CHEMICAL COMPOSITION OF *Hypericum rochelii* GRISEB. & SCHENK HEADSPACE VOLATILES

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The present study reports the chemical composition of *Hypericum rochelii* headspace volatiles, obtained from fresh roots, stems, leaves and fruits by HS-GC-MS technique. The volatiles obtained from fresh roots and fruits are reported for the first time. Forty-seven constituents were identified in total, 28 components in the fruit volatiles, 19 components in the leaf volatiles, 33 components in the stem volatiles, and 31 components in the root volatiles, accounting for 99.8, 97.9, 99.4 and 93.8% of the total volatiles, respectively. The components that were found in significant amounts in the analysed samples were (*E*)-2-hexenal, nonane, α -pinene, β -pinene and undecane.

Keywords: *Hypericum rochelii*, chemical composition, GC-MS, headspace volatiles

Introduction

Hypericum L. (Guttiferae/Clusiaceae/Hypericaceae) is a genus represented by more than 480 species, widespread in warm-temperate areas throughout the world [1]. Both extracts and essential oils of *Hypericum* species have been shown to possess significant antiviral, wound healing, antioxidant and antimicrobial properties [2,3], so the number of studies on *Hypericum* species has increased rapidly over the years.

Eighteen *Hypericum* species inhabit the territory of Serbia [4] with *Hypericum rochelii* as one of them. *Hypericum rochelii* is a perennial herb, 15-50 cm tall, erect to ascending, sometimes rooting, with few stems, unbranched below inflorescence or (very rarely) branched throughout when ascending. Stems are terete, eglandular, not glaucous with triangular-lanceolate or upper triangular-ovate leaves, and inflorescences 3–25-flowered, without lower branches or rarely with one to numerous flowering branches [5].

Up to now, there has been only one study dealing with essential oil composition of *H. rochelii* isolated from fresh aerial parts. [6]. Since the data on the chemical composition of *H. rochelii* volatiles are scarce, the aim of this study was to perform a compositional analysis of the volatiles obtained from fresh roots, stems, leaves and fruits by headspace (HS) technique. Data concerning the volatiles obtained from fresh roots and fruits are reported for the first time.

Experimental

Plant material

Plant material was collected in the fruiting stage from the area of Rtanj Mountain (southeastern Serbia) in June 2018. Voucher specimens were deposited in the Herbarium of the Faculty of Science and Mathematics, University of Niš, under the acquisition number 13859. The fresh plant was divided into parts: root, stem, leaf and fruit.

Headspace volatiles

For the HS experiment, chopped plant material (0.5 g) was put into 20 mL HS vial than soaked with 2 mL of distilled water. The sample was heated at 80 °C for 20 minutes with the following mixing program: shaking for 5 seconds, pausing for 2 seconds.

GC and GC-MS analyses

GC-MS analysis (three repetitions) was performed using an Agilent Technologies 7890B GC equipped with a fused silica capillary column (HP-5MS, 250 $\mu\text{m} \times 25\text{ m}$, film thickness 0.25 μm , Agilent Technologies, USA) and coupled with a 7890A flame ionization detector (FID) and 5977A MS detector of the same brand, recording at 70 eV. Full scan spectra were acquired over the range 35-500 amu (scan time 5 scans/second). The GC-MS was operated under the following conditions: injector temperature 250 °C; GC-MS interface temperature 300 °C; oven temperature programmed from 50° to 200 °C at 4 °C/minute, carrier gas He, 1.0 mL/min, constant flow mode, vacuum outlet (37 cm/second linear velocity); injected volume 500 μL .

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of HS volatiles, split ratio 40:1. The constituents were identified by comparison of their linear retention indices relative to the retention times of C8–C40 *n*-alkanes on the HP-5MS column [7] with literature values [8] and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST11, Agilent Mass Hunter Workstation B.06.00 software [9] and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils, and wherever possible, by co-injection with an authentic sample. GC-FID was carried out under the same conditions as described for the GC-MS. The percentage composition of the oil was computed from the GC peak areas without the use of any correction factors.

Results and discussion

The compounds identified in *H. rocheilii* HS volatiles obtained from fresh roots, stems, leaves and fruits are listed in Table 1. Forty-seven constituents were identified in total, 28 components in the fruit volatiles, 19 components in the leaf volatiles, 33 components in the stem volatiles, and 31 components in the root volatiles, accounting for 99.8, 97.9, 99.4 and 93.8% of the total volatiles, respectively (Table 1). Twelve components out of all were found to be present as mutual volatiles obtained from all plant parts (namely, nonane, α -thujene, α -pinene, sabinene, β -pinene, myrcene, *p*-cymene, limonene, (*Z*)- β -ocimene, (*E*)- β -ocimene, γ -terpinene and undecane). Twenty-six compounds were found in traces (with percentage less than 0.05%) at least in one of the HS samples.

