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Original article

# *In vitro* callus culture and these callus essential oil compositions of ten populations *Hypericum scabrum* L. from Turkey

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**Summary.** This study aims to develop in vitro callus from hypocotyl explants of ten different wild populations of H. scabrum growing in Turkey and evaluate the potential of these callus essential oils production with industrial application. Hypocotyl parts of in vitro growing plants used as explants sources. In vitro cultures were established on MS medium supplemented with 2 mg/L 2,4-D + 0.1 mg/L BAP. Some Hypericum taxa callus contains naphthodianthrones, phloroglucinols, tannins, xanthones, phenolic acids and essential oil. According to the HS-SPME/GC-MS analyses, a total of forty-one components were detected in ten H. scabrum calli with relatively high variation in their essential oil composition. Among constituents, α-pinene (7.68-40.20%), β-pinene (1.30-35.74%), limonene (0.02-32.21%), β-ocimene (0.0-37.90%) and germacrene D (0.15-30.55%) were found as the most abundant constituents in studied populations calli essential oils. Results showed that in vitro calli could be a good experimental system for further researches on essential oil production.

**Key words:** callus culture, essential oil, *Hypericum scabrum*

## Introduction

The genus *Hypericum* L. is the largest member of the Hypericaceae family, now usually included as subfamily (Hypericoideae) in Clusiaceae (Guttiferae) and comprises more than 470 species divided in 36 sections with worldwide distribution in warm temperate, subtropical and mountainous tropical regions (1). This genus is represented by nearly 100 taxa grouped under 19 sections in Turkey, among them, 45 taxa are endemic. In the traditional medicine of Turkey, the genus is known as "sarı kantaron, kantaron, binbirdelik otu, mayasıl otu" and most of them, have been used for the treatment of burns, wounds, haemorroids, diarrhorea, ulcers and psychological diseases such as neuralgia, anxiety, neurosis and depression (2-5). Moreover *Hypericum* taxa have been used in the traditional medicine for centuries and many of them have great economic importance as natural sources of active compounds (6). Nowadays, biological activity of different *Hypericum* species have been investigated and documented in number of studies (7-9). Morphologically, *Hypericum* is characterized by the presence of different types of secretory structures including translucent glands, black nodules and secretory canals. Essential oils are synthesized either in translucent glands or in secretory canals that may be localized in leaves, petals, sepals and pistil (10). Monographs for the crude drug, extracts of which are prepared from the aerial flowering portions of the plant, have been included in the European Pharmacopoeia (11). An infused oil of the flowers, which is prepared by macerating fresh flowers in olive or sunflower oil and exposing the mixture to sunlight for two to three weeks, has a history of traditional use in Europe for treatment of burns and ulcers (12). Oleum hyperici has a red color when either fresh flowers are extracted or heat is applied during the maceration process, although the naphthodianthrones (specifically hypericin

and pseudohypericin) are not extracted into the oil. It has been proposed that a related emodin-derivative(s), specifically a degradation product of hypericin upon exposure to sunlight, is responsible for this coloration, but these have not yet been isolated (13,14).

Recently, there has been increasing interest in the genus *Hypericum*, because it is a source of a variety of chemical compounds (15). Modern studies have been focused on the activity of extracts of these plants against certain viruses and bacteria and on their possible applications as medicines for various diseases (16). Many reports have been published for antimicrobial, antifungal, antiviral, antioxidant, antidepressant and anticonvulsant activities of *Hypericum* taxa (17). Previous reports showed that *H. scabrum* L. has antimicrobial, sedative effect, antiseptic, antidiarrhea, antihemorrhoid, antieczema, antipsoriasis, anthelmintic, antifungal and antiulcerogenic activities (18, 19). According to the phytochemical studies on *H. scabrum*, it has been reported that the essential oil constituents belong to different chemical classes with a considerable qualitative and quantitative variation in composition (20, 21). This variability could be related to the effect of variables such as genetic factors, developmental stages, types of plant materials, methods of extraction, environmental conditions, etc. Although, there are some comparative studies on the essential oil constituents of *H. scabrum* (20), to the best of our knowledge this is the first record on *in vitro* callus induced and these calli essential oil in Turkey populations. Therefore, the present study is investigate *in vitro* callus propagation and evaluate these calli essential oil from ten different wild populations of *H. scabrum* plants collected from Turkey. This study the first report on the essential oils of callus culture of *H. scabrum.*

