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Chemical composition of the essential oil of *Mentha pulegium L.* from Taftan Area by means of gas chromatography/mass spectrometry (GC/MS)

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The aerial parts of *Mentha pulegium* L. were collected during the flowering stage from the Taftan aera (Baluchestan, Iran) using hydro distillation. The essential oil was extracted from plants' aerial parts. The highest ratio of oil yield to dried plant weight was 2.642 (w/w) (when using hexane) and 2.053% w/w for the second oil (without using hexane). The oils were analyzed using gas chromatography/mass spectrometry (GC/MS) technique. In the first oil (when using Hexane), 35 constituents embracing 96.19% of total essential oil were recognized. Oxygen containing monoterpens was the main group of constituents of the oil. Pulegone (65.52%), 1,8 cineole (11.10%), isopulegone (2.53%), beta-pinene (2.45%) were the major components of the first oil extracted from *M. pulegium* L. In the second oil (without solvent), 42 constituents were recognized consisting of 82.94% of its total essential oil. The main constituents for this specie included; pulegone (48.88%), eucalyptol (3.99%), trans-ocimene (1.58%), camphene (2.29%), spathulenol (6.99%) and unknown compound (0.1%). The findings of the research revealed that the *M. pulegium* essential oil has a considerable percentage of oxygenated terpenoids with the anti-microbial property.

Key words: *Menthe pulegium* L., essential oil, gas chromatography/mass spectrometry (GC/MS), pulegone, 1,8 cineole, isopulegone.

INTRODUCTION

The genus *Mentha* (Lamiaceae) includes aromatic herbs of difficult taxonomic classification due to a great variability in their morphological characters and frequent hybridisation (Mozaffarian, 1966). *Menthe pulegium* L., commonly known as pennyroyal, is traditionally used in the treatment of flatulent dyspepsia and intestinal colic due to its carminative and antispasmodic properties (Newall et al., 1996). Previous reports (Lawrence, 1998; Hefendehl, 1970; Pino et al., 1996) on the composition of its essential oil showed that pulegone was the main constituent of *M. pulegium*, and its percentage ranged from 25 to 92%. The Labiates family has several members that have a significant proportion of essential oil, and some of them are used in perfumery or as spices in foods. In Iran, the genus mentha (Labiatae) is represented by five endemic varieties. *M. pulegium* L. (penny royal), that grows naturally now in Golestan, Gilan and Baluchestan province in Iran (Zargari, 1997), is strongly aromatic and has been used traditionally for digestion, liver and gallbladder disorders as well as gout,

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colds and increased micturition. It is also used externally for skin diseases (wald et al., 1998). Previous studies on the isolation of *M. pulegium* L. essential oils with hydro or steam distillation in different regions of the world resulted in the identification of the fact that the main constituents of the oil herbs are commonly used in folk medicine as food and beverage flavoring, as well and as fragrances in cosmetic products (Baser et al., 1999). In Iran, the genus mentha (Labiate) is represented by *Menthe aquatic*, *Menthe pulegium, Menthe spicata, Menthe longofolia* (with 7 varieties) and *Menthe mazaffariani,* where the latter one is endemic. A qualitative comparison of the oil constituents of *M. pulegium* L. was conducted in summer 2007 in Bander-Anzali (province of Gilan, Iran), The results indicated that the plant consisted of pulegone (37.8%), menthone (20.3%), 3/5/5-trim ethyl cyclohexane (3.2%) caryophyllene oxide (1.3%) (Peerzada,1997). The oil of *M. pulegium* L. analyzed in Uruguay consisted of (IR) – (+) – pulegone (73.4%), 1,8 cineole (0.1%), isopulegone (1.4%) (IR, 45) – (-) – menthone (3.6%) (Aghel et al., 2004).

METHODOLOGY

Plant

Flowering aerial parts of M. *pulegium* were collected from the Taftan area of Baluchestan (Iran) during June, 2000. The plant was identified in herbarium section of Tehran University, Faculty of Agriculture, Karaj.

