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CHEMICAL INVESTIGATIONS ON SOME *Hypericum* SPECIES GROWING IN TURKEY-I*

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The chemical composition of five taxa of the genus Hypericum *L. obtained from herbarium specimens has been investigated.* Namely, H. adenotrichum Spach *(endemic),* H. calycinum *L.,* H. cerastoides *(Spach) Robson,* H. montbretii *Spach, and* H. perforatum *L. were subjected to microdistillation to isolate the volatile compounds, which were analyzed by GC/MS.*

Key words: *Hypericum* species, *Guttiferae*, volatile compounds, microdistillation, GC/MS.

The *Hypericum* genus of *Guttiferae* is represented in Turkey by 89 species of which 43 are endemic. These are shrubs or herbs generally with translucent oil glands on their aerial parts. This genus is widespread in Turkey, and the most abundant and well known is *H. perforatum* [1–3].

Hypericum species are of great interest for mankind throughout many centuries; in particular, *H. perforatum* is used as a dye, in flavoring, in food, as a medicine in wound healing, ulcers, the common cold, diabetes mellitus, and as an astringent. It is also used as an antimicrobial, antiviral, and antidepressant has antihepatotoxic properties, and is used for many other ailments in Turkish folk medicine as well in the world [4-12].

Phytochemical investigations on *Hypericum* species have shown that they contain flavonols (catechins), xanthones, coumarins, glycosides, anthraquinones, phloroglucinols, flavonoids, flavonolglycosides, lactones, pyrones, lipids, triterpenes, tannins, and essential oils. Some of its constituents have been reported to be responsible for various biological activities [4, 5, 13].

Hypericum species are generally known in Turkey under the names «kantaron, binbirdelik otu, kan otu, koyunkiran, kuzukiran, kilicotu, mayasilotu, puren, sari kantaron, and yara otu» [14].

Although there are numerous investigations on the phytochemistry and biological activity of *Hypericum* species, there are only a few studies related to their essential oils [15–24].

Microdistillation was used in this study, for the first time, as a practical aid for the isolation of volatiles stored within five different *Hypericum* species (the endemic *H. adenotrichum* Spach, *H. calycinum* L., *H. cerastoides* (Spach), Robson, *H. montbretii* Spach, and *H. perforatum* L.).

In the course of the present study, steam volatiles of five of *Hypericum* species obtained by microdistillation were analyzed by GC/MS. Their compositions are given in Table 1.

Steam volatiles of the endemic *H. adenotrichum* (**A**) contained germacrene D (38%) as the major constituent. Forty five compounds were identified representing 93% of the total compounds.

The main volatile compounds of *H. calycinum* (**B**) were α-pinene (24%) and β-pinene (14%) among the 32 compounds characterized, representing 85% of the total amount of volatiles.

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TABLE 1. Composition of the Volatiles by Microdistillation of *Hypericum* Species, %

