New Menthone Type of *Mentha pulegium* L. Volatile Oil from Northwest Iran

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Abstract

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The constituents of the volatile oil of air-dried aerial parts of *Mentha pulegium* L. (Lamiaceae) plants wildly growing in Northwest Iran were analysed by GC/MS. 46 components were identified, comprising 96.6% of the essential oil. Monoterpenes (78.9%) were the main class of the identified components followed by a minor proportion of sesquiterpenes (11%). Oxygenated monoterpenes (75.3%) were the major subclass of volatile oil components with menthone (38.7%), menthol (11.3%), neomenthol (10.5%), and pulegone (6.8%) as major compounds. Sesquiterpene hydrocarbons (10.6%) were the highlighted subclass of 15 carbons sesquiterpenoidal compounds with (E)-caryophyllene (4.9%) and β-cubebene (2.5%) as their principle representatives. Furthermore, menthyl acetate (C_{12} acetylated monoterpene derived compound) was contained considerable amounts (5.2%) in the essential oil. In total, volatile oil composition of *M. pulegium* L. plants studied in the present experiment was characterised as a new menthone type with appreciable amounts of menthol and neomenthol, and it could be used as a potential source of these high value monoterpenes in pharmaceutical and food industries.

Keywords: *Mentha pulegium* L.; Lamiaceae; volatile oil; monoterpene compounds; menthone; menthol

Mentha pulegium L. (syn: *Mentha exiqua* Linn. or *Pulegium vulgare* Mill.) commonly known as pennyroyal/European pennyroyal is an aromatic perennial herbaceous plant reaching a height of 40 cm (Stengele & Stahl-Biskup 1993; Zarghari 1996). Pennyroyal grows wildly in humid and damp areas and water banks of central, southern, and western Europe, north Africa, western Asia, and the Mediterranean countries (Simon *et al.* 1984; Zarghari 1996). *M. pulegium* L. is a low, prostrate, and spreading plant with ovate to nearly orbicular leaves and bisexual lilac/pink coloured flowers in whorls at leaf axils. The flowers are in blossom from June to October (Simon *et al.* 1984; Zarghari 1996; Akhondzadeh 2002). From agronomical and climatological points of view, pennyroyal prefers fertile soils with partial shade and grows best in mild weather with soil pH of 4.8 to 8.3 (Simon *et al.* 1984). *M. pulegium* L. is commonly found in most of the temperate regions of the world (Weiss & Fintelmann 2000). In Iran, this plant is in common growth and use in Alborz Mountains, north and northwest of the country (Akhondzadeh 2002).

Aerial parts of this plant contain a wide diversity of secondary metabolites such as: tannins, resins, pectins, bitter principles, and essential oil (Simon *et al.* 1984; Zarghari 1996). Fresh or dried leaves and flowering tops are in common use for their healing and culinary properties. The whole plant and extracted essential oil have a characteristic and strong mint like odour (Simon *et al.* 1984).

In therapeutic applications, this plant and the preparations from it have traditionally been used for their antispasmodic, carminative, diaphoretic, sedative, stimulant, diuretic, antitussive, tonic, cholagogue, expectorant, antiseptic, bronchitis, and digestive effects (Simon *et al.* 1984; Zarghari 1996; Ben Fadhel *et al.* 2006; Mkaddem *et al.* 2007). It has been used to promote menstruation, cure headaches, relieve snake and scorpion bites, help against vomiting, and also for kidney ailments (Simon *et al.* 1984; Zarghari 1996). It has been also used as an insect repellent against fleas and other insects (Simon *et al.* 1984; Akhondzadeh 2002; Mkaddem *et al.* 2007). Externally this plant has a long-term history of use for the relief of acne and other dermatological disorders and gout (Akhondzadeh 2002). This plant has been used as a spice and flavouring agent in different foods, especially puddings (Zarghari 1996; Akhondzadeh 2002; Mkaddem *et al.* 2007).

Essential oil (*Pulegi folium aetheroleum*) of pennyroyal exhibits strong antibacterial activities against a wide diversity of Gram-positive and Gram-negative bacteria (Wagner & Bladt 1996; Ray *et al.* 2004). Essential oil of pennyroyal has been used as a fragrance in cosmetics (Simon *et al.* 1984; Mkaddem *et al.* 2007) and as a corrosion inhibitor for steel in chemical industries (Bouyanzer *et al.* 2006). In commercial medicinal plant markets, *M. pulegium* is sometimes traded as an adulterant of *M. piperita* and *M. arvensis* (Wagner & Bladt 1996).