## Material and Methods

### *Plant material source*

The seeds of ten taxa *Hypericum scabrum* collected from Yozgat: Akdağ Madeni, Ankara to Sivas, vicinity of Davutlu village, steppe, rocky and stony place, openning of *Quercus* foresty, 1150-1250 m, 18.06.2014, Kılıç 5424. Bingöl: Vicinity of Dikme village, rocky areas, 1650-1700 m., 18.06.2013, Kılıç 5286. Elazığ: Keban, north of Aslankaşı village, rocky slopes, 1300-1400 m., 25.06.2013, Kılıç 5327. Malatya: Malatya to Elazığ, exit of Sürgü, road edges, 1300-1350 m, 05.07.2015, Kılıç 5689. Sivas: Zara, between Halkalı and Korkut villages, steppe, gypsum areas, hill, slope, rocky and stony place openning of foresty, 1400-1500 m, 19.06.2014, Kılıç 5426. Tunceli: Ovacık, Munzur mountains, Yılanlı mountain, rocky and stony place, 1800-2000 m, 12.06.2015, Kılıç 5684. şanlıurfa: Between Siverek to şanlıurfa, 20. km, road edge, rocky areas, 600-700 m, 07.06.2014, Kılıç 5415. Adıyaman: Between Turuş village and Atatürk Dam, rocky-steppe areas, 600-700 m, 08.06.2014, Kılıç 5417. Bitlis: Tatvan, vicinity of Suboyu village, rocky and stony slopes, 1650-1750 m, 27.06.2015, Kılıç 5687. Muş: Muş-Bingöl 10. km roadside, steppe, 1300-1350 m, 28.06.2015, Kılıç 5688. Plant materials were identified with Flora of Turkey and East Aegean Islands (22). Voucher specimens were deposited in the Department of Park and Garden Plants of Technical Vocational College / Bingol University.

### *Callus culture*

The seeds of ten taxa *Hypericum scabrum* were treated with 100% commercial bleach (5% NaOCl Ace, Turkey) for 20 min. followed by 3 × 3 min. rinsing with sterilized distilled water. These were cultured on agar solidified MS medium (23) contained in Petri dishes (100 ×10 mm) supplemented with 3% sucrose to sprout them under 16 h light photoperiod (35 µmol $m^{-2}s^{-1}$) in Aralab versatile growth chamber at 24 ± 1 °C. Hypocotyl explants were obtained from 14 days old young seedlings. Hypocotyl explants were cultured on MS medium containing 2 mg/l 2,4-D plus 0.1 mg/l BAP suplemented with 3% (w/v) sucrose and 0.65% (w/v) plant agar (Duchefa). All media were autoclaved for 20 min. at 121°C and 1.4 kg $cm^{-2}$ pressure. The pH of all media was adjusted to 5.7± 0.1 with 1N NaOH or 1N HCl. Each treatment contained 30 explants that were divided into three equally distributed replications.

### *HS-SPME procedure*

Five grams powder each of callus samples were carried out by a (HS-SPME) head space solid phase

microextraction method using a divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber, with 50/30 um film thickness; before the analysis the fiber was pre conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of callus samples, previously homogenized, were weighed in to a 40 ml vial; the vial was equipped with a "mininert" valve. The vial was kept at 35˚C with continuous internal stirring and the sample was left to equilibratefor 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35˚C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analyses thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported (24).

*GC-MS analysis*

A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer (VarianSpa, Milan, Italy) was used with injector temperature, 260˚C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 lm film thickness (ChrompackItalys.r.l., Milan, Italy). The oven temperature was programmed as follows: 45˚C heldfor 5 min, then increased to 80˚C at a rate of 10˚C/min, and to 240˚C at 2˚C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250˚C; the onization mode, electron impact (EI); acquisit ion range, 40 to 200 m/z; scan rate, 1 us-1. The compounds were identified using the NIST (National Institute of Standardsand Technology) library (NIST/WILEY/EPA/NIH), mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz (25). The relative amounts were calculated on the basis of peak-area ratios. The dendograms and map of collected samples are seen in Figure 1,2 and identified constituents, major compounds of ten taxa *H. scabrum* calli and collect informations of studied *H. scabrum* populations are listed in Table 1.


