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Traceability of Volatile Organic Compounds from *Hypericum perforatum* in Fresh and Dried Form and in Essential Oil

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Abstract. *Microextraction in solid phase from headspace and GC-MS analysis was the method of studying the flowers of Hypericum perforatum in fresh and dry form.The essential oil obtained by hydrodistillation was also analyzed by GC-MS. Identification of the substances was made by comparison of mass spectra with NIST library and standard alkanes were used for the calculation of the linear retention index. The identified compounds were grouped into classes of substances: monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes, non-terpene hydrocarbons. The main common constituents were as follows: caryophyllene, β-ocimene, α-pinene, β-pinene, octane-2-methyl. The abundance of the majority compounds, common to the three forms of the plant: essential oil,fresh, and dried plants, was compared.*

Keywords:SPME, essential oil, Hypericum perforatum, volatile organic compounds.*

1.Introduction

Hypericum. Perforatum, as described by botanists [1], is a perennial flowering herb belonging to the Clusiaceae (Mangosteen family;alternatively, Hypericaceae and Guttiferae). The genus *Hypericum* consists of approximately 400 species of herbs and shrubs having yellow or coppery flowers with four to five petals, numerous stamens, and a single pistil.*H. perforatum* consist of freely branching shrubby herbs that typically range from 40 to 80 cm in height.

Hyperycum perforatum has traditional value as a medicinal plant. It has a long history of use as an herbal treatment for a variety of ailments. *H. perforatum* oil present antimicrobial [2,3] and antioxidant properties [4]. Over the past 20 years, it has become a mainstream alternative treatment for depression, as well as holding promise as a therapy for cancer, inflammation, bacterial and viral infections, as well as other disorders [5]**.** YAZAKI. K et al [6] and MILLER,N.D [7] describes the use for treating eczema and burns, neuralgia, ulcers, bedwetting and gastroenteritis .

Analysis of volatile organic compounds (VOCs) in plants is important by providing information on the content of biologically active compounds. These are analyzed by gas chromatography coupled with mass spectrometry. VOCs are involved in some physiological processes such as: attraction of pollinators, plant-plant interactions, indirect plant defence against insects, sources of new drugs, cosmetics, etc.

Microextraction in solid phase –SPME- was introduced for the first time by Arthur and Pawliszyn [8]. This technique is used in combination with various analytical instruments such as GC–MS for the analysis of volatile, semi-volatile, polar and non-polar plant compounds, vegetables, fruits, beverages, and dairy products [9].

Essential oils are natural products which consist of many volatile molecules. They have been used for several pharmaceutical and cosmetic application, agricultural and bioactivity example flowers. Extraction of essential oils could be carried out by various techniques. Hesham H. A. Rassem et al. [10] and Zyed Rouis etal. [11] studied volatile compounds from three species of *Hypericum: Humifusum*, *Perfoliatum* and *Ericoides*, using SPME-GCMS and identified 127 compounds, most of which are traces.These plants were dried.

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Abdollah Ghasemi Pirbalouti et al. [12] identified 48 volatile compounds in the volatile oil of *H. perforatum*. The major components were: alpha-pinene (12.5_1.0%), beta-pinene (8.3_1.7%), undecane (7.0_0.5) and germacrene-D (6.9_0.1%). Many studies on the essential oil content of *H. perforatum* indicate the enormous variability inherent inthe volatile chemistry of this species [13].

2. Materials and methods

Plant collection

The plants were collected, in the summer of 2019, from spontaneous flora on the hills near Cluj-Napoca.They were in the period of maximum flowering(during the full-bloom stage). Plant identities is according to the specialized literature in the library "Alexandru Borza" Botanical Garden from Cluj-Napoca [14]. They were analyzed in fresh form and the remainderwere dried for five days, at room temperature in a dark place. After drying, the flowers were analyzed SPME-GC-MS and subsequently by hydrodistillation the essential oil was obtained.

Chemical and reagents

An alkane mixture containing C8-C20 alkanes (40 mg/ml in hexane) was purchased from Merck. Helium 6.0 purity as carrier gas, was purchased from Linde Gas-Romania.

