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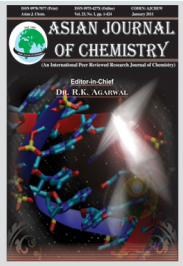

# Hydrodistillation, Solvent Free Microwave Assisted Extraction and Headspace-Solid Phase Microextraction for Analysis of Essential Oil of Flowers of *Helichrysum aucheri*

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In this study, the essential oil of flowers of *Helichrysum aucheri* is investigated. The essential oil of flowers of *Helichrysum aucheri* collected from Abasabad Valley of Hamedan, in June 2008, were isolated by solvent free microwave assisted extraction (SFME) and headspace-solid phase microextraction (HD-SPME) with polydimethylsiloxane/divinylbenzene fiber as solid phase and comparison the compositions of the essential oils extracted by these methods with hydrodistillation method. The volatile compounds which obtained by hydrodistillation, solvent free microwave assisted extraction and headspace-solid phase microextraction were analyzed by means of GC/MS. α-Pinene (38.5 %), β-caryophyllene (23.0 %), 1,8 cineole (12.0 %), were the major components in hydrodistillation method. Isoborneol (28.2%), β-caryophyllene (12.9 %), δ-cadinene (6.3 %), bornyl acetate (6.0 %) were found to be the main constituents in the solvent free microwave assisted extraction method. The main compounds which entered by headspace-solid phase microextraction were carvacrol (37.7 %), α-pinene (19.7 %) and β-caryophyllene (8.5 %).

**Key Words: *Helichrysum aucheri*, Essential oil, Solvent free microwave assisted extraction, Headspace-solid phase microextraction.**

## INTRODUCTION

Solid-phase microextraction is a successful solvent-free sampling technique. The analytes from the liquid or gaseous sample are directly absorbed onto an absorbent-coated fused silica fiber, which is part of the syringe needle and then either thermally desorbed directly into a gas chromatography (GC) injection port[1-4] or solvent desorbed into an high-performance liquid chromatography (HPLC) injection valve[5].

Solid-phase microextraction (SPME) is a modern analytical tool for easy effective extraction of various types of compounds. It is worthwhile to mention that headspace-solid phase microextraction (HD-SPME) is a non-destructive modern application for trapping/extracting volatiles, fragrances, emissions, *etc*. It is a fast, easy, convenient and effective technique with good future prospects[6-16].

Microwave heating has been recently used for the isolation and analysis of essential oils[17-19]. Solvent-free microwave extraction (SFME) is a new technique which combines microwave heating with dry distillation at atmospheric pressure for the isolation and concentration of the essential oils in fresh plant materials. In SFME method, there is no need to add any solvent or water if fresh plant material is used. If dry plant material is used, the sample is rehydrated by soaking in water for some time and then draining off the excess water[20].

In this investigation, the microwaves and SPME are applied for extraction of essential oils and compared the classic hydrodistillation method for the extraction of the essential oils with these methods. The essential oils obtained by SFME in 30 min and SPME in 10 min with the latter system were comparable, both from a qualitative and from a quantitative point of view, to those obtained after a 3 h hydrodistillation. The microwave method permitted a more efficient extraction of oxygenated compounds saving time and energy[18].

## EXPERIMENTAL

The aerial parts of *Helichrysum aucheri* were collected from Abasabad Valley of Hamedan, in June 2008. The flower and leave of the samples were separated and were dried in shade. A voucher specimen was deposited in the herbarium of Shahid Beheshti University.

### Isolation procedure

**Hydrodistillation:** The dried flower of samples (40 g) was subjected to hydrodistillation by using a Clevenger apparatus for 3 h. The oil were dried over anhydrous $Na_2SO_4$ and stored in sealed vials at 4 °C before analysis.

**HD-SPME analysis:** The dried flower of samples was subjected directly to HD-SPME. The manual SPME device (Supelco, USA), with a fiber precoated with a 65 μm-thick

layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB), was used for extraction of the plant volatiles. The fiber was pushed through the plastic film for exposure to the headspace of the material for 5 min at 70 °C. The fiber was then inserted into the injection port of the GC-MS for desorption (3 min) of the adsorbed volatile compounds for analysis. HD-SPME and subsequent analyses were performed in triplicate.

