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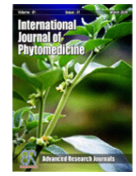

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Volatile constituents of *Hypericum asperulum* Jaub. & Spach aerial parts from Iran

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Abstract

The essential oil composition of flowering aerial parts of Iranian endemic *Hypericum asperulum* Jaub. & Spach (Hypericaceae) was analyzed for the first time by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Forty five compounds representing 97.8% of the total oil were identified. The main components were γ-Muurolene (13.1%), -Pinene (12.2%), Germacrene D (11.3%), β-Caryophyllene (9.8%) and Spathulenol (7.2%). The volatile oil of *Hypericum asperulum* Jaub. & Spach flowering aerial parts was characterized by high content of sesquiterpene hydrocarbons (57.4%).

Keywords: *Hypericum asperulum* Jaub. & Spach, Hypericaceae, Essential oil, γ-Muurolene, -Pinene

Introduction

Hypericum is one of nine genera forming the family Hypericaceae.[1] This genus consists of over 460 species which occurs throughout the world and is well represented in the Mediterranean region.[2] The genus *Hypericum* has been classified into 36 taxonomic sections.[3]

Plants of *Hypericum* genus have found numerous ethnopharmacological uses. The extracts, as well as the essential oils of *Hypericum* species have been shown to possess significant antiviral,[4] antimicrobial,[5,6] antifungal, [7,8] antidepressant,[9] wound healing,[10] antioxidant, [11] anxiolytic, [12] anti-inflammatory, [13] antitumoral [14] and anticonvulsant activities.[15]

Essential oil compositions of many species of this genus were previously informed which depend on genetic, geographic distribution, seasonal variation, phenological cycle, plant organ and analytical method used. Volatile constituents that have been most frequently reported from *Hypericum* species include monoterpenoid and sesquiterpenoid compounds as well as alkanes and aldehydes as the main components in the most of them.[16-50]

Seventeen species of the genus *Hypericum* are found in Iran which among them *Hypericum asperulum* Jaub. & Spach, *Hypericum fursei* N. Robson and *Hypericum dogonbadanicum* Assadi are endemic.[51, 52]

The literature survey revealed that there was no report on essential oil composition of *Hypericum asperulum* Jaub. & Spach and this article is the first study on this endemic species.

Materials & Methods

Plant Material

Flowering aerial parts of *Hypericum asperulum* Jaub. & Spach were collected in May 2012 from Arghavan Valley (Ilam Province, Iran). A voucher specimen has been deposited at the herbarium of Payame Noor University of Ilam, Iran.

Essential Oil Isolation

The air-dried crushed flowering aerial parts of *Hypericum asperulum* Jaub. & Spach were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hrs. The obtained volatile oil was dried over anhydrous sodium sulphate and stored at 4-6 C.

Essential Oil Analyses

Chemical composition of the oil was investigated by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

Hypericum asperulum Jaub. & Spach volatile oil was analyzed by GC–MS using a Hewlett-Packard 6890 gas chromatograph with DB-5 capillary column (30 m x 0.25 mm; film thickness 0.25 μm). The carrier gas was helium with a flow rate of l ml/min. The column temperature was programmed from 60ºC to 220ºC at 6ºC/min. The gas chromatograph was coupled to a Hewlett-Packard 5973 mass selective detector. The MS was operated at 70 eV ionization energy. The retention indices were calculated by using retention times of *n*-alkanes that were injected after the essential oil at the same conditions. The components were identified by comparison of retention indices with those reported in the literatures and also by comparison of their mass spectra with the published mass spectra or Wiley library.[53, 54]

Gas chromatography using flame ionization detection (GC–FID) analysis was carried out under the same experimental conditions with the same column as described for the GC–MS. The relative percentage of the identified compounds was computed from the GC peak area without applying correction factors.

Results and Discussion

The dried flowering aerial parts of *Hypericum asperulum* Jaub. & Spach yielded 0.15% V/W of a yellow volatile oil.

Forty five components comprising 97.8% of the total oil were identified. The detected compounds and their percentage are presented in Table 1.

