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SHORT REPORT

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Chemical composition analysis of the essential oil of *Mentha piperita* L. from Kermanshah, Iran by hydrodistillation and HS/SPME methods

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Abstract

Background: The volatile constituents from the aerial parts of *Mentha piperita* L. (peppermint) which were collected from cultivate growing plants in Kermanshah (Garreban; at the east of Kermanshah City) of Iran were extracted by hydrodistillation and headspace/solid-phase micro-extraction (HS/SPME) methods and were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Findings: A total of 39 and 41 compounds were identified in the essential oil by hydrodistillation and HS/SPME methods, respectively. The main components in the hydrodistillation method were menthol (45.34%), menthone (16.04%), menthofuran (8.91%), cis-carane (8.70%), 1,8-cineole (4.46%), neo-menthol (4.24%), and limonene (2.22%). The main components in the HS/SPME method were menthol (29.38%), menthone (16.88%), cis-carane (14.39%), menthofuran (11.38%), 1,8-cineole (9.45%), trans-caryophyllene (2.76%), neo-menthol (2.37%), β-Pinene (2.26%), α-Pinene (1.55%), germacrene-D (1.41%), trans-sabinene hydrate (1.28%), and neoisomenthyl acetate (1.02%).

Conclusions: The medicinal herb *Mentha piperita* L. (peppermint) would be changed during hydrodistillation method by chemical changes on the main essential oil of this herb before heat effects in presence of water vapor matrix (WVM).

Keywords: *Mentha piperita* L., Essential oil compounds, Hydrodistillation, HS/SPME method, Water vapor matrix (WVM), Medicinal herbs, Gas chromatography, GC-mass spectroscopy

Introduction

The *Mentha piperita* L. (peppermint) sample used in this study was collected from the west of Iran (Garreban, east of Kermanshah City, Kermanshah Province, Iran). A voucher specimen has been deposited in the Razi University, Research Center of Agriculture and Natural Resources, Kermanshah, Iran (Herbarium number: 2682). The local names of *Mentha piperita* L. is *Nana Felfeli* (NΛNΛ-FELFELI) in Iran. The aerial parts of this herb in crude or baked form was utilized. In terms of ancient medicine in Iran, folks in local medicine use of mint, including carminative, antiinflammatory, antispasmodic antiemetic, diaphoretic, analgesic, stimulant, emmenagogue, and anticatharrhal application. It is also used against nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints. Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes and for treating neuralgia, myalgia, headaches, and migraines (Foster 1990; Brown 1995; Bisset 1994; Tyler 1993; Baytop 1999; Hendriks 1998; Cowan 1999; Iscan et al. 2002; Kapp et al. 2013; McKay and Blumberg 2006; Peixoto et al. 2009; Rita and Animesh 2011; Saller 2004). The hydrodistillation method is one of the famous and routine methods to extract the essential oil of medicinal herbs. This is also the official standard method for extracting essential oils for quality control.

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Solid-phase micro-extraction (SPME) is a sampling technique based on the absorption of analysts on or into a polymeric material that coats a silica fiber. Recent advances in the development of analytical methods based on headspace/solid-phase micro-extraction (HS/SPME) of natural aroma compounds have been reviewed, with a special emphasis on increasing reproducibility (Barié et al. 2006; Cimato et al. 2006; Johnson et al. 2004; Stashenko and Martínez 2007; Taherpour et al. 2012; Taherpour et al. 2015). A simple, rapid, efficient, and inexpensive method for the determination of essential oil in different samples is headspace-solid phase micro-extraction/gas chromatography-mass spectrometry (HS/SPME-GC/MS). HS/SPME-GC/MS has been used as an alternative method to determine the essential oils in analytical samples and may be a potential tool for the quality assessment of medicinal herbs like *Mentha piperita* L (Barié et al. 2006; Cimato et al. 2006; Johnson et al. 2004; Stashenko and Martínez 2007; Taherpour et al. 2012; Taherpour et al. 2015). Compared with other volatile essential oil extraction studies, the results obtained by HS/SPME-GC/MS have shown high performance in other determining methods of the volatile constituents of the essential oil of *Mentha piperita* L. (Rohloff 1999; Moradi and Najafian 2015).

