadecane | 1900 | 1900 | 3.00 | 0.01 | | | 1.66 | 0.09 |
| n-eicosane | 2000 | | | | | | 0.35 | 0.06 |
| n- heneicosane | 2100 | 2100 | tr | | tr | | 1.06 | 0.09 |
| n- tricosane | 2300 | 2300 | | | | | 0.33 | 0.01 |
| n- pentacosane | 2500 | | | | | | 0.12 | 0.01 |
| **TOTAL** | | | **91.11** | | **91.66** | | **97.68** | |
| ***CHEMICAL CLASSES*** | | | | | | | | |
| ***hydrocarbons*** | | | 3.00 | | - | | 5.64 | |
| ***aldhehydes*** | | | 0.84 | | - | | 2.07 | |
| ***hydrocarbon monoterpenes (HM)*** | | | 0.06 | | 1.32 | | 10.08 | |
| ***oxygenated mononoterpenes (OM)*** | | | 37.41 | | 2.56 | | 4.70 | |
| ***hydrocarbon sesquiterpenes (HS)*** | | | 14.06 | | 75.44 | | 58.22 | |
| ***oxygenated sequiterpenes (OM)*** | | | 35.74 | | 11.71 | | 13.71 | |
| ***others*** | | | - | | - | | 2.53 | |

* Linear Retention Index on a non-polar (LRIa) and polar (LRIb) columns.

§ Percentage values are means of three values with *se* for the components below 5% in all cases.

*Tentative identification;

#tr = less than 0.01%.

**Captions to Figures**

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

**Figure 2.** The main volatile chemical classes in the analysed EOs (relative percentage composition; triplicate analysis: average values obtained by the identified volatiles belonging to the same chemical class)

**Figure 3.** PCA score plot of the three investigated species: *H. orientale*, *H. confertum*, and *H. lydium* (triplicate analysis for each plant sample)

