Essential Oil Comparison of *Hypericum perforatum* L. subsp. *perforatum* and subsp. *veronense* (Schrank) Ces. from Central Italy

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Abstract

Essential oil comparison of flowers from *Hypericum perforatum* L. subsp. *perforatum* and subsp. *veronense* (Schrank) Ces. from central Italy, performed by GC and GC/MS, led us to identify two chemotypes within *H. perforatum* taxon. α-Pinene and 2,6-dimethyloctane were the most abundant components in *H. perforatum* subsp. *veronense* flower oil, while β-caryophyllene and 2,6-dimethylheptane were predominant in the oil of *H. perforatum* subsp. *perforatum*. Monoterpenes hydrocarbons were also predominant in the oil of subsp. *veronense*, while sesquiterpenes and aliphatic hydrocarbons were found in the oil of subsp. *perforatum*. Such quantitative differentiation in the oil composition of the flowers may be useful as a chemotaxonomic intraspecific discriminatory character.

Key Word Index

Hypericum perforatum subsp. *perforatum*, *Hypericum perforatum* subsp. *veronense*, Hypericaceae, essential oil composition, α-pinene, 2,6-dimethyloctane, β-caryophyllene, 2,6-dimethylheptane.

Plant Name

Hypericum perforatum L. subsp. *perforatum*, *H. perforatum* subsp. *veronense* (Schrank) Ces. (syn. *H. perforatum* subsp. *angustifolium* (DC.) Gaudin), section Hypericum (1), Hypericaceae family (syn. Guttiferae).

Source

Appennino Umbro-Marchigiano (central Italy): *H. perforatum* subsp. *perforatum* was collected at Pian Grande (1260 m, near Norcia, Perugia district), in fat pastures on doline, while *H. perforatum* subsp. *veronense* was collected at Paganico (670 m, near Camerino, Macerata district), in dry uncultivated fields, both during their flowering periods, in June–July 2005. Voucher specimens were identified and deposited in the Herbarium Camerinensis (CAME, Dept. of Environmental Sciences, Sect. of Botany and Ecology), of the University of Camerino, under the accession number CAME 7987 and CAME 7981.

Plant Part

Fresh flowers were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus, in accordance with the method of the Italian Pharmacopoeia (2), to afford light yellow oils that were dried over anhydrous sodium sulfate and stored in sealed vials under refrigeration before GC and GC/MS analyses. The oil yield was 0.1% for the subsp. *perforatum* and 0.4% for the subsp. *veronense*.

Present Work

GC analysis of the oil was carried out using a Varian 3300 instrument equipped with an HP-InnoWax column (30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 3 min at 60°C, and subsequently at 4°C/min up to 210°C (for 10 min); injector and detector temperatures, 250°C; carrier gas, He (1 mL/min); injection volume of 1 μL, split ratio, 1:10. GC/MS analysis was performed using a Hewlett Packard 5890 GC/MS system operating in the EI mode at 70 eV, using two different columns: an HP InnoWax (30 m x 0.25 mm ID, film thickness 0.17 μm) capillary column, and a HP-5 (30 m x 0.25 mm, film thickness 0.25 μm) capillary column. The temperature program for HP InnoWax was 60–250°C at a rate of 4°C/min, and for the HP 5 was 60–300°C at a rate of 4°C/min. Injector and transfer line temperatures were 250°C and 280°C, respectively. Split ratio, 1:10. Helium was used as the

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Table I. Chemical composition (%) of the flower oils of *Hypericum perforatum* subsp. *veronense* and subsp. *perforatum* from central Italy

