



Original Article

Chemical Composition of the Essential Oils of Six *Hypericum* Species (Hypericaceae) from Iran

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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 six *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 between June and August 2007. The composition of the essential oils from *Hypericum* was investigated on flower and leaf. Essential oils were obtained by hydrodistillation method and then were analyzed by GC and GC/MS. Main components obtained in *H. dogonbadanicum* (endemic of Iran) on flower were phenyl ethyl octanoate (29.0%), terpin-4-ol (20.0%), and α -phellandrene (12.9%), and on leaf were β -pinene (54.3%), α -pinene (12.0%) and p-cymene (11.0%), in *H. helianthemoides* on flower were α -pinene (55.9%), Z- β -ocimene (8.7%) and β -pinene (7.5%), and in *H. hyssopifolium* on flower were α -pinene (49.5%), β -pinene (12.9%) and n-tetradecane (5.2%) and on leaf were E-nerolidol (21.0%), n-tetradecane (15.8%) and α -himachalene (13.3%), in *H. lysimachioides* on flower were α -pinene (55.0%), Z- β -ocimene (30.7%) and n-tetradecane (2.7%), in *H. perforatum* on flower were E- β -farnesene (14.7%), n-hexadecanal (9.1%) and E-nerolidol (7.8%), and in *H. triquetrifolium* on flower were n-tetradecane (21.3%), α -himachalene (14.2%) and α -pinene (10.7%), and on leaf were α -himachalene (27%), n-tetradecane (25.7%) and n-pentadecane (7.0%).

Key words: *H. dogonbadanicum*, *H. helianthemoides*, *H. hyssopifolium* subsp. *Elongatum*, *H. lysimachioides*, *H. perforatum*, *H. triquetrifolium*, Hypericin, Essential oils, Chemical composition, GC, GC/MS.

Introduction

The genus *Hypericum* (Guttiferae, 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 Iranica. *Hypericum* species are generally known locally in Iran with the names "Hofariqun" which Ebn Sina (or Bo Ali Sina) called it [2]. 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 [3].

Hypericum perforatum occupies a special position among the 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 [4-9]. 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 [10]. The content of the oil depends on the origin of the plant. Thus, α -pinene was the most abundant component of the oil of *H. perforatum* from Turkey (61.7%) [4] and β -caryophyllene of the oil from Uzbekistan (11.7%) [5]. Two monoterpenes (α - and β -pinene) made up to 70% of the leaf essential oil of *H. perforatum* from India [11]. Gudžic, study on essential oil composition and

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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 [12]. Maggi, et al. in 2010, the essential oil composition of nine taxa from seven sections of *Hypericum* L. (Guttiferae; *H. perforatum* subsp. *perforatum*, *H. perforatum* subsp. *veronense*, *H. calycinum*, *H. montanum*, *H. richeri* subsp. *richeri*, *H. hyssopifolium*, *H. hirsutum*, *H. hircinum* subsp. *majus*, and *H. tetrapterum*) occurring in central Italy (Appennino Umbro-Marchigiano) was analyzed by GC/FID and GC/MS. A total of 186 compounds were identified in the different species and subspecies, accounting for 86.9–92.8% of the total oils [28]. Schwob, study on the oil of *Hypericum hyssopifolium* ssp. *hyssopifolium* aerial parts was analysed, 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 [13]. Toker, study on the chemical compositions of essential oils obtained from *Hypericum hyssopifolium* var. *microcalycinum* and *Hypericum lysimachioides* var. *lysimachioides* were analysed by 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 µg/ml [14]. Smelcerovic, study on the essential oils of the aerial parts of nine species of *Hypericum* (*Hypericum barbatum*, *Hypericum hirsutum*, *Hypericum linarioides*, *Hypericum maculatum*, *Hypericum olympicum*, *Hypericum perforatum*, *Hypericum richeri*, *Hypericum rumeliacum* and *Hypericum tetrapterum*), collected from different locations in Southeast Serbia, were obtained by steam distillation and analyzed. 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*). The main conclusion from the above data is that genetic and environmental factors both play a role in determining the composition of essential oils of the *Hypericum* species studied [9]. Sajjadi, study on the essential oil of *Hypericum dogonbadanicum* Assadi (Hypericaceae) has been analyzed using GC and GC/MS. The oil contained more than 23 components. The major constituents were found to be α -pinene (34.7%), β -pinene (32.1%), limonene (12.1%) and camphene (6.6%) (10). The *H. perforatum* oils from Lithuania have been classified into three chemotypes: β -caryophyllene, caryophyllene oxide and germacrene D [8]. Considerable variation has already been reported in oil composition among different populations of *H. perforatum* from Serbia [15]. The essential oil content

of many other *Hypericum* species has been described: *Hypericum dogonbadanicum* [10], *Hypericum triquetrifolium* [16]. The aim of this paper was to determine the composition of the oil of six *Hypericum* species wild-growing in Iran.

