Analysis of the Essential Oils of two *Hypericum* species (*H. Lanuginosum* var. *lanuginosum* Lam. and *H. perforatum* L.) from Turkey

Türkiye'de Yetişen İki *Hypericum* Türünün (*H. lanuginosum* var. *lanuginosum* Lam. and *H. perforatum* L.) Uçucu Yağlarının analizi

Research Article

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ABSTRACT

The chemical composition of the essential oils of aerial parts of *Hypericum lanuginosum* var. *lanuginosum* Lam. and *Hypericum perforatum* L. were analyzed by GC and GC-MS. Forty one compounds were identified in the essential oils of *H. lanuginosum* with spathulenol (17.3%), caryophyllene oxide (13.1%), α–pinene (11.7%) and undecane (6.2%) as main constituents. Forty components were identified in the oil of *H. perforatum* with β–selinene (19.4%), bicyclogermacrene (15.3%), 2 tetradecene (8.2%) and α–amorphene (8.1%) as the most abundant components.

Key Words
Clusicaceae, *Hypericum lanuginosum* var. *lanuginosum*, *Hypericum perforatum*, spathulenol, β–selinene.

ÖZET

Hypericum lanuginosum var. *lanuginosum* Lam. ve *Hypericum perforatum* L. taksonlarının toprak üstü kısımlarının uçucu yağlarının kimyasal birleşimi GC ve GC-MS (Gaz kromatografisi-kütle spektrometresi) ile analiz edildi. *H. lanuginosum*'da 41 bileşen saptandı. Bu türde spathulenol (17.3%), caryophyllene oxide (13.1%), α–pinene (11.7%) ve undecane (6.2%) major bileşenleri tespit edildi. *H. perforatum* taksonunda 40 bileşen tanımlandı. Bu bileşenlerden β–selinene (19.4%), bicyclogermacrene (15.3%), 2 tetradecene (8.2%) ve α–amorphene (8.1%) en çok bulunan bileşenlerdir.

Anahtar Kelimeler
Clusicaceae, *Hypericum lanuginosum* var. *lanuginosum*, *Hypericum perforatum*, spathulenol, β–selinene.

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INTRODUCTION

The genus *Hypericum* belongs to the Clusicaceae and encompasses 460 species worldwide of which ca. 89 species are found in Turkey. *Hypericum lanuginosum* var. *lanuginosum* belongs to the Section Adenosepalum Spach and *Hypericum perforatum* to the Section *Hypericum* Robson in Flora of Turkey. Both taxa are black glands present on anters and intramarginally on leaves; seeds reticulate or foveolate to ribbed or rugulose. *Hypericum lanuginosum* var. *lanuginosum* has ovate to lanceolate or oblong and densely whitish-pubescent to scabrellous leaves. *Hypericum perforatum* has leaves narrowly ovate or lanceolate to elliptic-oblong or linear, sessile or subsessile, plane, and large pellucid dots. *Hypericum lanuginosum* var. *lanuginosum* differs from *H. perforatum* in having sepals broadly ovate to oblong or rarely lanceolate and usually obtuse to rounded [1].

Hypericum species have been reported to contain many bioactive compounds, namely naphthodianthrones, phloroglucinols, flavonoids, phenylpropanes, essential oils, amino acids, xanthones, tannins, procyanidins and other components, which possess a wide array of biological properties [2-4]. Major/high component of the *Hypericum* essential oils are 1-hexanal, α–pinene, pinene, 3-methylnonane, 3-methyldecane, caryophyllene oxide, spathulenol, germacrene D, camphor, limonene and *trans*-caryophyllene [5-9]. Variations in the essential oils composition of many species of this genus were previously reported, and depending on genetic and environmental factors, seasonal variation, plant organs and analytical methods used [10-12].

The flowering plant genus *Hypericum* (Hypericaceae) contains the well-known medicinally valuable species *Hypericum perforatum* (common St. John's wort). Species of *Hypericum* contain many bioactive constituents, including proanthocyanins, flavonoids, biflavonoids, xanthones, phenylpropanes and naphthodianthrones that are characterized by their relative hydrophilicity, as well as acylphloroglucinols and essential oil components that are more hydrophobic in nature [13].