RRI	Compound	A	B	C	D	E
965	3-Methylnonane	-	-	-	-	3.2
1017	4-Methyl-2-pentanone	-	0.5	-	-	-
1032	α-Pinene	15.5	24.1	57.7	25.7	50.3
1065	2-Methyldecane	-	-	-	-	3.0
1072	α-Fenchene	-	0.6	-	-	-
1076	Camphene	-	2.9	-	-	-
1093	Hexanal	0.3	0.4	0.2	-	-
1100	Undecane	16.5	0.2	5.0	4.8	3.4
1118	β-Pinene	1.0	14.2	3.3	18.8	1.0
1174	Myrcene	0.5	-	1.9	1.7	-
1188	α-Terpinene	-	-	-	Tr.	-
1203	Limonene	0.2	2.1	0.5	2.1	Tr.-
1225	(Z)-3-Hexenal	1.2	0.2	0.1	-	-
1244	Amylfuran (2-Pentylfuran)	Tr.	-	0.1	-	-
1246	(Z)-β-Ocimene	-	-	0.1	-	-
1255	γ-Terpinene	-	-	0.1	0.4	-
1266	(E)-β-Ocimene	-	-	1.4	-	-
1280	*p*-Cymene	-	2.3	0.1	-	Tr.
1290	Terpinolene	-	0.3	0.1	0.9	-
1300	Tridecane	0.3	-	-	-	-
1400	Nonanal	0.2	-	0.1	-	-
1450	*trans*-Linalool oxide (Furanoid)	-	0.4	-	-	-
1452	α,*p*-Dimethylstyrene	-	0.2	-	-	-
1452	1-Octen-3-ol	-	-	0.1	-	-
1466	α-Cubebene	-	-	0.2	-	-
1478	*cis*-Linalool oxide (Furanoid)	-	0.2	-	-	-
1495	Bicycloelemene	0.1	-	-	-	-
1497	α-Copaene	0.8	-	1.5	0.8	-
1499	α-Campholene aldehyde	-	0.9	-	-	-
1532	Camphor	-	1.0	-	-	-
1535	β-Bourbonene	0.3	-	0.1	-	-
1544	α-Gurjunene	-	-	0.3	-	-
1549	β-Cubebene	0.2	-	0.1	-	-
1586	Pinocarvone	-	1.8	-	-	-
1587	β-Funebrene	0.1	-	-	-	-
1589	β-Ylangene	0.3	-	0.1	-	-
1591	Fenchyl alcohol	-	3.3	-	0.5	-
1600	β-Elemene	-	-	0.2	-	-
1612	β-Caryophyllene	0.7	-	0.3	5.7	-
1614	Carvacrol methyl ether (=Methyl carvacrol)	-	-	-	-	Tr.
1628	Aromadendrene	-	-	0.2	-	-
1648	Myrtenal	-	4.5	0.1	-	-
1661	Alloaromadendrene	1.2	-	0.5	-	-
1664	*trans*-Pinocarveol	-	4.2	0.1	-	-
1668	(Z)-β-Farnesene	0.3	-	0.6	-	1.6
1674	*p*-Mentha-1,5-dien-8-ol	-	0.5	-	-	-
1687	α-Humulene	0.6	Tr.	0.3	-	-
1704	γ-Muurolene	1.1	-	1.5	4.3	-
1706	α-Terpineol	0.1	3.5	0.4	6.5	-
1719	Borneol	-	3.6	-	0.7	-

TABLE 1. (continued)

RRI	Compound	A	B	C	D	E
1725	Verbenone	-	4.5	-	-	-
1722	Dodecanal	-	-	0.3	-	-
1726	Germacrene D	37.7	-	2.5	5.9	-
1740	Valencene	-	-	0.3	-	-
1742	β-Selinene	-	-	-	-	6.6
1744	α-Selinene	-	-	0.4	-	3.5
1751	Carvone	-	0.6	-	-	-
1804	Myrtenol	-	2.6	-	-	-
1740	Valencene	-	-	-	1.0	-
1740	α-Muurolene	0.7	-	0.6	-	-
1743	α-Cadinene	-	-	-	1.3	-
1755	Bicyclogermacrene	4.8	-	0.2	0.7	-
1758	(E,E)-α-Farnesene	0.3	-	-	0.8	-
1773	δ-Cadinene	2.0	-	1.7	4.9	-
1776	γ-Cadinene	0.7	-	0.7	2.8	-
1799	Cadina-1,4-diene(=Cubenene)	0.1	-	0.1	0.3	-
1804	Myrtenol	0.6	-	-	-	-
1810	3,7-Guaiadiene	0.1	-	0.1	0.5	-
1827	(*E*,*E*)-2,4-Decadienal	0.1	-	-	-	-
1845	*trans*-Carveol	-	1.1	0.1	-	-
1853	*cis*-Calamenene	-	-	0.2	-	-
1864	*p*-Cymen-8-ol	-	1.0	-	-	-
1868	(*E*)-Geranyl acetone	-	-	0.1	-	-
1900	Nonadecane	0.3	-	0.1	-	-
1941	α-Calacorene	-	-	0.3	-	-
1945	1,5-Epoxy-salvial(4)14-ene	-	-	0.3	-	-
1973	Dodecanol	0.2	-	-	-	-
2004	*o*-Cresol	-	1.0	-	-	-
2008	Caryophyllene oxide	-	-	0.3	-	-
2037	Salvial-4(14)-en-1-one	-	-	0.2	-	-
2050	(*E*)-Nerolidol	-	-	-	1.1	-
2057	Ledol	-	-	0.9	-	-
2071	Humulene epoxide-II	-	0.8	0.3	-	-
2088	1-epi-Cubenol	-	-	-	0.6	-
2098	Globulol	0.3	-	-	-	-
2100	Heneicosane	0.5	-	0.2	-	-
2131	Hexahydrofarnesyl acetone	0.3	-	0.1	-	-
2144	Spathulenol	0.4	1.6	1.9	0.6	-
2148	(Z)-3-Hexen-1-yl benzoate	0.2	-	0.3	-	-
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	-	-	0.2	-	-
2179	Tetradecanol	-	-	-	-	1.2
2187	T-Cadinol	0.4	-	0.2	0.8	-
2198	Thymol	-	-	-	-	1.6
2209	T-Muurolol	0.5	-	0.2	0.7	-
2219	δ-Cadinol	0.1	-	-	-	-