Components	RI*	*H. perforatum* subsp. *veronense*	*H. perforatum* subsp. *perforatum*
octane	800	0.1	-
2,6-dimethylheptane	834	3.8	18.2
2-methyloctane	882	16.9	2.8
nonane	900	2.4	1.5
2-methyl-4-heptanone	992	0.6	2.3
α-pinene	1039	35.6	2.0
α-fenchene	1063	0.3	0.4
camphene	1082	1.3	1.8
2-methyldecane	1112	0.5	0.7
β-pinene	1125	0.9	2.2
sabinene	1135	4.4	tr
myrcene	1168	0.4	0.3
limonene	1207	0.5	0.1
1,8-cineole	1224	tr	tr
(Z)-β-ocimene	1240	-	0.5
γ-terpinene	1251	0.5	0.4
(E)-β-ocimene	1259	-	1.2
p-cymene	1275	0.5	0.6
2,3,5-trimethyldecane	1360	tr	tr
α-longipinene	1466	0.3	-
α-ylangene	1491	0.7	-
α-campholenal	1498	0.1	-
α-copaene	1515	0.2	-
camphor	1518	0.2	-
β-bourbonene	1530	3.5	-
linalool	1555	0.3	0.5
pinocarvone	1580	0.2	-
β-funebrene	1588	0.3	-
β-caryophyllene	1615	0.9	24.4
terpinen-4-ol	1631	0.4	0.4
ethyl benzoate	1647	0.2	tr
myrtenal	1648	0.2	1.2
α-himachalene	1649	0.5	4.5
trans-pinocarveol	1676	0.5	7.1
(E)-β-farnesene	1680	1.1	-
allo-aromadendrene	1683	1.2	0.8
α-humulene	1692	tr	tr
β-bisabolene	1714	1.7	1.1
α-terpineol	1718	1.3	0.6
γ-muurolene	1725	0.5	tr
verbenone	1733	0.3	-
valencene	1751	0.2	-
α-muurolene	1753	0.9	1.4
γ-cadinene	1770	0.3	tr
myrtenol	1794	0.2	-
cis-calamenene	1797	tr	-
p-cymen-8-ol	1846	tr	-
cis-carveol	1867	tr	0.1
α-calacorene	1928	-	0.1
(Z)-nerolidol	1961	0.4	0.7
caryophyllene oxide	1967	3.6	9.6
aristolene epoxide	1969	0.4	-
humulene epoxide-I	1972	0.4	0.5
(Z)-3-hexenyl benzoate	1983	0.3	tr
(E)-nerolidol	2044	0.3	-
viridiflorol	2069	0.5	tr
globulol	2104	0.3	tr
spathulenol	2153	0.2	1.1
T-cadinol	2155	1.3	1.7
carvacrol	2159	0.4	-
epi-α-muurulol	2171	1.4	1.5

Table I. Continued

Components	RI*	*H. perforatum* subsp. *veronense*	*H. perforatum* subsp. *perforatum*
thymol	2176	0.3	tr
α-cadinol	2224	0.3	0.4
(Z,E)-farnesol	2250	1.2	1.5
decanoic acid	2287	0.2	0.7
hexahydrofarnesyl acetone	2310	0.2	-
hexadecanol	2380	1.6	0.6
dodecanoic acid	2502	0.5	1.3
hexacosane	2600	0.3	0.5
(E)-phytol	2622	-	0,7
tetradecanoic acid	2640	-	0.5
hexadecanoic acid	2911	tr	0.6
Total (%)		**99.0**	**99.1**
Yield (% v/dry wt)		0.4	0.1
Class of constituents			
Monoterpenes		48.9	19.7
Sesquiterpenes		22.6	25.4
Aliphatic hydrocarbons		24.6	26.2

tr = traces, < 0.1%; * Retention indices, relative to n-alkane series on a HP-InnoWax column.

carrier gas, at a flow rate of 1 mL/min. The identification of the components was made for both the columns, by comparison of their retention time with respect to n-paraffin (C_6-C_{22}) internal standards. The mass spectra and retention indices (RI) were compared with those of commercial (NIST 98 and WILEY) and home-made library mass spectra built up from pure compounds and MS literature data (3–8). Area percentages were obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

The GC and the GC/MS analysis allowed the identification of 72 components (67 in the subsp. *veronense*, 53 in the subsp. *perforatum*), that are listed in Table I, following the elution order from an HP InnoWax column. The main component in the flower oil of subsp. *veronense* was α-pinene (35.6%) followed by the 2-methyloctane (16.9%). β-Caryophyllene (24.4%) was the principal constituent in flower oil of subsp. *perforatum*, followed by the 2,6-dimethylheptane (18.2%). Some noteworthy minor components were: 2-methyl-4-heptanone, β-pinene, sabinene, limonene, myrcene, p-cymene, α-himachalene, *trans*-pinocarveol, allo-aromadendrene, β-bisabolene, α-terpineol, α-muurolene, caryophyllene oxide, T-cadinol, spathulenol, α-cadinol. The monoterpenoid α-pinene was one of the main constituents in the oil of the *veronense* subspecies, while the sesquiterpenoid β-caryophyllene was the major component in the oil of *perforatum* subspecies, which lacks other sesquiterpenes like α-ylangene, α-copaene, β-bourbonene and α-gurjunene that were present in the *veronense* subspecies oil. Our data support those present in literature (9–12), however most of the studies about *H. perforatum* oil did not discriminate between the two subspecies. The observed differences in the flower oils, more quantitative than qualitative, mainly depending on genetic factors, are potentially of chemotaxonomic importance and may constitute a new discriminant intraspecific character within *H. perforatum* taxon.

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