Instruments and operating conditions (GC-MS, HS-SPME-GC-MS, UV-viz, LC-MS)

A manual SPME fiber holder provided by Supelco was used for the HS sampling. The SPME fiber used was 50/30 um DVB / Carboxen/ PDMS.1gr sample + 8mL distilled water, 10 min for eqilibrum, temp= 55 degree, followed 20 min collected on the fiber.The GC-MS analysis of the volatile compounds and essential oil was preformed using a Agilent Model 7890 & 5975 Series MSD, equipped with a HP-5MS column (30 m x 0.25 mm x 0.25 μM,). Volatile compounds adsorbed on the SPME fibre were immediately thermally desorbed in the injector port of a GC-MS and then separated on the GC column and identified using the MS detector. Injections were done using the splitless system. The temperature program was the following:Oven temperaturewas programmed as 40^0C for 1 min and an increase by 5^0C/min to 200^0C. From 200 to 240^0C increase with 20^0C/min. It is maintained at 240^0C for 5 min. Mass spectra: electron impact(EI $^+$) mode, 70eV and ion source temperature, 230^0C.Data acquisition and processing were performed using MSD Chem Station software.NIST library was used for identification of the components. C8-C10 standard alkanes (standard solution C_8-C_{10}, Sigma Aldrich) was used for calculation of the linear retention index (RI) for the purpose of comparison with data from the literature.

Hydrodistillation method

Essential oil can be obtained from the dried plants using a typical Clevenger circulatory hydrodistillation apparatus reported in the European Pharmacopoeia.The plant material is placed inside the glass containers along with water. Application of a heat source allows water to boil and extract EOs which are condensed along with water by the upper condensing system. The different density of EOs with respectto water allow separation andcollection. 100 gr dried flower in 1000 ml distilled water were distilled for 3 h.The distillate was extracted with hexane and dried over $MgSO_4$. The analysis was also performed on GC-MS with the following temperature program: oven temperature was programmed as 40^0C for 1 min and an increase by 5^0C /min to 200^0C. From 200 to 260^0C, increase with 20^0C/min. It is maintained at 260^0C for 10 min. For identification of the components from oil the NIST database was used and for the compliance with the literature data, retention indices were calculated using Alkane Standard Solution C8-C20, Sigma Aldrich.

3.Results and discussions

A total of 72 different compounds were identified.All of the compounds identified are in the Table 1 and Table 2.Identified volatile compounds from fresh flower: Total:47.Volatile compounds from dried flower: Total: 55.Volatile compounds from essential oil: Total: 35. For better characterization, we grouped these volatile compounds into the following classes:a) Non-terpene hydrocarbons, b) monoterpene hydrocarbons, c) sesquiterpene hydrocarbons, d) oxygenated monoterpenes, e) oxygenated sesquiterpenes.

Table 1. Compounds identified in fresh and dried *hypericum perforatumhs-spme-gc-ms*flowers