In the present study, we report on the yield and chemical composition of the essential oil isolated from *Hypericum lanuginosum* var. *lanuginosum* and *H. perforatum* plants obtained from aerial parts. Although the composition of *H. perforatum* oil has been the subject of previous studies [14-16], to the best of our knowledge, there is no publication on the composition of *H. lanuginosum* var. *lanuginosum* essential oil.

The aims of this study are to determine distribution of the essential oil constituents in genus and compare them with the other *Hypericum* taxon.

MATERIAL AND METHODS

Plant Material

The specimens of *H. lanuginosum* var. *lanuginosum* and *H. perforatum* were collected Gaziantep and Tunceli (Turkey) in 2011. Voucher specimens (FUH-9664 and FUH-9675) are kept at the Fırat University Herbarium (FUH), Elazig, Turkey.

Isolation of the Essential Oils

Air-dried aerial parts of the plant materials (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 *H.*

Gas Chromatographic (GC) Analysis

The essential oil was analysed using HP 6890 GC equipped with FID detector and HP- 5 MS (30 m x 0.25 mm *i.d.*, film tickness 0.25 μm) capillary column was used. The column and analysis conditions were the same as in GC-MS expressed as below. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis

The oils were analyzed by GC-MS, using a Hewlett Packard system. HP- Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Res. Lab. (BUBAL) in Firat University. HP-5 MS column (30 mx0.25 mm *i.d.*, film tickness 0.25 μm) was used with helium as the carrier gas. Injector temperature was 250oC, split flow was 1 ml/min. The GC oven temperature was kept at 70°C for 2 min. and programmed to

Table 1. Constituents of the essential oils from *Hypericum lanuginosum* var. *lanuginosum* (a) and *H. perforatum* (b).

Number	Compounds	RRI	% concentration	
			a	b
1	Octane	971	-	0.1
2	Nonane	996	0.5	-
3	α–pinene	1021	11.7	0.4
4	Verbenene	1037	0.1	-
5	Pinene	1056	0.5	-
6	p-Cymene	1091	0.1	-
7	Limonene	1095	0.1	-
8	2-methyldecane	1121	-	0.1
9	Undecane	1147	6.2	0.7
10	Alloocimene	1167	0.1	-
11	α–Terpineol	1215	0.4	0.2
12	Bicyclo(3,1,1) hept-3-en-2-one	1223	0.3	-
13	Myrtenol	1264	-	0.2
14	α–Cubebene	1360	0.4	0.2
15	Elemene	1370	0.5	1.0
16	Caryophyllene	1393	0.4	0.9
17	Cubebene	1400	0.1	0.2
18	Aromadendrene	1406	1.5	0.7
19	Trans Farnesene	1415	-	1.3
20	α–Humulene	1418	-	0.3
21	Neo alloocimene	1421	0.5	0.2
22	α–Amorphene	1430	1.9	8.1
23	Germacrene D	1435	1.1	3.2
24	β–selinene	1440	0.9	19.4
25	Valencene	1442	-	0.5
26	Bicyclogermacrene	1445	-	15.3
27	α–Muurolene	1446	0.4	-
28	Naphthalene	1456	1.2	1.0
29	Cadinene	1458	-	1.7
30	Cis-Calemenene	1460	-	0.8
31	α–Cadinene	1470	-	0.2
32	Dodecanoic acid	1484	-	1.1
33	Epiglobulol	1486	0.7	-
34	cis-3-Hexenyl benzoate	1490	-	0.4

Table 1. Constituents of the essential oils from *Hypericum lanuginosum* var. *lanuginosum* (a) and *H. perforatum* (b), (continue).