Nonane (45.8%), β -pinene (27.1%), α -pinene (19.1%) and undecane (4.7%) were the most abundant components of the fruit volatiles while the leaf volatiles were represented by (*E*)-2-hexenal (29.2%), β -pinene (22.5%), α -pinene (15.6%), nonane (6.1%) and hexanal (5.9%). The main stem volatiles were α -pinene (35.1%), β -pinene (33.5%), nonane (18.3%) and undecane (4.3%) (Table 1). Headspace volatiles of the root were characterized by undecane (76.1%), β -pinene (7.9%) and decane (4.0%). The fruit and leaf volatiles were almost evenly distributed between mono- (47.9 and 54.3%, respectively) and non-terpenoids (51.5 and 42.0%, respectively). Non-terpenoid fraction of the leaf volatiles was characterized by a large amount of aldehydes (42.0%), namely (*E*)-2-hexenal and hexanal. This compound class (aldehydes) was present in small quantities or was not present at all in HS volatiles of any other plant parts (Table 1). The root volatiles were characterized by a large amount of non-terpenoids (82.5%), mostly due to the main compound - undecane which made up about three quarters of the total percentage composition. In all samples under study, monoterpenoids were dominated by hydrocarbons (fruit, leaf, stem, root – 47.9, 52.1, 72.4 and 10.6% respectively), versus oxygenated components. Also, non-terpenoid compounds consisted mainly of *n*-alkanes except in the case of the leaf volatiles (Table 1).

Apart from our study, there is only one paper dealing

with *H. rocheilii* volatiles [6]. The authors reported *n*-nonane (24.7%), β -pinene (22.4%), germacrene D (7.5%), *n*-undecane (6.8%) and α -pinene (5.8%) as the main constituents of the essential oil composition of *H. rocheilii* isolated from fresh aerial parts. The above-mentioned mono- and non-terpenoids were in agreement with HS samples under study obtained from the corresponding plant parts (Table 1).

Conclusions

A detailed investigation on *H. rocheilii* headspace volatiles obtained from fresh roots, stems, leaves and fruits resulted in the identification of forty-seven constituents. The present findings improved the knowledge about the chemical composition of *H. rocheilii* species which has been insufficiently investigated up to now (some data herein were reported for the first time). Headspace technique proved to be a very fast and simple method for sample preparation (especially when plant material is scarce), and gives acceptable analyses results.

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Table 1. Percentage composition of *H. rochei* HS volatiles