Volatile oil compositional analysis of *M. pulegium* has been the subject of some studies conducted in the past (Stengele & Stahl-Biskup 1993; Chalchat 2000; Lorenzo *et al.* 2002; Aghel *et al.* 2004; Kokkini *et al.* 2004; Agnihotri *et al.* 2005; Stoyanova *et al.* 2005; Maroto-Diaz *et al.* 2007; Mkaddem *et al.* 2007; Mahboubi & Haghi 2008) (Table 1). Three definite chemotypes have been reported for this plant as pulegone, piperitenone/piperitone, and isomenthone/neoisomenthone types (Stoyanova *et al.* 2005). Despite these reports from different regions of the world and two independent studies in the north and west of Iran, there is no previous report on the chemical analysis of *M. pulegium* volatile oil from northwest Iran. Therefore, this experiment was aimed to characterise for the first time the volatile oil components of wild growing *M. pulegium* from northwest Iran.

MATERIAL AND METHODS

Plant material. The overground parts of 20 flowering individual plants representing the local population of spontaneously growing *Mentha pulegium* L. from northwest Iran (Maragheh district) were harvested in July 2008. A sample of the specimen was deposited in the Herbarium of Maragheh University, Iran. The harvested material was air-dried in a shaded place at ambient temperature of about 30°C. The air-dried material was mixed and pulverised to obtain homogenous fine grade powder.

Isolation of volatile oil. 50 grams of air-dried and ground plant material was subjected to hydrodistillation in an all-glass Clevenger type apparatus for 3 hours. The extracted essential oil was trapped with *n*-hexane as organic collecting solvent. The oil was dried over anhydrous sodium sulphate and kept in a refrigerator in sealed glass vials until analysis. Essential oil content was expressed as ml/100 g dry weight of the plant material.

GC/MS analysis. The compositional analysis of the volatile oil was carried out by a GC (Agilent Technologies 6890N) interfaced with a mass selective detector (MSD, Agilent 5973B) equipped with an apolar Agilent HP-5 ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm *i.d.* and 0.25 μm film thickness). The carrier gas was helium with a constant flow rate of 1 ml per minute. The oven temperature was set at 50°C for 2 min, then programmed up to 110°C at a rate of 10°C/min, then raised to 200°C at the 20°C/min rate, and finally increased at the rate 10°C/min to 280°C, isothermal at the temperature for 2 minute. The injector and detector temperatures were 300°C and 200°C, respectively. Injection mode, split; split ratio 1:100, volume injected 4 μl of the oil. The MS operating parameters were as follows: ionisation potential 70 eV; interface temperature 200°C and, acquisition mass range 50–800.

Identification and quantification of constituents. Relative percentage amounts of the volatile oil components were evaluated from the total peak area (TIC) by the apparatus software. The identification of the components in the volatile oil was based on the comparison of their mass spectra and retention times with those of the au-

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

No	Compound	RI	%
1	α-pinene	0939	0.2
2	sabinene	0975	0.2
3	β-pinene	0979	0.3
4	myrcene	0991	0.2
5	α-phellandrene	1003	0.1
6	α-terpinene	1017	0.1
7	*p*-cymene	1025	0.1
8	limonene	1029	0.3
9	**1,8-cineole**	**1031**	**1.1**
10	Δ-3-carene	1031	0.1
11	(Z)-β-ocimene	1037	0.2
12	γ-terpinene	1060	0.1
13	p-mentha-3,8-dien	1073	0.1
14	terpinolene	1089	0.1
15	**allo-ocimene**	**1132**	**1.4**
16	**neoisopulegol**	**1148**	**1.8**
17	**isopulegol**	**1150**	**1.5**
18	**menthone**	**1153**	**38.7**
19	**neomenthol**	**1166**	**10.5**
20	**menthol**	**1172**	**11.3**
21	nerol	1230	0.5
22	**pulegone**	**1237**	**6.8**
23	**chavicol**	**1250**	**1.2**
24	piperitone	1253	0.6
25	isopulegylacetate	1278	0.1
26	**menthylacetate**	**1295**	**5.2**
27	carvacrol	1299	0.5
28	**piperitenone**	**1343**	**1.7**
29	eugenol	1359	0.1
30	piperitenone oxide	1369	0.1
31	α-copaene	1377	0.1
32	β-bourbonene	1388	0.2
33	**β-cubebene**	**1388**	**2.5**
34	β-elemene	1391	0.2
35	**(E)-caryophyllene**	**1419**	**4.9**
36	α-humulene	1455	0.4
37	(E)-β-farnesene	1457	0.6
38	germacrene D	1485	0.6
39	bicyclogermacrene	1500	0.7
40	β-bisabolene	1506	0.1
41	γ-cadinene	1514	0.1
42	Δ-cadinene	1523	0.2
43	(Z)-nerolidol	1533	0.1
44	caryophyllene oxide	1583	0.2
45	α-cadinol	1654	0.1
46	phytol	1943	0.1
Total			96.6