**Figure 1.** Collection sites of the studied *Hypericum scabrum* populations from Turkey

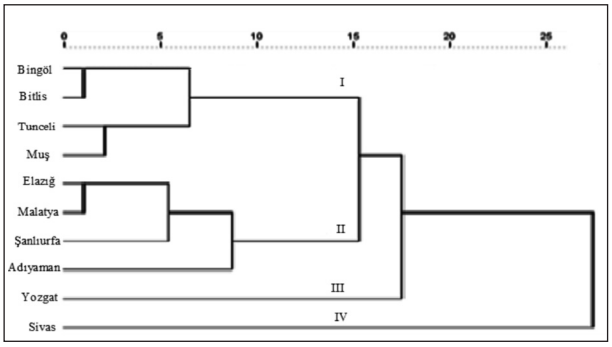


**Figure 2.** Average-linkage dendrogram of the ten *Hypericum scabrum* populations resulting from the cluster analysis of the calli essential oil components. Chemotype I (Limonene / Germacrene D), Chemotype II (α-pinene ), Chemotype III (β-ocimene) and Chemotype IV (β-pinene /α-pinene).

*Statistical analysis*

The statistical software Cropstat (IRRI 2005) was used to perform the ANOVA and pattern analysis. Standard analyses of variance (anova) were used to analyze the data obtained.

## Results

According to the HS-SPME/GC-MS analyses, a total of forty-one components were detected in the examined oils, accounting for 88.70-91.62% of the total compositions (Table 1). A great variability was found in the qualitative composition of the examined oils, since only six components, α-thujene, α-pinene, β-pinene, limonene, germacrene D, spathulenol, were

**Table 1.** The percentage calli essential oils composition of *Hypericum scabrum* growing in Turkey (%)

| Compounds | Bitlis | Muş | Bingöl | Tunceli | Malatya | Elazığ | Şanlıurfa | Adıyaman | Yozgat | Sivas |
|---|---|---|---|---|---|---|---|---|---|---|
| α–Thujene | 0.21 | 0.32 | 0.42 | 0.95 | 0.32 | 0.85 | 0.60 | 0.60 | 1.23 | 1.35 |
| Nonane | 1.05 | - | 0.12 | - | 2.57 | 4.05 | 2.95 | 3.75 | 4.45 | - |
| α-pinene | 7.68 | 15.50 | 12.40 | 10.45 | 33.80 | 33.25 | 40.20 | 38.40 | 8.85 | 25.20 |
| Camphene | - | 0.62 | - | - | 0.30 | 0.75 | 0.42 | 0.30 | - | - |
| β-pinene | 1.30 | 4.45 | 2.32 | 4.80 | 12.60 | 12.75 | 14.63 | 16.25 | 5.20 | 35.74 |
| β-myrcene | 0.07 | - | - | 0.45 | - | 0.63 | - | 0.15 | 0.05 | 0.04 |
| α-Phellandrene | 1.05 | 0.25 | 0.07 | 0.30 | 0.14 | - | 0.05 | - | 0.03 | 0.55 |
| β-phellandrene | 0.42 | 0.30 | 0.03 | 0.20 | 2.05 | - | 0.02 | 0.24 | 3.22 | 0.12 |
| α-terpinolene | 0.02 | - | 0.25 | 0.15 | 0.12 | 0.32 | 0.05 | 0.15 | - | 2.00 |
| p-cymene | 0.15 | - | - | 0.32 | 1.05 | 0.62 | 4.42 | - | 1.15 | 0.25 |
| α-cubebene | - | 0.35 | 0.23 | - | - | 0.42 | - | 0.15 | 0.14 | - |
| Copaene | 0.35 | 0.20 | 1.60 | 0.40 | 0.65 | 2.35 | 3.20 | - | - | 0.45 |
| Bicycloelemene | - | 0.20 | 0.10 | - | - | - | 0.04 | 0.12 | 0.05 | - |
| Benzene, 1-methyl-4 | 0.40 | - | - | 0.25 | 0.15 | 0.03 | - | - | - | 0.05 |
| Limonene | 29.50 | 30.32 | 28.15 | 32.21 | 4.06 | 2.32 | 1.14 | 0.02 | 1.10 | 0.54 |
| β-ocimene | - | 0.14 | - | 0.20 | - | - | 5.16 | 8.12 | 37.90 | 2.00 |
| β-Caryophyllene | 3.30 | 5.00 | 0.52 | 0.12 | 4.15 | 0.30 | 0.04 | - | 1.14 | 0.21 |
| γ-cadinene | 2.50 | 0.26 | 0.40 | 0.34 | 6.23 | 8.50 | - | 10.14 | 1.24 | 2.04 |
| Bornylacetate | 0.05 | - | - | - | 0.15 | - | 0.42 | - | 0.10 | - |
| 3-cyclohexen-1-ol | - | 0.04 | 0.05 | 0.25 | - | - | - | - | - | 7.04 |
| δ-cadinene | 3.42 | - | - | 0.30 | 0.52 | 1.05 | 1.01 | 0.54 | 0.05 | - |
| α-amorphene | - | 0.12 | - | - | - | - | 0.05 | 0.12 | - | - |

