

GC/MS ANALYSIS OF THE ESSENTIAL OILS OF *CUPRESSUS ARIZONICA* GREENE, *JUNIPERUS COMMUNIS* L. AND *MENTHA LONGIFOLIA* L.

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ABSTRACT. The chemical composition of the essential oils obtained by hydrodistillation from *Cupressus arizonica* Greene, *Juniperus communis* L. and *Mentha longifolia* L. were determined by gas chromatography-mass spectrometry (GC/MS) analysis. The chemical composition of the essential oils were identified by GC/MS. Eicosane (27.4%), umbellulone (13%) and α -pinene (10.51%) were the major components of *C. arizonica* oil; sabinene (32%), limonene (26%) and bornyl acetate (7.4%) were the major components of *J. communis* oil and pulegone (26%), *L*-menthone (13.4%) and *cis*-para-menthan-3,8-diol (10.2%) were the major components of *M. longifolia* oil. The percentage of monoterpenes in the three essential oils was compared. The percentage of monoterpenes in *C. arizonica* is about 5.2%, *J. communis* 46%, and *M. longifolia* is 50.1%. The percentage of terpenes in *C. arizonica* (16.3%), *J. communis* (5.2%) and *M. longifolia* (9.3%) were reported. But the percentage of cyclic monoterpenes in *C. arizonica* is about 6%, *J. communis* 26.1%, and *M. longifolia* is 3%. Monoterpenes are the most components that make up essential oils.

KEY WORDS: *Cupressus arizonica*, *Juniperus communis*, *Mentha longifolia*, Essential oil, Chemical composition

INTRODUCTION

Essential oils have been of the supreme importance for fine pharmacology applications, aroma and cosmetic preparations for a long time, because of their intense, pleasant, sweet, and floral as well as their fixative properties [1-4]. In the last years the number of procedures using extraction of organic compounds from different matrices has increased. Result of analyses involves pretreatment method of the samples, extraction and condensation method to increase the analytic concentration to within gadget sensitivity. The extraction method, qualitative and quantitative analyses by using gas chromatography-mass spectrometric technique (GC/MS) are the steps followed for compounds identification [5, 6]. The remedial possible of essential oils, like other plant-derived remedies, has yet to be fully realized. This is individually true of aromatic oils, which by their very nature have such a concentrated yet multifaceted approach make-up a small ratio of the world flora has been tested for pharmacologically active compounds, but with the ever-increasing danger of plants becoming extinguished, there is a real risk that many important plant sources may be lost [7-9]. The exercise of aromatherapy could be seen as part of the larger field of herbal medicine, since the essential oil is only one of many ways in which a plant can be prepared as a treatment. Since all essential oils are derived directly from plants, it can be exquisite to see them within a botanical context rather than as isolated products. In some ways the use of aromatic oils for therapeutic purposes interests gas chromatography columns with different polarities have been designed for the analysis of compounds with a corresponding range of polarities. To comprehensively characterize the composition of an essential oil sample, chromatographic columns with different polarities should be accessible [10-13]. In this project, the chemical composition of three different oils of *Cupressus arizonica* Greene, *Juniperus communis* L. and *Mentha longifolia* L. was investigated and their compositional components identified. For example, some compounds such as eicosane

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(27.4%, in Table 1, row 7 and column 2) were the main component in *C. arizonica* cultivated in North-West Iran while this compound is not the dominant component in other reports.

In other research reports, the most important components of essential oils from *M. longifolia* were menthol (33%), menthone (21%) and pulegone (18%) [14]. Main identified compounds of the *C. arizonica*, *J. communis* and *M. longifolia* essential oil in several studies obtained by GC/MS showed that α -pinene was the main components of the essential oil leaves of *C. arizonica* cultivated in Iran, Italy and Argentina [15,16]. α -Pinene dominated in the most leaf oils of *J. communis* growing in Estonia [17], China [18] and Bulgaria [19]. The active components of essential oils are alkaloids, glycosides, steroids and volatile oils [20, 21].

EXPERIMENTAL

Plants material

Aerial parts include leaves of *Cupressus arizonica* Greene (Cupressaceae), *Juniperus communis* L. (Cupressaceae) and *Mentha longifolia* L. (Lamiaceae) were collected from campus of Urmia University (West Azerbaijan Province, Urmia, Iran) in May 2017. These plants were dried in shadow (away from sunlight) and ventilated area at 32 ± 2 °C for 2-10 days. Each plant product was cut into small pieces using a blade and a chopping board then were kept in a refrigerator.

