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# **Original Article**

# Essential Oil Composition of Eight *Hypericum* species (Hypericaceae) from Iran: Part II

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## Abstract

The genus Hypericum is one of the most important medicinal plants that contain 17 species in Iran, three of them are endemics. This paper reports the essential oil composition of eight Hypericum species from Iran. The essential oil analysis of a number of the studied plants has already been reported but their report from Iran may be valuable for scientists. Samples collected from different places between June and August 2010. The composition of the essential oils from Hypericum was investigated on the flower head. Essential oils were obtained by hydrodistillation method and analyzed by GC and GC/MS. The essential oil yield and composition in *H. androsaemum* L.: oil yields (0.17%) and major components were longifolene 19.2%,  $\beta$ gurjunene 16%, and  $\gamma$ -gurjunene 8.4%, in *H. apricum* kar. & kir. oil yields (0.50%), and major components were cis-piperitol acetate 24.3%, p-cymenene 21% α-pinene 8.3%; in *H. armenum* Jaub. & Spach oil yields (0.20%) and major components were  $\gamma$ -cadinene 30.6%, longifolene 10.4%, and E-nerolidol 7.4%; in *H. asperulum* Jaub. & Spach oil yields (0.05%), and major components were  $\alpha$ -muurolol 17.6%, cis-sesquisablenen hydrate 12.5%, and germacrene B 9.8%; in H. hirsutum L. oil yields (0.05%), and major components were germacrene B 29.2%, citronellyl propanoate 7.9%, and  $\gamma$ -gurjunene 7.5%; in *H.linarioides* Bosse oil yields (0.15%), and major components were (E, E)-farnesyl acetate 16.5%, cis-cadinene ether 12.7%, and 1-tridecene 5.7%; and in *H. tetrapterum* Fries oil yields (0.08%), and major components were trans-linalool oxide 22.3%, p-cymenene 6.2% and (E, E)-farnesyl acetate 6%, and in H. vermiculare Boiss. & Hausskn. oil yields (1.74%), and major components were  $\alpha$ -pinene 61%, myrcyne 6% and E- $\beta$ -farnesene 5.3%.

**Key words :** Essential oils, Distillation, *Hypericum androsaemum*; *H. apricum*; *H. armenum*; *H. asperulum*; *H. hirsutum*; *H. linarioides*; *H. tetrapterum*; *H. vermiculare* 

## Introduction

The genus *Hypericum* (Hyperaceae, Hypericoideae) is a perennial plant, belonging to the Hypericaceae family is represented with around 400 species of herbs, widespread in warm-temperate areas throughout the world and well represented in the Mediterranean and the Near East area [1]. Seventeen *Hypericum* species are present in Iran of which 3 are endemic as recorded in the Flora of Iran [2]. (*H. fursei* N. Robson; *H. dogonbadanicum* 

Assadi; *H. asperulum* Jaub. & Spach). *Hypericum* species are generally known locally in Iran with the names "Hofariqun" which Ebn Sina (or Bo Ali Sina) called it [3]. Plants of the genus *Hypericum* have traditionally been used as medicinal plants in various parts of the world. *Hypericum perforatum* L., is the source to one of the most manufactured and used herbal preparations in recent years, especially as a mild antidepressant, and thus is the most studied *Hypericum* species [4]. *Hypericum perforatum* occupies a special position among the

