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Isolation and identification of the major chemical components found in Spearmint leaves (*Mentha viridis*) grown in Ahwaz city, Iran.

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ABSTRACT

Spearmint is grown for its aromatic and carminative oil, referred to as oil of spearmint. The main constituents of the essential oil obtained by hydrodistilltion of spearmint plant (*Mentha viridis*) grown in the city of Ahwaz, Iran, were isolated, purified and their chemical structures were determined by using various spectroscopic techniques. (-)- Carvone [(2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one)] and Limonene [(1-methyl-4-prop-1-en-2-yl-cyclohexene)] were the major components. GC-MS analysis of the essential oil revealed 17 components with Carvone as the most abundant constituents.

Keywords: Spearmint, *Mentha viridis*, Carvone, limonene, Phellandrene, dihydrocarveol acetate.

INTRODUCTION

Spearmint (*Mentha spicata*, syn *M. viridis*) is a species of mint native to central and southern Europe, where it grows in wet soils. [Scientific classification: Kingdom; Plantae: Division; Magnoliophyta: Class; Magnoliopsida: Order; Lamiales: Family; Lamiaceae: Genus; Mentha: Species; *M. spicata*]. There are three chief species of mint in cultivation and general use: Spearmint (*Mentha viridis*), Peppermint (*M. piperita*), and Pennyroyal (M. pulegium), the first being the one ordinarily used for cooking.[1,2]The various species of mint have much in common and have all been held in high medical repute. Mentha (mint) is a genus of about 25-30 species of flowering plants in the family Lamiaceae. They are aromatic perennial herbs, growing to 10-120 cm tall, with wide-spreading underground rhizomes and erect, branched stems, with leaves growing 5-9 cm long and 1.5-3 cm broad, having a serrated margin. The flowers are produced in slender spikes, each flower pink or white, 2.5-3 mm long and broad. Mints are generally vigorous, spreading plants that tolerate a wide range of conditions, but thrive where there is abundance of water.[1] If Spearmint is being grown as a medicinal herb, for the sake of the volatile oil to be extracted from it, the shoots should be gathered in August, when just coming into flower, and taken to the distillery as soon as possible after picking, the British Pharmacopceia directing that oil of Spearmint be distilled from the fresh, flowering plant. It is estimated that 350 lb. of Spearmint yield 1 lb. of oil. If the distillery is not on the ground or only a short distance away, and the crop has to be dispatched by train, the cutting should take place late in the afternoon on a fine day, before the dew falls, so as to be sent off by a night train to arrive at their destination next morning, having travelled in the cool, otherwise the leaves are apt to heat and ferment, losing colour. All the Mints yield fragrant oils by distillation.[3] Spearmint is grown for its aromatic and carminative oil, referred to as oil of spearmint. It grows well in nearly all temperate climates. The Ancients used mint to scent their bath water and as a restorative. In the fourteenth century, mint was used for whitening the teeth, and its distilled oil is still used to flavour tooth-pastes, etc., and in

America, especially, to flavour confectionery, chewing gums, and also to perfume soap. Nowadays, mint essential oils are used to flavor food, candy, teas, breath fresheners, antiseptic mouth rinses, and toothpaste.[1] Mint leaves are used in teas, beverages, jellies, syrups, and ice creams.[3]The taste and odour of the plant are very characteristic. Mint essential oil and menthol are extensively used as flavourings in drinks, chewing gum and desserts/ candies, as well. The substances that give the mints their characteristic aromas and flavours are:

Menthol

(2-(2-Propyl)-5-methylcyclohexanol)**(I)**: the main aroma of Spearmint, Peppermint, and Japanese Peppermint (a major commercial source). (ii) Pulegone; [R-(+)-5-methyl-2-(1-methyl ethylidene) cyclohexanone] **(II)**: in Pennyroyal and Corsican Mint.