Isolation of the essential oil

The essential oil was isolated from 20 g of dried herbal parts by hydro distillation method in a Clevenger type apparatus for 4 h. Affording essential oil yields were 2.642% ± 0.001 and 2.053% ± 0.001% w/w, respectively. The sample oil was yellowish green in color, then it was separated from the distillate by hexane. Normal liquid-liquid extraction oil was dried over anhydrous sodium sulphate and stored in a sealed glass vial at low temperature until analysis (Sateem GC/MS reference manual, 1992; Lorenzo et al., 2002).

GC/MS analysis

The essential oil was analyzed by gas chromatography using a thermo quest 2000 equipped with a flame ionization detector (FID) and later by GC/MS using a thermo Finningan 2000 equipment. The chromatography conditions were the same in both analyses: fused DB5 capillary column (30 m × 0.25 mm) with a phenyl methyl "siloxane" bonded phase (0.25 µm film thickness). Injector temperature 250°C and detector temperature 265°C, column program 50 to 265°C at 2.5°C/min. 265°C for 30 min, carrier gas, helium (1 ml/min), the injector volume was l0 µl. The MS data were recorded at 70 eV. Each component was identified by comparison of its mass spectra with those available from the equipment data base (Wiley) and from literature (Adams, 1995; McLafferty, 1991) and also by its retention. Index (RI) was determined using a calibration curve of n-alkanes (Van Den Dool and Kartz, 1963).

RESULTS AND DISCUSSION

35 constituents that comprised 96.19% of the total weight of the essential oil were analyzed and their components were identified. The yield of extraction for frst sample was 2.642% ± 0.001 w/w on dried weight basis. The results of the chromatographic analysis C > 0.05% of *M. pulegium* L. oil are listed in Table 1. The finding indicated that the oil was composed of 96.19% of the total plant's weight, the higher percentage are as follows (the fist oil in Hexane): beta- pinene (2.45%), trans-ocimene (1.58%), camphene (0.99%), 1,8 cineole (11.10%), menthe-furan (1.27%), isopulegone (2.53%), and pulegone (65.52%). The yield of extraction in second sample is 2.053% ± 0.001 w/w on dried weight basis. 42 components were identified in the oil (the product obtained without the use of organic solvent) representing 82.94% of the total oil with pulegone (48.88%), eucalyptol (3.99%), trans-ocimene (1.58%), camphene (2.29%), cis-Jasmone (1.55), 4-hydroxy-3-methoxy phenyl formate (1.67%), manoyl oxide (1.77%), spathulenol (6.99%) and unknown compound (0.1%). The major constituents are listed in order of elution from DB5 capillary column. The identification of the compounds was carried out by comparison of their MS spectra with the relative retention indices with those of standard compounds. The composition of *M. pulegium* L. oil (two samples) was rich in oxygenated monoterpenes (83.25 and 56.16%), especially the pulegone. Standard deviation and coefficient of variation (CV%) were 8.10×10^{-4} and 3.06×10^{-2}, respectively.

Conclusion

In essential oils, 21 similar compounds with high percentages were recognized. 87.26% of the total oil was the essential oil (when using hexane), and the percentage was 62.31% without using hexane. 24 components comprising 19.54% of the total oil were recognized in the essential oil without using hexane, but those components were not recognized when using hexane. 15 components compressing 6.66% of the total oil were identified in the essential oil when using hexane, but they were no identified essential oils whithout using hexane. In the first oil (when using hexane) *M. pulegium* L. was more than 90.10%. Terpenoids increase resulted in better quality of the essential oil; moreover the increase of oxygenated terpenoids increased anti-bacterial properties.

Table 1. Chemical composition of essential oils from *M. pulegium* L.