Materials and analytical method

Dried aerial parts of *Mentha piperita* L. were subjected to hydrodistillation and HS/SPME methods (headspace/solid-phase micro-extraction). The conditions for the HS/SPME method was SPME fiber (polydimethylsiloxane (PDMS) 100 μm, thickness (SUPELCO), sample weight 1.0 g, extraction temperature 60 °C; extraction time 20 min; sonication time 10 min, desorption time in injector port of GC-mass 3 min) to produce a yellow oil in 0.42% (w/w) yield. The essential oil of the aerial parts *Mentha piperita* L. (peppermint) for the hydrodistillation and HS/SPME methods were examined by GC/ MS (GC Agilent 6890N; MS: Agilent 5973) with an HP5-MS column (30 m × 0.25 mm fused silica capillary column, film thickness 0.25 μm). The temperature program ranged from 50 °C (5 min)–240 °C (2 min) at an increase rate of 5 °C/min (injection temperature 250 °C, carrier gas: helium (with purity 99.999%). The flow rate was 0.9 ml/min. The detector temperature was at 180 °C, the ionization energy in mass was 70 eV, the mass range was 10–300 amu, and the scan time was 1 s (Adams 1995).

The list of identified components is presented in Table 1. The constituents were identified by comparing their MS spectra with those in a computer library or with authentic compounds (similarity index). In the aerial parts of cultivate growing plants *Mentha piperita* L. (peppermint), the main identified components were (hydrodistillation) menthol (45.34%), menthone (16.04%), menthofuran (8.91%), *cis*-carane (8.70%), 1,8-cineole (4.46%), neo-menthol (4.24%), limonene (2.22%), and the main components were (HS/SPME) menthol (29.38%), menthone (16.88%), *cis*-carane (14.39%), menthofuran (11.38%), 1,8-cineole (9.45%), trans-caryophyllene (2.76%), neo-menthol (2.37%), β-Pinene (2.26%), α-Pinene (1.55%), germacrene-D (1.41%), trans-sabinene hydrate (1.28%), neoisomenthyl acetate (1.02%).

Results and discussion

In 1999, Monoterpene compounds of leaf pairs and flowers of *Mentha piperita* (Trondheim, Norway) were studied with direct headspace sampling using solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME-GC/MS) by Rohloff (Rohloff 1999). In accordance with the reported results, the content of peppermint-characteristic compounds such as menthol, menthyl acetate, and neomenthol increased in a basipetal direction (older plant parts), whereas menthone and isomenthone showed higher levels in the acropetal direction (younger plant parts) (Rohloff 1999). Higher levels of menthofuran had been found in peppermint flowers in contrast to the leaves (Rohloff 1999). The SPME sampling method resulted in relatively higher amounts of high-volatile monoterpenes and lower detection of less volatile compounds such as menthol and menthone, compared to solvent-based samples from essential oil distillation (Rohloff 1999).

In 2015, the aerial parts of *Mentha piperita* L. (Gachsaran in Kohgiluyeh and Boyer-Ahmad Province in Iran) were subjected to headspace and hydrodistillation techniques after drying, then headspace volatiles and the essential oil were analyzed by GC/MS (Moradi and Najafian 2015). The constituents were identified in hydro-distillation and CombiPAL system (Moradi and Najafian 2015). In the reported results, some of the main identified components in the applied *hydro-distillation* method were menthone (34.86%), iso-menthone (7.99%), mentofuran (5.11%), menthol (23.98), and germacrene D (2.20%) (Moradi and Najafian 2015) and also, some of the main identified compounds by applying the headspace method were α-pinene (8.06%), β-pinene (7.67%), myrcene (2.24%), limonene (13.36%), sabinene (5.03%), 1,8-cineole (17.85%), menthone (18.75%), iso-menthone (4.63%), and mentofuran (3.98%) (Moradi and Najafian 2015).