The chief constituent of Spearmint oil is Carvone [2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one] **(III)**. There are also present Phellandrene [α- phellandrene: 2-methyl-5-(1-methylethyl)-1,3-cyclohexadiene **(IV, a)**, β-phellandrene: 3-methylene-6-(1-methylethyl) cyclohexene **(IV, b)]**, Limonene (1-methyl-4-prop-1-en-2-yl-cyclohexene) **(V)** and dihydrocarveol acetate **(VI)**. Esters of acetic, butyric and caproic or caprylic acids are also present.[3]

EXPERIMENTAL

NMR spectra were recorded on Bruker NMR spectrophtometer AC 80 MHz (^{1}H) (Germany) using TMS as internal standard. GC-MS analysis was carried out by using Gas Chromatograph (Shimadzu 14A Japan) -Mass Spectrometer (quadruple, 1000 EX) instrument. Infra-red spectra were recorded using a JASCO, IR700 Infrared spectrophotometer. UV-Visible spectra were recorded using a JASCO, 810-UV spectrophotometer. All the chemicals were purchased from Merck.

1. Collecting spearmint plant

The plant was collected in March (the season that spearmint plant does not have any flower) and in June (the flowering season) in the city of Ahwaz, the capital city of Khuzestan province, Iran and identified as *Mentha viridis* by the Faculty of Agriculture, Shahid Chamran University of Iran. The upper parts (flowers, leaves) of the plant were separated from the other parts, then some were air dried and ground by using an electrical mill and some others were used without drying and as fresh.

2. Hydrodistillation of the ground plant (in the season without flowering)

The oil of air-dried and finely ground from the whole aerial parts of the spearmint plant (in the season without flowering) was obtained by hydrodistillation using a Clevenger-type hydrodistillation apparatus. Distillation was performed using 30 g of the dried plant material in 1 L distilled water for about 2 hours. The oil obtained was dried over anhydrous sodium sulphate and stored in a dark glass bottle at 4°C. The process was repeated several times to collect enough of the essential oil for further analysis. The yield was 0.55%.

3. Hydrodistillation of the fresh plant (in the season without flowering)

The oil from the whole aerial parts of the spearmint plant (in the season without flowering) was obtained by hydrodistillation using a Clevenger-type hydrodistillation apparatus. Distillation was performed using 30 g of the fresh plant in 1 L distilled water for about 2 hours. The oil obtained was dried over anhydrous sodium sulphate and stored in a dark glass bottle at 4°C. The process was repeated several times to collect enough of the essential oil for further analysis. The yield was 0.15%.

4. Hydrodistillation of the ground plant (in the season of flowering)

The oil of air-dried and finely ground from the whole aerial parts of the spearmint plant (in the season of flowering) was obtained by hydrodistillation using a Clevenger-type hydrodistillation apparatus. Distillation was performed using 30 g of the dried plant material in 1 L distilled water for about 2 hours. The oil obtained was dried over anhydrous sodium sulphate and stored in a dark glass bottle at 4°C. The process was repeated several times to collect enough of the essential oil for further analysis. The yield was 1.1%.

5. Hydrodistillation of the fresh plant (in the season of flowering)

The oil of from the whole aerial parts of fresh spearmint plant (in the season of flowering) was obtained by hydrodistillation using a Clevenger-type hydrodistillation apparatus. Distillation was performed using 30 g of the fresh plant in 1 L distilled water for about 2 hours. The oil obtained was dried over anhydrous sodium sulphate and stored in a dark glass bottle at 4°C. The process was repeated several times to collect enough of the essential oil for further analysis. The yield was 1.1%.

6. Thin Layer Chromatography analysis of the essential oil

TLC on silica gel with methylene chloride as the mobile phase was carried out. The spots were developed both by using a UV lamp, and by phosphomolibdic acid solution in ethanol (20% w/v), or vanillin solution in ethanol [(1% w/v), solution 1] , sulphuric acid solution in ethanol [(5% v/v), solution 2] reagents. The results showed two major spots with R_f = 0.97 (as the minor spot), and R_f = 0.55 (as the major spot).

7. Column Chromatography analysis of the essential oil (separation of the major fraction)

0.5 mL of the essential oil was column chromatographed on silica gel (35-70 mesh) with methylene chloride as the mobile phase. One major fraction with R_f = 0.55 was separated and characterized. Successive tlc on the fraction with R_f = 0.97 with n-hexane as the mobile phase, resulted in the purification of this fraction. IR, and ^{1}HNMR spectra of the fractions were taken. The whole process was repeated several times to obtain sufficient amount of each fraction for further identification.

