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Analysis of Volatile Constituents of *Perovskia abrotanoides* with Different Extraction Methods

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In this study, three new methods, solvent free microwave extraction (SFME), head-space solid-phase microextraction (HS-SPME) and microwave assisted head-space solid-phase microextraction (MW-HS-SPME), were developed for the analysis of essential oil compounds in aerial part and flower of *Perovskia abrotanoides*. Then these methods were compared with traditional extraction method of essential oil, hydrodistillation (HD). The extraction conditions of these methods were optimized. The optimum conditions for these methods were: 500 W power of microwave and 0.5 h the extraction time for SFME method, 70 ºC and 5 min exposure time for HS-SPME method (with PDMS fiber) and 400 W power and 5 min exposure time for MW-HS-SPME method.

Key Words: *Perovskia abrotanoides*, Essential oil, Solvent free microwave extraction, Head-space solid-phase microextraction, Microwave assisted head-space solid-phase Microextraction.

INTRODUCTION

Various methods can be used for the isolation and extraction of essential oils from plant materials which mainly include solvent extraction[1], supercritical fluid extraction (SFE)[2,3] and liquid-phase microwave-assisted process (MAP) extraction[4]. However, these methods always lead to the loss of some volatile compounds, low extraction efficiency, toxic solvent residues and are time-consuming.

Head-space solid-phase microextraction (HS-SPME) is a relatively new sampling and concentration technique for the extraction of plant essential oils[5-9].

Microwave heating has already been widely applied in solvent extraction because of its main advantages like rapidity and high efficiency. Many cases have already proved that microwave assisted extraction (MAE) is a viable alternative to conventional techniques for many kinds of samples[10-14].

Development of the novel technique combined the advantages of microwave extraction and SPME is very interesting. In 2003, Pawliszyn and co-workers developed microwave-assisted headspace solid-phase microextraction (MA-HS-SPME) as a simple and effective method for fast sampling of volatile compounds from *Eucalyptus citriodora* Hook (*E. citriodora*) leaves[15].

Solvent-free microwave extraction (SFME), a combination of microwave heating and dry distillation, is a new green technique developed in recent years. Conventional SFME performed at atmospheric conditions without adding any solvent or water provided a new idea in the extraction of volatile compounds from fresh plant materials or prior moistened dried materials and it made the whole process to be more simple, rapid and economic. Lucchesi and coworkers had applied SFME in extractions from aromatic herbs and spices[16-18].

In previous studies the *Perovskia abrotanoides* essential oil was isolated by hydrodistillation method[19,20]. In this study hydrodistillation method and also the tree new methods, HS-SPME, MW-HS-SPME and SFME, were used for isolation of the essential oil of *Perovskia abrotanoides*.

EXPERIMENTAL

Perovskia abrotanoides was collected from Yazd, in June 2009. The aerial parts and flower were dried in shade. A voucher specimen was deposited in the herbarium of Shahid Beheshti University, Iran.

Isolation procedure

Hydrodistillation: The dried aerial parts (100 g) and flower (50 g) of plant were subjected to hydrodistillation by using a Clevenger apparatus for 3 h. The oils were dried over anhydrous Na_2SO_4 and stored in sealed vials at 4 °C before analysis.

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 500 W for 10 min then the temperature achieved to 95 °C and then the extraction was done for 20 min.

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 *Perovskia abrotanoides* (aerial part and flower) 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 oils was collected in amber coloured vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4 °C until being analyzed.

HS-SPME Analysis: The dried aerial parts and flower of plant (1 g) were subjected directly to HS-SPME. The manual SPME device (Supelco, USA), with a fiber precoated with a 65 μm-thick layer of polydimethylsiloxane (PDMS), 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 optimum conditions). The fiber was then inserted into the injection port of the GC-MS for desorption (3 min) of the adsorbed volatile compounds for analysis. HS-SPME and subsequent analyses were performed in triplicate.

