International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2015 IJFAS Journal-2015-4-2/89-94/ 28 February, 2015
ISSN 2322-4134 ©2015 IJFAS

Comparative analysis of the aroma chemicals of *Mentha piperita* L. using hydrodistillation and CombiPAL system techniques

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ABSTRACT: Aerial parts of *Mentha piperita* L. (Peppermint) were subjected to headspace and hydro-distillation techniques after drying, then headspace volatiles and the essential oil were analyzed by GC/MS. 50 and 40 constituents were identified in hydro-distillation and CombiPAL system which represented 100% and 100% of the oils, respectively. *Hydro-distillation* method were β- pinene (1.29%), limonene (3.11%), 1,8-cineole (6.30%), cis- sabinene hydrate (1.85%), menthone (34.86%), iso-menthone (7.99%), mentofuran (5.11%), menthol (23.98), pulegone (1.99%), menthyl acetate (1.70 %), (E)- caryophyllene (1.95%), germacrene D (2.20%) and the main compounds by Headspace *techniques* were α-pinene (8.06%), β- pinene (7.67%), myrcene (2.24%), limonene (13.36%), sabinene(5.03%),1,8-cineole (17.85%), (Z)-β-ocimene (1.84%), cis-sabinene hydrate (1.49%), menthone (18.75%), iso-menthone (4.63%), mentofuran (3.98%) and menthol (9.28). The Headspace Technique is a new, rapid, simple and eco-friendly method for essential oil analysis of aromatic plants.

***Keywords*:** *Mentha piperita* L., CombiPAL system, Headspace, GC/MS, Volatile component

INTRODUCTION

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, and wood (Sanchez , 2010). Essential oils have been used for centuries in medicine, perfumery, and cosmetics, and have been added to foods as part of spices or herbs. Their initial application was in medicine, but in the nineteenth century, their use as aroma and flavor ingredients increased and became their major employment. Almost 3000 different essential oils are known and 300 are used commercially in the flavor and fragrances market (Burt, 2004). Essential oils are considered to be secondary metabolites and important for plant defense as they often possess antimicro- bial properties (Tajkarimi , 2010).

Species of Mentha genus are widespread except South America and Antarctic. Mentha spp. have been used as a folk remedy for treatment bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anticatharral activities (Gulluce, 2007).

Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin , 1998). *Mentha spicata* L. (spearmint) and *Mentha piperita* L. (peppermint) are commonly produced as a crop for their essential oils for food products, cosmetics and pharmaceuticals. Peppermint and spearmint also produce rosmarinic acid (RA), a naturally occurring and potent polyphenolic antioxidant, which plays a role in modulating inflammatory diseases including allergies, asthma and atherosclerosis (Makino , 2003; Inoue , 2005; Shixian , 2005) Techniques commonly used to extract the volatile

components include steam distillation, hydro distillation, dynamic and static headspace, supercritical fluid extraction and solvent extraction Headspace sampling for gas chromatographic analysis has many advantages. (Fakhraei 2005). The goal of this study was to investigate the composition of volatile components of *Mentha piperita* L. by HD and CombiPAL system (Headspace) Techniques from Iran.

MATERIALS AND METHODS

Plant material

Samples of *Mentha piperita* L. were collected from Basht city, near Gachsaran in Kohgiluyeh and Boyer-Ahmad Province in Iran. The plant specimen was identified by the herbarium of Fars Research Center for Agriculture and Natural Resources, Shiraz, Iran.

Hydro distillation essential oil extraction

The aerial parts were air-dried at ambient temperature in the shade. The dried samples of *M. piperita* were subjected to hydro-distillation using an all glass Clevenger-type apparatus for 3 hours, to extract essential oils, according to the method outlined by the European Pharmacopoeia (Anonymous 1997). The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) before gas chromatography-mass spectrometric (GC/MS) analysis (rowshan and najafian, 2012).

Headspace volatiles extraction

Up to 3 gr of each *M. piperita* dried sample was crushed and placed in 20 mL headspace vial, and immediately sealed with silicone rubber septa and aluminum caps. The vials were then transferred to the headspace tray. The headspace proceeded on the CombiPAL System which was provided with headspace auto-sampler, heater and agitator. The vial was heated up to 80° C and retained for 20 minutes while being agitated; the temperature of the sampling needle and transmission lines' temperature was 85° C. (rowshan and najafian, 2012).