	Compounds	**RT**	**RI**	***Hypericum perforatum*** **fresh flowers [area%]**	***Hypericum perforatum*** **dried flowers[area%]**
1	2-Hexenal (E)	10.124	848	X	1.10
2	**Octane-2-methyl**	10.508	863	**13.42**	**11.93**
3	Nonane	11.431	898	1.43	1.65
4	α-Thujene	12.345	929	0.78	2.50
5	α-Pinene	12.584	938	0.98	2.45
6	Benzaldehyde	13.388	965	X	0.11
7	Nonane-3-methyl	13.585	972	1.57	2.05
8	Cis-sabinene	13.777	978	1.23	3.06
9	**β-pinene**	13.922	983	2.37	**5.33**
10	β-Myrcene	14.202	993	0.89	2.82
11	3-Hexen-1-ol, acetate	14.649	1019	0.60	X
12	α-phellandrene	14.716	1021	X	0.47
13	4-Carene	15.058	1032	0.34	1.03
14	p-cymene	15.318	1039	0.41	1.48
15	β-phellandrene	15.458	1044	0.86	2.41
16	E- β-ocimene	15.603	1048	2.21	3.46
17	**Z- β-ocimene**	16.034	1060	**11.84**	**11.98**
18	**γ-terpinene**	16.205	1066	0.20	0.2
19	**Decane-2-methyl**	16.402	1072	**4.71**	**5,74**
20	p-Mentha-1,4(8)-diene	17.196	1096	0.24	0.68
21	Undecane	17.393	1102	0.95	1.12
22	Nonanal	17.544	1108	X	0.10
23	Phenylethyl alcohol	17.912	1122	X	0.30
24	Allo-ocimene (E,Z)	18.275	1125	0.67	1.07
25	1,3,8-p-menthatriene	18.358	1138	0.20	0.30
26	Trans-allo-ocimene (E,E)	18.649	1149	0.37	0.45
27	2,6-Nonadienal	18.971	1161	X	0.10
28	Dodecanal	19.412	1178	0.50	0.35
29	Terpinen-4-ol	19.796	1192	0.21	0.30
30	Methyl salicylate	20.273	1204	X	0.15
31	Cis-3-hexenyl-isovalerate	21.197	1239	0.11	0.18
32	Dodecane-2-methyl	21.959	1267	2.73	1.28
33	Tridecane	22.878	1302	0.54	0.24
34	α- Cubenene	24.403	1362	0.14	0.21
35	α- Longipinene	24.554	1368	0.42	0.38
36	E-2-Tetradecen-1-ol	24.766	1377	0.20	0.13
37	α-Ylangene	25.021	1387	0.37	0.31
38	α- copaene	25.135	1391	0.17	0.37
39	α-Bourbonene	25.394	1402	X	0.25
40	Aromadendrene	25.830	1420	0.13	0,21
41	β-Funebrene	26.178	1434	X	0.83
42	**Caryophyllene**	26.421	1444	**20.77**	**12.07**
43	Alloaromadendrene	26.754	1458	X	0.22
44	**β-Famesene**	26.940	1466	**7.87**	**1.82**
45	α- Himachalene	27.070	1472	1.39	0.78
46	Linalyl acetate	27.132	1474	1.18	X
47	Germacrene D	27.335	1483	0.46	X

48	γ-curcumene	27.548	1492	2.10	X
49	β- longipinene	27.734	1499	1.22	X
50	**Cis-α-bisabolene**	27.879	1506	**6.26**	**5.37**
51	α- farnesene	28.066	1514	1.11	1.50
52	β- Himachalene	28.212	1520	1.03	0.58
53	β- Humulene	28.404	1529	0.11	0.72
54	γ-Muurolene	28.461	1531	0.50	0.34
55	γ-cadinene	28.471	1532	X	0.67
56	δ-cadinene	28.632	1539	0.95	1.17
57	α- Amorphene	28.980	1554	0.15	0.20
58	Caryophyllene oxide	29.410	1573	X	0.11
59	Aromamadendrene	29.976	1598	X	0.17
60	2,2,4-trimethyl-1,3-pentanediol-diisobutyrate	30.142	1606	0.44	X
61	Caryophylla-4(12),8(13)-dien-5beta-ol	31.278	1659	X	0.17

Table 2
IDENTIFICATION OF ESSENTIAL OIL CONSTITUENTS USING: GC-MS

	Compounds	**RT**	**RI**	***Hypericum perforatum Oil* [area%]**	**QI**"quality index" [%]
1	2-Hexenal (E)	9.728	851	0.06	97
2	**Octane-2-methyl**	10.482	878	**14.21**	**87**
3	Nonane	11.113	901	0.99	91
4	α-Thujene	11.969	929	0.39	94
5	**α-Pinene**	12.296	940	**13.84**	**97**
6	Nonane-3-methyl	13.267	972	0.63	91
7	Cis-sabinene	13.427	977	0.19	94
8	**β-pinene**	13.682	986	**8.79**	**97**
9	D-limonene	15.134	1034	0.26	97
10	**Z- β-ocimene**	15.601	1050	**6.02**	**98**
11	Gamma -terpinene	15.996	1063	0.31	97
12	Decane, 2-methyl	16.074	1065	1.09	94
13	4-Carene	16.816	1090	0.13	98
14	Undecane	17.122	1100	0,26	91
15	Trans-allo-ocimene	17.973	1130	0.14	97
16	Terpinen-4-ol	19.659	1188	0.21	93
17	Terpineol	20.064	1203	0.52	86
18	Undecane, 2,10-dimethyl	21.750	1264	0.34	91
19	**Caryophyllen**e	26.238	1438	**16.74**	**99**
20	Cis-β-Famesene	26.358	1443	3.10	98
21	Humulene	27.006	1470	0.87	96
22	α- Himachalene	27.208	1478	0.21	81
23	γ- Muurolene	27.395	1486	0.91	99
24	**Germacrene D**	27.644	1496	**6.46**	**98**
25	α- farnesene	27.888	1507	1.3	95
26	δ-cadinene	28.308	1525	0.5	99
27	α- Amorphene	28.832	1547	0.13	98
28	Nerolidol	29.289	1567	1.28	91
29	**Caryophyllene oxide**	30.057	1601	**5.28**	94
30	α- cadinol	31.582	1670	0.84	99
31	α- bisabolol	32.122	1695	0.46	91
32	Tetradecanoic acid	33.388	1782	0.17	95
33	Benzyl benzoate	33.782	1783	0.14	96
34	2- Pentadecanone, 6,10,14- trimethyl	34.654	1845	0.15	99
35	n-Hexadecanoic acid	35.910	1962	0.25	97