MW-HS-SPME Analysis: The microwave oven used for MW-HS-SPME was Milestone MicroSynth, 2450 MHz with Maximum power 1000 W and ACTE0 sensor. The manual SPME device (Supelco, USA), with a fiber precoated with a 65 μm-thick layer of polydimethylsiloxane (PDMS), was used for extraction of the plant volatiles. 5 g of dried of flowers, stem and leaf of samples was soaked in 5 mL of distilled water at room temperature (25 °C) in order to hydrate the external layers of the plant material. The moistened plant material was placed in flat-bottom flask. The power of the oven was 400 W for 10 min then the temperature achieved to 95 °C and the optimum sampling time that the fiber exposure to the headspace was 5 min.

Gas chromatography-mass spectrmetry: Gas chromatography-mass spectrmetry (GC-MS) 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 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 mL/min; ionization energy, 70 eV.

Gas chromatography: Gas chromatography (GC) analysis of the oils was performed on a Shimadzu 15A gas chromatograph equipped with a split/split less injector (250 ºC). Nitrogen was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (30 m × 0.25 mm, film thickness 0.32 μm). The column temperature was kept at 60 °C for 3 min and then heated to 220 °C with 5 °C/min rates and kept constant at 220 °C for 5 min. Relative percentage amount were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Identification of components: The components of the oils 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

Optimization the conditions: Aerial part essential oil used for optimization condition because the aerial part and flower had the same volatile constituents.

SFME Method: For SFME method, the time of extraction and power of microwave were optimized. To optimization the conditions, 6 components that they are the main in the essential oil were selected (δ-2-crene, 1,8 cineole, camphor, β-caryophyllene and α-humulene). Extraction was done for 20, 30 and 40 min and in 400, 500, 600 and 700 W power of microwave. The best time for extraction these compounds were 0.5 h (Fig. 1). Figs. 2 and 3 show that the 500 W is the best power for extraction these components and the volume of the essential oil.

HS-SPME Method: In HS-SPME method the time and temperature of extraction were optimized.

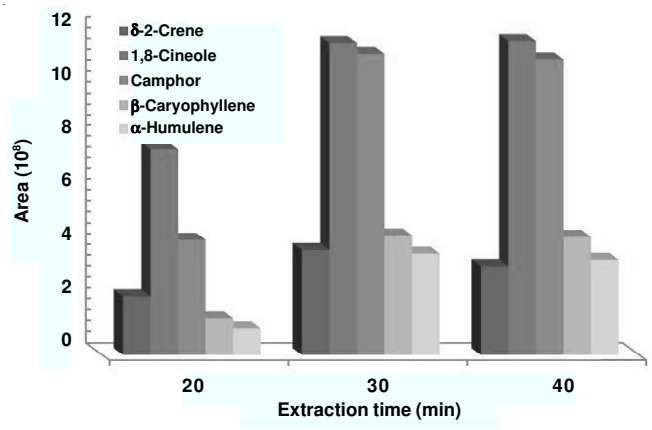

Fig. 1. Effect of the extraction time on sum of peak areas of some volatile compounds in aerial part of *Perovskia abrotanoides* essential oil isolated by SFME method. (Sample weight = 30 g and microwave power = 500 W)

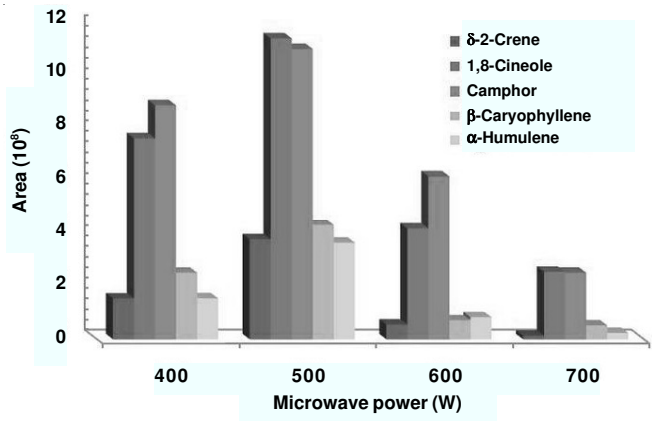

Fig. 2. Microwave power effect on the sum of peak areas of some volatile compounds in aerial part of *Perovskia abrotanoides* essential oil isolated by SFME method. (Sample weight = 30 g and extraction time = 0.5 h)