Compounds are reported according to their elution order on apolar column

thentic compounds, and on the computer matching with NIST and WILEY library as well as on the comparison of the fragmentation patterns of the mass spectral data with those reported in the literature (Stengele & Stahl-Biskup 1993; Chalchat 2000; Lorenzo *et al.* 2002; Aghel *et al.* 2004; Kokkini *et al.* 2004; Agnihotri *et al.* 2005; Stoyanova *et al.* 2005; Maroto-Diaz *et al.* 2007; Mkaddem *et al.* 2007; Mahboubi & Haghi 2008).

RESULTS AND DISCUSSION

Hydrodistillation of the aerial parts of *M. pulegium* L. gave a greenish yellow oil amounting to 1.2% (v/w) based on dry weight. The results obtained from the chemical analysis of the volatile oil of *M. pulegium* i.e. volatile constituents of the studied oil, percentage contents of the individual components, their elution order on apolar column as well as their retention indices, and the major classes, subclasses, and chemical groups of the identified and quantified components of the volatile oil are reported in Tables 1 and 2, respectively. In total, 46 constituents were identified in volatile oil of the aerial parts of *M. pulegium* from northwest Iran accounting for 96.6% of the total oil (Tables 1 and 2). Oxygenated monoterpenes (75.3%) were found to be the most abundant class of the components identified (Table 3). Menthone

Table 2. Main classes, subclasses and chemical groups of *Mentha pulegium* L. volatile oil constituents from Iran

Class, subclass and chemical group of compound		%
Monoterpenes		78.9
monoterpene hydrocarbons		3.6
oxygenated monoterpenes		75.3
Sesquiterpenes		11
sesquiterpene hydrocarbons		10.6
oxygenated sesquiterpenes		0.4
Others		6.6
Total identified		96.6
Chemical groups	ketones	47.2
	alcohols	27.7
	acetates	5.3
	oxides	1.4

(38.7%), menthol (11.3%), neomenthol (10.5%), and pulegone (6.8%) were the main constituents of oxygenated monoterpenes, respectively (Table 1). Some other oxygenated monoterpenes in amounts higher than 1% were neoisopulegol (1.8%), piperitenone (1.7%), allo-ocimene (1.4%), isopulegol (1.5%), chavicol (1.2%), and 1,8-cineol (1.1%). The majority of the oxygenated monoterpenes belonged to the C_3 oxygenated *p*-menthane skeleton compounds with great shares of menthone, menthol, neomenthol, and pulegone (together 70% of the total identified components) (Table 1). Considering the monoterpenoidal profile of the volatile oil, it seems that there exists a considerable chemical polymorphism between the findings of the present experiment and the reports of other scientists from elsewhere (Tables 1 and 3), because in most of the previous studies, the priority of volatile oil components was with biosynthetically intermediate ketone compound i.e. pulegone (Table 3). Despite previous reports, pulegone content in the volatile oil studied by us was low and this compound was ranked quantitatively below menthone, menthol, and neomenthol with only 7% from the total oil (Table 1). It is logical that the low content of pulegone in the studied plants could be considered as a premium criterion due to the extremely toxic properties, especially a high abortive potential of this compound (Simon *et al.* 1984; Wagner & Bladt 1996). Sesquiterpene compounds (11%) were the second class of essential oil components with the abundance of sesquiterenoidal hydrocarbons such as (E)-caryophyllene (4.9%) and β-cubebene (2.5%). Taking into account the sesquiterpene compounds, there were notable differences between our experiment and the reports of previous scientists (Tables 2 and 3). In particular, it seems that, like with nearly all of the previous experiments, the dominance of essential oil components was with monoterpenoidal compounds, especially C_3 oxygenated *p*-menthane compounds, and this trend indicates that biosynthetic paths of these compounds with the initiality of limonene and intermediacy of pulegone towards the end products of menthone, menthol, and neomenthol were dynamic and ongoing until the harvest time.