Essential oil extraction

Plant materials were milled into fine powder using a milling machine. 60 g of the plant samples to which 650 mL distilled water was added, were subjected to hydrodistillation using a modified Clevenger-type apparatus for 3 hours. Essential oils dried over anhydrous sodium sulfate and transferred into amber-colored vials at 4 °C for further tests [22].

Sample preparation

After the preparation of essential oils by hydrodistillation method, a quantity of 5 μ L from each of essential oil was mixed with 100 μ L hexane and a very small amount of sodium sulfate. Then 1 μ L from the new mixture was injected into the chromatograph.

GC/MS analysis

The analyses were performed in Jihad-e-Daneshgahi of Urmia, (West Azerbaijan Province, Urmia, Iran). The chemical composition of essential oils of *C. arizonica*, *J. communis* and *M. longifolia* were analyzed by GC/MS using a gas chromatography mass spectrometry system (Agilent 7890A/5975C GCMS System) equipped with HP-5 column (30 m \times 0.1 mm; 0.25 μ m film thickness). The oven temperature was held at 60 °C for 3 min, programmed at 20 °C/min to 240 °C and then held at this temperature for 8.5 min. The carrier gas was helium (99.99%) at a flow rate of 1 mL/min. Mass spectra were taken at 70 eV. The injector temperature was 280 °C. Identification of the constituents of the oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and those authentic samples [23]. Chemical compounds of essential oils include esters, aldehydes, alcohols, phenols, ketones and terpenes. Computer matching identified compounds used as references (Wiley and Mass Finder 3.1) [24, 25]. The comparative condensations of the separated compounds based on percentage were estimated based on chromatograms obtained from GC/FID/MS system. The chromatograms are shown in (Figures 1-3).

RESULTS AND DISCUSSION

In this research extracted the herbal essential oils of *C. arizonica*, *J. communis* and *M. longifolia* using the GC/MS technique and identified the constituents of them. Results of the compounds of *C. arizonica* are considered in Table 1, the most constituents of the compounds are terpene, terpenoids and acyclic alkanes. The chemical composition of the essential oils of *C. arizonica* has 23 compounds. The major of them include eicosane (C₂₀H₄₂): (27.4%), umbellulone (C₁₀H₁₄O): (13%) and α -pinene (C₁₀H₁₆): (11%) (Table 1). The chemical composition of the essential oils obtained from *J. communis* have 25 compounds, the major of them include sabinene (C₁₀H₁₆): (32%), limonene (C₁₀H₁₆): (26%) and bornyl acetate (C₁₂H₂₀O₂): (7.4%) (Table 2). The chemical composition of the essential oils obtained from *M. longifolia* have 29 compounds, the major of them include pulegone (C₁₀H₁₆O) (26%), *L*-menthone (C₁₀H₁₈O): (13.4%) and *cis-p*-menthan-3,8-diol (C₁₀H₂₀O₂): (10.2%) (Table 3).

Compositions of three essential oils

The compositions of *C. arizonica* oil contain terpene (16.3%), monoterpene (5.2%), cyclic monoterpene (6%), terpenoid (1.4%), monoterpene ketone (13%), terpineol (5.4%), sesquiterpene alcohol (1.2%), volatile terpene (4.1%) and acyclic alkanes (35.3%). Acyclic alkanes and sesquiterpene alcohols have the highest and the lowest percentage of compounds, respectively (Figure 4).

Table 1. Essential oil composition (%w/w) of leaves of *C. arizonica* cultivated in North-West Iran.

Component	RI	RT	Percentage	Component	RI	RT	Percentage
α -Pinene	934	5.2	11	Elemicin	1560	19	1
Sabinene	970	6	2	Cedrol	1608	20	1.2
β -Myrcene	990	6.3	1	1-Cubenol	1635	20.3	4.1
O-Cymene	1014	7.1	3	α -Acorenol	1638	20.4	1
Limonene	1030	7.2	6	α -Cadinol	1657	21	1
Camphor	1148	10	1.4	Eudesm-7(11)-en-4-ol	1694	21.4	4
Umbellulone	1176	11	13	Eicosane	2158	29	27.4
Terpinene-4-ol	1179	11	4	Tetatriacontane	2390	31.2	1
α -Terpineol	1193	11	1.3	Nonacosane	2606	34	5
Alloaromadrene	1467	17	3.3	Dotriacontane	3046	40	1.4
Is, <i>cis</i> -calamenene	1525	18.2	3.4	17-Pentatriacontene	3644	49	1
Candina-1(2),4-diene, <i>cis</i>	1538	18.4	1				