Materials and Methods

Present work

The oils were analyzed by capillary GC and GC/MS. The compounds identified in the oils are shown in Table 1.

Plant Names

Hypericum dogonbadanicum Assadi; *Hypericum helianthemoides* (Spach) Boiss.; *Hypericum hyssopifolium* Chaix subsp. *elongatum* (Ledeb.) Woron.; *Hypericum lysimachioides* Boiss. & Noe; *H. perforatum* L.; *Hypericum triquetrifolium* Turra.

Source

Flowering aerial parts were collected from different parts of Iran between 16 May up to 9 July 2007. All samples were collected by M. Golipour and identity of the plant was determined by V. Mozaffarian in Iranian Botanical Garden (IBG).

Hypericum dogonbadanicum growing wild in west of Iran, in Kuhgiluyeh and Boyer-Ahmad province: Ca. 10 Km from Gachsaran to Dehdast, between Abrigon and Genave, Alt. 1000 m, in 15 May 2007. The specimen is deposited in Central Herbarium of Iran 88275(TARI).

Hypericum helianthemoides growing wild in center of Iran, in Ghazvin, Takestan to Avaj, Alt. 1550 m, in 7 June 2007. The specimen is deposited in Central Herbarium of Iran 88278(TARI).

Hypericum hyssopifolium subsp. *elongatum* growing wild in west of Iran, Azarbaijan: Marand, Kuhkamar village, Alt. 2100-2700 m; in 21 June 2007. The specimen is deposited in Central Herbarium of Iran 88275 (TARI).

Hypericum lysimachioides growing wild in west of Iran, Kuhgiluyeh and Boyer-Ahmad; Ca. 10 Km from Gachsaran to Dehdast, between Abrigon and Genave, Alt. 1000 m, in 15 May 2007. The specimen is deposited in Central Herbarium of Iran 88276 (TARI).

Hypericum Perferatum growing wild in west of Iran, Kurdistan: Marivan, around Daryache-Zarivar, Alt. 1500 m, in 9 July 2007. The specimen is deposited in Central Herbarium of Iran 88283 (TARI).

Hypericum triquetrifolium growing wild in west of Iran, Kurdistan: Marivan, around Daryache-Zarivar, Alt. 1500 m, in 9 July 2007. The specimen is deposited in Central Herbarium of Iran 88282 (TARI). (See: Holmgren, Index Herbariorum).

Essential oil preparation

About 35 g flowers and leaves of *Hypericum* species were air-dried and subjected 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 anhydrous sodium sulfate.

