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Essential Oil Composition of Five Endemic *Hypericum* Species from Turkey

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Abstract

More than 465 *Hypericum* species exist on the Earth, widely spreading in Europe, Asia and North Africa. 42 of 80 species growing in Turkey are endemic. The chemical composition of the essential oils obtained from the aerial parts of the *H. uniglandulosum* Hausskn. ex Bornm., *H. scabroides* Robson & Poulter, *H. kotschyanum* Boiss., *H. salsugineum* Robson & Hub.-Mor. and *H. thymopsis* Boiss, five of the endemic species of Turkey by using the hydrodistillation method was identified by GC and GC/MS. The major components were identified as follows: 2,6-Dimethyl-3,5-heptadien-2-one (40.7%), nonacosane (3.2%), hexadecanoic acid (2.7%) and α-pinene (2.7%) in *H. uniglandulosum*; hexadecanoic acid (17.7%), spathulenol (5.3%), nonacosane (4.4%), dodecanoic acid (4.1%), baeckeol (4.1%) and γ-muurolene (3.9%) in *H. scabroides*; α-pinene (14.4%), nonacosane (11.1%), hexadecanoic acid (9.2%), β-pinene (8.7%), spathulenol (6.3%) and limonene (5.1%) in *H. kotschyanum*; nonacosane (42.7%), hexadecanoic acid (23.2%) and baeckeol (6.1%) in *H. salsugineum*; α-pinene (44.0%), baeckeol (32.9%), spathulenol (8.0%), limonene (7.6%) and camphene (5.2%) in *H. thymopsis*. Finally, the results are compared with each other. The differences between the results of the *H. thymopsis* and *H. scabroides* obtained in this study and the previous studies show that the chemical composition of the essential oils is different for the same species obtained at different locations. The essential oil composition of these species, except for the *H. thymopsis* and *H. scabroides* is identified for the first time.

Keywords: *Hypericum uniglandulosum; Hypericum scabroides*; *Hypericum kotschyanum*; *Hypericum salsugineum; Hypericum thymopsis;* Hypericaceae; Essential oil composition

Introduction

The genus *Hypericum* (Hypericaceae) 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, especially *H. perforatum*, have been used for the treatment of burns, wounds, hemorroids, diarrhorea and ulcers [1-5].

Moreover, aqueous extracts prepared from the flowering aerial parts of the *Hypericum* species are being used in the treatment of psychological diseases such as neuralgia, anxiety, neurosis and depression [6]. The preparative forms of the *Hypericum perforatum* (St. John's Wort) are sold for the treatment of mild to moderate depression in the USA and Europe. The chemical composition of some *Hypericum* species includes naphthodianthrones (especially hypericin and pseudohypericin), acylphloroglucinol derivatives (especially hyperforin and adhyperforin), flavonoids (especially quercetin, quercitrin, hyperoside and biapigenin), tannins, *n*-alkanes, xanthones and essential oil [7-9].

The essential oil compositions of about 50 different *Hypericum* species have so far been identified [10-13].

In this study, the oils of 5 endemic *Hypericum* species were obtained by hydro-distillation and analyzed by GC and GC/MS.

With the exception of *H. thymopsis* and *H. scabroides* the chemical composition of the essential oils of *H. uniglandulosum*, *H. kotschyanum* and *H. salsugineum* have not been reported to date [13,14].

The aim of this study was to investigate volatile constituents of five endemic Turkish *Hypericum* species, thus adding for the database of phytochemical knowledge for the genus. In addition to this, a comparison between the volatile oil composition of *H. thymopsis* and *H. scabroides* from different locations was performed.

Compounds were calculated automatically from peak areas of the total ion chromatogram (TIC). *n*-alkanes were used as reference points in the calculation of relative retention indexes (RRI). A library search was carried out using Wiley GC/MS Library, MassFinder, Adam's Library [15,16] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [17,18], was also used for the identification.

