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# AROMATIC AND MEDICINAL PLANTS OF MOROCCO: CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *Rosmarinus officinalis* AND *Juniperus Phoenicea*

E. Derwich[1*], Z. Benziane [2] and R. Chabir [3]

[1*] Unity of GC/MS and GC, Regional Center of Interface, University Sidi Mohamed Ben Abdellah, B. P. 2626, Road Immouzer, Fez, Morocco, Télé: 00.212. 45.756.052, E-mail: Elhoussinederwich@yahoo.fr

[2] Laboratory of Energy, Natural Resources and Modeling, University Sidi Mohamed Ben Abdellah, Faculty of Sciences, 30.000, Fez, Morocco

[4] Laboratory of Physiopathology, Faculty of Medicine and Pharmacy, Sidi Mohamed Ben Abdellah, 30.000, Fez, Morocco

**ABSTRACT:** The chemical composition of essential oils obtained from *Rosmarinus officinalis* (family Lamiaceae) and *Juniperus Phoenicea* (family Cupressaceae) were determined. Their essential oil was determined by hydro-distillation, analysed by GC/MS and GC-FID. The analyses for leaves of *Rosmarinus officinalis* resulted in the identification of twenty three compounds, representing 63.81% of the total oil and the yields were 0.54%. The major component was α-pinene (18.25%); other predominant components were camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%).

Twenty tree compounds were identified in leaves oil of *Juniperus phoenicea* representing 81.87% of the total oil composition. The yield of essential oil was 1.62% and the major compound in aerial parts was α-pinene (49.15%) followed by α-phyllandrene (7.39%), mycene (5.24%), β-pinene (3.58%), linalool (2.54%), piperitone (1.56%), γ-terpinene (1.28%), Trans-pinocarveole (1.23%) ρ-cymene (1.10%), α terpineol (1.02%) and γ-cardinene (1.01%).

**Key words**: *Rosmarinus officinalis, Juniperus Phoenicea,* Essential oils, GC/MS.

## INTRODUCTION

*Rosmarinus officinalis*, a member of the family Lamiaceae is a flowering plant that grows in Mediterranean countries, southern Europe and in the littoral region through Minor Asia areas wildly. Essential oils are also called volatile oils and are generally aromatic oils obtained by the steam or hydro-distillation of plants. Different parts of plants have been used to obtain essential oils. These include the flowers, leaves, seeds, roots, steams, bark, and wood though secretionary parts. Multiple studies have been reported on the chemical composition of the essential oils of *Rosmarinus officinalis* belonging to different regions in the world (Khorshidi et al., 2009; Pintore et al., 2002; Bicchi et al., 2000). The essential oil of *Rosmarinus officinalis* has been the object of several studies antioxidant activity (Moreno et al, 2006; Wang et al, 2008; Peng et al, 2005; Lo et al, 2002; Éva et al, 2003), antibacterial (Delcampo et al, 2000; Ouattara et al, 1997; Oluwatuyi et al, 2004; Rozman and Jersek, 2004; Moghtader, Afzali, 2009), Toxicity insecticidal (Papachristos and Stampoulos, 2004; Tunc et al, 2009), Anti-inflammatory and Antinociceptive (Takaki et al, 2008), antifungal (Ozcan and Chalchat, 2008; Pozzatti et al, 2008) and only, in recent years have these oils been commercialised as pest control products (Isman, 2000). In our previous studies on the toxicity of *Rosmarinus officinalis*, essential oil and blends of its major constituents against *Tetranychus urticae Koch* (Acari: Tetranychidae) on two different host plants (Miresmailli et al, 2006). In traditional medicine, Rosemary is used to treat different diseases including: depression, insomniac and arthritic pains (Zargari, 1995). Moreover volatile compounds obtained from plants, have known antimicrobial, antifungal and insecticidal activities (Janssen et al, 1987; Kurita et al, 1981; Oka et al, 2000). Essential oils have many therapeutic and they aid the distribution of drugs and antiseptics (Palevitch and Yaniv, 1991).