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species of Hypericum. The chemical composition of H. perforatum oil has been the subject of many publications. Hypericum perforatum (St. John's wort) has a wide range of uses such as a dye, flavoring, food, and as a medicine to treat nervous conditions [5-10]. It has also been used in wound healing, the treatment of gastric ulcers, as an antifungal, antiviral agent and for the treatment of several other diseases in Iranian folk medicine as well as in different parts of the world [11]. The content of the oil depends on the origin of the plant. Thus,  $\alpha$ -pinene was the most abundant component of the oil of *H. perforatum* from Turkey (61.7%) [5] and  $\beta$ -caryophyllene of the oil from Uzbekistan (11.7%) [6]. The two monoterpenes ( $\alpha$ - and  $\beta$ pinene) made up to 70% of the leaf essential oil of H. perforatum from India [12]. Gudzic et al. [32] study on essential oil composition and biological activities of Hypericum species influenced by seasonal variation, geographic distribution, phenological cycle and type of the organ in which EO are produced and/or accumulated have also been reported. Based on experimental work carried out in our laboratory we also mention possible biotechnology approaches envisaging EO improvement of some species of the genus [13]. Guedes et al. [29] the essential oil composition of nine taxa from seven sections of Hypericum L. (; H. perforatum subsp. perforatum, H. perforatum subsp. veronense, H. calycinum, H. montanum, H. richeri subsp. richeri, H. hyssopifolium chaix, H. hirsutum L., H. hircinum subsp. majus, and H. tetrapterum Fries) occurring in central Italy (Appennino Umbro-Marchigiano) were analyzed by GC/FID and GC/MS. A total of 186 compounds was identified in the different species and subspecies, accounting for 86.9-92.8% of the total oils. Schwob, study on the oil of Hypericum hyssopifolium ssp. hyssopifolium aerial parts were analyzed, it was found to be rich in sesquiterpenoids and characterized by spathulenol (19.5%) and two alkanols, tetradecanol (10.2%) and dodecanol (9.3%). Furthermore, the oil was screened for its antimicrobial activity against five microbial strains [14]. Toker evaluated the chemical compositions of essential oils obtained from Hypericum hyssopifolium var. microcalycinum and Hypericum lysimachioides var. lysimachioides using GC and GC-MS. Caryophyllene oxide was found to be the major component. The essential oils of both Hypericum species showed antimicrobial activity against nine microorganisms at a concentration of 60 to 80

 $\mu$ g/ml [15]. Smelcerovic et al. [10] studied on the essential oils of the aerial parts of nine species of Hypericum (Hypericum barbatum, Hypericum hirsutum L., Hypericum linarioides Bosse, Hypericum maculatum, Hypericum olympicum, Hypericum perforatum, Hypericum richeri, Hypericum rumeliacum and Hypericum tetrapterum Fries), collected from different locations in Southeast Serbia. The essential oils investigated were characterized by a high content of non-terpene compounds and a low content of monoterpenes. There were similarities in contents of non-terpenes and sesquiterpenes in oils of species that belong to the section Hypericum (H. maculatum, H. perforatum and H. tetrapterum Fries). The main conclusion from the above data is that genetics and environmental factors both play a role in determining the composition of essential oils of the Hypericum species studied [10]. Sajjadi et al. [11] evaluated the essential oil of Hypericum dogonbadanicum Assadi (Hypericaceae) using GC and GC/MS. The oil contained more than 23 components. The major constituents were found to be  $\alpha$ -pinene (34.7%),  $\beta$ -pinene (32.1%), limonene (12.1%) and camphene (6.6%) [11]. The *H*. perforatum oils from Lithuania have been classified into three chemotypes:  $\beta$ -caryophyllene, caryophyllene oxide and germacrene D [9]. Considerable variation has already been reported in oil composition among different populations of H. perforatum from Serbia [16]. The essential oil content of many other Hypericum species has been described: Hypericum dogonbadanicum [11], *Hypericum triquetrifolium* [17]. Jaimand et al. [12] determined the oil composition of six wild-growing Hypericum species in Iran. Main components obtained in *H. dogonbadanicum* (endemic of Iran) on flower were phenyl ethyl octanoate(29%), terpin-4-ol (20%), and  $\alpha$ -phellandrene (12.9%), and on leaf were  $\beta$ -pinene (54.3%),  $\alpha$ -pinene (12%) and p-cymene (11%), in *H. helianthemoides* on flower were  $\alpha$ -pinene (55.9%), Z- $\beta$ -ocimene (8.7%) and  $\beta$ pinene (7.5%), and in H. hyssopifolium on flower were  $\alpha$ -pinene (49.5%),  $\beta$ -pinene (12.9%) and ntetradecan (5.2%) and on leaf were E-nerolidol (21%), n-tetradecane (15.8%) and  $\alpha$ -himachalene (13.3%), in *H. lysimachioides* on flower were  $\alpha$ pinene (55%), Z-\beta-ocimene (30.7%) and ntetradecane (2.7%), in H. perforatum on flower were E- $\beta$ -farnesene (14.7%), n-hexadecanal (9.1%) and E-nerolidol (7.8%), and in H. triquetrifolium on flower were n-tetradecane (21.3%),  $\alpha$ -himachalene (14.2%) and  $\alpha$ -pinene (10.7%), and on leaf were  $\alpha$ -himachalen (27%), n-tetradecane (25.7%) and n-pentadecane (7%) [18]. The aim of this paper was to determine the oil composition of eight wild-growing *Hypericum* species in Iran.