Identification of the oil components by GC/MS

GC Analysis was carried out using an Agilent-technology chromatograph with HP-5 column (30m *0.32 mm i.d. x 0.25 pm). Oven temperature was performed as follows: 60° C to 210° C at 3°/min; 210° C to 240° C at 20 °/min and hold for 8.5 min, injector temperature 280° C; detector temperature, 290° C; carrier gas, N2 (1 ml/min); split ratio of 1:50. GC-MS analysis was carried out using a with Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30m x 0.25 mm i.d* 25pm) with He as the carrier gas and split ratio 1:50. Retention indices were determined using retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library data published mass spectra data (Adams, 2007; McLafferty, 1989; Joulain , 2001)

RESULTS AND DISCUSSION

GC/MS analysis resulted of samples extracted by HD and HS methods from *Mentha piperita* L. are presented in Table 1-2. In HS methods 40 compounds were identified in the essential oil which comprised 100.0% of that and 50 compounds comprising 100.0% of the HD method were identified. Hydro-distillation method were β- pinene (1.29%), limonene (3.11%), 1,8-cineole (6.30%), cis- sabinene hydrate (1.85%), menthone (34.86%), iso-menthone (7.99%), mentofuran (5.11%), menthol (23.98), pulegone (1.99%), menthyl acetate (1.70 %), (E)- caryophyllene (1.95%), germacrene D (2.20%) and the main compounds by CombiPAL techniques were α-pinene (8.06%), β- pinene (7.67%), myrcene (2.24%), limonene (13.36%), sabinene(5.03%),1,8-cineole (17.85%), (Z)-β-ocimene (1.84%), cis- sabinene hydrate (1.49%), menthone (18.75%), iso-menthone (4.63%), mentofuran (3.98%) and menthol (9.28). Our results showed that the number of components were different in these two methods. Many of the volatile compounds cannot always be recovered and often evaporate and also during the plants processing, Different chemical reactions take place so that its aroma no longer seems that of the actual plant and the product is different in composition that rarely happen in headspace technique (Handa , 2008). It could be concluded that HS could be the appropriate method for identifying lighter components

and the headspace technique is a new, rapid, simple and eco-friendly method for essential oil analysis of aromatic plants.

Table 1. Chemical composition of the essential oil of *Mentha piperita L.* by HD method

N	Compounds	RIa	Area,%
1	α-Thujene	*924.2*	0.05
2	α--Pinene	*931*	0.85
3	Camphene	*945.9*	0.03
4	Sabinene	*970.3*	0.75
5	β-Pinene	*974.5*	1.29
6	Myrcene	*987.8*	0.35
7	3-Octanol	*992.2*	0.20
8	α--Phellandrene	*1003*	0.03
9	α--Terpinene	*1014*	0.12
10	p-Cymene	*1022*	0.12
11	Limonene	*1026*	3.11
12	1,8-Cineole	*1029*	6.30
13	(Z)- β--Ocimene	*1034*	0.32
14	Benzene acetaldehyde	*1040*	0.07
15	(E)- β--Ocimene	*1044*	0.08
16	γ-Terpinene	*1055*	0.22
17	cis-Sabinene hydrate	*1064*	1.85
18	Terpinolene	*1086*	0.10
19	Linalool	*1098*	0.38
20	n-Amyl isovalerate	*1106*	0.06
21	allo-Ocimene	*1126*	0.02
22	Menthone	*1150*	34.86
23	iso-Menthone	*1163*	7.99
24	Menthofuran	*1164*	5.11
25	Menthol	*1175*	23.98
26	Terpinene-4-ol	*1177*	0.42
27	iso-Menthol	*1182*	0.29
28	α-Terpineol	*1188*	0.59
29	Myrtenol	*1194*	0.07
30	Pulegone	*1236*	1.99
31	Piperitone	*1251*	0.85
32	neo-Menthyl acetate	*1272*	0.08
33	Menthyl acetate	*1291*	1.70
34	iso-Menthyl acetate	*1305*	0.05
35	γ-Elemene	*1333*	0.04
36	α-Copaene	*1372*	0.02
37	β-Bourbonene	*1381*	0.17
38	β-Elemene	*1388*	0.14
39	(Z)-Jasmone	*1395*	0.03
40	(E)-Caryophyllene	*1415*	1.95
41	α-Humulene	*1449*	0.10
42	(E)-β-Farnesene	*1454*	0.27
43	Germacrene D	*1477*	2.20
44	Bicyclogermacrene	*1492*	0.26
45	γ-Cadinene	*1510*	0.01
46	δ-Cadinene	*1519*	0.04
47	Spathulenol	*1572*	0.01
48	Caryophyllene oxide	*1578*	0.03
49	Viridiflorol	*1587*	0.47
50	α-Cadinol	*1650*	0.04