In this study, a total of 39 and 41 compounds were identified in the essential oil of *Mentha piperita* L. by hydrodistillation and HS/SPME methods, respectively. See Table 1. The main components higher than 1% in the hydrodistillation methods were menthol (45.34%), menthone (16.04%), menthofuran (8.91%), *cis*-carane (8.70%), 1,8-cineole (4.46%), neo-menthol (4.24%), and limonene (2.22%). The main components higher than

Table 1 Essential oil constituents of *Mentha piperita* L. by HS/SPME and hydrodistillation methods of this study and the comparison of this study results (hydrodistillation and HS/SPME methods) with the previous studies (Ref. (Rohloff 1999) and (Moradi & Najafian 2015)) in this plant

No.	HS/SPME method			Hydrodistillation method			Results of other studies		
	Retention time (min)	Compound	%	Retention time (min)	Compound	%	Ref. (Rohloff 1999) SPME	Ref. (Moradi and Najafian 2015)	
								Hydro D.	HS
1	5.171	2-Hexenal	0.08	5.171	2-Hexenal	0.16	–	–	–
2	7.286	α-Pinene	1.55	7.286	α-Pinene	0.79	4.12	0.85	8.06
3	8.749	β-Pinene	2.26	7.645	Camphene	0.02	5.14	1.29	7.67
4	9.315	Myrcene	0.34	8.531	Sabinene	0.42	–	–	–
5	9.812	l-Phellandrene	0.15	8.749	β-Pinene	0.97	–	–	–
6	10.383	α-Terpinene	0.10	9.315	Myrcene	0.18	–	–	–
7	10.578	p-Cymene	0.03	9.56	3-Octanol	0.14	–	–	–
8	10.978	1,8-cineole	9.45	9.812	l-Phellandrene	0.06	20.29		17.85
9	11.601	**Δ**-3 Carene	0.07	10.383	α-Terpinene	0.29	–	–	–
10	12.047	γ-Terpinene	0.25	10.578	p-Cymene	0.09	–	–	–
11	12.595	trans-sabinene hydrate	1.28	10.692	Dl-limonene	2.22	9.32	3.11	13.36
12	13.264	Terpinolen	0.12	10.978	1,8-cineole	4.46	–	6.30	–
13	14.15	iso-amyl isovalerat	0.44	11.601	**Δ**-3 Carene	0.18	–[a]	–[a]	–[a]
14	15.093	Neo-allo-ocimene	0.03	12.047	γ-Terpinene	0.47	–	–	–
15	16.785	L-menthone	16.88	12.595	*trans*-sabinene hydrate	0.61	16.08	–	18.75
16	17.036	Menthofuran	11.38	13.264	Terpinolen	0.14	3.33	–	3.98
17	17.179	Neo-menthol	2.37	14.15	iso-amyl isovalerate	0.15	–[a]	–[a]	–[a]-
18	18.242	L-menthol	29.38	14.464	Thujone	0.01	16.65	–	9.28
19	20.94	Piperitone	0.46	16.785	L-menthone	16.04	–	34.86	–
20	21.694	3-menthene	0.92	17.036	Menthofuran	8.91	–	5.11	–
21	22.883	*cis*-carane	14.39	17.179	Neo-menthol	4.24	–	23.98	–
22	23.231	Neoisomenthyl acetate	1.02	18.242	L-menthol	45.34	–	–	–
23	24.226	Bicycloelemene	0.16	20.025	Pulegone	0.80	–	–	–
24	25.472	α-Farnesene	0.01	20.94	Piperitone	0.61	–	–	–
25	25.643	Ylangene	0.01	21.694	3-menthene	0.42	–	–	–
26	25.849	Copaene	0.09	22.883	*cis*-carane	8.70	–	–	–
27	26.278	β-Bourbonene	0.96	23.231	Neoisomenthyl acetate	0.51	–	–	–
28	26.449	β-Cubebene	0.05	23.157	*trans*-carane	0.18	–		–
29	26.586	β- Elemene	0.12	26.278	β-Bourbonene	0.21	–	–	–
30	27.615	*trans*-Caryophyllene	2.76	26.586	β-Elemene	0.06	–	–	–
31	27.958	Calarene	0.04	27.615	*trans*-Caryophyllene	0.79	–	–	–
32	28.438	α-Humulene	0.19	28.524	*trans*-β-Farnesene	0.18	–	–	–
33	28.524	*trans*-β-Farnesene	0.57	29.073	Germacrene-D	0.82	–	–	–
34	29.073	Germacrene-D	1.41	29.33	Bicyclogermacrene	0.15	–	–	–
35	29.33	Bicyclogermacrene	0.33	29.775	δ-Cadinene	0.03	–	–	–
36	29.775	δ-Cadinene	0.10	30.701	Caryophyllene oxide	0.05	–	–	–
37	30.016	α-Muurolene	0.03	30.861	Viridiflorol	0.23	–	–	–
38	30.701	Caryophyllene oxide	0.04	32.736	Eicosane	0.36	–	–	–
39	30.776	Hexadecane	0.03	33.456	Di-isobutyl phthalate	0.01	–	–	–

