



## Essential oil composition of Pennyroyal (*Mentha pulegium* L.) from Southern Iran

Ali Khalilipour, Mahmood Dejam\*

Department of Agriculture, Fasa Branch, Islamic Azad University, Fasa, Iran;

\*Email: [mahmooddejam@yahoo.com](mailto:mahmooddejam@yahoo.com)

### ARTICLE INFO

*Type:* Original Article

*Topic:* Phytochemistry

*Received* 05<sup>th</sup> April 2014

*Accepted* 14<sup>th</sup> June 2014

### Key words:

- ✓ *Mentha pulegium*
- ✓ *Volatile constituents*
- ✓ *Pulegone*
- ✓ *GC-MS*

### ABSTRACT

**Background & Aim:** Essential oil of pennyroyal (*Mentha pulegium* L.) has many applications in food, perfume and pharmaceutical industries. The aim of this study was to determine the content and chemical constituents of pennyroyal essential oil growing wild in Southern Iran.

**Experimental:** The aerial parts of the plant were collected around Hajiabad, Hormozgan province in 2013. The essential oil of dried aerial parts was extracted by hydro-distillation and analyzed using GC-MS.

**Results:** The oil yield of pennyroyal aerial parts was 0.6% (v/w). In total, 55 compounds were identified in the essential oil that oxygenated monoterpenes were dominant. The major oil constituents were pulegone (46.18%), piperitenone (19.56%), 1,8-cineole (4.55 %), and piperitenone oxide (4.23%).

**Recommended applications/industries:** *Mentha pulegium* is a good raw source of pulegone that has been widely used in food and drug industries.

### 1. Introduction

*Mentha* species belonging to the Lamiaceae family constitute one of the most popular essential oil crops (Lawrence, 2007). These species show considerable chemical diversity in the essential oil composition. Their essential oils contain a complex mixture of monoterpenoids that are extensively used in pharmaceutical, food, flavor, beverages and allied industries (Zargari, 1996; Lawrence, 2007). Pennyroyal (*Mentha pulegium* L.) is one of the *Mentha* species that grows wild in humid and damp areas and water banks of south Europe, north Africa and near east countries (Zargari, 1996). *Mentha pulegium* is used as antispasmodic, carminative, diaphoretic, sedative, stimulant, diuretic, antiseptic bronchitis and menstuate and externally for skin diseases in traditional medicine (Zargari, 1996; Akhondzadeh, 2002). Moreover, it has

been used as an insect repellent (Mkaddem *et al.*, 2007), spice and flavoring agent and fragrance in cosmetics (Akhondzadeh, 2002). The aerial parts of this plant contain a wide diversity of secondary metabolites such as tannins, resins, pectins, bitter principles and essential oils (Zargari, 1996; Lawrence, 2007). The essential oil of pennyroyal exhibits several biological properties particularly antibacterial (Mahboubi & Haghi, 2008; Derwich *et al.*, 2010; Boukhebt *et al.*, 2011), antifungal (Morteza-Semnani *et al.*, 2011), antioxidant (El-Ghorab, 2006; Teixeira *et al.*, 2012) and insecticidal (Zekri *et al.*, 2013) activities. The chemical composition of *M. pulegium* essential oil has been the subject of many studies (Chalchat *et al.*, 2000; Lorenzo *et al.*, 2002; Aghel *et al.*, 2004; Kokkini *et al.*, 2004; Agnihotri *et al.*, 2005; Stoyanova *et al.*, 2005; Mkaddem *et al.*, 2007; Hassanpouraghdam *et al.*, 2011; Shahmohamadi *et al.*, 2011; Hasniye *et al.*, 2012; Rodrigues *et al.*, 2013; Sardashti & Adhami, 2013) and

three chemotypes have been established, pulegone-type, piperitenone/piperitone-type and isomenthone/neoisomenthol-type (Kokkini *et al.*, 2004). Despite various reports from different regions of the world and some studies in north, west and east of Iran, there is no previous report on essential oil composition of *M. pulegium* from southern parts of Iran. Therefore, the aim of present study was chemical analysis of volatile constituents of *M. pulegium* from south of Iran.

## 2. Materials and Methods

### 2.1. Plant materials and essential oil extraction

Flowering aerial parts of *M. pulegium* L. were harvested from wild plants growing in a date palm orchard around Hajiabad (Hormozgan province, Iran) with latitude of 28° 19' N and longitude of 55° 54' E and 920 m above sea level in spring of 2013. After identification, a voucher specimen (IAUF-92-4) was deposited in the herbarium of Agriculture College of Islamic Azad University, Fasa Branch, Iran. The aerial parts were dried in shade and powdered using a grinder. The volatile oils were isolated by water distillation using a Clevenger type apparatus. Distillation was continued for about 2 h and then the oils were separated from aqueous layer and dried over anhydrous sodium sulfate. This procedure was repeated several times. Finally, the yield of extracted oil was expressed as v/w% of dry weight.