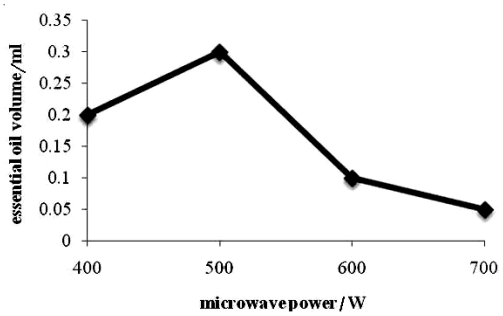

Fig. 3. Microwave power effect on the volume of aerial part of *Perovskia abrotanoides* essential oil isolated by SFME method. (Sample weight = 30 g and extraction time = 0.5 h)

For optimization the condition, α-pinene, camphene, β-pinene, δ-2-crene, limonene, β-caryophyllene and α-humulene were selected as main components for extraction by this method. The optimizations for each condition were done in 3, 5 and 7 min as extraction time and 60, 70 and 80 °C as temperature. The 5 min exposure time and 70 °C temperature were selected as the optimum conditions for HS-SPME method, as shown in Figs. 4 and 5.

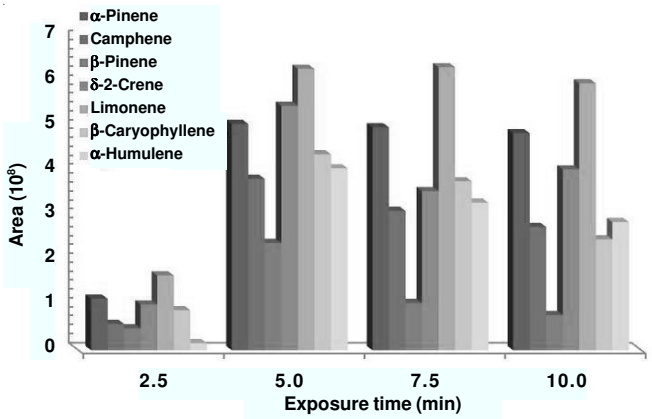

Fig. 4. Effect of the exposure time on sum of peak areas of some volatile compounds in aerial part of *Perovskia abrotanoides* essential oil isolated by HS-SPME method. (Sample weight = 2 g, PDMS fiber as extraction phase at 70 °C)

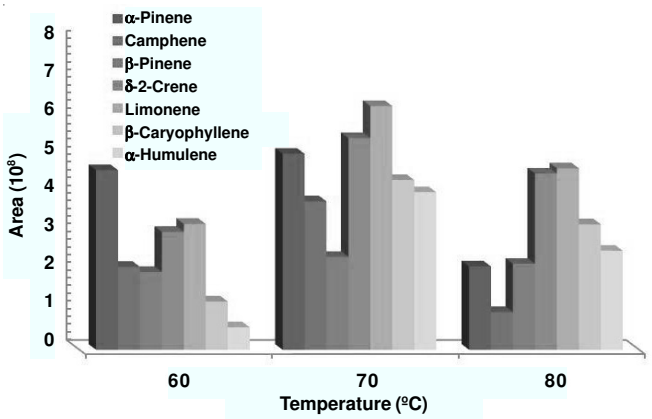

Fig. 5. Effect of the temperature on sum of peak areas of some volatile compounds in aerial part of *Perovskia abrotanoides* essential oil isolated by HS-SPME method. (Sample weight = 2 g, PDMS fiber as extraction phase and exposure time = 5 min)

Microwave assisted head-space solid-phase microextraction (MW-HS-SPME): In MW-HS-SPME method the time of extraction and power of microwave were optimized. For optimization the condition, α-pinene, camphene, β-pinene, δ-2-crene, 1,8-cineole and camphor were selected as main components for extraction by this method. The optimizations for each condition were done in 2.5, 5 and 7 min as extraction time and 400, 500 and 600 W as power of microwave. As shown in Figs. 6 and 7, the 5 min exposure time and 400 W power of microwave were selected as the optimum conditions for MW-HS-SPME method.