**(Continued...)**

**Table 1.** The percentage calli essential oils composition of *Hypericum scabrum* growing in Turkey (%) **(Continued...)**

| Compounds | Bitlis | Muş | Bingöl | Tunceli | Malatya | Elazığ | Şanlıurfa | Adıyaman | Yozgat | Sivas |
|---|---|---|---|---|---|---|---|---|---|---|
| Germacrene D | 28.25 | 27.45 | 30.55 | 28.42 | 4.85 | 6.45 | 8.56 | 2.01 | 0.22 | 0.15 |
| β-selinene | 0.32 | - | 0.12 | 0.15 | - | 0.41 | - | 0.12 | 0.25 | - |
| α-terpineol | - | 0.02 | - | - | 0.05 | - | 0.15 | - | – | 0.21 |
| Bicyclogermacrene | 0.04 | - | 0.05 | 0.20 | - | 0.21 | 0.20 | - | 0.12 | - |
| Borneol | - | 0.42 | 0.05 | - | - | - | - | 0.15 | 0.20 | 0.24 |
| Terpin 4 ol | 0.02 | - | 0.04 | 0.15 | - | - | 0.05 | - | - | - |
| Pulegone | - | - | - | 0.02 | 0.12 | - | - | 0.05 | - | 0.01 |
| Thymol | - | 0.05 | - | - | - | 0.05 | 0.12 | - | 0.12 | - |
| Carvacrol | 0.30 | - | 1.50 | 0.40 | 0.25 | 2.25 | 0.10 | 0.14 | 3.45 | 0.40 |
| β-Elemene | - | 0.40 | - | 0.30 | - | 0.20 | - | 1.15 | 0.05 | 0.40 |
| Aromadendrene | 0.20 | - | 0.40 | - | - | 0.02 | - | - | - | - |
| α-Humulene | 4.48 | 0.12 | 4.15 | 0.04 | 0.13 | - | 5.15 | 0.05 | 10.10 | 0.15 |
| Naphthalene | - | 0.10 | 0.21 | - | 1.10 | 1.15 | 0.02 | 0.14 | - | - |
| Caryophylleneoxide | 0.12 | - | - | 0.10 | 0.24 | - | 0.03 | - | 2.10 | - |
| Spathulenol | 3.15 | 0.05 | 2.12 | 1.05 | 4.55 | 3.28 | 2.34 | 5.54 | 2.20 | 1.45 |
| Isospathulenol | - | 0.12 | - | - | 0.32 | 0.47 | 0.25 | - | - | - |
| α-cadinol | - | 0.52 | 0.12 | - | - | 0.95 | - | 0.15 | 0.05 | 0.10 |
| β-Eudesmol | 0.40 | - | - | 0.30 | 0.12 | - | 0.25 | - | - | - |
| Phytol | - | 0.70 | 0.25 | - | - | 0.20 | - | 0.15 | 0.12 | 0.30 |
| Monoterpenes | 40.70 | 50.49 | 46.09 | 50.45 | 55.84 | 45.87 | 67.08 | 64.56 | 61.40 | 64.61 |
| Sesquiterpenes | 43.53 | 34.79 | 38.24 | 31.72 | 21.76 | 24.61 | 19.12 | 20.09 | 17.71 | 21.95 |
| Others | 5.39 | 3.74 | 5.92 | 8.65 | 12.99 | 19.35 | 5.42 | 4.05 | 5.92 | 4.55 |
| Total | 89.62 | 89.02 | 90.25 | 90.82 | 90.59 | 89.83 | 91.62 | 88.70 | 85.03 | 91.11 |

in common among all populations of *H. scabrum* calli analyzed. Moreover, the mentioned components showed a relatively high variation in levels. α-pinene (7.68-40.20%), β-pinene (1.30-35.74%), limonene (0.02-32.21%) and germacrene D (0.15%-30.55%) were found as the most abundant compounds (Table 1). This different behavior could be ascribed to the juvenile phase maintained in *in vitro* conditions (26) or to the artificial growing in an *in vitro* environment.

