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Chemical composition of the volatile fraction of *Perovskia abrotanoides* and *Nepeta glomerulosa* growing wild in Iran by different extraction methods

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ABSTRACT

Background & Aim: Essential oils are isolated from different sections of plants such as flowers, seeds, leaves, stems and roots. Essential oils are applied extensively in producing of perfumes, cosmetics, foods, beverages and confectioneries and as ingredients of disinfectants and insecticides.

Experimental: Essential oil are obtained by several isolation methods, such as hydro-distillation (HD), steam distillation (SD), solid phase micro extraction (SPME), microwave assisted distillation (MAD) and solvent free microwave extraction (SFME). Hydro-distillation, solvent free microwave extraction (SFME) and solid phase micro-extraction (SPME) methods has been applied to extraction of essential oil from *Perovskia abrotanoides* and *Nepeta glomerulosa* growing in Iran.

Results & Discussion: Camphor (15.2, 15.50 and 9.02%), 1,8-cineole (15.03, 14.21 and 9.87%) and §-carene (6.4, 7.19 and 9.78%) were the main compounds in the *Perovskia abrotanoides* essential oil, respectively. Geraniol (12.74%) and β-caryophyllene (10.05%) identified by hydro-distillation whereas carvacrol (70.52%) and β-caryophyllene (4.13%) identified by solvent free microwave extraction that were the main compounds in the *Nepeta glomerulosa* essential oil.

Industrial and practical recommendations: Hence, it can be High amount of sesquiterpenes and low quantity of hydrocarbon monoterpenes were identified in each essential oil.

1. Introduction

Essential oils are isolated from different sections of plants such as flowers, seeds, leaves, stems and roots. Essential oils are applied extensively in producing of perfumes, cosmetics, foods, beverages and confectioneries and as ingredients of disinfectants and insecticides. Essential oil are obtained by several isolation methods, such as hydrodistillation (HD), steam distillation (SD), solid phase micro extraction (SPME), microwave assisted distillation (MAD) and solvent free microwave extraction (SFME) (Aberoomand, 2013). Hydrodistillation has been

typical method to extract the essential oils from herbal plants. In order to optimize extraction yield operation time, costs and solvent depletion should be consider. So study on another method and their effects on the percentage composition of essential oil is necessary (Golmakani and Rezaei, 2008). Solid phase micro extraction is sample preparation methods in this method extraction and sample preparation. In this method, a fused silica fiber that coated with a liquid (polymer) or a solid (sorbent), is used for submerging into an aqueous solution sample or the headspace above the sample. Analytes which extracted by fiber are desorbed from the coated fiber to a chromatography column. Solid phase micro extraction has some advantages such as solvent less, fast sampling, low cost and sensitivity (Ruiz *et al*., 2003). Microwave usage for extraction is a research field in modern chemistry. Microwave-assisted extraction is appropriate alternative to conventional techniques. The basic advantage of using microwave energy is decreasing of extraction time and cost, faster energy transfer and deletion of process steps. Solvent free microwave extraction is performed out at atmospheric pressure and no need to add any water and solvent. In this method, herbal plant is put in microwave oven for internal heating of water within fresh plant or moisturized. Therefore essential oil is released and evaporated by the in situ water. The distillate condensed by the external cooling system (Lucchesi *et al*., 2004; Lucchesi *et al*., 2007) The genus *Nepeta* (Lamiaceae) with the common Persian name of pune-say comprises about 280 species in the world and 67 species that are found all over of Iran (Mozaffarian, 1996). Some species are applied in folk medicine as a fortifier, disinfectant, bacteriostatic and treating eczema type disorders (Rustaiyan and Nadji, 1999; Aberoomand, 2013). *Perovskia* genus, Lamiaceae family has seven species out of which three species such as *P. abrotanoides*, *P. atriplicifolia* and *P. artemisoides* grow in Iran (Mozaffarian, 1996). *P. abrotanoides* with vernacular name of Brazambal, Domou, and Gevereh is a perennial herb growing wild in Iran (Mozaffarian, 1996). Some of the pharmacological effects of plant such as leishmanicidal, antiplasmodial and cytotoxic activity as well as antinociceptive and antiinflammatory have been confirmed (Jaafari *et al*., 2007; Sairafianpour *et al*., 2001). In this work, analysis of the essential oils compounds of *Perovskia abrotanoides* and *Nepeta glomerulosa* were performed by GC and GC-MS. The essential oils were achieved with hydro distillation (HD), solid phase micro extraction (SPME) and solvent free microwave extraction (SFME) methods.

2. Materials and Methods

2.1. Plant material

Perovskia abrotanoides and *Nepeta glomerulosa* collected in July 2013, from the Bashm Area in Semnan Province. The voucher specimen of plants was kept in herbarium of science and research branch of Islamic Azad University. The aerial parts of each plant were dried in shade for one week.