**Figure 4.** Cluster analysis of the analysed *Hypericum* spp. samples (triplicate analysis for each plant sample)

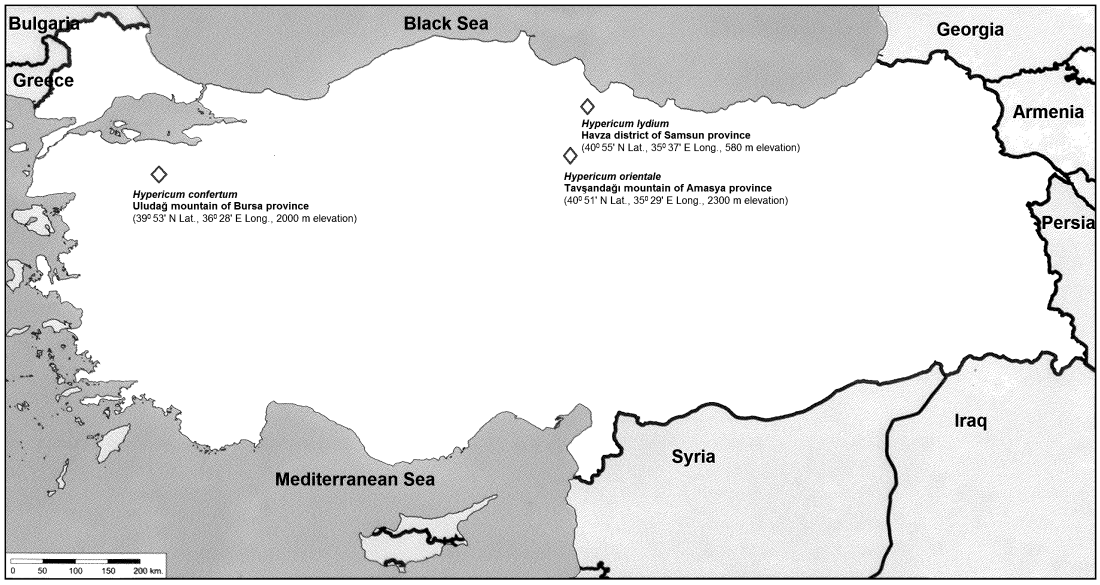


Figure 1

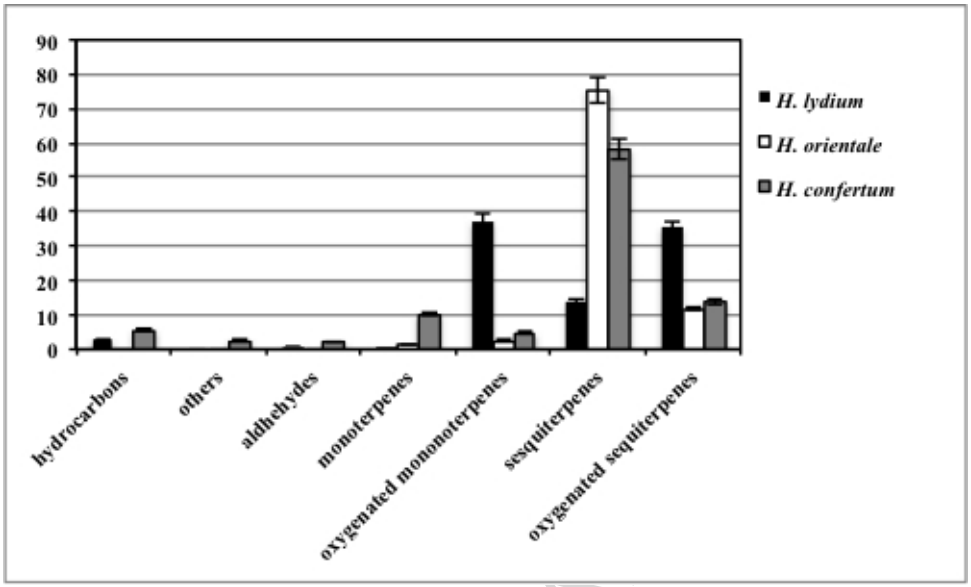


Figure 2

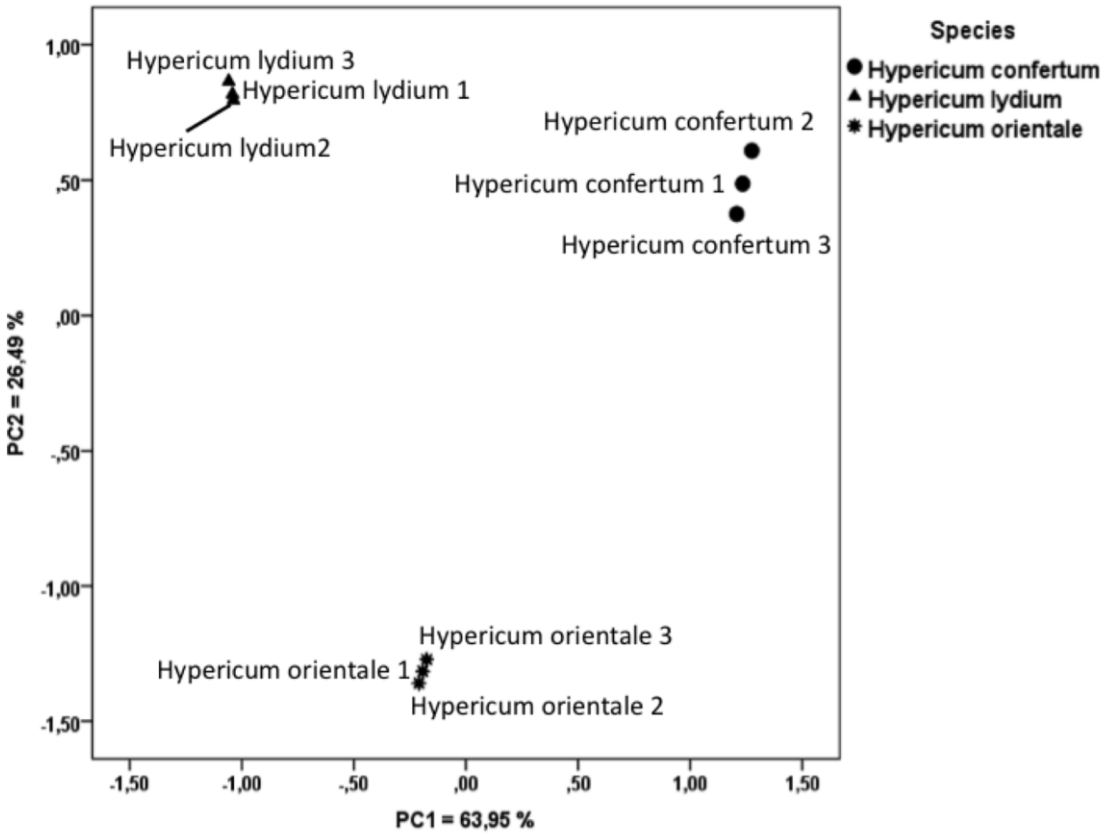


