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Isolation and Determination of the Major Chemical Compounds Present in Essential Oil of the Leaves of Myrtus Plant Grown in Khuzestan Province of Iran

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Myrtus plant has a variety of therapeutic properties. Regarding the abundance of the Myrtus plant grown around the city of Izeh, North East of Khuzestan province of Iran and its various therapeutic effects which have been used widely in the traditional medicine. The isolation of the main chemical components in the crude extracts obtained from the leaves of the myrtus shrub by maceration and Soxhlet extraction methods in various polar and non-polar solvents was carried out. The isolation and identification of the chemical components of the essential oil obtained by hydrodistillation from the leaves of Myrtus plant was done by using the advanced gas chromatography-mass spectrometry (GC-MS) technique. Maceration of the crushed leaves in toluene and Soxhlet extraction of the crushed leaves in toluene resulted in an oily material. TLC and column chromatography resulted in the separation of two fractions with R_f = 0.47 and 0.78. On the basis of various spectra (^{1}H NMR, ^{13}C NMR, UV-Visible, IR and MS), the fraction with R_f = 0.47 was identified as linalool (**I**) and the fraction with R_f = 0.78 was identified as 1,8-cineol (**II**). On the basis of the results obtained from GC-MS analysis of the volatile oil, it was concluded that the essential oil is consisted of four major components (α-pinene, 1,8-cineol, linalool and α-terpineol) totally 92.3 %, one minor component (linalyl acetate 1.1 %) and 23 other components with small amounts.

Key Words: Myrtus, *Myrtus communis* Lam, Myrtaceae.

INTRODUCTION

The Myrtle (Myrtus) is a genus of one or two species of flowering plants in the family of Myrtaceae, native to the Mediterranean regions, North Africa and Iran. Myrtles are aromatic evergreen shrubs with glossy dark-green leaves, white fragrant flowers and later bluish berries or small trees, growing to 5 m tall. The fresh or dried leaves, flowers and fruits are astringent and antiseptic and are used as a condiment

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and in cosmetics[1]. The leaves are about 3-5 cm long, with a pleasant fragrant essential oil. The flowers have five petals and sepals and an amazingly large number of stamens. Petals are usually white, with globose blue-black berries containing several seeds. The flowers are pollinated by insects and the seeds dispersed by birds which feed on the berries. The common Myrtle, *Myrtus communis* is widespread in the Mediterranean region and is also by far the most commonly cultivated. The plants are grown for myrtle oil used in perfume manufacture and as a condiment and as ornamental shrubs used in xeriscaping, where they are valued for their tolerance of hot and dry summers. On the Italian island of Sardinia, a digestive liquor called mirto is made by macerating myrtle berries in alcohol[2]. The leaves are aromatic, balsamic, haemostatic and tonic[3,4]. Recent research has revealed a substance in the plant that has an antibiotic action[3]. The active ingredients in myrtle are rapidly absorbed and give a violet-like scent to the urine within 15 min[5]. The plant is taken internally in the treatment of urinary infections, digestive problems, vaginal discharge, bronchial congestion, sinusitis and dry coughs[5,6]. In India it is considered to be useful in the treatment of cerebral affections, especially epilepsy[7]. Externally, it is used in the treatment of acne (the essential oil is normally used here), wounds, gum infections and haemorrhoid[5]. The leaves are picked as required and used fresh or dried[5]. An essential oil obtained from the plant is antiseptic[7]. It contains the substance myrtol-this is used as a remedy for gingivitis[3,7]. The oil is used as a local application in the treatment of rheumatism[7]. The fruit is carminative[7]. It is used in the treatment of dysentery, diarrhoea, haemorrhoids, internal ulceration and rheumatism[7].

EXPERIMENTAL

All the chemicals were purchased from Merck. NMR spectra were recorded on Varian FT-NMR spectrophtometer 500 MHz (^{1}H) and 125 MHz (^{13}C) using TMS as internal standard. Mass spectra were measured on HP (Agilent Technology) 5973 mass spectrometer. GC-MS analysis was carried out by using gas chromatograph-mass spectrometer Thermoquest-Finnigan instrument. Infrared spectra were recorded using a JASCO, IR700 Infrared spectrophotometer. UV-Visible spectra were recorded using a JASCO, 810-UV spectrophotometer.

Collection and drying the leaves of Myrtus plant: The leaves of the wild-growing Myrtus plant were collected from the surroundings of the city of Izeh, North East of Khuzestan province of Iran in the spring season. The plant was identified as *Myrtus communis* L. by the Research Institute of Medicinal Plants of Shahid Beheshti University, Tehran, Iran. The leaves of the plant were dried under the sunshine then powdered by using an electrical mill.