Essential oil of *Hypericum asperulum* Jaub. & Spach flowering aerial parts was characterized by high content of sesquiterpene hydrocarbons (57.4%) with γ-Muurolene (13.1%), Germacrene D (11.3%) and β-Caryophyllene (9.8%) as main constituents, other ingredients with considerable quantities were -Cadinene (3.9%), -Muurolene (3.8%) and Bicyclogermacrene(3.3%). Monoterpene hydrocarbons represented 23.2% of the total volatile oil which dominated by -Pinene (12.2%), another compound with appreciable amount was β-Pinene (3.7%). *Hypericum asperulum* Jaub. & Spach aerial parts oil contained 13.9% oxygenated sesquiterpenes. Spathulenol (7.2%) and Caryophyllene oxide (3.7%) constituted the principle components of this fraction. Oxygenated monoterpenes amounted to only 2.1%.

A search through the literature showed that volatile compositions of different species of *Hypericum* genus have been extensively studied in numerous regions from all over the world. Table 2 shows the origin, plant organ and major components of the essential oil from *Hypericum* species which have been previously reported.[16-50] In general, this genus species have high amounts of sesquiterpenoids such as β-Caryophyllene, Germacrene D, Caryophyllene oxide and Spathulenol in their essential oils. Likewise, -Pinene and β-Pinene were the two mainly represented monoterpenes in hypericum species volatile oils. The yield and composition of *Hypericum* essential oils depend upon the organ type and plant origin.

Despite the various researches on *hypericum* species oils, there was no report on *Hypericum asperulum* Jaub. & Spach and in this investigation volatile constituents of this endemic species is studied for the first time.

Table 1. Essential oil composition of *Hypericum asperulum* Jaub. & Spach aerial parts

No.	Compound	RI[a]	Content (%)
1	*n*-Nonane	900	0.7
2	-Pinene	937	12.2
3	Camphene	953	1.1
4	β-Pinene	980	3.7
5	Myrcene	991	0.9
6	*n*-Decane	999	0.1
7	-Terpinene	1019	0.2
8	*p*-Cymene	1026	0.8
9	Limonene	1031	2.8
10	(*Z*)-β-Ocimene	1038	0.3
11	(*E*)-β-Ocimene	1049	0.9
12	γ-Terpinene	1061	0.3
13	*n*-Undecane	1099	0.1
14	Linalool	1102	0.7
15	-Terpineol	1190	1.4
16	-Cubebene	1352	Tr.[b]
17	-Copaene	1376	0.4
18	-Gurjunene	1409	0.8
19	β-Caryophyllene	1419	9.8
20	β-Copaene	1430	0.1
21	-Guaiene	1439	0.5
22	-Humulene	1454	1.2
23	(*E*)-β-Farnesene	1458	0.9
24	*n*-Dodecanol	1472	0.2
25	γ-Muurolene	1477	13.1
26	-Amorphene	1479	0.6
27	Germacrene D	1482	11.3
28	β-Selinene	1486	2.4
29	-Selinene	1494	0.2
30	Bicyclogermacrene	1497	3.3
31	-Muurolene	1500	3.8
32	(*Z*)- -Bisabolene	1505	0.1
33	Germacrene A	1509	2.9
34	γ-Cadinene	1514	1.9
35	-Cadinene	1524	3.9
36	-Cadinene	1538	0.2
37	(*E*)-Nerolidol	1563	0.8
38	Spathulenol	1577	7.2
39	Caryophyllene oxide	1583	3.7
40	Tetradecanal	1612	Tr.
41	β-Eudesmol	1650	0.7
42	-Cadinol	1655	1.5
43	Tetradecanol	1671	0.1
44	*n*-Heptadecane	1700	Tr.
45	*n*-Octadecane	1800	Tr.

[a] Retention indices; relative to *n*-alkane series on DB-5 capillary column.

[b] trace (<0.05%).