2.2. Isolation methods

***2.2.1. Hydro-distillation*:** 100 g *of dried aerial part* of *Perovskia abrotanoides* and *Nepeta glomerulosa* were subjected to hydrodistillation using a Clevenger type apparatus for 4 h, separately each oil was dried on anhydrous sodium sulphate and kept in 4 ºC until further analysis.

2.2.2. Solvent free microwave extraction (SFME): A Milestone Microsynth microwave apparatus, 2450 MHz and ACTE0 sensor was applied. The maximum power was 1000 W. 35g of the air-dried of *Perovskia abrotanoides* and *Nepeta glomerulosa* was immersed in distilled water for 1 h due to moisturized of the plant. The moistened plant was heated by an optimize power 600 w and time 20 min. The extracted oils were dried on anhydrous sodium sulphate and stored in 4 ºC until to be analyzed.

2.2.3. Head space solid phase micro extraction (HS-SPME): 1.5 g of dried powdered plant tissue was placed in 20 mL SPME vial sealed with rubber septum caps which purchased from supelco (Bellefonte, USA). The vial was heated at 70 ºC for 15 min then a 65 µm polydimethyl siloxne divinyl benzene (PDMS-DVB) fiber (supelco, USA) was subjected to head space of sample for 15 min. Analytes desorption was performed at 250 ºC for 3 min in a splitless GC injector.

2.2.4. Gas chromatography: Analytical GC was carried out on a Shimadzu 15A gas chromatography equipped with a split/ splitless injector (250 ºC) and a flame ionization detector (250 ºC). DB-5 capillary column (30 m × 0.25 mm, film thickness 0.32 µm) and type of carrier gas was N2 (1 ml/min). The column

temperature was held at 60 ºC for 3 min and then heated to 250 ºC with a 6 ºC/min rate and held constant at 250 ºC for 5 min.

2.2.5. Gas chromatography-mass Spectrometry: GC-MS analyses were accomplished by Hewlett-Packard (HP-6890/ 5973) GC-MC system coupled with an HP-5MS column (30 m × 0.25 mm, film thickness 0.32 µm). The column temperature was as like as GC condition. Helium (99.999%) was used as carrier gas (1 mL/min) beside ionization energy in MS was 70 eV, mass range 40-300 amu and scan time was 1 s.

***2.2.6. Components identification*:** Identification of components was performed by comparison of their MS spectra and GC retention indices with those of authentic references (Adams, 2004) and Wiley 257 mass spectra database. The retention indices were figured out using homologous series of normal alkanes.

3. Results and discussion

The percentage composition of essential oil from *Perovskia abrotanoides* and *Nepeta glomerulosa* with three isolation methods is shown in table 1 and 2, respectively. As seen in table1 camphor (15.21, 15.50 and 9.02%), 1, 8-cineole (15.03, 14.21 and 9.87%) and §-Carene (6.47, 7.19 and 9.78%) were the main compounds in the volatile fraction of *Perovskia abrotanoides* that achieved by hydrodistillation, solvent free microwave extraction and headspace-solid phase micro extraction, respectively. The oils were obtained by these methods has high amount of sesquiterpenes and low quantity of hydrocarbon monoterpenes and the volatile fraction which extracted by SFME method has higher quantity of oxygenated compounds and lower amount of monoterpenes hydrocarbons than the essential oil extracted by HD method due to adsorption of microwave irradiation by the molecules which have dipolar moment such as oxygenated compounds. In *Nepeta glomerulosa*, geranial (12.74%) and □-caryophyllene (10.05%) were identified as the main constituents in the oil which obtained by hydrodistillation. Carvacrol (70.52%) and β-caryophyllene (4.13%) were characterized as the major components obtained by SFME that were the main compounds in the *Nepeta glomerulosa* essential oil. The oils were obtained by these methods has high amount of sesquiterpenes and low quantity of hydrocarbon monoterpenes. The essential oil obtained by SFME method has higher quantity of oxygenated compounds and lower amount of monoterpenes hydrocarbons than the essential oil extracted by HD method. Solvent free microwave extraction uses rapid heating of polar solvent (water), smaller quantity of water, time and energy consuming compared with traditional HD method which leads to reduction in decomposition of oxygenated compounds by thermal and hydrolytic effects (Lucchesi *et al.*, 2004).

Table1. Percentage composition of *Perovskia abrotanoides* volatile compounds extracted by three methods.