## Discussion

The highest amounts of these constituents were found in the oils from şanlıurfa, Sivas, Tunceli and Bingöl populations, respectively. The calli of *H. scabrum* specimens of ten populations from different region of Turkey were found to contain between 30 and 35 compounds in their essential oils, making up between 84 and 90% of the total compounds present (Table 1). β-ocimene (35.74%), α-humulene (10.10%), α-pinene (8.85%) in Yozgat population; Limonene (28.15%), germacrene D (30.55%), α-pinene (12.40%) in Bingöl population; α-pinene (33.25%), β-pinene (12.75%), γ-cadinene (8.50%) in Elazığ population; α-pinene (33.80%), β-pinene (12.60%), γ-cadinene (6.23%) in Malatya population; β-pinene (35.74%), α-pinene (25.20%), γ-cadinene (7.04%) in Sivas population; Limonene (32.21%), germacrene D (28.42%), a-pinene (10.45%) in Tunceli population; α-pinene (40.20%), germacrene D (8.56%), β-ocimene (5.16%) in şanlıurfa population; α-pinene (38.40%), β-pinene (16.25%), β-ocimene

**Table 2.** Origins and geographical characteristics of studied *H. scabrum* populations

| Number | Area of sampling collection | Populations | Altitude (m) | Collection Time | Habitat | Voucher number |
|---|---|---|---|---|---|---|
| 1 | Yozgat: Akdağ Madeni, Davutlu village | Davutlu village | 1150-1250 | 18.06.2014 | Steppe, rocky and stony place, openning of Quercus foresty | 5424 |
| 2 | Bingöl: Vicinity of Dikme village | Dikme village | 1650-1700 | 18.06.2013 | Rocky areas | 5286 |
| 3 | Elazığ: Keban | Aslankaşı village | 1300-1400 | 25.06.2013 | Rocky slopes | 5327 |
| 4 | Malatya: Sürgü region | Sürgü region | 1300-1350 | 05.07.2015 | Road edges | 5689 |
| 5 | Sivas: Zara region | Halkalı and Korkut villages | 1400-1500 | 19.06.2014 | Steppe, gypsum areas, hill, slope, rocky and stony place openning of foresty | 5426 |
| 6 | Tunceli: Ovacık, Munzur-Yılanlı mountain | Munzur-Yılanlı mountain | 1800-2000 | 12.06.2015 | Rocky and stony place | 5684 |
| 7 | Şanlıurfa: Between Siverek to Şanlıurfa | Between Siverek to anlıurfa, 20. km | 600-700 | 07.06.2014 | Road edge, rocky areas | 5415 |
| 8 | Adıyaman: Between Turuş village and Atatürk | Between Turuş village and Atatürk Dam | 600-700 | 08.06.2014 | Rocky-steppe areas | 5417 |
| 9 | Bitlis: Tatvan | Suboyu village | 1650-1750 | 27.06.2015 | Rocky and stony slopes | 5687 |
| 10 | Muş: Muş-Bingöl 10. km | Muş-Bingöl 10. km | 1300-1350 | 28.06.2015 | Roadside, steppe | 5688 |

(8.12%) in Adıyaman population; Limonene (29.50%), germacrene D (28.25%), α-pinene (7.68%) in Bitlis population; Limonene (30.32%), germacrene D (27.45%), α-pinene (15.50%) in Muş population; were determined major compounds (Table 1).

The identification of particular chemotypes of *H. scabrum* calli in this study, displaying a dominant production of α- and β-pinene, limonene, germacrene D, β-ocimene has led to the development of a hypothesis that particular populations (or chemotypes) of this species calli are rich in α-/β-pinene (e.g. monoterpene hydrocarbons), but not produce both groups of compounds in higher amounts simultaneously (Table 1). This study evidences that *in vitro* conditions influenced the quali quantitative compositions of the calli essential oils of *H. scabrum* in comparision with the *in vivo* grown mother plants.

