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Composition of the essential oils of *Hypericum perforatum* L. from southeastern France

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Abstract – The composition of the volatile oils from the aerial parts of *Hypericum perforatum* L. collected in six localities from southeastern France was analysed by GC–MS. Twenty-nine to 41 compounds have been identified in these volatile oils. The main constituents were sesquiterpene hydrocarbons, and minor variations were pointed out in the oil composition among the six populations. However, the composition of all the analysed oils greatly varied from that of the previous studies, carried out on *H. perforatum* essential oils from other localities, in which monoterpenoids were the major constituents, particularly, the α-pinene. ***To cite this article: I. Schwob et al., C. R. Biologies 325 (2002) 781–785.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

***Hypericum perforatum* / Guttiferae / essential oil / sesquiterpene hydrocarbons**

Résumé – Composition des huiles essentielles de *Hypericum perforatum* L. du Sud-Est de la France. La composition des huiles essentielles des parties aériennes de *Hypericum perforatum* L., récoltées dans six stations du Sud-Est de la France, a été analysée par CG–SM. De 29 à 41 composés ont été identifiés dans ces huiles essentielles. Les composés majoritaires sont des sesquiterpènes. Une variabilité réduite de la composition des huiles obtenues pour les six populations a pu être mise en évidence. Cependant, la composition de l'ensemble de ces huiles est très différente de celles, précédemment publiées dans la littérature, de spécimens de *H. perforatum* provenant d'autres localités et très riches en monoterpènes, notamment en α-pinène. ***Pour citer cet article : I. Schwob et al., C. R. Biologies 325 (2002) 781–785.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

***Hypericum perforatum* / Guttifère / huile essentielle / sesquiterpènes**

Version abrégée

Plus de 300 espèces du genre *Hypericum* sont représentées dans les zones chaudes et tempérées du globe, mais seulement 17 espèces sont présentes en France. Parmi ces espèces, le millepertuis, *Hypericum perforatum*, est le plus connu, étant utilisé depuis longtemps dans la pharmacopée traditionnelle. Cette plante pérenne des habitats perturbés croît actuellement sur les cinq continents.

Cette espèce a récemment fait l'objet de nombreuses études sur ses constituants chimiques à forte activité biologique. Sa composition en constituants lourds, tels que l'hypéricine, a été très étudiée, mais les composés volatils ont été le sujet d'un nombre restreint de travaux. La présente étude concerne les particularités

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des huiles essentielles de cette espèce dans le Sud-Est de la France. Des populations récoltées dans six stations distinctes ont été étudiées. Les rendements en huile essentielle varient de 0,03 à 0,12 %. De 29 à 41 composés ont pu être identifiés dans les huiles. Dans l'ensemble des huiles analysées, les sesquiterpènes, oxygénés ou non, sont la classe de composés la plus abondante. La proportion de monoterpènes représente, au plus, 5,9 % des huiles, donc une proportion réduite de ces huiles. Une variabilité réduite de la composition de ces huiles a pu être mise en évidence entre les diverses populations provenant des habitats de plaine, variété *perforatum*, c'est-à-dire de Val-d'Arc, de Pertuis, de Saint-Cyr et de Mérindol. Les populations de Bandol, variété *angustifolium*, et de Méailles, habitat en altitude, se distinguent des autres par les plus faibles proportions de sesquiterpènes oxygénés et les plus fortes concentrations de monoterpènes oxygénés. La variété *angustifolium* de Bandol ne contient ni α-, ni β-himachalène et elle est particulièrement pauvre en β-caryophyllène. Une variation de la composition chimique des huiles essentielles de millepertuis en fonction de la variété est donc envisageable. Par ailleurs, pour une même variété, l'altitude peut influencer la composition chimique de l'huile essentielle.

Ces huiles du Sud-Est de la France ont en commun, dans leur ensemble, d'une part, leur richesse en sesquiterpènes et, d'autre part, une quasi-absence d'α- et de β-pinènes. Ces derniers représentent, au plus, 0,3 % de l'huile. Ces huiles se distinguent donc des huiles essentielles de millepertuis d'autres localités, riches en monoterpènes, dont la composition a été précédemment publiée dans la littérature.