## Methods

#### Plant Name

*H. androsaemum* L.; *H. apricum* kar. & kir.; *H. armenum* Jaub. & Spach ; *H. asperulum* Jaub. & Spach ; *H. hirsutum* L.; *H.linarioides* Bosse ; *H. tetrapterum* Fries ; *H. vermiculare* Boiss. & Hausskn.

#### Source

Flowering aerial parts were collected from different parts of Iran between mid of May up to early July 2010. All samples were collected by M. Golipoor and identification of the plants was determined by V. Mozaffrian and R. Azadi in Iranian Botanical Garden (IBG).

*H. androsaemum* L. grows wild in north of Iran, Gilan: Siahkal to Dilaman, Alt. 800 m, in 11 July 2010. The specimen is deposited in the Central Herbarium of Iran (TARI) 98967.

*H. apricum* Kar. & Kir. grows wild in west of Iran, East Azarbaijan: Tabriz to Ahar, 15 km to Ahar, Alt. 1600 m, 22 June 2010,. The specimen is deposited in the Central Herbarium of Iran (TARI) 98960. *H. armenum* Jaub. & Spach grows wild in central of Iran, Semnan: Hpco, Alt. 2275 m, 12 July 2010,. The specimen is deposited in the Central Herbarium of Iran (TARI) 98965.

*H. asperulum* Jaub. & Spach grows wild in west of Iran, Sanandaj: High Abidar upper of Noreh village, Alt. 2500 m, 27 June 2010. The specimen is deposited in the Central Herbarium of Iran (TARI) 98959.

*H. hirsutum* L. grows wild in North West of Iran, Arasbaran: Kaliber to Klaleh, Alt. 1720 m, 22 June 2010,. The specimen is deposited in the Central Herbarium of Iran (TARI) 98961.

*H. linarioides* Bosse grows wild in North West of Iran, Arasbaran: Alt. 1760 m, 22 June 2010. The specimen is deposited in the Central Herbarium of Iran (TARI) 98962.

*H. tetrapterum* Fries grows wild in north of Iran, Nooshar: beside of Khir rode, Alt. 180 m, 10 August 2010,. The specimen is deposited in the Central Herbarium of Iran (TARI) 98963. *H. vermiculare* Boiss. & Hausskn. grows wild in west of Iran, Sanandaj: between Bostam and Doo Ab Marivan Road to Baneh, Alt. 1620 m, 19 July 2010,. The specimen is deposited in the Central Herbarium of Iran (TARI) 98964.

## Plant Part

About 35 g flowers and leaves of Hypericum were air-dried and subjected species to hydrodistillation for 2 hours using a Clevenger type apparatus. The oils were separated from the water by decantation and were dried by filtration over sodium sulfate. Oil anhydrous yield for H. androsaemum L. (0.17%), H. apricum kar. & kir. (0.50%), H. armenum Jaub. & Spach (0.20%), H. asperulum Jaub. & Spach (0.05%), H. hirsutum Н. L. (0.05%), linarioides Bosse (0.15%), Fries (0.08%), H. vermiculare H. tetrapterum Boiss. & Hausskn. (1.74%).