Table 1 Percentage composition of the oil of six *Hypericum* species from Iran

Compound	^a RI	<i>H. dogonbadanicum</i>		<i>H. hyssopifolium</i>		<i>H. triquetrifolium</i>		<i>H. helianthemoides</i>	<i>H. lysimachoides</i>	<i>H. perforatum</i>
		Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower	Flower	Flower
Tricycline	927	-	-	-	-	10.7	2.4	0.3	-	-
α -pinene	937	7.0	12.7	49.5	-	-	-	55.9	55.0	-
3-methyl-cyclohexane	949	8.3	-	-	-	-	-	0.2	-	-
Thuja-2,4(10)[diene	960	-	-	-	-	8.5	2.9	-	-	-
<i>n</i> -heptanol	966	-	-	-	-	0.6	-	-	-	-
β -pinene	973	-	54.3	12.9	-	0.6	-	7.5	1.7	-
Myrcene	982	2.9	-	2.0	-	-	-	2.7	2.0	-
α -phellandrene	1006	12.9	-	-	-	-	-	0.9	-	-
Hexyl acetate	1010	-	-	-	-	-	-	0.8	-	-
α -terpinene	1015	-	1.8	2.7	0.6	-	-	1.1	0.6	1.4
<i>p</i> -cymene	1020	-	11.0	0.9	-	-	-	6.7	1.0	-
1,8-cineole	1027	-	-	3.6	1.1	-	-	0.6	-	-
(<i>Z</i>)- β -ocimene	1035	-	-	-	-	3.7	0.9	8.7	30.7	-
2-heptyl acetate	1044	-	-	-	-	-	-	2.2	-	-
<i>n</i> -octanol	1066	-	-	-	-	1.1	0.6	0.7	-	0.8
<i>trans</i> -linalool oxide	1071	-	2.6	3.8	0.3	-	-	0.5	0.7	-
Dihydro myrcenol	1076	-	2.3	-	-	-	-	-	-	-
Phenyl ethyl alcohol	1108	-	2.7	-	-	-	-	-	-	-
β -pinene oxide	1160	-	2.7	-	-	-	-	-	-	-
Terpin-4-ol	1177	29.9	100	-	-	-	-	0.3	-	-
Myrtenyl acetate	1321	6.1	-	-	-	-	-	0.3	-	-
<i>cis</i> -piperitol acetate	1332	-	-	0.6	-	0.8	0.6	-	0.5	-
Neryl acetate	1358	-	-	0.7	1.5	1.5	1.3	0.4	-	0.5
Carvacrol acetate	1368	-	-	-	0.8	-	-	0.2	-	-
<i>n</i> -tetradecane	1400	-	-	5.2	15.8	21.3	25.7	3.0	2.7	4.6
Longifolene	1407	-	-	-	-	1.2	1.5	-	-	2.3
β -gurjunene	1427	-	-	2.5	1.9	3.0	3.8	-	-	2.2
Citronellyl propanoate	1447	-	-	0.9	-	3.9	-	0.4	0.5	4.2
α -himachalene	1451	-	-	0.9	13.3	14.2	27.0	3.6	-	-
E- β -farnesene	1457	-	-	-	1.8	-	-	-	-	14.7
Allo-aramadendrene	1460	-	-	1.3	3.0	4.3	3.8	1.4	-	3.2
α -acoradiene	1464	-	-	-	1.7	1.5	1.0	-	-	-
γ -gurjunene	1475	-	-	-	0.9	4.0	3.8	0.3	-	0.8
α -cyclogeraniol acetate	1482	-	-	-	-	7.6	-	0.7	-	-
<i>n</i> -pentadecane	1501	-	-	1.1	2.6	-	7.0	-	-	-
γ -cadinene	1514	-	-	-	-	0.9	0.9	-	-	-
Methyl dodecanoate	1528	4.3	-	-	-	-	-	-	-	-
<i>cis</i> -cadinene ether	1552	-	-	-	-	0.6	1.1	0.3	0.9	1.2
germacrene B	1557	-	-	-	-	-	-	-	-	3.0
E-nerolidol	1564	-	-	-	21.0	-	-	-	-	7.8
Spathulenol	1573	-	-	-	1.5	-	-	-	-	0.9
Viridiflorol	1591	-	-	-	1.5	-	-	-	-	1.5
<i>n</i> -tetradecanal	1612	4.7	-	-	-	1.4	1.6	-	-	-
β -cedrene epoxide	1621	-	-	-	1.9	-	-	-	-	1.6
Citronellyl pentanoate	1625	-	-	-	1.1	1.6	2.1	-	-	-
β -acorenol	1634	-	-	-	3.8	-	-	0.4	0.4	4.6
α -muurolol	1645	-	-	-	1.1	-	-	-	-	-
α -cadinol	1657	-	-	-	-	-	-	-	-	6.6
<i>n</i> -heptadecane	1700	-	-	-	-	-	-	-	0.4	-
Longifolol	1720	-	-	-	1.2	-	-	-	-	-
Curcumenol	1733	-	-	-	-	-	-	-	-	1.8
α -sinesal	1756	-	-	-	-	-	-	-	-	1.5
Z-nuciferol acetate	1833	4.9	-	-	-	-	-	-	0.5	1.3
Phenyl ethyl octanoate	1855	29.0	-	-	-	-	-	-	2.0	0.9
<i>n</i> -hexadecanol	1873	-	-	-	-	-	-	-	-	9.1
Nootkatin	1950	-	-	0.9	7.6	-	-	-	-	5.9
<i>n</i> -octadecanol	2077	-	-	-	-	-	-	-	-	5.8
<i>n</i> -hencosane	2106	-	-	-	-	-	1.0	-	-	-
Laurenan-2-one	2117	-	-	-	1.6	-	-	-	-	-
Grandiflorene	2175	-	-	-	1.2	-	-	-	-	-

^aRI: retention indices on DB-5 column.