35	Spathulenol	1495	17.3	3.9
36	Caryophyllene oxide	1498	13.1	-
37	Azulene	1500	3.8	0.4
38	Salvial- 4(14)-en-1-one	1505	2.7	-
39	Cyclododecane	1511	0.9	-
40	Ledol	1514	1.3	0.3
41	1H-3a.7-Methanazulene	1518	-	1.3
42	Copaene	1528	-	2.6
43	Bicyclo(4,4,0)dec-1-ene	1532	1.2	1.2
44	Selinene	1534	0.9	-
45	α–Cadinol	1539	4.4	1.8
46	-Methanazulene	1541	-	4.5
47	α–Calacorene	1544	0.5	-
48	Caryophyllene-II	1548	1.3	-
49	2 Tetradecene	1550	-	8.2
50	12-Norcyercene-B	1558	-	1.4
51	Ledene oxide	1574	0.3	-
52	6-Isopropenyl-4	1576	-	0.6
53	α–Cyperone	1586	-	0.2
54	Benzilbenzoate	1596	0.4	0.3
55	2- Pentadecanone	1631	1.2	0.3
56	1,2-Benzenedicarboxylic acid	1639	0.7	-
57	Cyclotetradecane	1650	-	0.5
58	Pentacosane	1671	1.4	-
59	Longipinocarvone	1678	0.3	-
60	n-Hexadecanoic acid	1691	0.2	-
61	Tricosane	1903	0.1	-
	Total		81.6	85.6

150°C at a rate of 10°C/min and then kept constant at 150°C for 15 min to 240°C at a rate of 5°C/min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). MS were taken at 70 eV and a mass range of 35-425. Component identification was carried out using spectrometric electronic libraries (WILEY, NIST).

RESULTS AND DISCUSSION

The compositions of the oils isolated from two species of *Hypericum* are reproduced in Table 1. The oils were complex mixtures of non-terpenes, monoterpenes and sesquiterpenes: 62 components were identified in two essential oils under study. Both oils were characterised by a high content of sesquiterpenes. The essential oil analysis showed that sesquiterpenes concentrations were higher than those of monoterpenes. Similarly essential oil compositions of five *Hypericum* species (*Hypericum caprifoliatum* Cham. & Schlecht., *Hypericum*

ternum A. St. Hil., *Hypericum carinatum* Griseb., *Hypericum* polyanthemum Klotzsch ex Reichardt and *Hypericum myrianthum* Cham. & Schlecht.) from southern Brazil showed that sesquiterpenes are present in higher concentrations [17].

A total of forty components of *H. lanuginosum* var. *lanuginosum* were identified, representing 81.6% of the total oil. It is observed that spathulenol (17.3%), caryophyllene oxide (13.1%), α–pinene (11.7%) and undecane (6.2%) are the most predominant of the forty compounds. The first major compound of *H. lanuginosum* var. *lanuginosum* is spathulenol, which is a major constituent of many *Hypericum* species like, *H. perforatum*, *H. maculatum* and *H.* olypicum [18], *H. carinatum* [17], *H.* capitatum var. capitatum and var. *luteum* [8], *H. thymbrifolium* and *H. pseudolaeve* [6], *H. avicularifolium* subsp. *depilatum* var. *depilatum* [9].

Forty components of *H. perforatum* were identified, representing 85.6% of the total oil. The most abundant constituents were β–selinene (19.4%), bicyclogermacrene (15.3%), 2 tetradecene (8.2%) and α–amorphene (8.1%). Comparison of the composition of the *H. perforatum* essential oil with literature data on the essential oil of other *H. perforatum* origins showed that the first major component was α–pinene, [14-16] except in one report from Yugoslavia, where the main constituent was (E)-caryophyllene (14.2%) [18,19]. Recent studies have pointed out caryophyllene and caryophyllene oxide to be the principal constituents of *H. perforatum* essential oil collected in South-East France [20] and Serbia [19]. Therefore, a large variability in the essential oil composition of *H. perforatum* due to the origin of plant material has to be considered.

Spathulenol, α–amorphene, germacerene D, naphthalene, bicyclo(4,4,0)dec-lene and α–cadinol were found as main constituents in both oils. The major components caryophyllene oxide, caryophyllene-II, salvial-4(14)-en-1-one and pentacosane, determined in the essential oils of *H. lanuginosum* var. *lanuginosum*, were not determined in the essential oils of *H. perforatum* (Table 1).

In conclusion, this study demonstrates the occurrence of spathulenol/caryophyllene oxide chemotype of *H. lanuginosum* var. *lanuginosum* and β–selinene/bicyclogermacrene chemotype of *H. perforatum* in Turkey. All studies of this genus, variations in the typical essential oil constituents are related to plant organ, genetic, enviromental and seasonal factors.

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