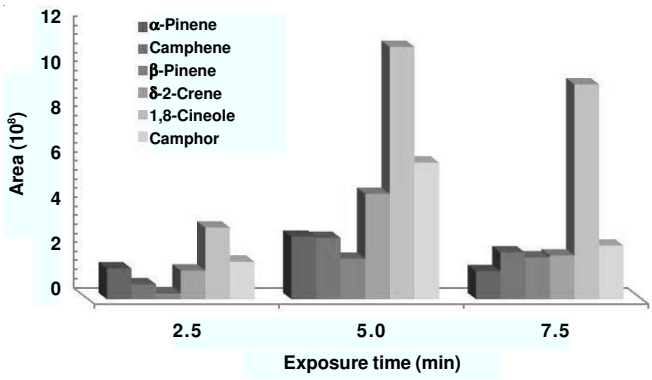

Fig. 6. Effect of the exposure time on sum of peak areas of some volatile compounds in aerial part of *Perovskia abrotanoides* essential oil isolated by MW-HS-SPME method. (Sample weight = 5 g, PDMS fiber as extraction phase and microwave power = 400 W)

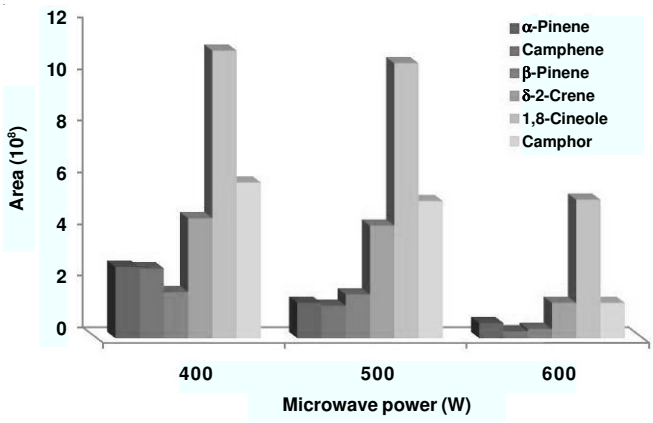

Fig. 7. Microwave power effect on sum of peak areas of some volatile compounds in aerial part of *Perovskia abrotanoides* essential oil isolated by HS-SPME method. (Sample weight = 5 g, PDMS fiber as extraction phase and exposure time = 5 min)

Analysis of essential oil in *Peroveskia abrotanoides* by conventional HD, SFME, HS-SPME and MW-HS-SPME: The compounds which found in the aerial part and flower essential oil of *Perovskia abrotanoides* are listed in Table-1. Their relative contents were calculated in relation to the extracts.

α-Pinene (5.0 and 9.9 %), limonene (11.0 and 8.6 %), 1,8-cineole (6.9 and 13.8 %) and camphor (12.4 and 14.7 %) which isolated by HD method were the main components in aerial part and flower essential oils, respectively. The major components of aerial part and flower essential oils were 1,8-cineole (18.6 and 10.6 %), camphor (17.8 and 14.8 %), β-caryophyllene (7.1 and 8.2 %), α-hummulene (6.0 and 8.3 %) and epi-α-cadinol by SFME method. Arial part and flower essential oils mainly included α-pinene (10.6 and 20.1 %), camphnene (8.0 and 8.6 %), β-pinene (5.0 and 10.0 %), δ-2-cerene (11.4

TABLE-1
IDENTIFICATION OF CHEMICAL COMPONENTS IN THE ESSENTIAL OIL OF Perovskia abrotanoides (AERIAL PART AND FLOWER) WITH HD, SFME (500 W POWER OF MICROWAVE FOR 20 min EXTRACTION TIME), HS-SPME (PDMS FIBER AT 70 ºC FOR 5 min EXPOSURE TIME) AND MW-HS-SPME (PDMS FIBER IN 400 W POWER OF MICROWAVE FOR 5 min EXPOSURE TIME)