### 2.2. Essential oil analysis

The composition of essential oil was analyzed by GC and GC/MS. GC analysis was done on an HP7890 A series gas chromatograph (Agilent Technologies) equipped with Flame Ionization Detector (FID) and with a HP-5 silica capillary column (30 m × 0.25 mm ID, film thickness of 0.25 µm). The oven temperature was held at 60 °C for 5 min and then programmed from 60 to 210 °C at a rate of 3 °C /min and from 210 °C to 240 °C at 20 °C /min and held for 8.5 min. The carrier gas was helium at a flow rate of 1 ml/min. Injector and detector temperatures were 280 °C. GC/MS was performed by a HP5975A system (Agilent Technologies). An HP-5 MS capillary column (30 m × 0.25 mm ID, film thickness 0.25 µm) was directly coupled to mass spectrometer. The carrier gas was helium with a flow rate of 1 ml/min. The oven

**Table 1.** Chemical composition of the essential oil of *Mentha pulegium* L. from southern Iran.

No	Compounds <sup>a</sup>	RI <sup>b</sup>	%
1	Tricyclene	921	0.009
2	$\alpha$ -Thujene	924	0.058
3	$\alpha$ -Pinene	931	1.317
4	Camphene	946	0.365
5	Sabinene	970	0.664
6	$\beta$ -Pinene	974	1.685
7	Myrcene	988	0.482
8	3-Octanol	992	0.551
9	$\alpha$ -Phellandrene	1003	0.058
10	$\delta$ -3-Carene	1009	0.027
11	$\alpha$ -Terpinene	1014	0.122
12	<i>p</i> -Cymene	1022	0.328
13	Limonene	1026	2.294
14	1,8-Cineole	1029	<b>4.552</b>
15	(Z)- $\beta$ -Ocimene	1034	0.097
16	$\epsilon$ - $\beta$ -Ocimene	1044	0.031
17	$\gamma$ -Terpinene	1055	0.405
18	<i>cis</i> -Sabinene hydrate	1064	0.062
19	Terpinolene	1086	0.098
20	Linalool	1098	0.173
21	1-Octen-3-yl acetate	1109	0.057
22	3-Octanol acetate	1121	0.183
23	<i>trans</i> -Pinocarveol	1136	0.209
24	Camphor	1141	0.108
25	Menthone	1150	0.319
26	<i>iso</i> -Menthone	1161	0.848
27	Borneol	1163	0.953
28	$\alpha$ -Terpineol	1189	0.155
29	Myrtenal	1193	0.129
30	Pulegone	1237	<b>46.178</b>
31	Piperitone	1252	0.458
32	Bornyl acetate	1283	0.272
33	Thymol	1295	0.184
34	Carvacrol	1303	2.139
35	Piperitenone	1342	<b>19.558</b>
36	Piperitenone oxide	1365	<b>4.230</b>
37	$\alpha$ -Copaene	1372	0.043
38	$\beta$ -Bourbonene	1381	0.149
39	4a- $\alpha$ ,7- $\beta$ ,7a- $\alpha$ -Nepeta lactone	1397	0.313
40	$\beta$ -Caryophyllene	1416	2.325
41	$\alpha$ -Humulene	1449	0.353
42	(E)- $\beta$ -Farnesene	1453	0.213
43	Germacrene D	1476	0.302
44	(E)- $\beta$ -Ionone	1482	0.041
45	Phenyl ethyl 3-methyl butanoate	1488	0.042
46	Bicyclogermacrene	1492	0.17
47	$\gamma$ -Cadinene	1509	0.033
48	$\delta$ -Cadinene	1519	0.067
49	(E)- $\gamma$ -Bisabolene	1539	0.043
50	(E)-Nerolidol	1560	0.042
51	Spathulenol	1573	0.451
52	Caryophyllene oxide	1579	2.457
53	Salvial-4(14)-en-1-one	1589	0.037
54	Humulene epoxide II	1604	0.2
55	Dill apiol	1620	0.129

temperature program was the same as GC conditions. The samples were injected manually with a split ratio of 1:50. For GC/MS detection, an electron ionization system with ionization energy of 70 eV and mass range of 50-480 m/z was used.

### 2.3. Identification and quantification of components

Chemstation software was used for handling of mass spectra and chromatograms. Retention indices were calculated by using retention times of *n*-alkanes (C<sub>9</sub>-C<sub>18</sub>) which were injected at the same chromatographic conditions. The identification of compounds was made by comparison of their mass spectra and relative retention indices either with those given in the literature or with authentic samples (Adams, 2007). For quantification, the relative area percentage obtained for each constituent from GC-FID analysis of the oil was used to calculate the mean values without using correction factors.