8. Characterization of the component with R_f = 0.55

Its IR (neat liquid) had $\bar{\nu}$ (cm^{-1}): 3078 (=C-H, m); 2920 (C-H, s), 1673 (C=O, s), 1650 (C=C , m-w); 1447 (CH_2, m), 1433, 1365 (CH_3, m), 896 (=CH_{oop}, s); its ^{1}HNMR ($CDCl_3$, 80 MHz) had δ(ppm): 1.70 (s, 6H, 2xCH_3), 2.35 (m, 5H, 2xCH_3 , 1xCH), 4.71 (s, 2H, =CH_2), 6.66 (m, 1H, =CH). Based upon these results and comparison of the spectra with the corresponding standard spectra of Carvone, this fraction was identified as Carvone **(III)**.

9. Characterization of the component with R_f = 0.97

Its IR (neat liquid) had $\bar{\nu}$ (cm^{-1}): 3068 (=C-H, m); 2922 (C-H, s), 1632 (C=C , m-w); 1453 (CH_2, m), 1381 (CH_3, m), 884 (=CH_{oop}, s); its ^{1}HNMR (C_6D_6 , 80 MHz) had δ(ppm): 0.90 (s, 3H, CH_3), 1.20 (m, 3H, CH_3), 1.5-2.3 (m, 3xCH_2 + 1xCH), 4.63 (t, 2H, =CH_2), 5.2 (s, 1H, =CH). Based upon these results and comparison of the spectra with the corresponding standard spectra of limonene, this fraction was identified as Limonene **(V)**.

10. GC-MS Analysis of the essential oil

The crude volatile (essential) oil was analyzed by Gas Chromatography–Mass Spectrometry (GC-MS) QP-1000 EX instrument equipped with a CBD1 fused silica column (25m x 0.22 mm i.d) (Fig. 2). The oven temperature was held at 60 °C for 5 minutes then programmed to 250 °C at a rate of 12 °C/minute. The injector and detector (FID) temperatures were kept at 200 °C. Helium was used as the carrier gas at the constant flow rate of 1 mL/min. The quadrupole mass spectrometer was scanned over the 40 - 300 amu with an ionizing voltage of 70 eV. Some of the constituents of the volatile oil were identified by comparison of their mass spectra with those of the internal reference mass spectra library. Quantitative data was reported as area percentages without the use of correction factors (Table 1). Five major components were identified as discussed in the discussion section of this article (Table 2).

DISCUSSION

Spearmint (*Mentha viridis* L.) plant is fairly cultivated throughout Iran. It is used medicinally as carminative, diaphoretic, stimulant, antispasmodic and diuretic.[4] Regarding these therapeutic effects, it was decided to isolate and identify the major chemical components of the essential oil obtained from the upper parts of the plant by using Gas Chromatography-Mass Spectrometry (GC-MS) technique and various spectroscopic techniques. The oil of the air-dried, finely ground, and the fresh whole aerial parts of *Spearmint viridis* L. before and after flowering season of the plant was obtained by hydrodistillation using a Clevenger-type apparatus. The oil obtained by hydrodistillation, was analyzed by GC/MS technique. The mass spectra were

Table - 1: GC-MS data of the essential oil obtained from the upper parts of Spearmint plant grown in the city of Ahwaz, Khuzestan Province, Iran.

Peak No.	TIC (Begining)	TIC (Top)	TIC (End)	Retention time (min)	Relative area %
1	34	37	41	1.62	9.3
2	52	53	57	1.89	0.4
3	129	132	139	3.21	0.4
4	198	202	207	4.37	0.4
5	246	249	253	5.16	1.1
6	288	292	297	5.87	17.0
7	303	305	310	6.09	0.8
8	480	485	490	9.09	2.7
9	492	498	512	9.31	6.8
10	545	560	570	10.34	100.0
11	625	628	632	11.47	0.8
12	645	650	654	11.84	9.6
13	707	709	712	12.82	0/8
14	737	742	746	13.37	3.7
15	786	790	793	14.17	0.5
16	866	871	876	15.52	0.6
17	1143	1146	1151	20.11	0.5

Table - 2: The major components of the essential oil obtained from the upper parts of Spearmint plant grown in the city of Ahwaz, Khuzestan Province, Iran.