1. Introduction

More than 300 species of the genus *Hypericum*, belonging to the Hypericaceae (Guttiferae family), grow in the warm and temperate regions of the Earth, but only 17 species are known to be present in France [1, 2]. Among these species, *Hypericum perforatum* L., also known as St. John's Wort, is traditionally used as a medicinal plant [3]. It is a perennial herb that is often found in disturbed areas. St. John's Wort is native to Europe, West Asia, North Africa, Madeira and the Azores, and is now naturalised in many parts of the world, notably North America and Australia [4].

St. John's Wort has been extensively examined for its biological activities. This species has been found to be effective in treating mild to moderate depression [5], as well as anxiety and insomnia [6]. While *H. perforatum* has been found to contain flavonoids, phloroglucinols, xanthones [6, 7], and biflavonoids [7], the main constituents associated with the biological activity of the plant are the napthodianthrones hypericin and hypericin-like [6, 8, 9]. Works on the biological properties of species from the genus *Hypericum* are currently carried on in our Laboratory. A previous work dealt with hypericin and hypericin-like synthesis potential of *in vitro* shoot cultures of St. John's Wort [10].

Currently studied for the hypericin and hyperforin contents, *H. perforatum* is not the subject of many studies on the essential oil composition. However, volatile compounds in plant chemistry are often valuable in cosmetology and pharmacology. Essential oils of *H. perforatum* have been investigated previously from material collected in France [11–14], Italy [15], India [16], Turkey [17] and, Serbia [18]. Specialised in essential oils of various aromatic plants from South-Eastern France, our laboratory works on chemical markers helpful to study *Hypericum* taxons [19–21]. To reach this purpose, this study was done to examine the composition of volatile oils of *H. perforatum* populations from southeastern France.

2. Materials and methods

2.1. Plant material

The plant material was collected during the summer 2000 in Provence–Alpes–Côte d'Azur (southeastern France), at flowering developmental stage, in different wild populations of *Hypericum perforatum* L. var. *perforatum* and var. *angustifolium* D.C. of various ecological conditions. Distinction between the two different varieties was estimated according to the morphological description in Flora Europaea [22] based on the leaves size. Two localities were along riverside wasteland, namely Val-d'Arc (V.) and Pertuis (P.), one, in a dried basin in Saint-Cyr (S.), two, in 'garrigues': one in Mérindol (M.) and the other in Bandol (B.); the last locality was situated in a mountain meadow in Meailles (Me.). Sampling was done by a randomised collection of 30 individuals in each population.

The voucher specimens were deposited in the Herbarium of the University of Provence, Marseille, France.

2.2. Isolation of the volatile oils

Oil samples were isolated from freshly air-dried and powdered aerial parts by hydrodistillation for 2 h, using a Clevenger-type apparatus. Oil yields were then esti-

Table 1. Essential oil yield.

Locality	*H. perforatum* L. variety	Yield (%, w/w)
Val-d'Arc (V.)	*perforatum*	0.10
Pertuis (P.)	*perforatum*	0.03
St Cyr (S.)	*perforatum*	0.08
Mérindol (M.)	*perforatum*	0.03
Bandol (B.)	*angustifolium* DC.	0.12
Meailles (Me.)	*perforatum*	0.05

mated and the oil composition analysed by GC–MS (Hewlett-Packard, Model 5972, capillary GC–quadrupole MS system (EI, 70 eV) fitted with a 25 m × 0.2 mm i.d. fused silica column coated with DB5). Temperature programme was 3 °C min^{-1} from 60 to 220 °C. Helium was used as carrier gas at a flow of 1 ml min^{-1}.

2.3. Identification of the components

Identification of the components of the volatile oils was based on retention indices [23] and computer matching with the NBS 75K and WILEY 138 libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [24].

3. Results and discussion

The hydrodistillation of the aerial parts gave yellowish oils with a yield (Table 1) from 0.03% (P. and M.) to 0.12% (B.).

Thirty-three, 29, 41, 29, 34 and 39 compounds were identified in the essential oil of *Hypericum perforatum* from V., P., S., M., B., and Me. respectively. The composition of the volatile oils is given in Table 2. Fourteen compounds were present in the essential oil of each tested population, namely β-caryophyllene, caryophyllene oxide, (E)-β-farnesene, γ-cadinene, δ-cadinene, *ar*-curcumene, *cis*-calamenene, branched tetradecanol, spathulenol, nerolidol, α-cadinol, 2-methyldodecane, dodecanol, and one unidentified compound; however, these compounds were present at different rates. For example, the amount of β-caryophyllene was up to 28% in P. oil but was only 0.2% in B. oil.