## 3. Results and discussion

In this investigation, the essential oil yield of pennyroyal aerial parts was 0.6 % (v/w) based on dry weight. The essential oil yield in this study is similar to that obtained by Aghel *et al.* (2004), although higher (Lorenzo *et al.*, 2002; Boukhebt *et al.*, 2011; Hassanpouraghdam *et al.*, 2011; Hasniye *et al.*, 2012; Zeki *et al.*, 2013) and lower (Chalchat *et al.*, 2000; Mahboubi & Haghi, 2008) oil yields have also been reported for wild *M. pulegium*. These differences in oil yield of *M. pulegium* may be due to different geographical origins, climates, growth stages and/or extraction procedures.

**Table 2.** Main classes and subclasses of *Mentha pulegium* L. volatile oil components from southern Iran

Class and subclass of compound	%
Monoterpenes	88.88
monoterpene hydrocarbons	8.10
oxygenated monoterpenes	80.78
Sesquiterpenes	7.06
sesquiterpene hydrocarbons	3.70
oxygenated sesquiterpenes	3.36
Others	0.83
Total identified	96.77

GC-MS analyses of hydro-distilled essential oil of *M. pulegium* allowed the identification of 55

compounds representing 96.77% of the total oil (Table 1).

The chromatographic analysis of extracted volatile oil of this plant showed a complex mixture consisting mainly of monoterpenes. It was dominated by oxygenated monoterpenes (80.78%), while sesquiterpenes were only present in small percentage (Table 2). The major compounds of pennyroyal essential oil in this study were pulegone (46.18%), piperitenone (19.56%), 1,8-cineole (4.55 %), piperitenone oxide (4.23%), caryophyllene oxide (2.45%),  $\beta$ -caryophyllene (2.33%), limonene (2.29%), carvacrol (2.14%),  $\beta$ -pinene (1.69%) and  $\alpha$ -pinene (1.32 %) (Table 1). The most similar composition to the results of present investigation is the report of Stoyanova *et al.* (2005) from Bulgaria that pulegone (42.9% - 45.4%) and piperitenone (21.7% - 23.1%) were major oil constituents of *M. pulegium*. In addition, similar to the results of this study, pulegone (> 40%) has been the main component of pennyroyal essential oil from Iran (Morteza-Semnani *et al.*, 2011; Shahmohamadi *et al.*, 2011; Sardashti & Adhami, 2013) and other countries (Lorenzo *et al.*, 2002; Agnihotri *et al.*, 2005; El-Ghorab, 2006; Mkaddem *et al.*, 2007; Rodrigues *et al.*, 2013; Zekri *et al.*, 2013), although there are great variation in other oil constituents of *M. pulegium* among these reports. While it has been reported that menthone (15.1% - 38.7%) is one of the major oil components of Iranian *M. pulegium* (Aghel *et al.*, 2004; Hassanpouraghdam *et al.*, 2011; Morteza-Semnani *et al.*, 2011; Shahmohamadi *et al.*, 2011), the amount of this compound was very low (0.32%) in present study. In total, great quantitative and qualitative variations in volatile composition of *M. pulegium* were seen between this and other studies. These variations may be due to the influence of geographical differences, environmental conditions, physiological differences, different extraction and analytical procedures and genetic factors (Kokkini *et al.*, 2004; Hassanpouraghdam *et al.*, 2011). Moreover, since major oil constituents such as pulegone, menthone, isomenthone, piperitone and piperitenone have a common biosynthetic pathway (Davis *et al.*, 2005); therefore, the existence of different chemotypes is a common feature in *Mentha* species and hybrids.

## 4. Conclusion

The major constituents of *M. pulegium* essential oil from southern Iran were pulegone (46.18 %) and piperitenone (19.56 %). The oil composition in this study was different with most of previous reports. These variations reflect the influence of different geographical origins, climatic conditions, physiological and biochemical states of plants and many other factors.