No.	Retention time (t_R)(min)	Component	Relative percentage (%)
1.	6.12	Cineole	0.8
2.	5.85	?	17
3.	10.34	Carvone **(III)**	100
4.	11.84	2-Methylene-3-(1-methylethyl)-cyclohexanol acetate (cis)**(VI)**	9.6
5.	13.37	Caryophyllene **(VIII)**	3.7

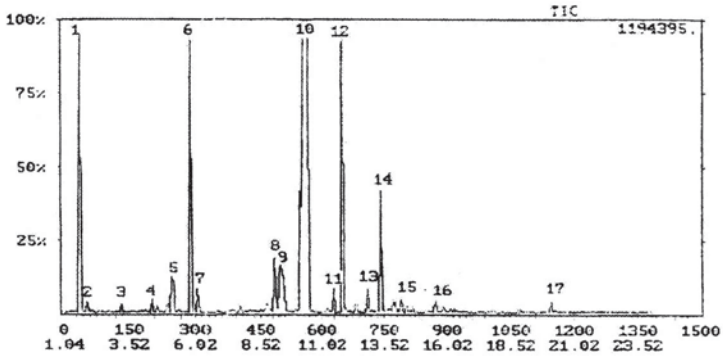
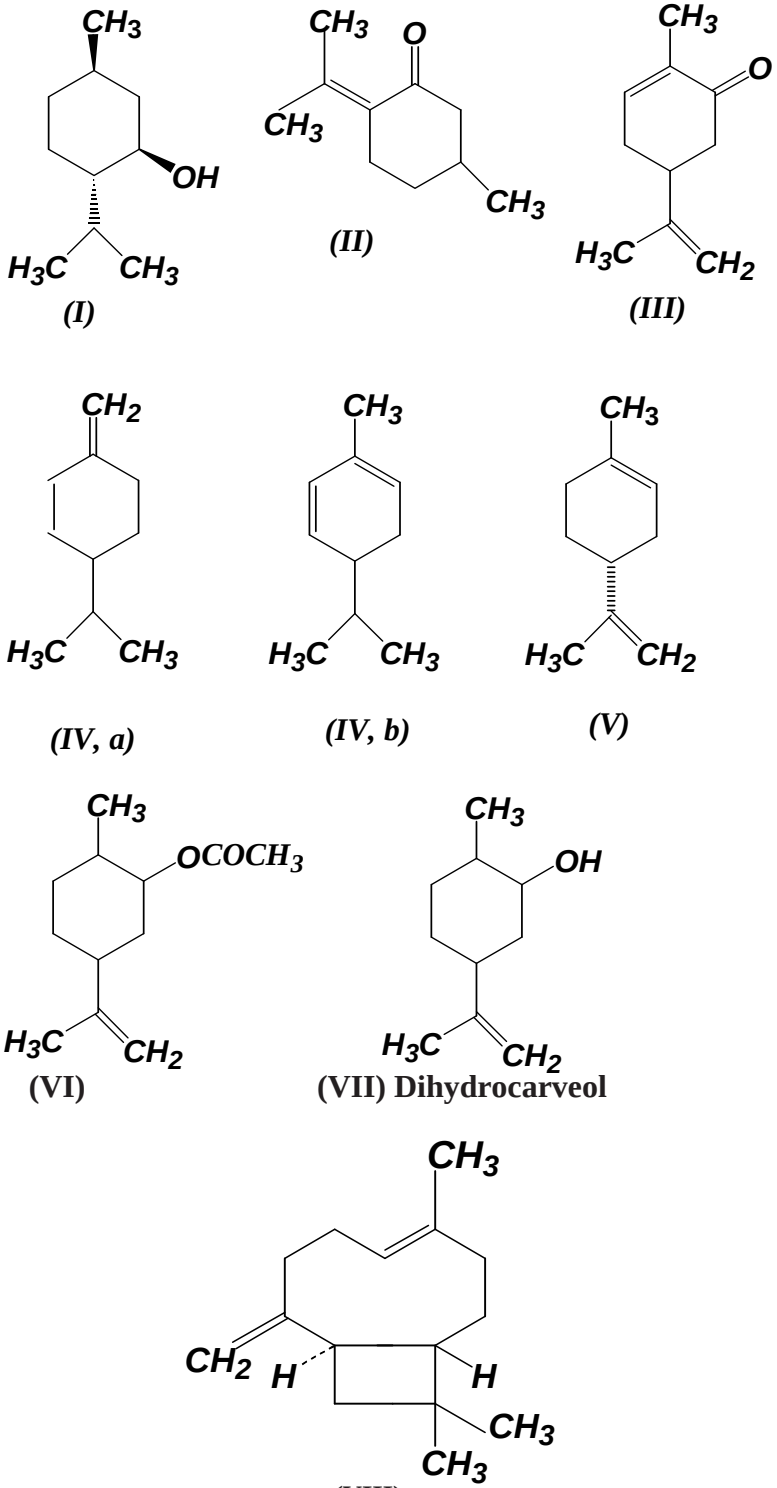