Material and Methods

Plant material

Flowering aerial parts of *H. uniglandulosum* were collected from east Anatolia, namely, Erzincan: Erzincan-Eski Çayırlı road, 10 km to Eski Çayırlı, 1450 m, 15.07.2006, that of *H. scabroides* were collected from east Anatolia, namely, Erzincan: Erzincan-Kelkit, 15 km to Kelkit, 1550 m, 14.07.2006, that of *H. kotschyanum* were collected from south Anatolia, namely, İçel: North-west of Arslanköy, 1840 m, 15.06.2006, that of *H. salsugineum* were collected from central Anatolia, namely, Konya: around The Salt Lake, on 01.07.2005, *H. thymopsis* were collected from

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Compound	RRI	UN	SB	KC	SG	TP
α-pinene	1032	2.7	3.1	**14.4**	-	**44.0**
Camphene	1076	-	-	0.5	-	5.2
Undecane	1100	1.9	0.3	0.1	tr	-
β-pinene	1118	-	-	**8.7**	-	1.7
Limonene	1203	0.2	1.0	**5.1**	-	**7.6**
γ-terpinene	1255	-	-	0.7	-	-
p-cymene	1280	-	0.3	1.5	-	-
6-methyl-5-hepten-2-one	1348	0.9	-	-	-	-
2,6 Dimethyl-3,5 heptadien-2-one*	1377	**40.7**	-	-	-	-
Nonanal	1400	0.1	-	-	-	-
trans-linalool oxide (furanoid)	1450	-	-	-	0.2	-
cis-linalool oxide (furanoid)	1478	0.9	-	-	0.1	-
Bicycloelemene	1495	-	-	-	0.7	-
α-copaene	1497	0.1	0.5	0.6	0.3	-
α-campholene aldehyde	1499	0.2	0.2	0.6	-	-
β-bourbonene	1535	-	-	-	0.3	-
Linalool	1553	1.2	-	-	0.1	-
Pinocarvone	1586	-	-	0.3	-	-
Fenchylalcohol	1591	-	-	0.5	-	-
β-ylangene	1589	-	-	-	0.3	-
β-copaene	1597	-	-	-	0.5	-
β-elemene	1600	-	-	-	0.3	-
β-caryophyllene	1612	-	-	-	0.2	-
Aromadendrene	1628	0.1	1.2	-	0.3	-
Myrtenal	1648	0.2	-	0.7	-	-
(E)-2-Decenal	1655	-	-	-	0.1	-
γ-Gurjunene	1659	-	-	-	0.1	-
Alloaroma dendrene	1661	-	-	-	0.1	-
trans-pinocarveol	1670	-	-	1.0	-	-
Acetophenone	1671	-	1.5	-	-	-
trans- Verbenol	1683	0.2	-	0.4	-	-
Drima-7,9(11)-diene	1694	tr	-	-	-	-
γ-Muurolene	1704	1.0	3.9	1.8	0.4	-
Borneol	1719	0.2	-	0.3	0.1	tr
Verbenone	1725	0.3	0.8	-	0.2	-
Germacrene-D	1726	-	-	0.9	1.4	tr
Valencene	1740	0.2	-	-	-	-
α-Muurolene	1740	0.2	0.8	0.3	0.2	-
Geranial	1740	0.1	-	-	-	-
Carvone	1751	0.1	-	-	-	-
Bicyclogermacrene	1755	-	-	-	1.0	-
Decanol	1766	-	-	0.3	-	-
δ-Cadinene	1773	tr	1.6	0.6	0.4	tr
γ-Cadinene	1776	0.4	2.2	0.6	0.3	tr
Myrtenol	1804	0.2	-	0.4	-	-
Methyl dodecanoate	1815	-	-	-	0.2	-
Trans-carveol	1845	0.3	0.9	0.3	-	-
Calamenene	1849	1.0	1.8	0.9	0.3	-
Geraniol	1857	1.0	-	-	-	-
(E)-Geranyl acetone	1868	0.2	-	0.4	0.1	-
α-calacorene	1941	0.1	1.2	0.6	-	-
1,5-Epoxysalvial-4(14)-ene	1945	-	-	1.0	-	-
(E)-β-Ionone	1958	0.1	-	-	-	-
1-Dodecanol	1973	0.5	-	-	1.5	-
Eicosane	2000	0.1	-	-	-	-
Caryophyllene oxide	2008	0.3	1.7	1.3	0.1	-
Salvial-4(14)-en-1-one	2037	0.6	-	-	tr	-
1-Tridecanol	2077	-	-	-	0.2	-
Octanoic acid	2084	0.1	1.0	-	-	-
Globulol	2098	0.2	-	-	-	-
Heneicosane	2100	-	-	-	0.2	-
Viridiflorol	2104	-	-	-	0.2	-
Hexahydrofarnesyl acetone	2131	0.6	1.4	0.8	1.1	tr
Spathulenol	2144	1.5	**5.3**	**6.3**	-	**8.0**
1-Tetradecanol	2179	0.3	-	-	0.5	-
T-cadinol	2187	-	tr	-	-	-
Nonanoic acid	2192	0.3	2.2	-	-	-
Docosane	2200	0.1	-	-	-	-
T-Muurolol	2209	0.2	-	-	0.1	-
Methyl hexadecanoate	2226	0.1	-	-	0.2	-
Carvacrol	2239	-	-	2.4	-	-
α-cadinol	2255	0.3	1.8	0.8	0.2	tr
Cadalene	2256	0.4	3.0	0.8	0.2	0.5
Ethyl hexadecanoate	2262	0.2	-	-	-	-
Decanoic acid	2298	0.6	-	-	-	-
Tricosane	2300	0.9	0.6	0.8	0.6	-
Eudesma-4(15),7-dien-1β-ol	2369	-	-	0.9	0.7	tr
1-Hexadecanol	2384	-	-	-	0.1	-
Undecanoic acid	2400	0.2	-	-	-	-
Tetracosane	2400	-	-	-	0.4	-
Pentacosane	2500	0.2	2.6	3.9	1.9	-
Dodecanoic acid	2503	2.3	4.1	3.0	0.1	tr
Hexacosane	2600	-	-	-	0.2	-
Phytol	2622	-	-	tr	0.7	-
Benzyl benzoate	2655	0.1	-	1.8	-	-
Baeckeol	2668	0.9	4.1	2.4	**6.1**	**32.9**
Tetradecanoic acid	2670	0.8	2.9	2.4	2.5	-
Heptacosane	2700	0.1	1.2	1.0	2.2	-
Octacosane	2800	-	-	-	1.3	-
Pentadecanoic acid	2822	-	-	-	1.2	-
Nonacosane	2900	3.2	4.4	**11.1**	**42.7**	tr
Hexadecanoic acid	2931	2.7	**17.7**	**9.2**	**23.2**	tr
Total		**72.7**	**75.3**	**92.4**	**96.9**	**99.9**