	Compd.	KI	Aerial part				Flower			
			HD	SFME	HS-SPME	MW-HS-SPME	HD	SFME	HS-SPME	MW-HS-SPME
1	α-Thujene	930	0.7	0.5	0.5	0.4	0.3		0.8	0.5
2	α-Pinene	939	5.0	3.9	10.6	7.8	9.9	3.3	20.1	13.8
3	Camphene	954	4.5	4.0	8.0	7.7	4.3	0.7	8.6	6.7
4	Sabinene	975	Trace	Trace	Trace	0.3	Trace	Trace	Trace	0.4
5	β-Pinene	979	5.2	4.0	5.0	5.1	3.9	0.9	10.0	8.0
6	δ-2-Crene	1002	7.6	6.2	11.4	13.2	5.5	2.2	9.7	10.1
7	α-Phellendrene	1003	Trace	Trace	Trace	Trace	1.5	Trace	0.5	0.6
8	α-Terpinene	1017	0.6	0.5	0.2	Trace	0.4	Trace	Trace	Trace
9	*ortho*-Cymene	1026	Trace	Trace	Trace	Trace	0.6	0.3	Trace	Trace
10	Limonene	1029	11.0	Trace	13.2	Trace	8.6	4.0	10.5	Trace
11	1,8-Cineole	1031	6.9	18.6	Trace	31.5	13.8	10.6	7.0	20.1
12	γ-Terpinene	1060	1.0	0.8	0.3	1.1	0.8	Trace	1.2	2.4
13	*cis*-Sabinene hydrate	1070	Trace	0.7	Trace	0.1	0.2	Trace	0.1	1.0
14	Terpinolene	1089	1.7	1.2	0.6	1.5	0.9	0.2	1.9	3.8
15	Linalool	1097	Trace	Trace	Trace	Trace	0.2	Trace	Trace	1.1
16	*trans*-Thujone	1114	Trace	Trace	Trace	0.5	Trace	Trace	Trace	0.1
17	α-Campholenal	1126	Trace	Trace	Trace	0.1	Trace	Trace	Trace	
18	*allo*-Ocimene	1132	Trace	Trace	Trace	Trace	Trace	Trace	Trace	1.6
19	Sabinol	1143	0.5	Trace	Trace	Trace	Trace	Trace	Trace	
20	Camphor	1146	12.4	17.8	6.2	17.1	14.7	14.8	5.9	15.7
21	Borneol	1169	3.5	5.2	0.6	0.5	5.3	4.2	1.2	2.1
22	Terpinene-4-ol	1177	0.7	0.7	Trace	Trace	0.4	Trace	Trace	0.4
23	α-Terpineol	1189	0.8	1.0	Trace	Trace	1.5	0.3	0.1	0.2
24	Iso-Bornyl formate	1239	Trace	Trace	Trace	Trace	Trace	Trace	Trace	0.1
25	Linalyl acetate	1257	0.5	1.7	0.6	0.4	0.2	0.3	0.4	0.6
26	Bornyl acetate	1289	3.3	4.3	2.7	2.8	4.7	5.4	2.2	3.2
27	Thymol	1290	Trace	Trace	Trace	Trace	0.3	Trace	Trace	Trace
28	α-Terpinyl acetate	1349	4.7	4.9	2.7	0.9	4.6	6.3	2.0	1.2
29	α-Cubebene	1351	Trace	Trace	0.9	0.2	Trace	Trace	0.1	0.2
30	Neryl acetate	1362	Trace	Trace	Trace	Trace	Trace	Trace	Trace	0.1
31	α-Copaene	1377	0.6	0.8	1.8	0.4	0.2	0.2	0.6	0.4
32	α-Gurjunene	1410	1.5	1.8	4.0	0.7	0.4	0.7	1.6	0.6
33	β-Caryophyllene	1419	6.6	7.1	9.2	4.5	4.0	8.2	5.5	2.5
34	α-Humulene	1455	6.1	6.0	8.5	2.2	4.0	8.3	5.3	1.6
35	*cis*-Muurola-4(14),5-diene	1467	Trace	Trace	0.5	Trace	Trace	Trace	Trace	0.0
36	*trans*-Cadina-1(6),4-diene	1477	Trace	Trace	0.5	Trace	Trace	Trace	0.1	0.1
37	Germacrene D	1485	Trace	1.8	Trace	Trace	Trace	Trace	Trace	Trace
38	*epi*-Cubebol	1494	0.9	Trace	Trace	Trace	Trace	Trace	Trace	Trace
39	α-Muurolene	1500	Trace	Trace	0.7	Trace	Trace	0.3	Trace	0.1
40	Bicyclogermacrene	1500	Trace	0.5	Trace	Trace	Trace	Trace	Trace	Trace
41	γ-Cadinene	1514	2.5	1.6	2.9	0.2	1.0	3.3	1.8	0.2
42	δ-Cadinene	1523	3.3	1.3	3.4	0.1	0.9	3.1	1.3	0.2
43	*trans*-Calamenene	1529	Trace	Trace	Trace	Trace	Trace	0.7	Trace	Trace
44	Caryophyllene oxide	1583	Trace	Trace	Trace	Trace	0.4	1.1	Trace	Trace
45	1,10-di-*epi*-Cubenol	1619	1.2	0.5	0.2	Trace	0.7	2.4	Trace	Trace
46	*epi*-α-Cadinol	1640	4.5	1.6	1.2	Trace	4.5	11.6	0.7	Trace
	Total		97.5	98.9	96.4	99.1	98.3	93.3	99.0	99.5