Hydrodistillation of the dried leaves: Hydrodistillation of the powdered leaves was done by using Clevenger apparatus and resulted in the formation of a clear yellow volatile oil which turned dark on standing at room temperature (1.5 mL). The oil was dried over anhydrous sodium sulphate and kept in refrigerator (4 °C).

Analysis: The crude volatile oil was analyzed by gas chromatography and gas chromatography-mass spectrometry. Gas chromatography analysis of the oil was conducted using a Thermoquest-Finnigan instrument equipped with a DB-1 fused silica column (30 m × 0.32 mm i.d., film thickness 0.25 μm) (Fig. 2). The oven temperature was held at 40 °C for 5 min then programmed to 250 °C at a rate of 4 °C/min. The injector and detector (FID) temperatures were kept at 250 °C. Helium was used as the carrier gas at the constant flow rate of 1.1 mL/min.

GC-MS analysis was carried out on a Thermoquest-Finnigan trace GC-MS instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 50 to 250 °C at a rate of 4°C/min, transfer line temperature 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min; split ratio, 1/50. The quadrupole mass spectrometer was scanned over the 40-300 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA (Fig. 3). The constituents of the volatile oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_6-C_{24}) and the oil on a DB-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature[7,8] as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature. Quantitative data was obtained from FID area percentages without the use of correction factors (Table-1).

Extraction of the dried leaves by maceration method: Maceration of 200 g of the dried leaves in 2000 mL of toluene followed by filtration of the mixture and removal of the solvent, gave 3.8 g of an oily material.

Soxhlet extraction of the dried leaves: Soxhlet extraction of 30 g of the dried leaves in 600 mL of toluene for 8 h followed by removal of the solvent gave 1.41 g of an oily material.

Thin layer chromatography analysis of the extracted oil: Thin layer chromatography analysis on silica gel with dichloromethane as the mobile phase was carried out. The spots were developed by using a UV lamp and Iodine crystals. The results showed two coloured spots with R_f = 0.47 and 0.78. Preparative TLC on the oily material was carried out and the two fractions with R_f = 0.47 and 0.78 separated and characterized. IR, UV-Visible, ^{1}H NMR and MS spectra of each fraction was taken.

Characterization of the component with R_f = 0.47 (linalool): IR (ν_{max}, cm^{-1}, neat liquid): 3450 s (OH); 2968 s, 2922 s (C-H, aliphatic), 1637 m-w (C=C), 1450 (CH_2), 1374 (CH_3); UV CH_2Cl_2 λ_{max} = 227 and 220 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.27 [CH_3(C-3), s], 1.57 [CH_2(C-4), m], 1.60 [CH_3(C-7), s], 1.67 [CH_3(C-7), s], 1.92 [OH(C-3), s], 2.04 [CH_2(C-5), m], 5.05 [H(C-1), d], 5.11 [H(C-6), t], 5.21 [H(C-1), d], 5.91 [H(C-2), dd]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 17.692

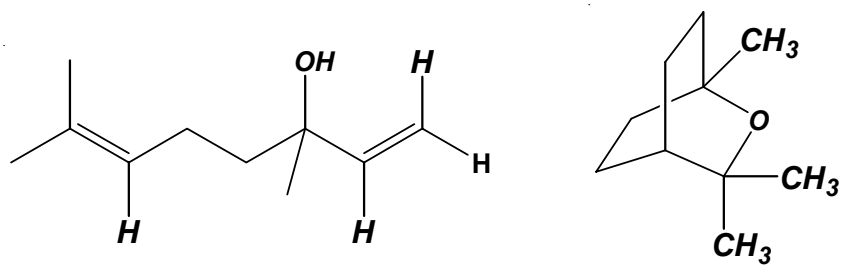
TABLE-1
COMPOSITION OF THE ESSENTIAL OIL OBTAINED FROM THE LEAVES OF MYRTUS PLANT GROWN AROUND THE IZEH CITY OF KHUZESTAN PROVINCE, IRAN