Germacrene D and bicyclogermacrene were present in great amount in the V., P., S., M. and V., P., M. oils, respectively, but were not identified or poorly represented (0.3%) in the B. and Me. oils. Spathulenol was present in great amount in the B. and Me. oils and poorly represented in the other oils. Germacrene D and bicyclogermacrene, known as fragile molecules, may be converted in other compounds as spathulenol. This difference of composition between these analysed oils may only reveal oxidation processes of the oils.

It is interesting to notice the presence of α- or β-himachalene, rare in plant chemistry, in all the *H. perforatum* var. *perforatum* oils but not in the *angustifolium* variety. The oil of the variety *angustifolium* is poor in farnesene forms, unlike the other oils, which are rich in (E)-β-, (E,E)-α- or (Z,E)-α-farnesene. In the oil of this variety, there is little content of β-caryophyllene and cayophyllene oxide. Hence, a chemical difference between the two varieties may be revealed as demonstrated between *H. perforatum* var. *perforatum* and var. *angustifolium* from Serbia [18].

The populations from B. and Me. localities should be distinguished from the others by both, fewer content in sesquiterpene hydrocarbons but higher content in oxygenated sesquiterpenes, and more oxygenated monoterpenes, than in the other populations.

Little variability in oil composition among the *H. perforatum* var. *perforatum* populations was pointed out in lowland (V., P., S., M.). However changes in oil composition occurred between *perforatum* (V., P., S., M.) and *angustifolium* (B.) varieties, and between the population of lowland (V., P., S., M.) and highland (Me.). But, these variations were minor.

In all the analysed oils, sesquiterpenes hydrocarbons and oxygen-containing sesquiterpenes were the main classes of compounds (Table 3). Monoterpenes, oxygenated or not, represented, at the most, 5.9% of the oil (Me.).

Moreover, compared with previous reports [12–17] on *H. perforatum* essential oils from other localities, which are rich in monoterpenoids, particularly, the α-pinene, the composition of all the oils that we analysed greatly differed (Table 4). It is possible that particular features characterise the essential oils from *H. perforatum* populations of southeastern France. Other research will be done to know whether these particularities could be extended to the composition in hypericin and hypericin-like compounds of the same populations.

Table 2. Constituents of the essential oil of *Hypericum perforatum* L.