QI, "quality index", reflects the fit comparison of experimental mass spectrum and NIST library mass spectrum

The essential oil is a mixture of different compounds that reflect: processes in the plants (before harvest and distillation), processes that occur during distillation (due to chemical modifications enhanced by the presence of water and heat) and post-distillation processes (due to reactions caused by light and oxygen).

By collecting the volatiles from the fresh and dried plants and the corresponding essential oils we see the percentage variation of the majority volatile compounds (Table 3).

Table 3. The main common volatile compounds from *Hypericum perforatum*, flowers and oil

	Oil by SPME Area[%]	**Dried** flower by SPME Area [%]	**Fresh** flowers by SPME Area [%]
Octane -2 methyl	**14.21**	**11.93**	**13.42**
α-pinene	13.84	2.45	0.9
β-pinene	8.79	7.88	2.37
β- ocimene	**6.02**	**11.98**	**16.85**
Decane -2-methyl	1,09	5.74	4.71
Caryophylene	**16.74**	**12.64**	**20.77**
Germacrene D	7.48	0.14	0.46
γ-terpinene	0.31	5.74	0.2
Caryophyllene-oxide	5.28	0.67	-
Cis-beta-Farnesene	3.10	1.82	7.87
undecane	0.26	1.64	1.13

2-methyl octane or Isononane is a major constituent for flowers and oil of *Hypericum perforatum*, and this could be a potential marker for this plant. Not for fragrance and flavor use.(The Good Scents Company-TGSC-Information System).

The most abundant compound, **Caryophylene -** is a sesquiterpene widely distributed in essential oils of various plants, it is mainly used for therapeutic purposes. Caryophyllene, the main have strong antioxidant activity[15]. The cytotoxicity of caryophyllene, a major compound along with other components in some essential oils, has been tested and proven on human tumor cell linesand antitumor activity [16].

D-germacrene, has been known to exert cytotoxic activity against cancer cell lines, fungicidal activity, and antibacterial properties against both gram-positive and gram-negative bacteria [17,18]

β-ocimene has multiple relevant functions in plants. There is strong indication that β-ocimene can play very relevant roles in the attraction of several types of pollinators to the flowers of a diverse array of plants.

Caryophyllene oxide, an oxygenated sesquiterpene has antifungal significant activity [19] and anticancer activities via the suppression of cellular growth and induction of apoptosis [20].

Abundance the main classes of volatile compounds from flowers and oil is represented in Figure 1 and Figure 2.

A comparison of our results with other reports from literature on the chemical composition of *Hypericum perforatum* suggests some differences in the volatile composition. This could be attributed to genetic (genus, species, sub species and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases and extraction and quantification methods [21].

Depending on the state of the plant, dried or fresh, one class or another of hydrocarbons predominates.

In the fresh flowers, most of the volatiles belong to **sesquiterpene hydrocarbons**, respectively 45.38% and the least belong to the oxygenated sesquiterpenes, respectively 0.15%.Sesquiterpenes are less volatile than terpenes and have stronger odors.

In the dried flowers, most of the volatiles belong to **monoterpene hydrocarbons**, respectively 40.76% and the least belong to the oxygenated sesquiterpenes, respectively 0.35%.

E- β- ocimene is representative of the class of monoterpenes identified. It is used in perfumery for its sweet herbal scent, and are believed to act as plant defense and have anti-fungal properties. We thus conclude that β-ocimene is a key plant volatile with multiple relevant functions in plants, depending on the organ and the time of emission. β-ocimene is a generalist attractant of a wide spectrum of pollinators [19].