Compd. No.	Compound	Retention time (RT)	Retention index (RI)	%
1	α-Pinene	9.00	938	42.1
2	Camphene	9.27	950	0.2
3	β-Pinene	9.90	976	0.5
4	Myrcene	10.00	983	0.1
5	α-Pinene oxide	10.40	998	0.2
6	3-Carene	10.70	1010	0.1
7	α-Terpinene	10.80	1015	0.1
8	*para*-Cymene	10.90	1017	0.1
9	1,8-Cineol	11.20	1028	38.8
10	*trans*-Ocimene	11.50	1038	0.1
11	γ-Terpinene	11.80	1052	0.3
12	*trans*-Linalool oxide	12.46	1075	0.1
13	Linalool	12.70	1087	7.7
14	exo-Fenchol	13.23	1105	0.1
15	α-Campholenal	13.39	1111	0.1
16	*trans*-Pinocarveol	13.90	1131	0.3
17	δ-Terpineol	14.50	1153	0.1
18	Borneol	14.60	1157	0.1
19	*trans*-3-Pinanone	14.85	1164	0.1
20	4-Terpineol	15.00	1168	0.2
21	α-Terpineol	15.20	1178	3.7
22	Carvone	16.40	1223	0.1
23	Geraniol	16.80	1237	0.2
24	Linalyl acetate	16.90	1240	1.1
25	Terpinyl acetate	19.50	1337	0.2
26	Neryl acetate	19.70	1344	0.1
27	Geranyl acetate	20.10	1362	0.4
28	β-Caryophyllene	21.80	1427	0.1

(C_5), 22.825 [CH_3(C-7)], 25.705 (C_8), 27.812 [CH_3(C-3)], 42.116 (C_4), 73.447 (C_3), 111.690 (C_1), 124.407 (C_6), 131.822 (C_7), 145.094 ($C2_3$); MS (EI) showed m/z: 137 [$(M\text{-}OH)^+$, 2.1 %], 136 [$(M\text{-}H_2O)^+$, 4.3 %], 121{[$M\text{-}(CH_3+H_2O)]^+$, 15.1 %}, 83{[CH_2=CH-C(OH)(CH_3)$CH_2]^+$, 12.2 %}, 71 {[$(CH_3)_2$C=CH-$CHCH_2]^+$, 82.2 %}, 69 {[CH_2=CH-C(OH)(CH_3)]$^+$, 38.8 %}, 55 {[$(CH_3)_2$C=CH]$^+$, 60.4 %}, 51 {[69-$H_2O]^+$, 5 %}, 43 {[CH_2=CH-C(CH_3)]$^+$, 100 %}. On the basis of these results, this component was identified as linalool (**I**).

Characterization of the component with R_f = 0.78 (1,8-cineol): Its IR (ν_{max}, cm^{-1}, neat liquid): 2966 s, 2942 s, 2922 s (C-H, aliphatic), 1464 s, 1445 s (CH_2), 1374 s, 1359 s (CH_3), 1214 s, 1166 s (C-O); UV CH_2Cl_2 λ_{max} = 227 and 212 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.048 [CH_3(C-1), s], 1.24 [2 × CH_2(C-3), s], 1.41 [H(C-4), s], 1.53 [4H(C-7, C-8), q], 1.66 [2H(C-5/C-4), t], 2.04 [2H(C-4/C-5), t]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 22.85 [2 × CH_3 (C_3)], 27.59 [CH_3(C-1)],

28.90 [(C_7 + C_8)/(C_5 + C_6)], 31.52 [C_5 + C_6)/(C_7 + C_8)], 32.94 (C_4), 69.75 (C_3-O), 73.59 (C_1-O); MS (EI) showed m/z: 154 [M^+, 89 %], 140 [(M-CH_2)$^+$, 7.8 %], 134 [(M-CH_3)$^+$, 83.5 %], 126 {[140-(CH_2)]$^+$, 10.7 %}, 111 [98 %], 96 [52.8]$^+$, 68 [45.5 %]$^+$, 67 [29.2 %]$^+$, 58 {[(CH_3)$_2$C=O]$^+$, 29.2 %}, 43 {[CH_3CO]$^+$, 100 %}. On the basis of these results, this component was identified as 1,8-cineol (**II**).



(**I**) Linalool (**II**) 1,8-Cineol

Fig. 1. Chemical structures of the isolated components from the extracted oil obtained from the leaves of Myrtus plant

RESULTS AND DISCUSSION

Myrtus is an evergreen shrub or small tree with the scientific name of *Myrtus communis* Lam which is grown wildly in various parts of Iran. Myrtus is also cultivated as an ornamental plant and has a beautiful appearance. Its leaves, flowers and hulls have a very pleasant odour and are used in making perfume. In general, all different parts of the plant and its essential oil have been used for their therapeutic effects. Myrtus plant has a variety of therapeutic effects including antiseptic, antiparasitic, cardic tonic, stomach tonic, coagulant and also is used for the treatment of the respiratory and urinary system diseases. Regarding the abundance of the myrtus plant grown around the city of Izeh, North East of Khuzestan province of Iran and its various therapeutic effects which have been used widely in the traditional medicine, it was decided to investigate the following objectives in this research: (i) Isolation of the main chemical components found in the crude extracts obtained from the leaves of the myrtus shrub by maceration and Soxhlet extraction methods in various polar and non-polar solvents, (ii) Identification and determination of the chemical structures of the above mentioned isolated components by using various spectroscopic techniques, (iii) Isolation and identification of the chemical components of the essential oil obtained from the leaves by the advanced gas chromatography-mass spectrometry (GC-MS) technique. After collecting the plant from the surroundings of the city of Izeh, it was identified as *Myrtus communis* Lam, then air dried and finally the leaves were crushed. Maceration of the crushed leaves was carried out in various polar and non-polar solvents such as chloroform, dichloromethane, methanol, ethanol, toluene and water. However, the best solvent was found to be