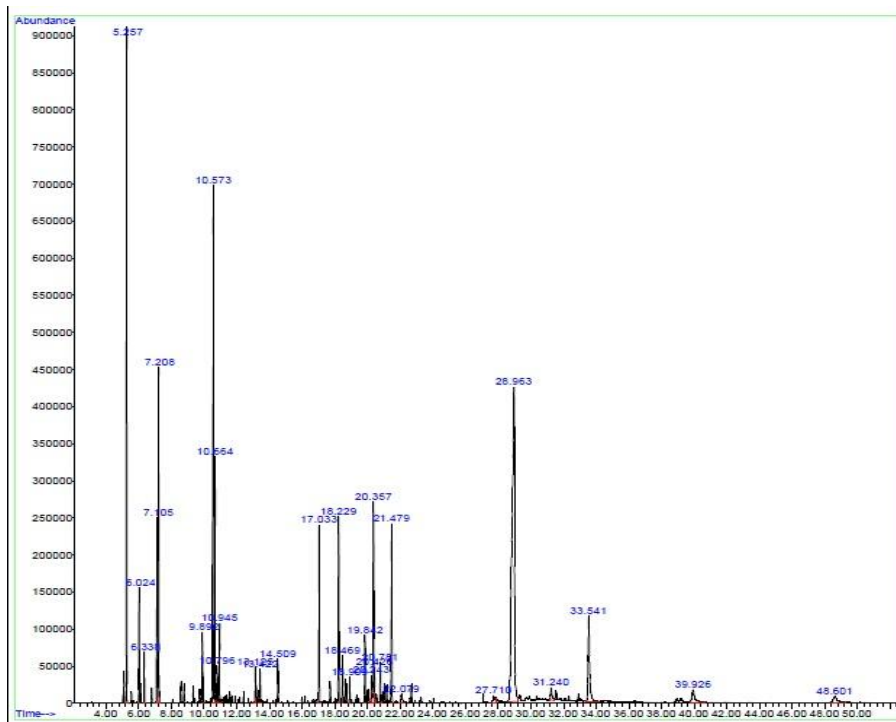
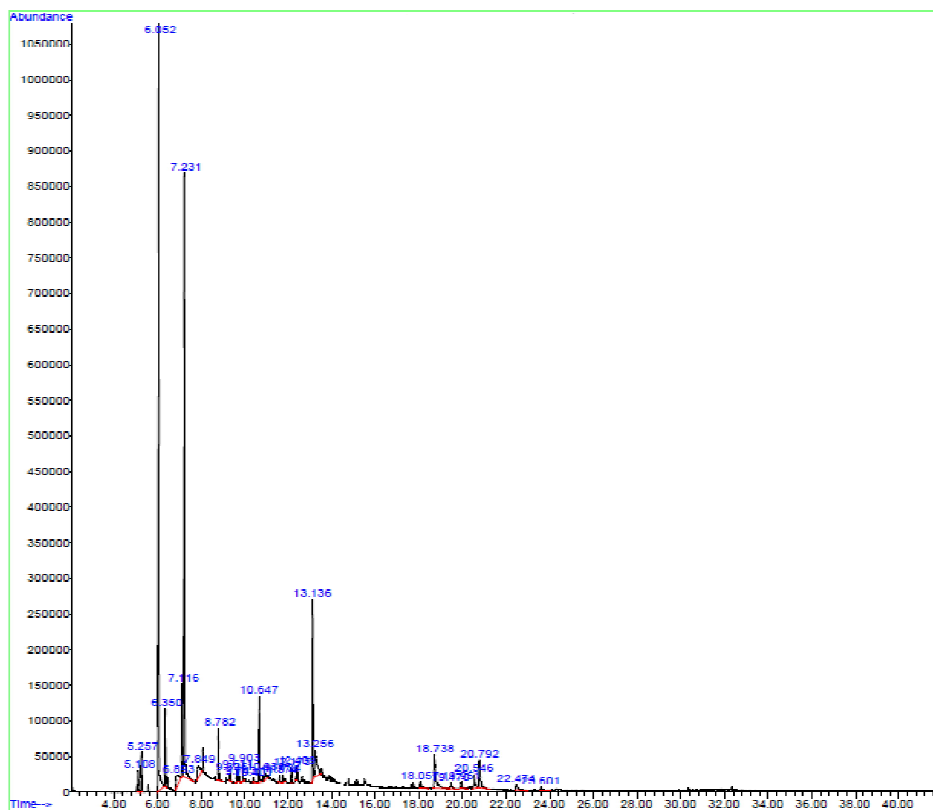