RIa, retention indices

Table 2. Chemical composition of the essential oil of *Mentha piperita* L. by HS method

N	Compounds	RIa	Area,%
1	α-Thujene	*924.9*	0.53
2	α-Pinene	*932.2*	8.06
3	Camphene	*946.5*	0.18
4	Sabinene	*971.2*	5.03
5	β-Pinene	*975.6*	7.67
6	Myrcene	*988.8*	2.24
7	3-Octanol	*992.5*	0.35
8	α-Phellandrene	*1004*	0.11
9	α-Terpinene	*1015*	0.53
10	p-Cymene	*1022*	0.35
11	Limonene	*1027*	13.36
12	1,8-Cineole	*1030*	17.85
13	(Z)-β-Ocimene	*1034*	1.84
14	(E)-β-Ocimene	*1044*	0.42
15	γ-Terpinene	*1055*	0.67
16	cis-Sabinene hydrate	*1064*	1.49
17	Terpinolene	*1086*	0.24
18	Linalool	*1098*	0.23
19	n-Amyl isovalerate	*1106*	0.11
20	3-Octanol acetate	*1121*	0.03
21	allo-Ocimene	*1126*	0.07
22	Menthone	*1153*	18.75
23	iso-Menthone	*1160*	4.63
24	Menthofuran	*1161*	3.98
25	Menthol	*1171*	9.28
26	Terpinene-4-ol	*1175*	0.15
27	iso-Menthol	*1180*	0.16
28	α-Terpineol	*1188*	0.10
29	Pulegone	*1236*	0.09
30	Piperitone	*1251*	0.09
31	neo-Menthyl acetate	*1272*	0.01
32	Menthyl acetate	*1291*	0.25
33	iso-Menthyl acetate	*1305*	0.01
34	β-Bourbonene	*1381*	0.10
35	β-Elemene	*1389*	0.01
36	(E)-Caryophyllene	*1415*	0.61
37	α-Humulene	*1449*	0.01
38	(E)-β-Farnesene	*1454*	0.03
39	Germacrene D	*1477*	0.37
40	Bicyclogermacrene	*1492*	0.03

RIa, retention indices

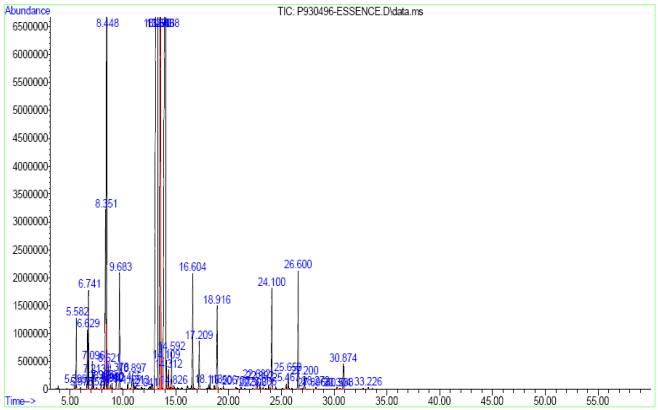

Figure 1. Chromatogram profile of *Mentha piperita* L. by HD method

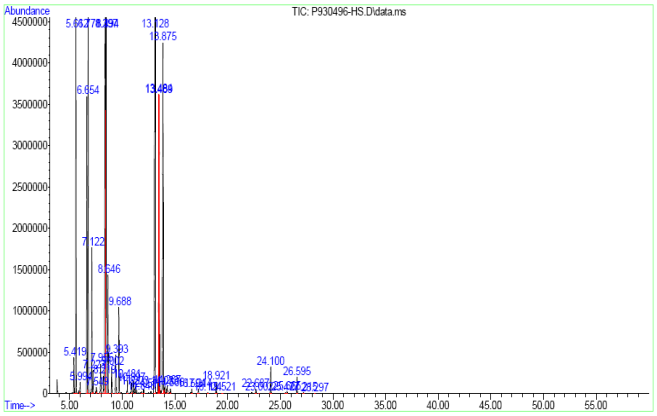

Figure 2. Chromatogram profile of *Mentha piperita* L. by HS method

Acknowledgment

First of all, I would like to thank the grace of God for completing this work and the author wish to thank to thank superintendent of Department of Natural Resources, Fars Research Center for Agriculture and Natural Resources Dr Vahid Rowshan for his kindness and providing the laboratory facilities.

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