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.

_Hypericum 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 et al. [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 in the 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 remainder were 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 equilibrium, 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 temperature was programmed as 40°C for 1 min and an increase by 5°C/min to 200°C. From 200 to 260°C increase with 20°C/min. It is maintained at 260°C for 5 min. Mass spectra: electron impact (EI +) mode, 70eV and ion source temperature, 230°C. 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 C8-C10, 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 respect to water allow separation and collection. 100 gr dried flower in 1000 ml distilled water were distilled for 3 h. The distillate was extracted with hexane and dried over MgSO4. The analysis was also performed on GC-MS with the following temperature program: oven temperature was programmed as 40°C for 1 min and an increase by 5°C /min to 200°C. From 200 to 260°C, increase with 20°C/min. It is maintained at 260°C 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 perforatum* L.-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 | 10.308 | 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        | β-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-terpine | 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        | Alto-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        | Allosiformadendrene | 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 |
**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 2-Methyl                                | 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-oicimene                          | 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-αllo-oicimene                    | 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 Caryophyllene                          | 26.238 | 1438 | **16.74**                         | **99**                |
| 20 Cis-β-Farnesene                        | 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- Murolene                            | 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**

| Compound                  | 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                           |
| Caryophyllene             | 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, Caryophyllene - 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 lines and 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](image1)

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

![Figure 2. Distribution of volatile compounds in the Hypericum perforatum oil](image2)

**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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