#### Gas Chromatography

GC analyses were performed using a Shimadzu-9A gas chromaph equipped with a flame ionization detector, and quantitation was carried out on Euro Chrom 2000 from Knauer by the area normalization method neglecting response factors. The analysis was carried out using a DB-5 fused-silica column (30m x 0.25 mm, film thickness 0.25  $\mu$ m, J & W Scientific Inc., Rancho Cordova, CA, USA). The operating conditions were as follows: injector and detector temperature, 250 °C and 265 °C, respectively; carrier gas, Helium. The oven temperature program was 40-250 °C at the rate of 4 °C/min.

Gas Chromatography - Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used . The column was same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V.

The identity of the oil components was established from their GC retention indices, relative to  $C_{7}$ - $C_{25}$ n-alkanes, by comparison of their MS spectra with those reported in the literature [19-21], and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

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Compound	R.T.	H. androsaemum	H. apricum	H. armenum	H. asperulum	H. hirsutum	H. linarioides	H. tetrapterum	H. vermiculare
2-heptanone	882	-	-	5.1	-	-	-	-	-
<i>n</i> -nonane	897	-	1.0	-	-	-	-	-	0.8
α-pinene	937	0.5	8.3	-	-	-	-	-	61.0
β-pinene	973	-	0.7	-	-	-	-	-	-
6- methyle-5-heptanone	982	3.1	1.4	-	-	-	-	-	6.0
3-octanone	991	-	-	-	-	-	-	-	2.1
$\alpha$ -phellandrene	1001	-	0.7	-	-	-	-	-	-
p-cymene	1020	-	0.4	-	-	-	-	-	-
1,8-cineole	1027	5.3	0.9	-	-	-	-	-	3.2
(Z)-β-ocimene	1035	-	0.5	-	-	-	-	2.2	0.5
trans-linalool oxide	1071	-	-	-	-	-	-	22.3	-
Dihydro myrcenol	1076	-	-	5.8	-	-	-	-	-
Terpinolene	1084	-	-	-	-	-	-	4.8	-
p-cymenene	1093	6.1	21.0	-	-	-	1.0	6.2	0.5
<i>n</i> -undecane	1100	-	0.6	-	-	-	-	4.7	-
trans-thujone	1119	-	0.7	-	-	-	-	-	-
Neo-allo-ocimene	1141	-	-	-	-	-	-	3.6	-
<i>n</i> -dodecane	1200	-	-	-	-	0.8	-	-	-
cis-myrtanol	1248	-	-	-	1.2	-	-	-	-
trans-carvone oxide	1274	-	0.5	-	0.8	1.0	-	-	-
γ-terpinen-7-al	1287	-	-	-	-	0.8	-	1.3	-
1-tridecene	1291	-	3.3	-	7.6	5.9	5.7	0.8	-
<i>n</i> -tridecane	1300	-	0.5	-	0.8	-	-	-	-
cis-piperitol acetate	1335	-	24.3	-	-	0.7	2.8	-	4.0
trans-piperitol acetate	1347	-	0.5	-	-	-	-	-	-
α-terpnyl acetate	1351	0.5	2.9	0.7	-	1.2	-	-	0.4
Nervl acetate	1361	0.7	-	-	2.7	-	1.1	5.0	-
α- copaene	1372	0.6	3.8	-	-	0.7	-	-	-
<i>n</i> -tetradecane	1400	-	-	-	-	1.6	2.4	2.7	-
Longifolene	1408	19.2	1.5	10.4	-	-	-	5.4	2.7
β-guriunene	1420	16.0	1.4	-	-	-	-	0.9	0.8
β-humulene	1438	-	0.5	0.8	-	-	-	-	-
Citronellyl propanoate	1446	4.0	2.2	4.0	-	7.9	3.7	-	0.4
$\alpha$ -himachalene	1451	6.6	4.8	-	-	-	1.5	4.0	5.3
Allo-aramadendrene	1460	_	_	-	-	-	1.0	_	1.1
α-acoradiene	1466	2.2	-	1.4	-	0.5	0.9	1.4	0.5
v-guriunene	1475	8.4	1.5	4.0	-	7.5	1.8	0.9	2.0
a-cyclogeraniol acetate	1482	3.7	0.8	1.7	_	-	13	-	17
B- himachalene	1489	16	-	-	_	_	27	22	-
n-pentadecane	1500	3.6	- 28	- 1.4	0.6	-	13	0.8	2 1