Table 2. Reported main volatile constituents of various *Hypericum* species

Species	Origin	Plant organ	Major components (%)	Reference
H. acmosepalum N. Robson	China	flowering aerial parts	β-Selinene (16.3), *ar*-Curcumene (12.6), Caryophyllene oxide (9.0), γ-Muurolene (8.7)	[16]
H. adenotrichum Spach	Turkey	aerial parts	Germacrene D (37.7), Undecane (16.5)	[17]
H. aegypticum L. subsp. *aegypticum*	Libya	flowering aerial parts	Ishwarane (14.4), Eudesm-11-en-4-ol stereoisomer (10.7), Eudesm-11-en-4-ol stereoisomer (9.6)	[18]
H. aegypticum L. subsp. *marrocanum* (Pau) N. Robson	Northwestern Africa	flowering aerial parts	Caryophyllene oxide (29.2), β-Caryophyllene (15.1), Caryophylladienol-II (9.7)	[18]
H. alpinum Waldst. & Kit.	Serbia	flowering aerial parts	β-Pinene (13.3), γ-Terpinene (7.7), (*E*)-Caryophyllene (6.5), Caryophyllene oxide (4.8), -Cadinene (4.3)	[19]
H. androsaemum L.	Portugal	aerial parts	$C_{15}H_{24}$ (27.6), β-Caryophyllene (14.0), Germacrene D (12.3)	[20]
H. androsaemum L.	Portugal	Leaves	(*E*)-Caryophyllene (9.0-17.0), γ-Elemene (9.3-17.3), β-Gurjunene (7.9-14.8)	[21]
H. androsaemum L.	Iran	Leaves	Caryophyllene oxide (35.8), Ishwarane (30.5), Humulene epoxide II (5.6)	[22]
H. androsaemum L.	Iran	Flowers	-Guaiene (40.2), Caryophyllene oxide (28.0)	[22]
H. balearicum L.	Balearic Islands	flowering aerial parts	-Pinene (28.5), β-Pinene (20.4), β-Eudesmol (11.2)	[18]
H. barbatum Jacq.	Serbia	flowering aerial parts	(-)- -Pinene (17.1), (-)-β-Pinene (17.0), Caryophyllene oxide (12.2), β-Caryophyllene (8.0), (-)-Limonene (6.0)	[19]
H. beanii N. Robson	China	flowering aerial parts	Caryophyllene oxide (18.7), β-Selinene (16.3), γ-Muurolene (11.3)	[16]
H. brasiliense Choisy	Brazil	whole plant	β-Caryophyllene (29.5), -Humulene (12.7), Caryophyllene oxide (9.9)	[23]
H. bupleuroides Griseb.	Turkey	aerial parts	β-Sesquiphellandrene (33.2), β-Caryophyllene (20.2), Selina-3,7(11)-diene (7.0)	[24]
H. calycinum L.	Turkey	aerial parts	-Pinene (24.1), β-Pinene (14.2)	[17]
H. calycinum L.	China	flowering aerial parts	β-Pinene (29.2), -Terpineol (11.5)	[16]
H. caprifoliatum Cham & Schlecht	Brazil	flowering aerial parts	*n*-Nonane (55.8), β-Caryophyllene (5.9), *n*-Undecane (5.0)	[25]
H. carinatum Griseb.	Brazil	flowering aerial parts	β-Caryophyllene (21.0), -*trans*-Bergamotene (10.0), Caryophyllene oxide (9.5), *n*-nonane (9.0)	[25]
H. cerastoides (Spach) Robson	Turkey	aerial parts	-Pinene (57.7), Undecane (5.0)	[17]
H. choisyanum Wall. ex N. Robson	China	flowering aerial parts	*cis*-Eudesma-6,11-diene (11.4), Allo-Aromadendrene (8.1), γ-Muurolene (7.8)	[16]
H. connatum Lam.	Brazil	flowering aerial parts	Caryophyllene oxide (40.1), β-Caryophyllene (13.1), Humulene epoxide II (10.5)	[25]
H. coris L.	France	aerial parts	-Curcumene (40.1), γ-Cadinene (14.7), -Cadinene (6.6)	[26]
H. delphicum Boiss. & Heldr.	Arabian Peninsula	flowering aerial parts	Caryophyllene oxide (31.5), β-Caryophyllene (18.2), *n*-Undecane (17.5)	[18]
H. dogonbadanicum Assadi	Iran	flowering aerial parts	-Pinene (34.7), β-Pinene (32.1), Limonene (12.1), Camphene (6.6)	[27]
H. dogonbadanicum Assadi	Iran	aerial parts	-Pinene (12.8), Limonene (8.2), β-Pinene (4.7) Camphene (3.9)	[28]
H. foliosum Aiton	Azorean Islands	aerial parts	*n*-Nonane (28.7-72.6), Limonene (6.9 - 45.8), Terpinolene (0.5-18.8)	[29]
H. forrestii (Chitt.) N. Robson	China	flowering aerial parts	Caryophyllene oxide (12.7), -Pinene (10.4)	[16]
H. helianthemoides (Spach) Boiss.	Iran	aerial parts	β-Caryophyllene (23.3), Spathulenol (17.4)	[28]
H. heterophyllum Vent.	Turkey	flowering aerial parts	Isocaryophyllene (17.1), -Pinene (11.6), -Cadinene, (9.5), γ-Muurolene (8.2)	[7]
H. 'Hidcote'	Italy	flowering aerial parts	β-Pinene (11.9), -Humulene (7.4), β-Caryophyllene (6.5), -Selinene (5.4)	[30]
H. hirsutum L.	Serbia	flowering aerial parts	Nonane (24.8), Undecane (13.3), (*E*)-Caryophyllene (5.4)	[19]
H. hirsutum L.	Serbia	aerial parts	*n*-Undecane (32.2), Patchoulene(11.8), Caryophyllene oxide (9.3)	[31]
H. hirtellum Boiss.	Iran	aerial parts	β-Caryophyllene (14.1), Spathulenol (12.3)	[28]
H. humifusum L.	Portugal	aerial parts	-Pinene (44.7-77.2), β-Pinene (4.7-7.7), β-Caryophyllene (1.2-9.3), Germacrene D (1.9-6.1)	[32]
H. hyssopifolium Chaix	Turkey	flowering aerial parts	-Pinene (57.3), β-Pinene (9.0), Limonene (6.2)	[7]
H. hyssopifolium Chaix subsp.	France	aerial parts	Spathulenol (19.5), Tetradecanol (10.2), Dodecanol (9.3),	[33]