α-pinene was determined in the cali essential oils of all investigated populationits proportion ranging from 8 to 40% (Tab. 1). This compound had previously also been identified as a major component of the essentials oils of *H. scabrum* (40.9%) from Alamut Mountain (27). Regarding the qualitative pattern of the essential oils of some *Hypericum* species, there are similar results for α-pinene, major/high component reported (28, 29). Nevertheless a large differences occured in the amounts of some compounds. It is noteworthy that in the composition of Sivas pupulation β-pinene (35.74%) was determined more than other populations; this compound showed different chemical behavior from all the other studied populations, (Table 1). α-Pinene (37.20%) and β-Pinene (12.77%) were found as the major components in the essential oil of *H. scabrum* from Turkey as well (30). β-ocimene was absent from the essential oil of Bitlis, Bingöl, Malatya, Elazığ populations or present only in low percentages in the calli essential oil of Muş (0.14%), Tunceli (0.20%), Sivas (2.00%) populations, but it is noteworhy that β-ocimene (35.74%), was the major component only in Yozgat population (Table 1). The calli essential oils of Bitlis, Muş, Bingöl, Tunceli populations had a chemical composition different from that of

**Table 3.** The main compounds of studied calli *H. scabrum* populations

| Population name | Major compounds (content [%]a) |
|---|---|
| Yozgat: Akdağ Madeni, Davutlu village | β-ocimene (37.90), α-humulene (10.10), α-pinene (8.85), β-pinene (5.25), nonane (4.45) |
| Bingöl: Vicinity of Dikme village | Limonene (31.15), germacrene D (30.55), α-pinene (12.40), α-humulene (4.15) |
| Elazığ: Keban, Aslankaşı village | α-pinene (36.25), β-pinene (15.75), γ-cadinene (8.50), germacrene D (6.45), nonane (4.05) |
| Malatya: Sürgü region | α-pinene (38.80), β-pinene (15.60), γ-cadinene (6.23), limonene (6.06), β-caryophyllene (4.15) |
| Sivas: Zara region, Halkalı and Korkut villages | β-pinene (37.74), α-pinene (32.20), γ-cadinene (7.04) |
| Tunceli: Ovacık, Munzur-Yılanlı mountain | Limonene (32.21), germacrene D (28.42), α-pinene (18.45), β-pinene (4.80) |
| Şanlıurfa: Between Siverek to Şanlıurfa, 20. km | α-pinene (40.20), germacrene D (6.56), β-ocimene (5.16), α-humulene (5.15), p-cymene (4.42) |
| Adıyaman: Between Turuş village and Atatürk Dam | α-pinene (38.40), β-pinene (16.25), β-ocimene (8.12), spathulenol (5.54) |
| Bitlis: Tatvan, Suboyu village | Limonene (29.50), germacrene D (25.25), α-pinene (10.68), α-humulene (4.48) |
| Muş: Muş-Bingöl 10. km | Limonene (35.32), germacrene D (27.45), α-pinene (15.50), β-pinene (4.45) |

*a) All compounds with a content >4% of the total oil composition were considered as major compounds; their contents in % are given in parentheses.*

all the other species, producing high amounts of limonene (29.50%, 30.32%, 28.15%, 32.21%, respectively), and germacrene D (28.25%, 27.45%, 30.55%, 28.42%, respectively); whereas these compounds were not detected as major component in some studied populations or absent (Table 1). Germacrene D is also detected as main compouds of *H. perforatum* growing wild in Tajikistan (31). In addition twenty-six components were identified in the oil of *H. scabrum* with α-pinene (44.8%) and spathulenol (7.1%), as the most abundant components (31); α-pinene is also as the most abundant component of all studied populations calli, whereas spathulenol were detected low percentages almost all studied populations (Table 1). Germacrene D was one of the major constituents of the calli essential oils of Bingöl population (30.55%) (Table 1). In the calli essential oil of Adıyaman-Yozgat and Sivas populations Germacrene D was determined in low percentages (Table 1); this compound was also main constituents of *H. perforatum* essential oil (32).