toluene. Soxhlet extraction of the crushed leaves in toluene also resulted in an oily material. TLC and column chromatography on silica gel with methylene chloride as the mobile phase resulted in the separation of two fractions with R_f = 0.47 and 0.78. ^{1}H NMR, ^{13}C NMR, UV-Visible, IR and MS spectra of these fractions were taken. On the basis of their spectra, the fraction with R_f = 0.47 was identified as linalool (**I**) and the fraction with R_f = 0.78 was identified as 1,8-cineol (**II**). Hydrodistillation of the powdered leaves of Myrtus plant by using Clevenger hydrodistillation apparatus gave a clear yellow oil with an ardent odour. GC-MS analysis of the volatile oil obtained by hydrodistillation, was carried out and identification was based on the retention indices relative to reference library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature[8,9]. On the basis of the results obtained it was concluded that the essential oil obtained from the leaves of Myrtus plant is consisted of four major components (α-pinene, 1,8-cineol, linalool and α-terpineol), totally 92.3 %, one minor component (linalyl acetate 1.1 %) and 23 other components with tiny amounts (Table-1). The structures of some of the isolated components are given in Fig. 2.

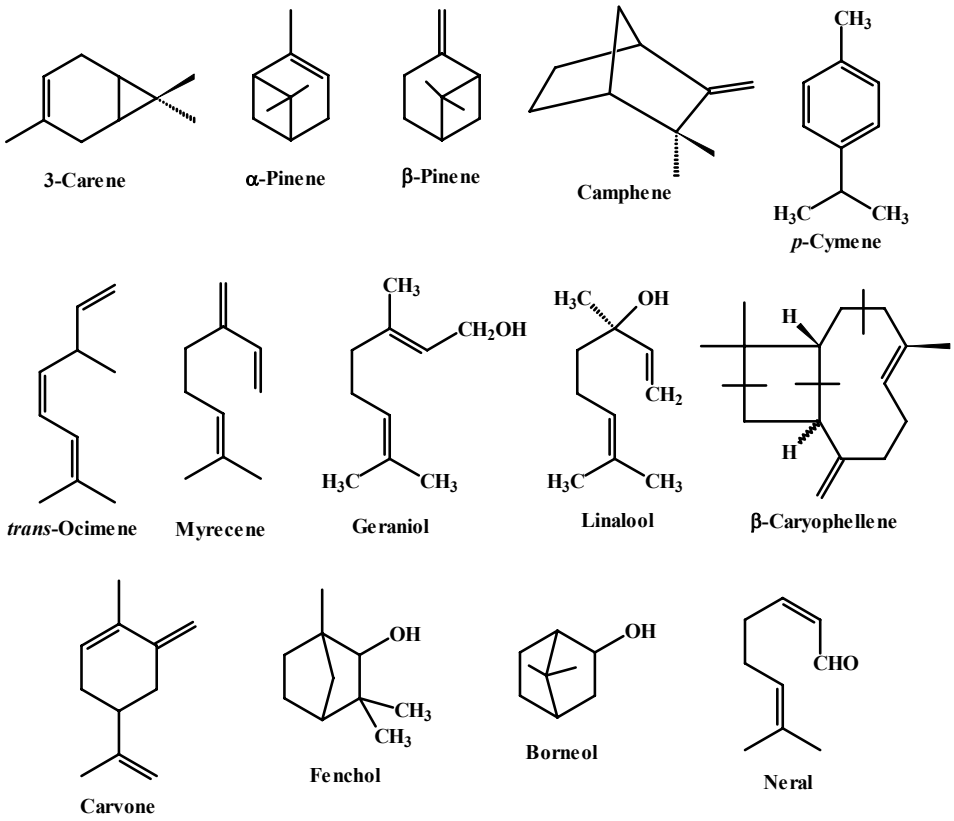


Fig. 2. Chemical structures of some of the components found in the essential oil obtained from the leaves of Myrtus plant grown in Izeh, Khuzestan province of Iran

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