UN: *H. uniglandulosum*; **SB:** *H. scabroides;* **KC:** *H. kotschyanum;* **SG:** *H. salsugineum;* **TP:** *H. thymopsis*
RRI: Relative retention indices calculated against *n*-alkanes
% calculated from FID data
tr: Trace (<0.1%)
* tentative identification

Table 1: Percentage of volatiles of 5 endemic *Hypericum* species.

central Anatolia, namely, Sivas: Sivas-Malatya road, Ziyaret Tepe, 1350 m, 10.07.2005. Specimens were identified and vouchers were deposited in the Herbarium of Istanbul University, Faculty of Pharmacy (İstanbul Üniversitesi Eczacılık Fakültesi Herbaryumu, İstanbul, Turkey) under code numbers of ISTE 85344, ISTE 85343, ISTE 83979, ISTE 85341 and ISTE 85342, respectively.

Isolation of the essential oil

Air dried and powdered plant materials were subjected to hydro-distillation in a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [19]. The oils obtained were stored at +4°C until analyzed.

Gas chromatography (GC)

The GC analysis was carried out using an Agilent 6890N GC system.

FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in table 1.

Gas chromatography/mass spectrometry (GC/MS)

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m×0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Injection volume was 1 µL (10%) in hexane. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram (TIC). *n*-Alkanes were used as reference points in the calculation of relative retention indexes (RRI). A library search was carried out using Wiley GC/MS Library, MassFinder, Adam's Library [15,16] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [17,18], was also used for the identification.

Results and Discussion

Volatile oils were obtained from the aerial parts of the *H. uniglandulosum, H. scabroides, H. kotschyanum, H. salsugineum and H. thymopsis* with yields of 0.67% (v/w), trace (in hexane), 0.67% (v/w), trace (in hexane), 0.67% (v/w), respectively. The analyses were performed and are given in table 1.

Fifty-eight constituents corresponding to the 72.7% of the oil from the *H. uniglandulosum,* thirty-two constituents corresponding to the 75.3% of the oil from *H. scabroides,* forty-five constituents corresponding to the 92.4% of the oil from the *H. kotschyanum,* fifty-four constituents corresponding to the 96.9% of the oil from the *H. salsugineum,* seventeen constituents corresponding to the 99.9% of the oil from the *H. thymopsis* were identified.