Table 1 Essential oil constituents of *Mentha piperita* L. by HS/SPME and hydrodistillation methods of this study and the comparison of this study results (hydrodistillation and HS/SPME methods) with the previous studies (Ref. (Rohloff 1999) and (Moradi & Najafian 2015)) in this plant *(Continued)*

40	30.861	Viridiflorol	0.15	–	–	–	–	–	–
41	33.456	Di-isobutyl phthalate	0.01	–	–	–	–	–	–
42	–	*iso*-menthone	–[a]	–[a]	–[a]	–	2.98	7.99	4.63

[a]The starred components (*iso*-amyl isovalerat and *iso*-menthone) were not found in the addressed studies

1% in the HS/SPME methods were menthol (29.38%), menthone (16.88%), *cis*-carane (14.39%), menthofuran (11.38%), 1,8-cineole (9.45%), trans-caryophyllene (2.76%), neo-menthol (2.37%), β-Pinene (2.26%), α-Pinene (1.55%), germacrene-D (1.41%), trans-sabinene hydrate (1.28%), and neoisomenthyl acetate (1.02%).

The results have demonstrated that the components in hydrodistillation and HS/SPME methods were different because of the reactions on the essential oil components of this herb under the high temperature of the water vapor matrix (WVM) in hydrodistillation method. The different components in the essential oils of *Mentha piperita* L. (peppermint) due to the heat effect (baked form of the herb in hydrodistillation method) has made different medicinal properties for this herb. So, the medicinal herb *Mentha piperita* L. (peppermint) would be changed during hydrodistillation method by chemical changes on the essential oil of this herb (before heat effects in presence of WVM). The comparison of Table 1 shows that the main identified components (higher than 5%) by HS/SPME method (without WVM effect) were menthol (29.38%), menthone (16.88%), *cis*-carane (14.39%), menthofuran (11.38%), 1,8-cineole (9.45%), and the main identified components (higher than 5%) by hydrodistillation (with WVM effect) were menthol (45.34%), menthone (16.04%), menthofuran (8.91%), and *cis*-carane (8.70%). The components menthol, menthone, and *cis*-carane (29.38, 16.88, and 14.39%, respectively, in the essential oil of this herb without WVM effect; extracted by HS/SPME method) have the main roles in the medicinal properties of the none baked *Mentha piperita* L.

The components menthol (45.34%), menthone (16.04%), menthofuran (8.91%), and *cis*-carane (8.70%) in the essential oil of this herb with the effect of WVM (extracted by hydrodistillation method) have the main roles in the medicinal properties of the baked *Mentha piperita* L. (peppermint). In addition, some of the important

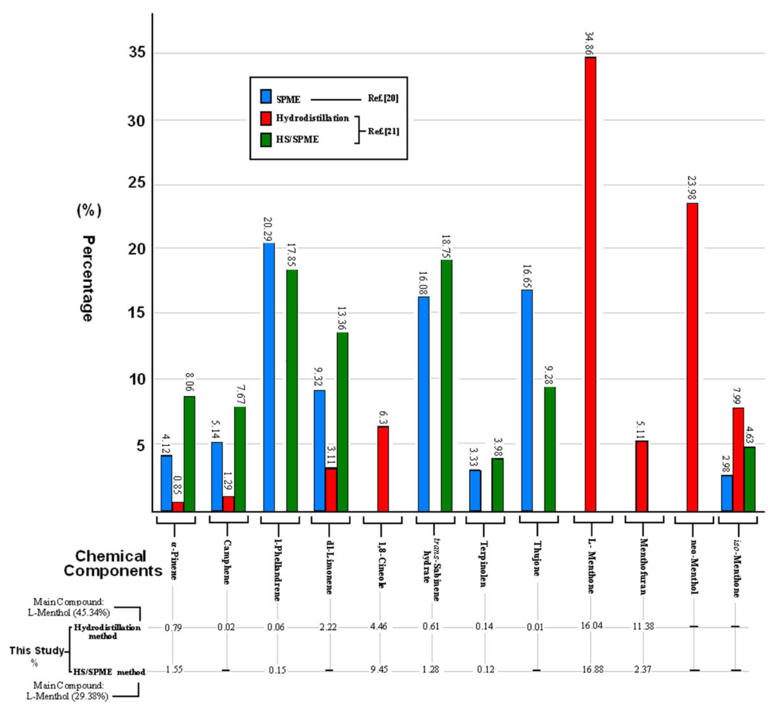

Fig. 1 The comparative diagram of the main essential oil constituents of *Mentha piperita* L. by HS/SPME and hydrodistillation methods of this study and the previous studies (Ref. (Rohloff 1999) and (Moradi and Najafian 2015)) in this plant

