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

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**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 wildly 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 menstruate 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; Boukhebti *et al*., 2011), antifungal (Morteza-Semnani *et al*., 2011), antioxidant (El-Ghorab, 2006; Teixeria *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, pulegune-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 [a] | RI [b] | % |
|---|---|---|---|
| 1 | Tricyclene | 921 | 0.009 |
| 2 | α-Thujene | 924 | 0.058 |
| 3 | α-Pinene | 931 | 1.317 |
| 4 | Camphene | 946 | 0.365 |
| 5 | Sabinene | 970 | 0.664 |
| 6 | β-Pinene | 974 | 1.685 |
| 7 | Myrcene | 988 | 0.482 |
| 8 | 3-Octanol | 992 | 0.551 |
| 9 | α-Phellandrene | 1003 | 0.058 |
| 10 | δ-3-Carene | 1009 | 0.027 |
| 11 | α-Terpinene | 1014 | 0.122 |
| 12 | *p*-Cymene | 1022 | 0.328 |
| 13 | Limonene | 1026 | 2.294 |
| 14 | 1,8-Cineole | 1029 | **4.552** |
| 15 | (Z)-β-Ocimene | 1034 | 0.097 |
| 16 | €-β-Ocimene | 1044 | 0.031 |
| 17 | γ-Terpinene | 1055 | 0.405 |
| 18 | *cis*-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 | *trans*-Pinocarveol | 1136 | 0.209 |
| 24 | Camphor | 1141 | 0.108 |
| 25 | Menthone | 1150 | 0.319 |
| 26 | *iso*-Menthone | 1161 | 0.848 |
| 27 | Borneol | 1163 | 0.953 |
| 28 | α-Terpineol | 1189 | 0.155 |
| 29 | Myrtenal | 1193 | 0.129 |
| 30 | Pulegone | 1237 | **46.178** |
| 31 | Piperitone | 1252 | 0.458 |
| 32 | Bornyl acetate | 1283 | 0.272 |
| 33 | Thymol | 1295 | 0.184 |
| 34 | Carvacrol | 1303 | 2.139 |
| 35 | Piperitenone | 1342 | **19.558** |
| 36 | Piperitenone oxide | 1365 | **4.230** |
| 37 | α-Copaene | 1372 | 0.043 |
| 38 | β-Bourbonene | 1381 | 0.149 |
| 39 | 4a-α,7-β,7a-α-Nepeta lactone | 1397 | 0.313 |
| 40 | β-Caryophyllene | 1416 | 2.325 |
| 41 | α-Humulene | 1449 | 0.353 |
| 42 | (E)-β-Farnesene | 1453 | 0.213 |
| 43 | Germacrene D | 1476 | 0.302 |
| 44 | (E)-β-Ionone | 1482 | 0.041 |
| 45 | Phenyl ethyl 3-methyl butanoate | 1488 | 0.042 |
| 46 | Bicyclogermacrene | 1492 | 0.17 |
| 47 | γ-Cadinene | 1509 | 0.033 |
| 48 | δ-Cadinene | 1519 | 0.067 |
| 49 | (E)-γ-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 ($C_9$-$C_{18}$) 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; Boukhebti *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-distillated 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%), β-caryophyllene (2.33%), limonene (2.29%), carvacrol (2.14%), β-pinene (1.69%) and α-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.

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