Figure 1. GC Chromatogram of *C. arizonica* leaves, run on a HP-5 capillary column.

Table 2. Essential oil composition (%w/w) of leaves of *J. communis* cultivated in North-West Iran.

Component	RI	RT	Percentage	Component	RI	RT	Percentage
Thujene	928	5.1	1	Terpinene-4-ol	1179	10.64	3.37
α -Pinene	934	5.2	1.2	α -Terpineol	1193	12	1
Sabinene	970	6	32	Trans-carveol	1219	12	0.3
β - Myrcene	989	6.3	3	Isobronylformate	1226	12	0.3
1,4-Cineole	1014	7	2	Caryone	1245	12.1	1
O-Cymene	1014	7.1	3.3	Bronyl acetate	1287	13.1	7.4
Limonene	1030	7.2	26	Laranduly acetate	1293	13.2	3.3
C-Terpinene	1057	8	2	A-Selinene,7-epi-	1517	18	1
Linolool	1098	9	2.1	Germacrene B	1551	19	3.4
Limonene oxide, trans-	1122	10	1	Cubenol,1,10-di-epi-	1614	20	1
Terpen-1-ol	1134	10	0.4	A-Muurolol	1645	21	1.2
camphor	1148	10	1	A-Cadinolii	1658	21	2.3
3-Hexenyl butanoate,(z)-	1168	10.4	0.4				

Figure 2. GCChromatogram of *J. communis* leaves, run on a HP-5 capillary column.Table 3. Essential oil composition (%w/w) of leaves of *M. longifolia* cultivated in North-West of Iran.

Component	RI	RT	Percentage	Component	RI	RT	Percentage
α -Pinene	934	5.2	5.4	<i>cis</i> -Piperitone oxide	1257	12.4	4
Sabinene	970	6	1	Thymol	1280	13.1	1
β -myrcene	990	6.3	1	<i>p</i> -Cymen-7-ol	1294	13.2	1
O-Cymene	1014	7.1	1.1	Piperitenone	1343	14.3	1
Limonene	1030	7.2	3	α -Terpinyl acetate	1350	15	9.4
1,8-Cineole	1032	7.3	1	Piperitenone oxide	1368	15	2.1
Linalool <i>l</i>	1099	9	0.3	<i>trans</i> -Caryophyllene	1425	16.1	1
Camphor	1148	10	1	alloaromadendrene	1467	17	1.3
<i>l</i> -Menthone	1157	10.1	13.4	1 <i>s,cis</i> -Calamene oxide	1525	18.2	2.1
Isomenthone	1166	10.3	2	Caryophyllene oxide	1589	19.4	1
<i>cis-p</i> -Menthan-3,8-diol	1175	11	10.2	Cedrol	1608	20	1
Terpinene-4-ol	1179	11	3	1-Cubenol, epi-	1635	30.3	2.4
α -Terpinneol	1193	11	1	α -Cadinol	1657	21	1
<i>cis</i> -Dihydrocarryone	1202	11.1	1	Eudesm-7(11)-en-4-ol	1694	21.4	3
Pulegone	1240	12.1	26				