*n*-eicosane

Methyl linoleate

Laurenan-2-one

E-phytol acetate

*n*-henicosane

Grandiflorene

1-docosene

*n*-docosane

*n*-tricosane

Total %

4-epi-abietol

#### Table 1 (continue)

Table 1 (continue)									
γ-cadinene	1519	5.3	-	30.6	-	-	-	-	-
Methyl dodecanoate	1523	4.7	-	-	1.0	0.6	-	-	-
(Z)- nerolidol	1536	0.8	-	-	12.5	1.3	-	-	-
cis-sesquisabinene	1545	-	-	-	-	4.5	-	-	-
cis-cadinene ether hydrate	1552	-	-	-	1.0	1.1	12.7	-	-
Germacrene B	1555	2.1	5.5	6.0	9.8	29.2	1.4	2.9	2.4
E-nerolidol	1564	-	-	7.4	-	1.3	3.2	0.7	-
Spathulenol	1577	0.4	-	0.4	1.6	1.8	2.0	2.0	-
Viridiflorol	1591	-	-	1.6	-	-	-	-	-
Humulene epoxide II	1604	-	-	-	-	-	1.1	-	-
β-cedrene epoxide	1621	-	-	-	1.4	-	-	-	-
Citronellyl pentanoate	1625	-	-	0.6	4.3	1.7	-	-	-
α-muurolol	1645	-	-	2.8	17.6	6.5	3.0	0.8	0.6
α-cadinol	1657	-	-	-	4.0	-	1.5	-	-
Dihydro-eudesmol	1663	-	1.0	1.3	-	5.4	2.7	1.1	-
Acorenone	1676	0.5	-	-	-	-	4.5	-	-
Longiborneol	1683	0.6	0.5	5.3	0.9	2.2	-	2.5	-
Caryophyllene acetate	1694	0.8	-	-	-	1.5	0.9	-	-
<i>n</i> -heptadecane	1700	1.1	-	0.5	-	-	1.9	-	-
Longifolol	1720	-	-	-	-	-	0.9	-	-
Curcumenol	1733	-	-	0.5	-	0.6	-	-	-
(E,Z)-farnesol	1748	0.7	-	-	-	0.8	-	-	-
α-sinesal	1756	-	-	1.0	-	0.6	-	2.0	-
γ-eudesmol acetate	1773	-	-	-	-	-	-	0.5	-
Z-nuciferol acetate	1833	-	-	0.8	2.7	-	-	0.5	-
(E,E)-farensyl acetate	1839	-	-	-	3.4	3.8	16.5	6.0	-
Phenyl ethyl octanoate	1855	-	-	0.4	0.7	-	-	0.5	-
<i>n</i> -hexadecanol	1873	-	-	-	0.6	-	-	2.0	-
<i>n</i> -nonadecane	1900	-	0.5	-	2.0	1.9	3.5	2.1	-
Nootkatin	1950	-	-	0.9	3.0	1.4	3.5	1.0	-
Occidol acetate	1961	-	-	-	-	-	-	2.5	-

0.7

3.5

2.5

5.2

0.6

-

-

-

2.4

0.8

-

-

-

-

-

-

-

-

-

0.5

1.4

1.6

-

-

-

-

1.0

1.2

4.1

0.7

1.4

-

3.2

-

-

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-

1.9

1.7

-

-

-

-

-

 ${}^{a}R.I. =$  retention indices on DB5 column

2000

2097

2106

2117

2175

2184

2200

2224

2300

2338

-

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## **Results and discussion**

Interest in essential oils has revived in recent decades, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in carrier oil and used in massage or burned as incense. About 300 essential oils out of 3000 known are commercially important mainly for their flavors and fragrances [21].