Compound	KI	HD (%)	SFME (%)	SPME (%)
Artemisia triene	930	0.25	-	0.35
Thujene<alpha->	930	0.48	0.36	0.85
Pinene<alpha->	939	4.20	4.23	7.57
Camphene	954	3.33	2.99	5.01
Pinene<beta->	979	2.48	2.17	3.50
Myrcene	991	2.71	2.46	5.39
Carene<delta->	1002	6.47	7.19	9.78
Terpinene<alpha->	1017	0.42	0.28	0.52
Cymene<ortho->	1026	-	0.48	0.78
Sylvestrene	1031	-	1.20	2.47
Cineole<1,8->	1031	15.03	14.21	9.87
Terpinene<gamma->	1060	0.88	0.69	1.04
Sabinene hydrate<cis->	1070	0.50	0.56	0.25
Isoterpinolene	1088	0.19	0.49	0.30
Terpinolene	1089	0.63	-	0.99
Linalool	1097	-	-	0.67
Campholenal<alpha>	1126	0.34	-	-
Camphor	1146	15.21	15.50	9.02
Isoborneol	1162	3.66	3.42	1.24
4-terpineol	1177	0.96	0.56	-
Terpineol<alpha->	1189	0.58	0.47	0.29
Linalyl acetate	1257	1.03	1.09	1.23
Isobornyl acetate	1286	2.45	2.51	1.99
Terpinyl acetate<alpha->	1349	2.90	3.11	2.53
Cubebene<alpha->	1351	0.62	0.56	0.85
Eugenol	1359	-	0.30	-
Copaene<alpha->	1377	1.33	1.11	1.84
Cubebene<beta->	1388	0.16	-	-
Gurjunene<alpha->	1410	2.47	2.23	3.09
Caryophyllene<beta->	1421	5.75	6.51	6.95
Copaene<beta->	1432	0.31	-	0.43
Caryophyllene<alpha->	1455	-	5.79	-
Humulene<alpha->	1455	4.89	-	5.68
Muurolene<gamma-	1480	0.64	0.58	0.84

Germacrene-D	1485	0.27	-	0.33
Muurola-4(14),5-diene<trans->	1494	0.49	0.45	0.49
Cadina-1,4-diene	1496	0.18	-	-
Muurolene<alpha->	1500	1.13	1.14	0.94
Cadinene<gamma->	1514	2.03	2.07	2.27
Cadinene<delta->	1523	3.19	3.37	3.10
Calacorene<alpha->	1546	0.40	0.43	0.43
Germacrene D-4-ol	1576	0.35	0.43	-
Humulene epoxide	1608	0.78	1.05	0.44
Cadinol<epi-alpha>	1640	1.86	2.71	1.50
Eudesmol<alpha->	1654	0.30	0.42	0.13
Cedranol<5-neo->	1685	1.86	2.61	1.45
Longifolol	1715	0.32	0.45	-
Drimenol	1767	0.31	0.43	-
Bisabolol<alpha->	1686	0.63	0.86	0.31
Total		94.9%	97.4%	96.8%

Table 2. Percentage composition of *Nepeta glomerulosa* volatile compounds extracted by three methods.

Compound	**KI**	**HD (%)**	**SFME (%)**
Octen-3-ol<1->	979	3.70	1.05
Octanone<3->	984	0.88	-
Octanol<3->	991	2.45	0.74
Butyl butanoate	995	5.94	3.04
Ocimene<cis->	1037	1.35	0.42
Fenchocamphorone<alpha->	1106	0.72	-
Octen-3-yl acetate<1->	1113	-	0.70
Terpineol<cis-beta->	1144	1.17	-
Borneol	1169	1.00	-
Dill ether	1187	0.88	-
Cresol<2-methoxy-para->	1190	2.39	0.61
Methylalpha-cyclo geranate	1198	0.96	-
Nerol	1230	4.62	0.71
Ocimenon<E->	1238	0.93	-
Limonol	1253	12.74	2.19
Bornyl acetate	1289	4.74	1.51
Carvacrol	1299	1.23	70.52
Eugenol	1353	1.57	3.01
Neryl acetate	1362	2.31	1.33
Geranyl acetate	1381	0.84	-
Caryophyllene<(E)->	1409	5.93	1.64
Humulene<alpha->	1455	0.87	-
Farnesene<trans-beta->	1457	0.83	-
Sesquisabinene	1460	1.13	-
Bisabolene<beta->	1506	0.78	0.99
Sesquiphellandrene<beta->	1523	2.62	0.76
Caryophellene<beta->	1583	10.05	4.13
Thujopsanone<3->	1655	1.28	-
Bisabolol<beta->	1675	1.19	-
Total		75.02%	93.37%

4. Conclusions

In this study, the essential oils of *Perovskia abrotanoides* and *Nepeta glomerulosa* were extracted by hydro distillation (HD), solvent free microwave extraction (SFME) and solid phase micro extraction (SPME) methods. Fifty nine compounds for the essential oil of *Perovskia abrotanoides* and thirty two compounds for the essential oil of *Nepeta glomerulosa* were identified by gas chromatography and gas chromatography-mass spectrometry. The essential oil obtained by SFME method has higher content of oxygenated compounds and smaller amount of monoterpenes hydrocarbons than the essential oil extracted by HD method. The oxygenated compounds are very odoriferous and more important than monoterpenes hydrocarbons. Compared to many extraction methods SFME is simple, fast, green and solvent free for extraction of essential oil from material plants.

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