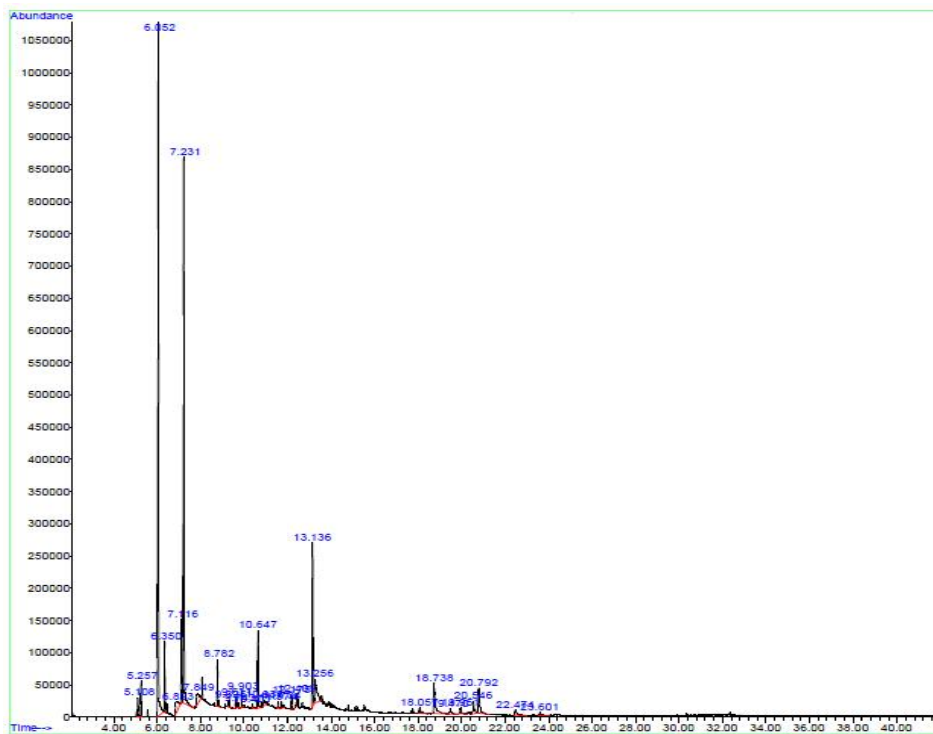


Figure 3. GC Chromatogram of *M. Longifolia* leaves, run on a HP-5 capillary column.

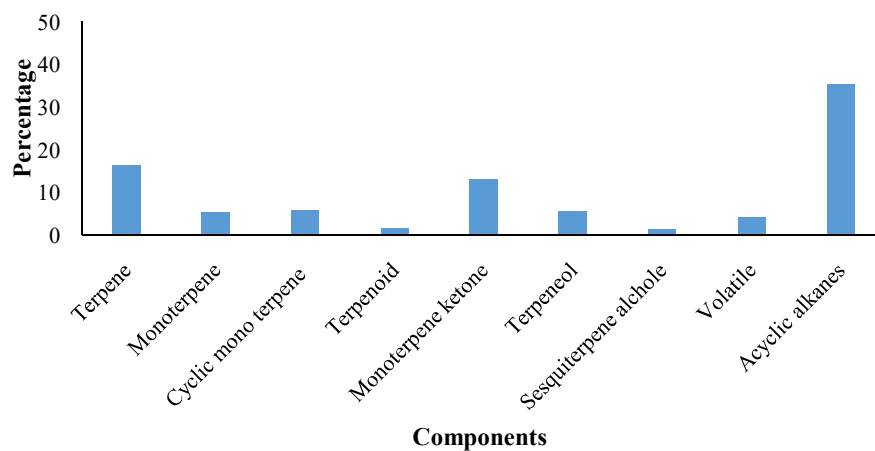


Figure 4. The chart of effective percentage composition from leaves of *C. Arizona* essential oil.

The compositions of *J. Communis* oil contain terpene (5.2%), monoterpene (46%), cyclic monoterpene (26.1), terpineol (4), terpinoid (1.2%), monocyclic monoterpinoic alcohol (0.3%), volatile organic hydrocarbons (4%) and sesquiterpenoid alcohol (2.3%). Monoterpenes and monocyclic monoterpinoic alcohol have the highest and the lowest percentage of compounds, respectively (Figure 5).