Fig. - 1: GC-MS Chromatogram of the essential oil obtained from the upper parts of Spearmint plant grown in the city of Ahwaz, Khuzestan Province, Iran.



Caryophyllene {4, 11, 11-trimethyl-8-methylenebicyclo[7.2.0.] undec-4-ene}

Fig. - 3: Chemical structure of the major components found in the essential oil obtained from the upper parts of Spearmint plant grown in the city of Ahwaz, Khuzestan Province, Iran

generally recorded over 40-400 amu full-scan mode that revealed the total ion current (TIC) chromatograms. A linear temperature program was adapted to separate the different oil components. It was reported that Spearmint oil contains mainly (-)-Carvone with a smaller amount of limonene and very small amounts of the lower-boiling terpenes α- and β-phellandrene. The (-)-Carvone could be easily separated from the oil by distillation.[5]Chemical composition of the essential oil obtained by hydrodistillation from the aerial parts of Spearmint viridis L. grown in the city of Ahwaz, capital city of Khuzestan province, Iran was determined both by GC-MS analysis and by using the various spectra of the separated components. Successive TLC and column chromatography of the various essential oils obtained on silica gel with methylene chloride as the mobile phase resulted in the separation of two fractions with R_f = 0.97 (as the minor fraction), and R_f = 0.55 (as the major fraction). IR, and ^{1}HNMR spectra of the fractions were taken and compared with the corresponding standard spectra.

The spectra were carefully analyzed. It was concluded that the fraction with R_f = 0.55 was (-)-Carvone [(2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one)] and the fraction with R_f = 0.97 was Limonene **[**(1-methyl-4-prop-1-en-2-yl-cyclohexene)]. GC-MS analysis of the essential oil revealed 17 components (table 1). It was found that the essential oil contained five major components with (-)-Carvone as the most abundant constituent. Four of the 5 major components were identified as Cineole, 2-Methylene-3-(1-methylethyl)-cyclohexanol acetate (cis) **(VI),** and Caryophyllene **(VIII)** (Table 2). For the identification of the other minor components, a more advanced GC-MS instrument equipped with a more efficient column and software program is needed.

REFERENCES

1. http://en.wikipedia.org/wiki/Spearmint; Accessed on 24th Feb. 2007.
2. http://mung.pittsfieldrr.net/Botany/Plants/LAMIACEAE%20-%20Mint/Mentha%20viridis%20-%20Spearmint.html; Accessed on 26th Feb. 2007.
3. http://www.botanical.com/botanical/mgmh/m/mints-39.html; Accessed on 26th Feb. 2007.
4. http://www.myherbals.net/Spearmint.htm; Accessed on 27th Feb. 2007.
5. Pavia DL, Lampman GM, and Kriz GS,"Introduction to Organic Laboratory Techniques, a contemporary approach", Saunders College Publising, 2nd edition, USA, 163-169 (1982).