## 5. References

- Adams, R.P. 2007. *Identification of essential oil components by gas chromatography/mass spectrometry*. 4<sup>th</sup> ed. Allured Publ., Carol Stream, IL.
- Aghel, N., Yamini, Y., Hajiakhoondi, A. and Pourmortazavi, S.M. 2004. Supercritical carbon dioxide extraction of *Mentha pulegium* L. essential oil. *Talanta*, 62: 407-411.
- Agnihotri, V.K., Agarwal, S.G., Dhar, P.L., Thappa, R.K., Kapahi, B., Saxena, R.K. and Qazi, G.N. 2005. Essential oil composition of *Mentha pulegium* L. growing wild in the north-western Himalayas, India. *Flavour and Fragrance Journal*, 20: 607-610.
- Akhondzadeh, S. 2002. *Encyclopedia of Iranian Medicinal Plants*. Arjmand Publication, Tehran, Iran. (In Persian)
- Boukhebt, H., Chaker, A.N., Belhadj, H., Sahli, F., Ramdhani, M., Laouer, H. and Harzallah, D. 2011. Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils. *Der Pharmacia Letters*, 3: 267-275.
- Chalchat, J., Gorunovic, M.S., Maksimovic, Z.A. and Petrovic, S.D. 2000. Essential oil of wild growing *Mentha pulegium* L. from Yugoslavia. *Journal of Essential Oil Research*, 12: 598-600.
- Davis, E.M., Ringer, K.L., Mc Conkey, M.E. and Croteau, R. 2005. Monoterpene metabolism: Cloning, expression and characterization of menthone reductase from peppermint. *Plant Physiology*, 37: 873-881.
- Derwich, E., Benziane, Z. and Boukir, A. 2010. GC/MS analysis and antibacterial activity of the essential oil of *Mentha pulegium*, *Journal of Agriculture and Biological Sciences*, 6: 191-198.
- El-Ghorab, A.H. 2006. The chemical composition of *Mentha pulegium* L. essential oil from Egypt and its antioxidant activity. *Journal of Essential Oil Bearing Plants*, 9: 183-195.
- Hasniye, Y., Onar, H.C. and Yusufoglu, A.S. 2012. Chemical composition of the essential oil of *Mentha pulegium* L. from Bodrum, Turkey. *Journal of Essential Oil Bearing Plants*, 15: 1040-1043.
- Hassanpouraghdam, M.B., Akhgari, A.B., Aazami, M.A. and Emarat-Pardaz, J. 2011. New menthone type of *Mentha pulegium* L. volatile oil from Northwest Iran. *Czech Journal of Food Sciences*, 29: 285-290.
- Kokkini, S., Hanlidou, E., Karousou, R. and Lanaras, T. 2004. Clinal variation of *Mentha pulegium* essential oils along the climatic gradient of Greece. *Journal of Essential Oil Research*, 16: 588-593.
- Lawrence, B.M. 2007. *Mints: The Genus Mentha*, CRC Press, Boca Raton, FL, 170 pp.
- Lorenzo, D., Paz, D., Dellacassa, E., Davies, P., Vila, R. and Calgueral, S. 2002. Essential oil of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay. *Brazilian Archives of Biology and Technology*, 45: 519-524.
- Mahboubi, M. and Haghi, G. 2008. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *Journal of Ethnopharmacology*, 119: 325-327.
- Mkaddem, M., Boussaid, M. and Ben Fadhel, N. 2007. Variability of volatiles in Tunisian *Mentha pulegium* L. (Lamiaceae). *Journal of Essential Oil Research*, 19: 211-214.
- Morteza-Semnani, K., Saeedi, M. and Akbarzadeh, M. 2011. Chemical composition and antimicrobial activity of essential oil of *Mentha pulegium* L. *Journal of Essential Oil Bearing Plants*, 14: 208-213.
- Rodrigues, L., Pova, O., Teixeira, G., Figueiredo, A.C., Moldao, M. and Monteiro, A. 2013. Trichomes micromorphology and essential oil variation at different developmental stages of cultivated and wild growing *Mentha pulegium* L. populations from Portugal. *Industrial Crops and Products*, 43: 692-700.
- Sardashti, A. and Adhami, Y. 2013. Chemical composition of the essential oil of *Mentha pulegium* L. from Taftan area by means of gas chromatography/mass spectrometry (GC/MS). *Journal of Medicinal Plant Research*, 7: 3003-3007.

- Shahmohamadi, R., Sariri, R., Rasa, M., Ghafoori, H., Aghamol, M., Nasuti, S. and Tahery, M. 2011. Chemical composition and antimicrobial activity of flowering aerial parts of *Mentha pulegium* from Gilan. *Pharmacology Online.*, 3: 651-659.
- Stoyanova, A., Georgiev, E., Kula, J. and Majda, T. 2005. Chemical composition of the essential oil of *Mentha pulegium* L. from Bulgaria. *Journal of Essential Oil Research.*, 17: 475-476.
- Teixeria, B., Marques, A., Ramos, C., Batista, I., Serrano, C., Matos, O., Neng, N.R., Nogueira, J.M.F., Saraiva, J.A. and Nunes, M.L. 2012. European pennyroyal (*Mentha pulegium*) from Portugal: chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. *Industrial Crops and Products.*, 36: 81-87.
- Zargari, A. 1996. *Medicinal Plants*, Tehran University Publication, Iran. (In Persian)
- Zekri, N., Amalich, S., Boughdad, A., El Belghiti, M.A. and Zair, T. 2013. Phytochemical study and insecticidal activity of *Mentha pulegium* L. oils from Morocco against *Sitophilus oryzae*. *Mediterranean Journal of Chemistry.*, 2: 607-619.