Compound	RI	Percent of compound (when using Hexane)	Percent of compound (without using hexane)
2-Butanic acid methyl-ethyl ester	850	0.23	0.25
Furan,2-5 diethyl tetra hydro	896	0.10	0.11
trans-Ocimene	934	1.58	1.58
Camphene	950	0.99	2.29
beta-Pinene	976	2.45	-
Methyl-hepten-2 one	985	0.39	-
beta-Myrcene	990	0.35	0.33
Linalool	997	0.50	0.08
Myrcenol	1002	-	0.10
1,8 Cineole	1034	11.10	0.05
Eucalyptol	1044	-	3.99
Alpha-Terpinene	1058	0.13	0.06
(IR)-(t)-trans-isolimonene	1069	0.32	0.10
Alpha-Terpinolene	1085	0.13	-
Terpinolene	1088	0.13	-
Iso-Pino campheol	1102	0.13	-
1-Terpineol	1153	0.55	-
Mentho-Furan	1164	1.27	0.56
Iso-Pulegone	1177	2.53	0.35
1-Bor neol	1183	0.41	-
3-Cyclo hexane-1-methanol	1209	0.67	-
Pulegone	1231	65.52	48.88
3-Ethyliden/Heptan-2,6-Dione	1270	0.25	-
1-(2,4,6-Trihydrx phenyl)-3-(3-hydroxy)	1291	0.13	-
Endobornyl Acetate	1313	-	0.19
Trans-Geraniol	1324	-	0.23
Caran-4-on,3-Hydroxy	1341	-	0.12
2-Cyclohexen-1-one-3-methyl-6-(1-methyl ethylidene)	1350	0.85	0.94
7-oxabicyclo[4.1.0]heptan-2-one-3methyl-6-(1-methyl)	1355	0.95	-
4-Hydroxy-3-methoxy phenyl formate	1372	-	1.67
Piperitenone oxide	1379	-	0.12
beta-Damascenone	1392	-	0.24

Table 1. Contd.

Cis-Jasmone	1397	0.79	1.55
Trans-Caryophyllene	1414	0.36	0.74
Neryl-Acetone	1454	0.12	0.30
(E)-1- Methoxy,phenyl-2-methoxy	1491	0.22	-
Minet Furan 2	1494	0.23	0.55
2-(1-methoxy carbony-ethyl)-5-methyl	1510	0.25	-
Dihydro-beta-agro furan	1516	-	0.68
Ethanone-2-Cyclopentyl-1(1 Himidazol-4-yl)	1534	-	0.13
(+)Spathulenol	1588	-	6.99
Rosifoliol	1597	-	0.27
Humuladienone	1608	-	0.18
Torreyol	1643	-	0.47
(-)Caryophellene oxide	1676	0.05	0.42
(E)-4-(1-hydreperdxy,2-2-Dimethyl	1723	-	0.18
Farnesyl Acetone	1919	-	0.11
1,2-Benzene dicarboxylic acid/dibutyl ester	1963	0.24	0.59
Manoyl oxide	1983	0.47	1.77
Noname	2055	-	0.10
Sclareol	2095	0.24	0.81
1,2-Dihgdro-1-methyl-2-trifuoro acetyl methylene quinoline	2110	0.1 7	-
3,5-Octadiene,4/5 Diethyl	2166	-	0.26
3-Ketomanoyloxide	2189	-	0.18
Solanesol	2231	-	0.21
2,2-Dimethyl-3-(3,7,16,20-teramethyl-Heneicosa-3,7,11,15,19-pentaenyl)-oxirane	2249	0.47	-
Contortadiol	2259	-	2.21
2-Benzo furan methanol	2266	-	0.73
(E)-4-(1-hydroperoxy-2-2-dimethyl-6-methyllidene-cyclohexyl)pent-3-en-2-one	-	-	0.18
Monoterpene hydrocarbons	6.23	-	5.06
Monoterpene Oxygenated	83.25	-	56.16
Sesquiterpene hydrocarbons	0.45	-	0.74
Oxygenated Sesquiterpene	0.17	-	9.10
Other compounds hydrocarbon	0.31	-	0.31
Other compounds Oxygenated	5.65	-	11.44
Other compounds	0.13	-	0.13

Table 1. Contd.

Unknown compound	-	-	0.10
Terpenoid hydrocarbons	6.68	-	5.80
Oxygenated terpenoids	83.42	-	65.26
Total terpenoids	90.10	-	71.06
Total	96.19	-	83.04
Oil yield (w/w per dry)	2.642	-	2.053
Number of compounds	35	-	42

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