In this research thymol, a rare compound in plant essential oil, or was not found in all *H. scabrum* populations calli. The same result has been previously reported by Mohammed Reza *et al.*, (33) in *H. perforatum* populations growing in Iran. In agreement to our results, α-pinene was also previously reported as a major component essential oil of *H. capitatum* var. *capitatum* from Turkey (34). Contrarily, constituents such as germacrene D, limonene (35), γ-muurolene (36) and carvacrol (37), which have been previously reported as main components essential oils of some *Hypericum* taxa, were not detected at all in the investigated calli samples in our research. All populations calli essential oils were characterized by a high content of monoterpenes (54.7%). The calli essential oil analysis showed that monoterpene concentrations were higher than those of sesquiterpenes.Whereas essential oil composition of five *Hypericum* species from southern Brazil showed that sesquiterpenes are present in higher concentrations (38). Among the monoterpenic major components, α-pinene and β-pinene determined in the calli essential oils of both our studied populations were reported in the essential oils of *H. myrianthum* (6.5%, 3.7%) (38), *H. perfoliatum* (64.3%, 3.2%), *H. linarifolium* (31.2%, 11.0%) and *H. pulchrum* (46.8%, 12.5%) (28). Essential oils *in vitro* cultured plants was predominantly composed of hydrogenated and oxygenated monoterpenes in comparison of *in vitro* plant materials, in respect to the propagated one, could be the consequence of the different ontological stage. This hypothesis is supported by the fact that *in vitro* cultured plants are considered, by definition, at juvenile stage and it is well known that accumulation of the highest capasity of biosynthesis (39, 40) It's remarkable that the essential oils profile of the *in vivo* sample does not correspond with that reported in the literature (41, 42)

An interesting fact is that, although studied *H. scabrum* populations are some quantitive and qualitive different in their essential oil compositions. In planta, culture conditions are crucial factor for growth and development directly affecting the photosynthetic rate and influence the quali quantitative composition of *in vitro*. These similarities or differences may be dependent from local, climatic, seasonal factors and regulated by plant growth regulators during *in vitro* cultures. All species growing in close habitats and near locations. Further investigations of the essential oil compositions of larger number of relative and distant populations of different and close taxa, along with more data about *Hypericum* taxa, could be helpful in chemotaxonomy.

Nevertheless the populations growing in eastern Iran, including Bitlis, Muş, Bingöl and Tunceli populations, showing different temperature and altitudes, constituted a same group (Chemotype I; Fig. 1). It has already been explained that the distribution of calli essential oil chemotypes seemed to be linked with the local selective forces acting on the chemotype variety. In fact, moisture, temperature, topography, and edaphic factors and/or fauna and flora acts on terpene-biosynthesis pathways and contribute to the emergence of different chemical oil profiles (43). The first chemotype (I) was characterized by high content of limonene (28.15%-32.21%) and germacrene D (27.45%-30.55%); α-pinene (7.68%) was other main component of this chemotype. Chemotype II was composed of four populations (Malatya, Elazığ, şanlıurfa and Adıyaman) characterized by high content of α-pinene (33.80% - 33.25% - 40.20% - 38.40%, respectively); α-pinene chemotype was also recorded in southeastern France. Chemotype III was composed of one population (Yozgat) with β-ocimene (37.90%) as

the major compound. The lower amount of monoterpenoids such as α-pinene and β-pinene in this population made it a separate chemotype. This chemotype contained the lowest amount of sesquiterpene hydrocarbons (17.71%) compared to the other chemotypes (19.12% - 43.53%). The high content of β-pinene (35.74%) and α-pinene (25.20%) differentiated the population from Sivas as a distinct chemotype (chemotype IV: β-pinene/α-pinene). The calli essential oil variation observed among *H. scabrum* populations in accordance with their geographical and bioclimatic distribution imposes that conservation strategies should be made appropriately, taking into account these factors; conservation strategies should concern all populations representing the different chemotypes (44).

*In vitro* technology is well recognized for biodiversity preservation and may represents an alternative method to satisfy the increasing demand for both volatile (e.g. essential oils) and non volatile bioactive secondary metabolites. Moreover, since the products of secondary metabolism have always been considered as plant responses to biotic and abiotic stress, several physical and chemical elicitors, can be applied to stimulate plants to produce high concentrations of a desired compound or group of compounds.

In conclusion, aroma and biological activities of essential oils is determined by the type of compounds present and their relative percentages, such variability in the calli essential oil profile of studied populations enabled selection of those with specific aromas or activities of their calli essential oils for use in relevant industries and sectors. In addition the chemical results from this study might be helpful chemotaxonomy and potential usefulness of *Hypericum* taxa. Besides, due to their various bioactivities, further researchs should be carried out on the drug development of *Hypericum* extracts and their constituents.

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