Hyssopifolium			β-Caryophyllene (8.4), γ-Muurolene (8.0)	
H. kouytchense H. Lév.	China	flowering aerial parts	γ-Muurolene (12.4), *cis*-β-Guaiene (10.7), Caryophyllene oxide (9.0)	[16]
H. lancasteri N. Robson	China	flowering aerial parts	β-Selinene (11.4), Eudesmadienone (10.8), γ-Muurolene (8.9)	[16]
H. leschenaultii Choisy	China	flowering aerial parts	Cuparene (24.8), γ-Muurolene (16.8), *ar*-Curcumene (10.0)	[16]
H. linarifolium Vahl	Portugal	aerial parts	-Pinene (19.9-31.2), β-Pinene (5.0-11.0), β-Caryophyllene (6.6-11.6), Germacrene D (4-7)	[32]
H. linarioides Bosse	Turkey	flowering aerial parts	-Cadinene (6.9), γ-Muurolene (5.5), (*Z*)-β-Farnesene (5.2), Spathulenol (5)	[34]
H. lysimachioides var. *lysimachioides* Boiss. & Noë	Turkey	aerial parts	Caryophyllene oxide (30.8), β-Selinene (6.7), -Longifolene (6.4)	[35]
H. maculatum Crantz	Serbia	flowering aerial parts	β-Farnesene (10.0), *n*-Undecane (8.2), β-Caryphyllene (7.6)	[5]
H. monogynum L.	China	flowering aerial parts	Tricosane (13.3), Myrcene (10.4)	[16]
H. montbretii Spach	Turkey	aerial parts	-Pinene (25.0), β-Pinene (18.8)	[17]
H. myrianthum Cham. & Schltdl.	Brazil	flowering aerial parts	Undecane (20.7), Nonane(17.5), Dehydro-aromadendrene (8.6)	[25]
H. olympicum L.	Serbia	aerial parts	(*E*)-Anethole (30.7), β-Farnesene (12.4), -Cadinene (8.7)	[36]
H. olympicum L.	Greece	flowering aerial parts	Germacrene D (16.0), (*E*)-Caryophyllene (7.4), Spathulenol (6.7)	[37]
H. patulum Thunb.	China	flowering aerial parts	β-Selinene (14.7), *ar*-Curcumene (8.0), Spathulenol (6.7)	[16]
H. perfoliatum L.	Greece	aerial parts	-Pinene (48.6), *n*-Nonane (8.5), -Cadinene (4.6)	[38]
H. perfoliatum L.	Greece	aerial parts	-Pinene (34.2), β-Pinene (9.2), -Cadinene (8.1)	[38]
H. perfoliatum L.	Algeria	aerial parts	Thymol (22.1), -Cadinol (18.5), 4,5-Dimethyl-2-ethylphenol (13.0)	[39]
H. perfoliatum L.	Portugal	aerial parts	-Pinene (39.4-64.3), *n*-Nonane (11.9-23.8), β-Pinene(1.9-3.2)	[32]
H. perforatum L.	India	Leaves	-Pinene (67.3), β-Caryophyllene (5.2)	[40]
H. perforatum L.	Turkey	flowering aerial parts	-Pinene (61.7), 3-Carene (7.5), β-Caryophyllene (5.5)	[41]
H. perforatum var. *perforatum* L.	Serbia	aerial parts	1-Tetradecanol (5.08-23.75), *cis*-β-Caryophyllene (0.64-19.23), 10-Methyl-1-undecene (0-14.66)	[42]
H. perforatum L.	Portugal	aerial parts	-Pinene (23.6-2.1), Germacrene D (5.1-13.4), β-Caryophyllene (3.7-10.0)	[43]