TABLE 1. (continued)

RRI	Compound	A	B	C	D	E
2239	Carvacrol	0.1	-	-	-	21.9
2255	α-Cadinol	0.9	-	0.3	1.9	-
2300	Decanoic acid	-	-	0.1	-	-
2300	Tricosane	-	-	0.3	-	-
2503	Dodecanoic acid	0.2	-	-	-	-
2931	Hexadecanoic acid	-	-	-	0.4	-
	Total Identified Compounds	45	32	61	31	14
	Total Percentage	93.0	85.1	90.8	97.2	97.3

A: *H. adenotrichum* Spach, B: *H. calycinum* L., C: *H. cerastoides* (Spach) Robson, D: *H. montbretii* Spach, E: *H. perforatum* L.

RRI: Relative retention indices calculated against *n*-alkanes.

%: calculated from TIC data.

Tr: Trace (<0.1 %).

The volatiles of *H. cerastoides* (**C**) contained α-pinene (58%), undecane (5%), and β-pinene (3%) as major compounds. These were among the 61 compounds representing 91% of the total volatiles.

The microdistillation of *H. montbretii* (**D**) resulted in the characterization of 31 compounds representing 97% of the total oil. α-Pinene (26%), β-pinene (19%), and undecane (5%) were identified as major compounds.

Fourteen of the *H. perforatum* (**E**) steam volatiles obtained by microdistillation were identified by GC/MS to represent 97% of the total. α-Pinene (50%) and carvacrol (22%) were identified as major constituents. α-Pinene was reported as the main component in several *H. perforatum* essential oils [15, 16, 18-21].

Microdistillation is a useful technique in obtaining volatiles from small amounts of plant materials. Chemical profiling using volatiles may be useful in taxonomical classifications. Further investigations on the chemical composition of Turkish *Hypericum* species are ongoing.

EXPERIMENTAL

Plant Materials: *H. adenotrichum* Spach (**A**) (ESSE 13761) A2: Bursa: Inegol – Keles (Bogaz), 1200 m, June 2001; *H. calycinum* L. (**B**) (ESSE 9151) A2: Bursa: Guneybostanci Village, 14 June 1991; *H. cerastoides* (Spach) Robson (**C**) (ESSE 13759) A2: Bursa: Huseyinalan Village, 700 m, May 2001; *H. montbretii* Spach (**D**) (ESSE 13758) A2: Bursa: Inegol-Kocayayla, June 2001 and *H. perforatum* L. (**E**) (ESSE 13340) B3: Eskisehir: Dagkuplu Village, 700-1000 m, July 1997, dated Herbarium specimens of the Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey (ESSE) were subjected to chemical investigation using the microdistillation method [25, 26].

Microdistillation: Crushed dry plant material (~500 mg) was placed in the sample vial of the MicroDistiller® (Eppendorf-Germany) together with 10 ml of water. 2.5 g of NaCl and 0.5 ml of water was added into the collection vial to break any possible emulsion formation. 300 ml hexane was also added into the collecting vial to trap the volatile components. The sample vial was heated to 108°C at a rate of 20°C/min and then kept at 108°C for 90 min, then heated to 112°C at a rate of 20°C/min and kept at this temperature for 30 min. Later, the sample was subjected to post-run for 5 min under the same condition. The collecting vial was cooled to –1°C during the distillation. After the distillation was completed, the volatiles were analyzed.

Analysis of Steam Volatiles: The samples were analyzed by GC/MS using a HP-G1800A GCD system. HP-Innowax FSC column (60 m × 0.25 mm i.d., with 0.25 mm film thickness). Helium (1 ml/min) was used as carrier gas. The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and then maintained constant at 220°C for 10 min to 240°C at rate of 1°C/min. Mass range was recorded from *m/z* 35 to 425. MS were taken at 70 eV. Relative

percentages of the individual compounds were calculated automatically from peak areas of the total ion chromatogram (TIC). *n*-Alkanes were used as reference points for the calculation of relative retention indices (RRI). Library search was carried out using both the "Wiley GC/MS Library" and the "TBAM Library of Essential Oil Constituents" for the identification (Table 1).

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