properties will appear in the essential oil of *Mentha piperita* L. (peppermint) under extraction conditions by hydrodistillation method and WVM effect because of the high percentage of menthol (45.34%). By comparing the results of the two methods (HS/SPME and hydrodistillation) on the essential oil components' high extent of menthol (29.38%), menthone (16.88%), *cis*-carane (14.39%), menthofuran (11.38%), 1,8-cineole (9.45%) and other obtained components, it is possible to predict the different medicinal properties of *Mentha piperita* L. in the backed form (by hydrodistillation method).

In Table 1 has shown the comparison between the results of this study on *Mentha piperita* L. (from Trondheim, Norway and Gachsaran, Kohgiluyeh and Boyer-Ahmad, Iran, respectively) by SPME, hydrodistillation, and headspace (CombiPAL system) methods and the reported results on *Mentha piperita* L. (Moradi and Najafian 2015; Adams 1995) for the main components. Figure 1 has shown the comparative diagram of the main essential oil constituents of *Mentha piperita* L. by HS/SPME and hydrodistillation methods of this study and the previous studies (Ref. (Rohloff 1999) and (Moradi and Najafian 2015)) in this plant. Figure 1 has shown the comparative diagram of the main essential oil constituents of *Mentha piperita* L. by HS/SPME and hydrodistillation methods of this study and with the previous studies (Ref. (Rohloff 1999) and (Moradi and Najafian 2015)) in this plant. The different medicinal properties of the essential oils of the herbs return to the differences of the chemical components. The results have demonstrated that the main component of *Mentha piperita* L. (peppermint) (this study) by hydrodistillation and HS/SPME methods is menthol with 45.34 and 29.38%, respectively, and while for *Mentha piperita* L. of the previous study (Rohloff 1999; Moradi and Najafian 2015) are 1,8-cineole (20.29%)(Rohloff 1999) and menthone (34.86%); by hydrodistillation and; by CombiPAL system (18.75%) (Moradi and Najafian 2015). In *Mentha piperita* L. (Rohloff 1999) and (Moradi and Najafian 2015) some of the components did not exist such as: *cis*-carane and *trans*-caryophyllene, and also in *Mentha piperita* L. (this study) some of the components like *iso*-menthone did not exist.

Conclusions

Mentha piperita L. (peppermint), the family Labiatae, was collected from the west of Iran (Kermanshah Province). It has been utilized as a medicinal herb for various purposes in local and traditional medicine by folks in Iran. Thirty nine (39) and 41 compounds in the essential oil by hydrodistillation and HS/SPME methods, respectively, were identified in the essential oil of *Mentha piperita* L. (peppermint) by GC and GC/MS techniques. In this herb, the main identified components (higher than 5%) by hydrodistillation were menthol (45.34%), menthone (16.04%), menthofuran (8.91%), *cis*-carane (8.70%), and the main identified components (higher than 5%) by HS/SPME method were menthol (29.38%), menthone (16.88%), *cis*-carane (14.39%), menthofuran (11.38%), and 1,8-cineole (9.45%). The different components in the essential oils of *Mentha piperita* L. (peppermint) due to the heat effect (baked form of the herb in hydrodistillation method) has made different medicinal properties for this herb. So, the medicinal herb *Mentha piperita* L. (peppermint) would be changed during hydrodistillation method by chemical changes on the main essential oil of this herb before heat effects in presence of water vapor matrix (WVM).

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Authors' contributions
AAT, Professor of Organic Chemistry-Ph.D.; SK, Ph.D. Candidate; AY, Ph.D. Candidate; SN, Ph.D. Candidate; MF, M.Sc. of Organic Chemistry; SG, M.Sc. of Organic Chemistry. All authors read and approved the final manuscript.

Competing interests
The authors declared that they have no competing interests.

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