Table 3. *Mentha pulegium* L. studies reported from different countries with their main volatile oil constituents.

Reference	Method of identification	Main component(s) (%)	Volatile oil extraction	Plant part	Origin
Stengele & Stahl-Biskup (1993)	GC, GC/MS	pulegone (7–85.3), menthone (2.7–32.3), neomenthol (0.4–35.5)	hydrodistillation	different plant parts	European countries
Chalchat (2000)	GC, GC/MS	menthone (31), pulegone (14). neomenthol (13.8), caryophyllene oxide (9)	steam distillation	aerial parts	Yugoslavia
Lorenzo *et al.* (2002)	GC-FID, GC/MS	pulegone (73.4), isomenthone (12.9)	hydrodistillation	leaves	Uruguay
Aghel *et al.* (2004)	GC/MS	pulegone (52), menthone (30.3)	SC-CO_2	aerial parts	Iran
		pulegone (37.8), menthone (20.3), piperitenone (6.8)	hydrodistillation		
Kokkini *et al.* (2004)	GC, GC/MS	pulegone(0.1–90.7), piperitone (0–97.2), menthone (0.2–53.4), isomenthone (0.1–45.1), piperitenone (0.1–39.8), isopiperitenone (0–23.5)	hydrodistillation	aerial parts	Greece
Agnihotri *et al.* (2005)	GC, GC/MS, ^{1}H-NMR, ^{13}C-NMR	pulegone(65-83), menthone (8), isomenthone (4), neomenthol (0.7–13)	hydrodistillation	aerial parts	India
Stoyanova *et al.* (2005)	GC, GC/MS	pulegone (42.9-45.4), piperitenone (21.7–23.1), isomenthone (11.3–12.8)	water & steam distillation	aerial parts	Bulgaria
Maroto-Diaz *et al.* (2007)	GC/MS, GC/Olfactometry	pulegone, piperitone oxide, isopulegol	hydrodistillation	aerial parts	Spain
Mkaddem *et al.* (2007)	GC, GC/MS	pulegone (42), isomenthone (11.3), carvone (6.2), menthofuran (3.7)	hexane extract	aerial parts	Tunisia
Mahboubi & Haghi (2008)	GC, GC/MS	piperitone (38), piperitenone (33%), α-terpineole (4.7), pulegone (2.3)	hydrodistillation	aerial parts	Iran

From the chemical point of view, ketones (47.2%) were the most abundant group of volatile oil components with menthone, pulegone, and piperitone as their representatives (Tables 1 and 2). Alcohols (27.7%) were the second main group of the identified constituents with menthol, neomenthol, neoisopulegol, isopulegol, and chavicol as the main ones (Tables 1 and 2). Acetates, the less frequent components of volatile oil, contained only two C_{12} compounds with prevalence of menthyl acetate (5.2%). Furthermore, this is the first report on the high occurrence of this compound in *M. pulegium* essential oil. Oxides (1.4%) were another chemical group of volatile oil components with 1,8-cineole as the only compound of this group amounting above 1%. In total, taking into account the chemical profile (main classes, subclasses and dominant compounds) of the essential oil of the wild growing *M. pulegium* plants from Maragheh district in northwest Iran and the reports of other scientists, it seems that there are significant qualitative and quantitative differences between the chemical profiles of the essential oils (Tables 1–3). Finally, chemical profile of the volatile oil of the studied plant was drastically different from the previously introduced chemotypes and contained a high amount of menthone followed by menthol and neomenthol. The comparative survey of different reports and the present experiment guides us to the suggestion to call this chemotype as a menthone type with appreciable amounts of menthol and neomenthol. It is likely that these chemical variations are due to the diverse climatic and geographical differences between *M. pulegium* wild habitats in different countries as well as the divergent genetic potential of plants for compartmentalisation of different biochemical pathways leading to a wide variety of oil components. Additionally, it seems that different volatile oil extraction procedures and also analytical and instrumental parameters had inevitable effects on the chemical profile of volatile oil (Tables 2 and 3). In conclusion, the chemical composition of the volatile oil of *M. pulegium* plants spontaneously growing in northwest Iran was characterised by the presence of high amounts of menthone and was introduced as menthone chemotype. Ultimately, it can be postulated that *M. pulegium* plants studied in the present experiment could be a hopeful source of menthone and menthol for substituting other sources of these commercial oxygenated monoterpenes or for submitting to the high demands of pharmaceutical and food industries for these monoterpenes.

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