Entry	RI ^{a)}	AI ^{a)}	Component	Fruit	Leaf	Stem	Root	Identification ^{b)}
1.	800	800	Octane	0.9	-	3.1	0.3	RI, MS, Col
2.	803	801	Hexanal	-	5.9	-	-	RI, MS, Col
3.	850	846	(E)-2-Hexenal	-	29.2	0.3	-	RI, MS
4.	900	900	Nonane	45.8	6.1	18.3	0.2	RI, MS, Col
5.	924	924	α -Thujene	0.3	0.4	0.5	0.2	RI, MS
6.	932	932	α -Pinene	19.1	15.6	35.1	1.9	RI, MS, Col
7.	948	946	Camphene	0.1	-	0.2	0.1	RI, MS
8.	970	969	Sabinene	tr	0.5	0.2	tr	RI, MS
9.	975	974	β -Pinene	27.1	22.5	33.5	7.9	RI, MS, Col
10.	990	988	Myrcene	0.5	1.1	0.8	0.1	RI, MS, Col
11.	998	998	Octanal	tr	-	-	-	RI, MS
12.	1000	1000	Decane	0.1	-	0.3	4.0	RI, MS, Col
13.	1005	1002	α -Phellandrene	tr	-	-	-	RI, MS
14.	1015	1014	α -Terpinene	0.1	-	0.1	-	RI, MS
15.	1020	1020	<i>p</i> -Cymene	tr	2.2	0.2	0.1	RI, MS, Col
16.	1025	1024	Limonene	0.6	1.2	0.7	0.1	RI, MS, Col
17.	1026	1026	1,8-Cineole	tr	0.9	-	0.1	RI, MS, Col
18.	1035	1032	(Z)- β -Ocimene	tr	3.2	0.4	tr	RI, MS
19.	1045	1044	(E)- β -Ocimene	tr	2.9	0.4	0.1	RI, MS
20.	1055	1054	γ -Terpinene	0.1	2.5	0.2	0.1	RI, MS
21.	1090	1086	Terpinolene	tr	-	0.1	-	RI, MS
22.	1100	1100	Undecane	4.7	0.8	4.3	76.1	RI, MS, Col
23.	1100	1100	Nonanal	tr	tr	tr	-	RI, MS
24.	1143	1141	Camphor	-	0.6	-	-	RI, MS, Col
25.	1160	1155	Isoborneol	-	0.7	-	-	RI, MS
26.	1190	1186	α -Terpineol	tr	-	-	-	RI, MS
27.	1200	1200	Dodecane	-	-	-	0.4	RI, MS, Col
28.	1201	1201	Decanal	tr	-	tr	-	RI, MS
29.	1300	1300	Tridecane	tr	-	0.1	1.5	RI, MS, Col
30.	1348	1345	α -Cubebene	-	-	tr	tr	RI, MS
31.	1350	1350	α -Longipinene	0.3	-	0.1	0.1	RI, MS
32.	1376	1373	α -Ylangene	-	-	-	0.2	RI, MS
33.	1380	1374	α -Copaene	tr	-	tr	0.3	RI, MS
34.	1390	1387	β -Cubebene	-	-	-	tr	RI, MS
35.	1400	1400	β -Longipinene	tr	-	-	-	RI, MS
36.	1410	1409	α -Gurjunene	-	-	-	tr	RI, MS
37.	1420	1417	(E)-Caryophyllene	0.1	-	0.1	tr	RI, MS, Col
38.	1431	1430	β -Copaene	-	-	tr	-	RI, MS
39.	1480	1475	<i>trans</i> -Cadina-1(6),4-diene	-	-	tr	tr	RI, MS
40.	1482	1478	γ -Murolene	-	-	0.1	tr	RI, MS
41.	1485	1484	Germacrene D	-	1.6	0.1	-	RI, MS
42.	1500	1500	Pentadecane	-	-	-	tr	RI, MS, Col
43.	1502	1495	<i>cis</i> -Cadina-1,4-diene	-	-	tr	tr	RI, MS
44.	1515	1513	γ -Cadinene	-	-	0.1	-	RI, MS
45.	1526	1521	<i>trans</i> -Calamenene	-	-	-	tr	RI, MS
46.	1530	1522	δ -Cadinene	-	-	0.1	tr	RI, MS
47.	1538	1533	<i>trans</i> -Cadina-1,4-diene	-	-	tr	-	RI, MS
Total				99.8	97.9	99.4	93.8	
Monoterpenoids				47.9	54.3	72.4	10.7	
Monoterpene				47.9	52.1	72.4	10.6	
hydrocarbons								
Oxygenated monoterpenes				tr	2.2	-	0.1	
Sesquiterpenoids				0.4	1.6	0.6	0.6	
Sesquiterpene				0.4	1.6	0.6	0.6	
hydrocarbons								
Oxygenated				-	-	-	-	
sesquiterpenes								
Non-terpenoids				51.5	42.0	26.4	82.5	
n-Alkanes				51.5	6.9	26.1	82.5	
Aldehydes				tr	35.1	0.3	-	

a) RI: Experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes C₈-C₂₀ and C₂₁-C₄₀; AI: Literature retention indices [8].

b) Identification method: RI, retention indices matching with literature data; MS, mass spectra matching with literature data; Col, coinjection with an authentic sample.

tr: Trace (< 0.05%).

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Izvod

HEMIJSKI SASTAV "HEADSPACE" ISPARLJIVIH KOMPONENTI BILJNE VRSTE *Hypericum rochelii* GRISEB. & SCHENK

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U ovom radu objavljen je hemijski sastav "headspace" isparljivih komponenti izolovanih iz svežeg korena, stabla, lista i ploda biljne vrste *Hypericum rochelii*, tehnikom "headspace"-gasna hromatografija-masena spektrometrija (HS-GC-MS). "Headspace" isparljive komponente izolovane iz korena i ploda objavljene su po prvi put. Identifikovano je ukupno 47 komponenti, od toga, 28 u plodu, 19 u listu, 33 u stablu i 31 u korenu, što iznosi 99,8%, 97,9%, 99,4% i 93,8% od ukupnih isparljivih komponenti. Komponente, identifikovane u značajnom procentu u svim analiziranim uzorcima su (*E*)-2-heksenal, nonan, α -pinen, β -pinen i undekan.

Ključne reči: *Hypericum rochelii*, hemijski sastav, GC-MS, "headspace" isparljiva jedinjenja