and 9.7 %), limonene (13.2 and 10.5 %), β-caryophyllene (9.2 and 5.5 %) and α-humulene (8.5 and 5.3 %) isolated by HS-SPME and α-pinene (7.8 and 13.8 %), δ-2-cerene (13.2 and 10.1 %), 1,8-cineole (31.5 and 20.1 %) and camphor (17.1 and 15.7 %) isolated by MW-HS-SPME.

Conclusion

The obtained results from both aerial part and flower essential oils in this study are shown as following:

(1) The comparison of essential oil extracted by different methods showed the methods based on a microwave heating were more able to extract the oxygenated compounds (both mono and sesquiterpenes). For example, although the PDMS fiber, as a nonpolar extraction phase, was used for both solid-phase microextraction methods (HS-SPME and MW-HS-SPME), the percentages of oxygenated compound in HS-SPME assisted by microwave were more than HS-SPME heated by convection bath. Also the comparison of the

hydrodistillation and SFME methods showed the same results that described above, as shown in Fig. 8.

(2) Fig. 8 showed that the usage of PDMS fiber as a non-polar solid phase caused to increase the extraction the non-polar compounds (mono and sesquiterpenes) in comparative with HD and SFME methods.

(3) The main components in both aerial part and flower essential oils of *Perovskia abrotanoides* were almost the same.

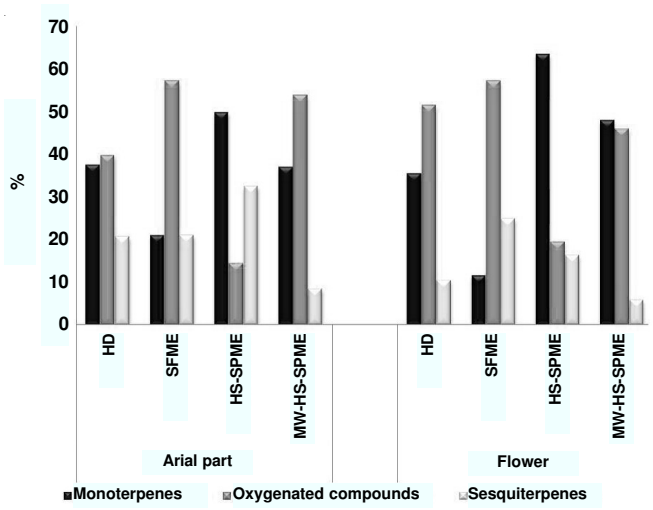

Fig. 8. Variation in the composition of aerial part and flower of *Perovskia abrotanoides* essential oils with respect to different extraction methods (HD, SFME, HS-SPME and MW-HS-SPME)

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