H. perforatum L.	Turkey	flowering aerial parts	-Pinene (50.3), Carvacrol (22.0)	[17]
H. perforatum L.	Serbia	aerial parts	β-Caryophyllene (14.2), 2-Methyl-octane (13.1), 2-Methyl-decane (7.9)	[36]
H. perforatum L.	Uzbekistan	aerial parts	β-Caryophyllene (11.7), Caryophyllene oxide (6.3), Spathulenol (6.0)	[44]
H. perforatum L. var. *angustifolium* DC.	Italy	aerial parts	2-Methyl-octane (21.1), Germacrene D (17.6), -Pinene (15.8)	[45]
H. perforatum L.	Serbia	aerial parts	Nonane (63.8), *p*-Cymene (4.8), 3-Methylnonane (4.5)	[46]
H. perforatum L.	Greece	aerial parts	-Pinene (21.0), 2-Methyl-octane (12.6), γ-Muurolene (6.9)	[37]
H. perforatum L.	Serbia	flowering aerial parts	-Pinene (8.6), Germacrene D (6.8), (*Z*)-β-Farnesene (6.6)	[19]
H. polyanthemum Klotzsch ex Reichardt	Brazil	flowering aerial parts	Benzopyran HP_1 (26.7), Benzopyran HP_2 (13.2), Undecane (7.9)	[25]
H. pseudohenryi N. Robson	China	flowering aerial parts	β-Selinene (18.5)	[16]
H. pulchrum L.	Portugal	aerial parts	-Pinene (49.8), β-Pinene (12.5), Germacrene D (5.4)	[32]
H. richeri Vill.	Italy	flowering aerial parts	(*Z*)-β-Ocimene (19.5), *n*-Nonane (13.8), β-Bisabolene (8.7)	[47]
H. roeperanum Schimp. ex A. Rich.	East Africa	flowering aerial parts	γ-Curcumene (15.6), (2*E*,6*E*)-Farnesol (7.8), *ar*-Curcumene (7.7)	[18]
H. scabrum L.	Turkey	flowering aerial parts	-Pinene (71.6), β-Caryophyllene (4.8), Myrcene (3.8)	[41]
H. scabrum L.	Uzbekistan	aerial parts	-Pinene (11.2), Spathulenol (7.2), *p*-Cymene (6.1)	[44]
H. scabrum L.	Iran	flowering aerial parts	-Pinene (45.3), *n*-Nonane (5.6), Thymol (5.3)	[48]
H. scabrum L.	Iran	aerial parts	-Pinene (59.3), β-Pinene (4.1), Limonene (2.1)	[28]
H. ternum A. St. Hil.	Brazil	flowering aerial parts	β-Caryophyllene (12.0), Bicyclogermacrene (10.0), β-Cadinene (5.0)	[25]
H. tetrapterum Fries	Greece	flowering aerial parts	-Copaene (11.3), -Longipinene (9.7), Caryophyllene oxide (8.9)	[37]
H. thymopsis Boiss.	Turkey	aerial parts	Spathulenol (10.8), -Cadinene (7.1), Germacrene D (6.1), γ-Muurolene (5.9)	[49]
H. tomentosum L.	Portugal	aerial parts	β-Caryophyllene (12.6), *n*-Undecane (7.5), -Humulene (5.2)	[20]
H. triquetrifolium Turra	Italy	Leaves	Myrcene (16.4), -Pinene (13.3), Sabinene (13)	[50]
H. triquetrifolium Turra	Italy	Flowers	Nonane (14.7), Germacrene D (12.7)	[50]
H. x moserianum auct.	China	flowering aerial parts	γ-Muurolene (10.7), -Cadinene (10.2)	[16]

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