In the present study, eight Hypericum species were collected from different parts of Iran between mid of May up to early July 2010, with native Iran and subjected distributions in to hydrodistillation and analyzed for their volatile constituents by GC/MS. Their compositions are given in Table 1. Several major volatile compounds (representing >10% of the total amount isolated) were identified in these samples in amounts ranging from 11.2-31.5%. Some compounds, such as  $\alpha$ pinene, β-pinene, undecane, β-caryophyllene, and caryophyllene oxide, have been previously reported as major volatile constituents of other Hypericum species [22-25].

In the current study, main components obtained in species viz. each Hypericum the essential oil yields and compositions were as follows. In the species H. androsaemum L., oil yield was 0.17% and major components were longifolene (19.2%),  $\beta$ -gurjunene (16%), and  $\gamma$ -gurjunene (8.4%); in H. apricum Kar. & Kir., oil yield was 0.50% and major components were cis-piperitol acetate (24.3%), p-cymenene (21%),  $\alpha$ -pinene (8.3%); in H. armenum oil yield was 0.20% and major components were  $\gamma$ -cadinene (30.6%), longifolene (10.4%), and E-nerolidol (7.4%); in H. asperulum oil yield was 0.05% and major components were a-muurolol (17.6%), cissesquisabienen hydrate (12.5%), and germacrene B (9.8%); in H. hirsutum L. oil yield was 0.05% and major components were germacrene B (29.2%), citronellyl propanoate (7.9%), and  $\gamma$ -gurjunene (7.5%); in H. linarioides oil yield was 0.15% and major components were (E,E)-farnesyl acetate (16.5%), *cis*-cadinene ether (12.7%), and 1tridecene (5.7%); in *H. tetrapterum* oil yield was 0.08% and major components were translinalool oxide (22.3%), p-cymenene (6.2%) and (E,E)-farnesvl acetate (6%); and in H. vermiculare oil yield was 1.74% and major components were  $\alpha$ -pinene (61%), myrcyne (6%) and E- $\beta$ -farmesene (5.3%). Our work on H. androsaemum L. with same species from Iran by Saroglou et al. [30] showed different components viz. caryophyllene oxide (35.8%), ishwarane (30.5%), humulene epoxide II (5.6%),  $\beta$ -guaiene (40.2%), caryophyllene oxide (28.0%), khusinol (4.2%), and also in comparison with same species from Portugal by Guedes et al. [27] main components were (E)-caryophyllene (9.4-15.1%), γ-elemene (8.0-17.9%), β-gurjunene (6.1-15.5%) and again by Guedes et al. [28] main components were (E)-caryophyllene (9.0- 17.0%),  $\gamma$ -elemene (9.3-17.3%),  $\beta$ -gurjunene (7.9-14.8%), and also by Nogueira et al. [26] main components were C<sub>15</sub>H<sub>24</sub> (27.6%), germacrene D (12.3%),  $\beta$ -caryophyllene (14.0%),. The different results obtained can be related to condition of cultivation, time of collection and essential oil extraction methods. In H. hirsutum L. varied results were reported by two different authors from Serbia. Saroglou et al. [30] reported that main components were nonane (24.8%), undecane (13.3%), (-)-(*E*)-caryophyllene (5.4%) and Gudžic et al. [31] reported main components as *n*-undecane (32.2%), patchoulene (11.8%), caryophyllene oxide (9.3%), which is not in concurrence with our results. In H. linarioides Bosse., also results of Cakir et al. [32] from Turkey with main components δ-cadinene (6.9%),  $\gamma$ muurolene (5.5%), (Z)- $\beta$ -farnesene (5.2%), were different from our results. In H. tetrapterum Fries from Greece Pavlović et al. [33] reported that main were  $\alpha$ -copaene (11.3%), components αlongipinene (9.7%), caryophyllene oxide (8.9%), which are not similar with our results.

Overall, our results on chemical composition of *Hypericum* species from Iran were completely different from the other reports; it could be suggested that the essential oils content and composition can be greatly affected by several parameters including season [28], phenological cycle [34] and geographic distribution.

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