Components	RI	Locality					
		Val-d'Arc (V.) (%)	Pertuis (P.) (%)	Saint-Cyr (S.) %	Mérindol (M.) (%)	Bandol (B.) (%)	Meailles (Me.) (%)
α-pinene	936	0.3					
β-pinene	975	0.3					
limonene	1028						0.3
(E)-β-ocimene	1045	0.3		traces			
2-methyldecane	1061	0.5	0.1	0.2			
cis-linalyl oxide	1069						0.1
trans-linalyl oxide	1085						0.1
n-undecane	1098	0.3	0.1	0.2	0.1		0.2
linalol	1099						0.5
campholenal	1123					0.3	0.4
octanol	1134					0.2	0.5
pinocarveneol	1138					0.3	0.7
p-mentha-1,5-dien-8-ol	1167			traces		0.6	0.5
terpinen-4-ol	1173		0.1	traces	0.1		
p-cymen-8-ol	1186					0.3	0.3
α-terpineol	1192	0.2				0.3	1.5
myrtenol	1198						0.4
safranal	1207					0.3	0.3
cis-carveol	1222					0.5	0.6
2-methyldodecane	1266	**0.6**	**0.4**	**0.6**	**0.3**	**4.0**	**1.7**
n-tridecane	1303			traces			0.3
σ-butyl benzoate	1329					0.5	
α-cubebene	1351		0.1	0.7	0.1		
α-longipinene	1355	2.8					
α-copaene	1379	0.4	0.1	0.2		0.9	0.8
β-bourbonene	1385			0.1			
isobutyl isobutyrate	1392			traces	0.1		0.6
β-elemene	1393			0.1			
β-funebrene	1414		0.5	0.1	0.6		
β-caryophyllene	1425	**14.8**	**28.4**	**26.1**	**24.1**	**0.2**	**13.3**
β-copaene	1431	0.4	0.2	0.4	0.1		
isoamyl benzoate	1437					1.7	0.6
aromadendrene	1441	0.3		0.1		0.1	0.2
α-himachalene	1449	0.6	traces	traces	0.1		2.6
α-humulene	1454	0.4	0.5	0.4	0.3		
(*E*)-β-farnesene	1459	**7.1**	**3.0**	**3.6**	**4.1**	**0.9**	**2.4**
dodecanol	1478	**3.8**	**3.0**	**7.5**	**3.6**	**0.4**	**0.8**
γ-muurolene	1480			4.3	1.7	7.7	6.9
ar-curcumene	1484	**13.0**	**2.5**	**0.6**	**2.9**	**1.3**	**0.9**
germacrene D	1486	17.8	37.3	6.3	29.1		
β-selinene	1492		0.7	1.2	6.0		
(*Z*,*E*)-α-farnesene	1493	1.1		1.3			
α-selinene	1499	2.3	0.8	15.5	3.1	0.5	
bicyclogermacrene	1499	5.7	3.8		5.8	0.3	
α-muurolene	1501	0.7	0.4	4.8	0.3		
β-himachalene	1501	0.3					0.5
(*E*,*E*)-α-farnesene	1507	1,0	0.3	1.3	0.7		8.4
γ-cadinene	1513	**2.2**	**3.0**	**1.3**	**0.8**	**2.8**	**1.6**
cis-calamenene	1522	**0.4**	**0.1**	**0.1**	**0.9**	**2.6**	**0.5**
δ-cadinene	1522	**4.9**	**2.7**	**2.9**	**0.9**	**3.2**	**2.1**
α-cadinene	1534	0.2		0.1			
calacorene	1538			traces		0.9	0.4
nerolidol	1559	**0.7**	**0.6**	**0.5**	**0.1**	**6.5**	**1.2**
hexenyl benzoate	1563		0.2	0.2		0.9	0.3
spathulenol	1574	**0.5**	**2.5**	**0.5**	**2.6**	**21.1**	**21.5**
caryophyllene oxide	1577	**0.5**	**2.3**	**1.1**	**2.0**	**4.4**	**18.4**
humulene II oxide	1601			0.2		0.6	0.5
branched tetradecanol	1629	**1.3**	**0.8**	**0.4**	**0.8**	**9.1**	**2.3**
T-cadinol	1631			0.4	0.1	0.5	
α-cadinol	1642	**0.4**	**0.4**	**0.5**	**0.7**	**1.2**	**0.3**
benzyl benzoate*	1738					0.5	
hexahydrofarnesylacetone*	1813					0.7	
Identified components (%)		83	91	84	83	76	75

* Stereochemistry not determined. Traces: < 0.1%

Table 3. Percentage of particular classes of compounds in *H. perforatum* L. essential oil.

Grouped components	Locality					
	Val-d'Arc (V.)	Pertuis (P.)	Saint-Cyr (S.)	Mérindol (M.)	Bandol (B.)	Meailles (Me.)
Monoterpene hydrocarbons	1	0	traces	0	0	0.3
Oxygen-containing monoterpenes	0.2	0.1	traces	0.1	2.6	5.6
Sesquiterpene hydrocarbons	76.4	84.4	71.4	81.7	21.5	40.8
Oxygen-containing sesquiterpenes	3.5	6.6	3.5	6.2	44.1	44
Alkanes	1.4	0.7	1	0.4	4	2.2
Alkanols	3.8	3	7.5	3.6	0.6	1.3
Others	14.7	5.2	16.2	8	27.3	6.1

Table 4. Plant material, origin, main class of component, and main components of the essential oils of *Hypericum perforatum* L. previously reported.

Reference	Plant material	Origin	Main class of component	Main components
[7–9]	aerial parts	northeastern France	alkanes	2-methyl-octane (45%) α-pinene (24%)
[10]	aerial parts	middle France	monoterpene hydrocarbons	α-pinene (15.3%) caryophyllene oxide (10.4%)
[11]	aerial parts	Italy	alkanes	2-methyl-octane (16.4%) α-pinene (10.6%)
[12]	leaves	India	monoterpene hydrocarbons	α-pinene (67.3%) β-caryophyllene (5.2%)
[13]	aerial parts	Turkey	monoterpene hydrocarbons	α-pinene (61.7%) 3-carene (7.5%)
[14]	aerial parts	Serbia	Non-terpene compounds (alkanes, alcohols, esters, and acids)	1-tetradecanol (5.1 to 23.8%) β-caryophyllene (1.1 to 19.8%)

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