Oil yield for *H. dogonbadanicum* Assadi were (flower 0.07% and leaf 0.02%); *H. helianthemoides* (flower 0.2%); *H. hirsutum* L. (flower 0.2% and leaves 0.06%); *H. hyssopifolium* Chaix subsp. *elongatum* (flower 0.3% and leaves 0.2%); *H. lysimachioides* (flower 0.08%); *H. triquetrifolium* (flower 0.2% and leaves 0.1%).

Gas Chromatography

GC analyses were performed using a Shimadzu-9A gas chromatograph 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 (30 m × 0.25 mm, film thickness 0.25 µm, 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. Oven temperature programme 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 C₇-C₂₅ n-alkanes, by comparison of their MS spectra with those reported in the literature [17-19], and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results and Discussion

In the present study, six *Hypericum* species was collected from different parts of Iran between 16 May to 9 July 2007, with native distributions in Iran and subjected to hydrodistillation and analyzed for their volatile constituents by GC/MS. Their compositions are given in Table 1. Several major volatile compounds (representing >10.0% of the total amount isolated) were identified in these samples in amounts ranging from 11.2%-31.5%. Some compounds, such as α-pinene, β-pinene, undecane, β-caryophyllene, and caryophyllene oxide, have been previously reported as major volatile constituents of other *Hypericum* species [20-23].

In the current study, Main components obtained in *H. dogonbadanicum* (endemic of Iran) on flower were phenyl ethyl octanoate(29.0%), terpin-4-ol (20.0%),

and α-phellandrene (12.9%), and on leaf were β-pinene (54.3%), α-pinene (12.0%) and p-cymene (11.0%), Javidnia, et al. 2008, worked on *H. dogonbadanicum*, the major constituents were α-pinene (34.7%), β-pinene (32.1%), limonene (12.1%) and camphene (6.6%) [24], in *H. helianthemoides* on flower were α-pinene (55.9%), Z-β-ocimene (8.7%) and β-pinene (7.5%), and in *H. hyssopifolium* on flower were α-pinene (49.5%), β-pinene (12.9%) and n-tetradecane (5.2%) and on leaf were E-nerolidol (21.0%), n-tetradecane (15.8%) and α-himachalene(13.3%), Schwob,et al. 2006, in *H. hyssopifolium* was found to be rich in sesquiterpenoids and characterized by spathulenol (19.5%) and two alkanols, tetradecanol (10.2%) and dodecanol (9.3%) [13], and also Cakir, et al. 2004, the composition of the hydrodistilled essential oils obtained from the aerial parts of *Hypericum hyssopifolium* subsp. *elongatum* var. *elongatum*, and 66 compounds were determined in total [25]. The oils showed remarkable differences in chemical composition. The oil of *H. hyssopifolium*, which is rich in monoterpenes, consists primarily of α-pinene (57.3%), β-pinene (9.0%), limonene (6.2%) and α-phellandrene (4.4%), Cakir,et al. 2004, in *H. lysimachioides* the composition of oil in flower were α-pinene (55.0%), Z-β-ocimene (30.7%) and n-tetradecane (2.7%), Zuhail,et al. 2006, the chemical compositions of essential oils obtained from *Hypericum hyssopifolium* var. *microcalycinum* and *Hypericum lysimachioides* var. *lysimachioides* were caryophyllene oxide which was found to be the major component [26], in *H. perforatum* the constituents of the oil from flower were E-β-farnesene (14.7%), n-hexadecanal (9.1%) and E-nerolidol (7.8%), and in *H. triquetrifolium* the constituents of flower were n-tetradecane (21.3%), α-himachalene (14.2%) and α-pinene (10.7%), and on leaf were α-himachalene (27.0%), n-tetradecane (25.7%) and n-pentadecane (7%), the flower and leaf oil of *H. triquetrifolium* from Calabria (Italy) were studied by Alessandra, et al. 2003, the major components identified in each oil were n-nonane (8.0%, 15.0%), β-pinene (8.0%, 4.0%), α-pinene (13.0%, 10.0%), myrcene (16.0%, 5.0%), β-caryophyllene (5.0%, 11.0%), germacrene-D (10.0%, 13.0%), sabinene (13.0%, 3.0%) and caryophyllene oxide (5.0%, 12.0%) in the leaf and flower oils, respectively [27].

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