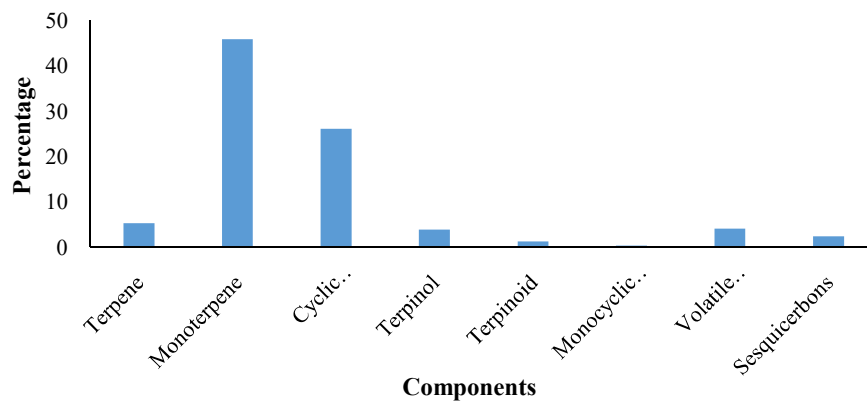


Figure 5. Effective percentage composition from leaves of *J. communis* essential oil.

The compositions of *M. longifolia* oil contain terpenes 9.3%, monoterpenes (50.1%), cyclic monoterpene (3%), monoterpinoic alcohol (2%), terpinoid (21.2%), terpineol (12.3%), monoterpinoic alcohol (2.1%) and aromatic terpene (1%). Monoterpenes and aromatic terpenes have the highest and the lowest percentage of compounds, respectively (Figure 6). Representatively, molecular structures of some terpenes are shown in Figure 7.

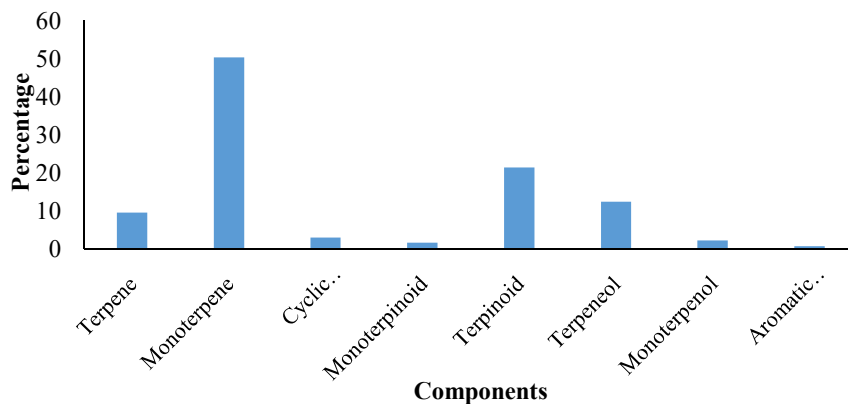


Figure 6. Effective percentage composition from leaves of *M. longifolia* essential oil.

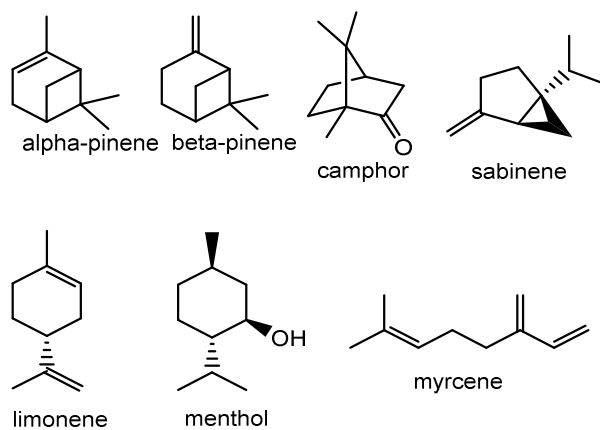
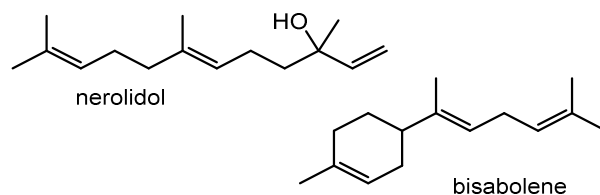
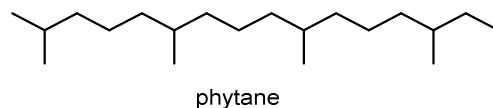
Examples of monoterpene**Sesquiterpene****Diterpene**

Figure 7. Molecular structures of some terpenes.