**Solvent-free microwave extraction (SFME):** The microwave oven used for SFME was Milestone MicroSynth, 2450 MHz with Maximum power 1000 W and ACTE0 sensor. The power of the oven was 800 W for 2 min then the temperature achieved to 95 °C the power of the oven set at 500 W for 0.5 h. Flat bottom flask having a capacity of 500 mL was placed in the oven and connected to Clevenger apparatus through, the hole. For SFME, 30 g of dried of flowers of *Helichrysum aucheri* was soaked in 20 mL distilled water at room temperature (25 °C) for 1 h in order to hydrate the external layers of the plant material. The moistened plant material was placed in flat-bottom flask combined to a Clevenger apparatus. During the process, the vapour passed through the condenser outside the microwave cavity where it was condensed. The SFME process was performed for 0.5 h. The essential oil was collected in amber coloured vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4 °C until being analyzed.

**Gas chromatography-mass spectrometry (GC-MS):** Gas chromatography-mass spectrometry analyses were carried out on a Hewlett-Packard (HP) 6890 GC-MS system equipped with a DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 μm), oven temperature program was 60 °C to 220 °C at a rate of 6 ºC/min; transfer line temperature, 280 °C; injector temperature, 250 °C; carrier gas, helium; flow rate, 1.5 mL/min; ionization energy, 70 eV.

**Identification of components:** The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

## RESULTS AND DISCUSSION

The oils isolated by hydrodistillation from the flower of *Helichrysum aucheri* were found to be yellow liquid. Fifteen compounds were identified in the essential oil of the flower of *Helichrysum aucheri*, representing more than 99.28 % of the oil. The major components were found to be α-pinene (38.45 %), (*E*)-caryophyllene (22.98 %), 1,8-cineole (12.03 %) and α-humulene (6.6 %), β-pinene (5.35 %), bisabolene (2.19 %), palmitic acid (2.1 %) and limonene (2.04 %), while in SFME

TABLE-1
CHEMICAL COMPOSITION OF THE FLOWER OF *Helichrysum Aucheri* OILS OBTAINED BY HYDRODISTILLATION, SOLVENT-FREE MICROWAVE EXTRACTION (SFME) AND HEAD SPACE MICROEXTRACTION (HS-SPME)