In the essential oil, most of the volatiles belong to **sesquiterpene hydrocarbons**, respectively 30,55% and closely followed by monoterpene hydrocarbons, 30.05%.

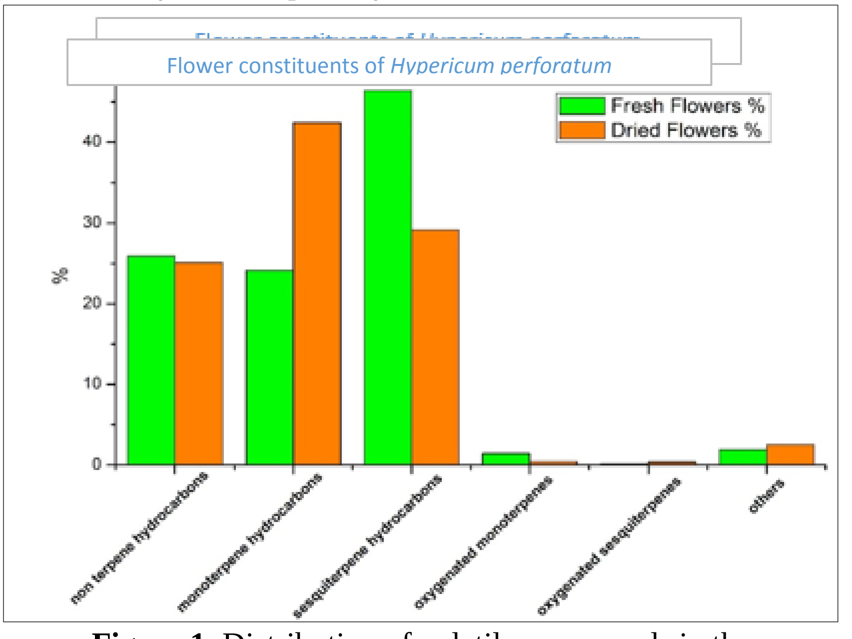

Figure 1. Distribution of volatile compounds in the studied flowers samples

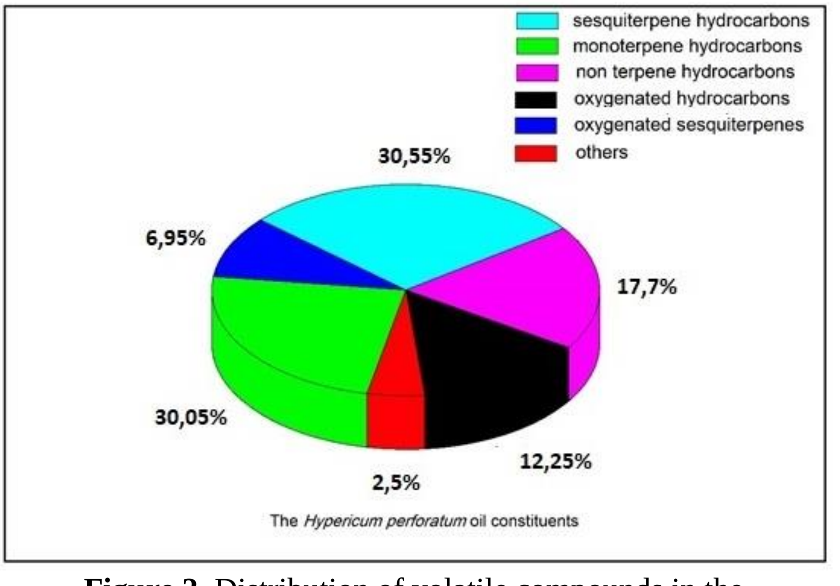

Figure 2. Distribution of volatile compounds in the *Hypericum perforatum* oil

4. Conclusions

-Analysing the volatiles from the fresh and dried plants and their corresponding essential oils we see the most common compounds whose abundance varies.

-The essential oil contains traces of some substances that are missing in the flower volatilom.

-*2-methyl octane* could be a potential marker for *Hypericum perforatum* flowers and essential oil.

-According to the data from the mentioned literature, the high content of terpenes in flowers and essential oils studied gives them biological activity.

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