GC/MS is a structural analysis tool which plays a key role in medical, pharmacy, agriculture, the analysis of essential oils, pesticides and other classes of compounds. The results of the analysis of three essential oils by gas chromatography in this survey with other researchers from different places show that there are differences between the percentages of the ingredients. The chemical constituents of *M. longifolia* oil collected by [26] from Tehran province in Iran (located in central Iran), extracted by the same conditions as described in this study were comprised of carvone (62%) and limonene (19.4%) as the major constituents with 23 compounds. According to [27] reports from Taif in Saudi Arabia, with 38 compounds, pulegone (33.2%), and menthone (29%) were comprised the major constituents. The essential oil composition of *M. longifolia*, *cis*-epoxy piperitone (18.4%), pulegone (15.5%) and piperitenone oxide (15%) were as main components in Turkey [28]. The other research by [29] from five regions in South-West of Iran the major compounds were piperitenone oxide (7.4–60%),

pulegone (4–49.4%). The present research was conducted in North-West of Iran with 29 compounds, pulegone (26%) and *L*-menthone (13.4%) were the major constituents. The essential oil from *M. longifolia* species including secondary metabolite components such as menthol, menthone and limonene studied for their insecticidal activity against several stored product pests [30, 31].

Angioni *et al.* [32] reported that the essential oils from leaves of *J. communis* from Italy with 35 components, sabinene with 61% was dominated. Also, according to Shahmir *et al.* [33] reported that sabinene was the dominant compound in Northern Iran (19.2%) and the effective compositions of *J. communis* included monoterpene (83.2%) and sesquiterpene (1.2%). Results of this present study from North-West of Iran showed that the essential oil composition of *J. communis* with 25 components, sabinene (32%), limonene (32%) and bronyl acetate (7.4%) were as main components and the effective compositions included monoterpene calculated (46%). The present results showed that sabinene was the dominant component with difference in percentage, therefore is in agreement with sample from Italy and Iran [32, 33]. Carroll *et al.* [18] reported the major component of the essential oils from leaves of *J. communis* oil from China was α -pinene (27%). Sela *et al.* [34] reported that the chemical composition of fifteen samples of juniper essential oil locations in Republic of Macedonia showed that the major components were α -pinene (16–43.1%), β -pinene (2%-5.3%), β -myrcene (3%-26.5%) and sabinene (3–12%). Buci *et al.* [35] reported the chemical composition of the essential oils of *J. communis* from Albania there were 56 compounds and the principal were: α -pinene (36%), β -myrcene (45%), sabinene (10%) and germacene D (5%). Also, the effective composition of *J. communis* oils was monoterpene (72%). The composition of the essential oil from the leaves of *J. communis* in present research is different from that of the essential oil of China, Republic of Macedonia and Albania, but in the other studies α -pinene was dominant.

Cheraif *et al.* [36] reported that the essential oil from leaves of *C. arizonica* from Tunisia was dominated α -pinene (20%), umbellulone (18.4%) and limonene (5.8%). Analysis of volatile compounds from *C. arizonica* leaves has been the subject of several investigations in six other countries. These previous studies showed that α -pinene was the main components of the oil obtained from the leaves of *C. arizonica* cultivated in Isfahan (located in central region), Iran (19.2%) [15], Argentina (23%) [16], Italy (8%) [37]. Our result showed that the eicosane with (27.4%) was the main components of this essential oil from Iran. The result of this research is different from the results of other researchers.

There are differences among the major compounds, the number of compounds and the percentage of the essential oils in our study compared to other similar reports. Comparing the results obtained from GC/MS analysis, herbal essential oils showed remarkable difference from the same species cultivated in other countries. These differences can be related to several factors like the geographic and climate conditions, plant organs used and methods for extraction of essential oils, season of sampling and soil composition [38-40]. Characterization of the compounds by comparison of mass spectra and retention indices with literature records showed that *C. arizonica*, *J. communis* and *M. longifolia* are composed mainly of monoterpene (18.1, 46 and 50.1%) and cyclic monoterpene (6, 26.1 and 3%), respectively.

CONCLUSION

In summary, in this study, the GC-MS analysis of the percentage chemical composition of essential oils extracted from leaves of *C. arzonica*, *J.communis* and *M. longifolia* in North-West Iran including the major components of three essential oils eicosane (27.4%), sabinene (32%) and pulegone (26%), respectively. Also, the eicosane, sabinene and pulegone were dominant components of *C. arzonica*, *J.communis* and *M. longifolia* oils, respectively. In literature there are differences among the major compounds and the number and percentages of compounds in essential oils of plants. These differences can be due to factors such as geographic location,

plant organs used, extraction methods, season of sampling and soil composition. Therefore, all these factors affected the results of present study.

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