| | Retention time | Compound | KI | Hydrodistillation (%) | HD-SPME (%) | SFME (%) |
|---|---|---|---|---|---|---|
| 1 | 5.46 | α-Pinene | 939 | 38.5 | 19.7 | 4.2 |
| 2 | 5.76 | Camphene | 954 | 1 | 0.6 | 4.4 |
| 3 | 6.39 | β-Pinene | 979 | 5.4 | 1.1 | 1.6 |
| 4 | 6.7 | Myrcene | 991 | 1.5 | 1.0 | Not detected |
| 5 | 7.49 | *p*-Cymene | 1019 | Not detected | 0.4 | Not detected |
| 6 | 7.59 | Limonene | 1029 | 2.0 | 2.1 | 5.9 |
| 7 | 7.67 | 1,8-Cineole | 1031 | 12.0 | 7.4 | Not detected |
| 8 | 8.28 | γ-terpinene | 1060 | 0.7 | Not detected | Not detected |
| 9 | 9.31 | *n*-Nonanal | 1097 | Not detected | 0.9 | Not detected |
| 10 | 10.77 | Isoborneol | 1162 | Not detected | 3.7 | 28.2 |
| 11 | 11.32 | α-Terpineol | 1189 | Not detected | 1.2 | Not detected |
| 12 | 11.52 | *n*-Decanal | 1196 | Not detected | 0.6 | Not detected |
| 13 | 12.52 | Thymoquinone | 1251 | Not detected | 1.3 | Not detected |
| 14 | 12.61 | Geraniol | 1255 | Not detected | 0.9 | Not detected |
| 15 | 13.2 | Bornyl acetate | 1288 | Not detected | Not detected | 6.0 |
| 16 | 13.71 | Carvacrol | 1314 | Not detected | 37.7 | Not detected |
| 17 | 14.45 | α-Terpinylacetate | 1349 | Not detected | 1.8 | 3.3 |
| 18 | 15.23 | β-Cubebene | 1385 | Not detected | Not detected | 3.1 |
| 19 | 15.57 | *n*-Tetradecane | 1402 | Not detected | 1 | Not detected |
| 20 | 15.84 | β-Caryophyllene | 1419 | 23.0 | 8.5 | 12.9 |
| 21 | 16.03 | α-*trans*-Bergamotene | 1435 | 0.8 | 0.4 | Not detected |
| 22 | 16.36 | z-β-Farnesene | 1445 | Not detected | 0.7 | Not detected |
| 23 | 16.44 | α-Humulene | 1455 | 6.6 | 1.8 | 4.1 |
| 24 | 16.92 | Germacrene D | 1477 | Not detected | Not detected | 4.3 |
| 25 | 17.02 | β-Selinene | 1490 | 1.8 | 1.3 | 2.1 |
| 26 | 17.34 | β-Bisabolene | 1506 | 2.2 | 1.4 | 1.8 |
| 27 | 17.62 | δ-Cadinene | 1516 | Not detected | 0.6 | 6.3 |
| 28 | 18.28 | (*E*)-Nerolidol | 1563 | 1.2 | Not detected | Not detected |
| 29 | 18.52 | Caryolan-8-ol | 1573 | 0.7 | 0.6 | Not detected |
| 30 | 18.74 | Caryophyllene oxide | 1583 | Not detected | 0.6 | 5.2 |
| 31 | 24.43 | Palmitic acid | 1969 | 2.1 | Not detected | Not detected |
| Total | | | | 99.4 | 97.4 | 93.4 |

are isoborneol (28.21 %), β-caryophyllene (12.86 %), δ-cadinene (6.26 %), bornyl acetate (6.01 %), limonene (5.95 %), caryophyllen oxide (5.19) and in HD-SPME are carvocrol (37.73 %), α-pinene (19.66%), β-caryophyllene (8.47 %), 1,8-cineole (7.35 %), isoborneol (3.67 %) and limonene (2.13 %). The chemical composition of major components of the flower of *Helichrysum aucheri* oils of three different methods are given in Table-1.

As seen in Table-1, carvacrol has been obtained only by HD-SPME and is the major compound of this method. As the same the major compound of SFME, isoborneol, has been not obtaind by hydrodistillation.

Table-2 shows the chemical classes of compounds of the essential oils in three methods. The highest percentages of each class of compounds are monoterpene hydrocarbons (49.01 %) obtained by hydrodistillation, sesquiterpene hydrocarbons (34.56 and 34.37 %) by SFME and hydrodistillation, oxygenated monoterpenes (54.04 %) by HS-SPME and oxygenated sesquiterpenes (5.19 %) by SFME. Oxygenated compounds (both of the mono and sesquiterpenes are the main composition of the essential oil in HS-SPME (55.24 %) and followed in decreasing order in SFME (42.71 %) and then hydrodistillation (13.9 %). Therefore, it is true that the microwave is able to extract the oxygenated compounds than hyrodistillation[18]. As PDMS-DVB fiber (a solid phase suitable for polar components) used in HS-SPME method, the percentage of extracted oxygenated compounds were more than hydrodistillation and SFME.

TABLE-2
CHEMICAL CLASSES OF COMPOUNDS OF ESSENTIAL OILS OBTAINED BY THREE DIFFERENT METHODS

| | HD (%) | HD-SPME (%) | SFME (%) |
|---|---|---|---|
| Monoterpene hydrocarbons | 49.0 | 26.2 | 16.2 |
| Sesquiterpene hydrocarbons | 34.4 | 14.8 | 34.6 |
| Oxygenated monoterpenes | 12.0 | 54.0 | 37.5 |
| Oxygenated sesquiterpenes | 1.9 | 1.2 | 5.2 |

HD = Hydrodistillation

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