2,6-Dimethyl-3,5-heptadien-2-one (40.7%), nonacosane (3.2%), hexadecanoic acid (2.7%) and α-pinene (2.7%) were characterized as the main components of the *H. uniglandulosum*. Hexadecanoic acid (17.7%), spathulenol (5.3%), nonacosane (4.4%), dodecanoic acid (4.1%), baeckeol (4.1%) and γ-muurolene (3.9%) were characterized as the main components of the *H. scabroides* (SB). α-pinene (14.4%), nonacosane (11.1%), hexadecanoic acid (9.2%), β-pinene (8.7%), spathulenol (6.3%) and limonene (5.1%) were characterized as the main components of the *H. kotschyanum*. Nonacosane (42.7%), hexadecanoic acid (23.2%) and baeckeol (6.1%) were characterized as the main components of the *H. salsugineum*. α-pinene (44.0%), baeckeol (32.9%), spathulenol (8.0%), limonene (7.6%) and camphene (5.2%) were characterized as the main components of the *H. thymopsis*. The chemical class distribution of the volatile oils of 5 different species is given in table 2.

It has been observed that, the oil of *H. uniglandulosum* was rich in terms of carbonylic compounds and fatty acids. The oil of *H. scabroides* was rich in terms of sesquiterpene hydrocarbons and fatty acids. The oil of *H. kotschyanum*, on the other hand, was dominated by monoterpene hydrocarbons, alkanes, oxygenated sesquiterpene hydrocarbons and fatty acids. The oil of *H. salsugineum* contained alkanes and fatty acids. The oil of *H. thymopsis* was found to be rich in monoterpene hydrocarbons and a phenolic-compound.

Chemical Class	UN (#/%)	SB (#/%)	KC (#/%)	SG (#/%)	TP (#/%)
Monoterpene Hydrocarbons	2 / 2.9	3 / 4.4	6 / 30.9	-	4 / 58.5
Oxygenated Monoterpenes	12 / 4.3	4 / 5.0	11 / 7.5	6 / 0.7	2 / Tr
Sesquiterpene Hydrocarbons	11 / 3.5	9 / 16.2	9 / 7.1	17 / 7.3	4 / 0.5
Oxygenated Sesquiterpenes	6 / 3.0	4 / 7.2	5 / 10.1	5 / 1.1	2 / 8.0
Alkanes+Alkenes	7 / 6.5	5 / 9.1	5 / 16.9	9 / 49.5	1 / Tr
Fatty acids	8 / 7.9	5 / 27.9	3 / 14.6	6 / 27.3	2 / Tr
Others	12 / 44.6	2 / 5.5	5 / 5.3	11 / 11.0	2 / 32.9
Total	58 / 72.7	32 / 75.3	45 / 92.4	54 / 96.9	17 / 99.9

UN: *H. uniglandulosum*; **SB:** *H. scabroides;* **KC:** *H. kotschyanum;* **SG:** *H. salsugineum;* **TP:** *H. thymopsis*
#: number of compound
% relative percentage

Table 2: The chemical class distribution of the oil components of 5 endemic *Hypericum* species.

As a result of this research α-pinene-2,6-dimethyl-3,5-heptadien-2-one, baeckeol, nonacosane and hexadecanoic acid were identified as major volatile constituents (>10%) in *Hypericum* species.

Comparing the main constituent of the *H. uniglandulosum* oil to the other studies, the following are observed: 2,6-Dimethyl-3,5-heptadien-2-one was found to be the major component only in *H. tetrapterum* from Serbia [20].

Hexadecanoic acid and spathulenol were detected in high amount in *H. scabroides* in this study, however, the δ-3-carene and sabinene were reported as the main constituents of the *H. scabroides* collected from different location [14].

Nonacosane was found to be the major component only in two species, namely *H. salsugineum* and *H. davisii* [21].

It was observed that α-pinene was found to be the main component in *H. kotschyanum* and *H. thymopsis*. Among the previous studies about *Hypericum* essential oils from Turkey, many taxa were characterized by the high amount of α-pinene [11,20,22-30], namely *H. calycinum*, *H. cerastoides*, *H. montbretii*, *H. scabrum*, *H. perforatum* [10,31], *H. hyssopifolium* subsp. *elongatum* var. *elongatum, H. capitatum* var. *capitatum*, *H. aviculariifolium* subsp. *depilatum* var. *depilatum* and *H. apricum* [21].

α-pinene, baeckeol, limonene and spathulenol were identified as major components in *H. thymopsis*, although baeckeol and limonene were not determined in a previous study. α-pinene, germacrene D, δ-cadinene, γ-cadinene, spathulenol, α-cadinol, eudesma-4(15),7-dien-1β-ol, nonacosane and hexadecanoic acid were identified both in this study and the previous one [13].

Essential oil compositions of species show difference in the sense of collecting regions and dates. Chemical profiling using volatiles may be useful in taxonomical classifications.

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