Figure 3

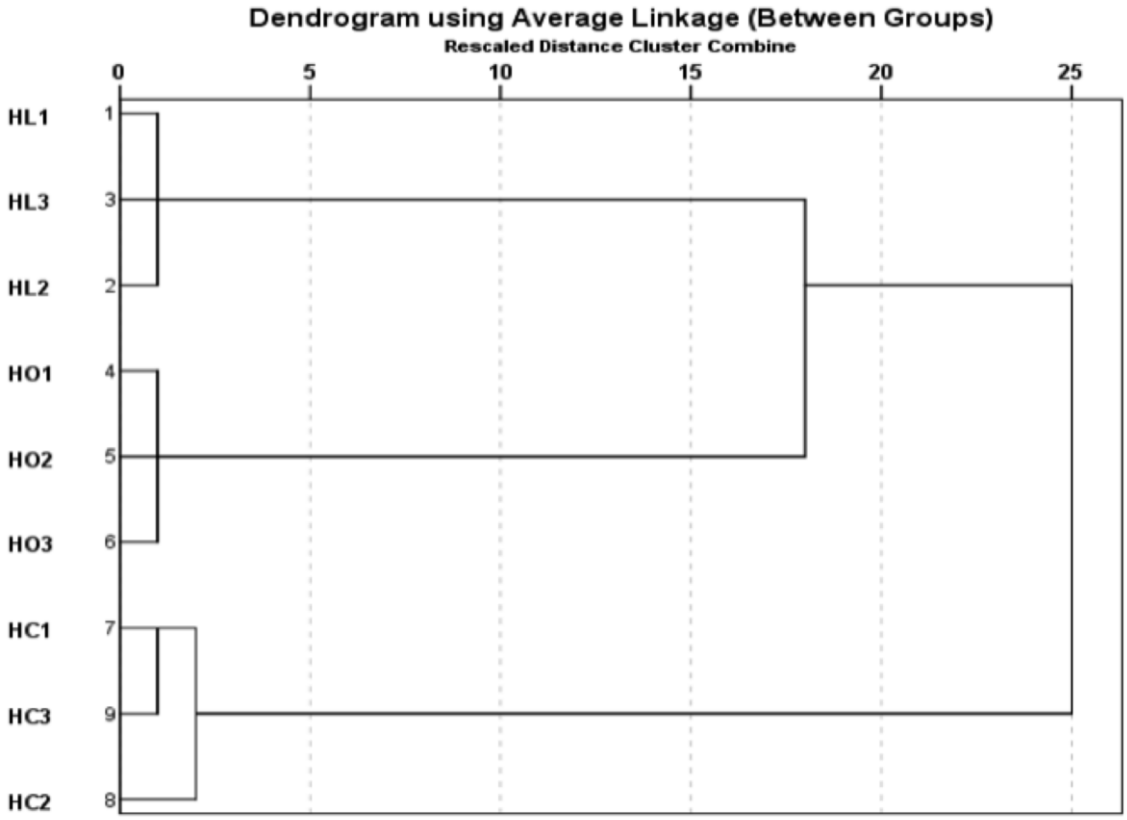


Figure 4

Highlights

- Multi-targeted screenings of new valuable *Hypericum* spp.
- Selection of inter-species and collection place discriminating factors
- Volatile constituents as potential markers in EU plant provision