*Juniperus phoenicea*. (Fam. Cupressaceae) is the species found in Morocco. It extends to Egypt (Elsawi et al., 2007) and Central Arabia (Boulos, 1999). The leaf essential oil of *Juniperus phoenicea* has been reported in varying details from Saudi Arabia (Dawidar et al., 1991), France (Tabacik and Laporte, 1971; Tabacik and Poisson, 1971) and from Greece and Spain (Adams et al., 1996). Also, there are some reports on the analysis of fruit essential oils (Ramic and Murko, 1983; Delitala, 1980). Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro et al., 2000). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli, 2009). Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition (Buchbauer, 2000). Also the essential oils are used in traditional medicine for their antiseptic action, are constituted 1% of plant secondary metabolites and are mainly represented by terpenoids, phenypropanoids or benzenoids, fatty acid derivatives and amino-acid derivatives (Dudareva et al., 2006). *Juniperus phoenicea*, is small tree that is native to the northern lands bordering the Mediterranean Sea from Portugal. It is also native to North Africa in Algeria and Morocco as well as the Canary Islands (Gaussen, 1968). The essential oils which were utilised centuries ago in cosmetics usually show interesting biological features. The oils also help increase the flow of digestive fluids, improve digestion and eliminate gas and stomach cramping (Uphof, 1968). Juniper has been used for centuries as a steam inhalant for bronchitis and to control arthritis. The oil is also irritating to microbes, so much so that it kills many of them (Watt et al., 1962; Stassi et al., 1996). In the light of this work we have determined and comparative the chemical composition of essential oils isolated from leaf of *Rosmarinus officinalis* and *Juniperus Phoenicea* from Morocoo.

## MATERIALS AND METHODS

### Vegetal material

The leaves of *Rosmarinus officinalis* and *Juniperus Phoenicea* have been collected during March 2009 in the region of Atlas median (Tafersoust) from Morocco, 90 km in the south east of Sefrou city (latitude: 25° 31 '11" longitude: 5° 22' 21"; altitude: 1000 m); the climate is semi-humid with strong continental influence having an annual average temperature of 20°C. The collected leaves were then dried in the open air. The leaves were then isolated from the other specimen and conserved for extraction.

### Essential oil extraction

The essential oils were extracted by hydro-distillation using an apparatus of Clevenger type. The extraction took 3.5 hours for mixing 200g of plants in 1600 ml of distilled water. After filtration the solvent is eliminated by pressure distillation reduced in rotary evaporator at 35°C and pure oil was stored at 4°C in obscurity till the beginning of analysis.

### Gas chromatography analysis (GC-FID and GC/MS)

The chemical composition of leaf oil from *Rosmarinus officinalis* and *Juniperus Phoenicea* in Morocco was determined by GC-MS and GC-FID.

The GC (TRACE GC ULTRA, Thermo Fischer) analysis equipped with flame ionisation detector (FID), Varian capillary column Test Report CP 7770 (CP-SIL- 5 CB; 50m length, 0.32mm of Inside diameter, 0.45mm Outside diameter and Film thickness 1.20 µm). The column temperature was programmed from 40 to 280°C for 4°C/min. The temperature of the injector was fixed to 240°C and the one of the detector (FID) to 270°C. The debit of gas vector (nitrogen) was fixed to 1.5ml/min.The volume of injected specimen was 1µl of diluted oil in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

The identification of different chemical constituents was done by gas phase chromatography (TRACE GC ULTRA, Thermo Fischer) coupled with mass spectrometry (GC/MS) (PolarisQ, Thermo Fischer). The utilised column was; Varian capillary column Test Report CP 7770 (CP-SIL- 5 CB; 50m length, 0.32mm of Inside diameter, 0.45mm Outside diameter and Film thickness 1.20 µm). The column temperature was programmed from 40 to 280°C for 3°C/min. The temperature of the injector was fixed to 250°C and the one of the detector (PolarisQ) to 200°C. Electrons impact: 70ev. The debit of gas vector (Helium) was fixed to 1.5ml/min.The volume of injected specimen was of 1µl of diluted oil in hexane solution (10%). The constituents of essential oils were identified in comparison with their Kovats Index, calculated in relation to the retention time of a series of lineary alkanes ($C_5$- $C_{29}$) with those of reference products and in comparison with their Kovats Index with those of the chemical components gathered by Adams (2001) and in comparison with their spectres of mass with those gathered in a library of (NIST-MS) type.

**Table.1.** Chemical composition of essential oils of *Rosmarinus officinalis* and *Juniperus Phoenicea.*

| *KI | **Mass range (m/z) | Method of identification | Compounds | ***Area (%) | |
|---|---|---|---|---|---|
| | | | | *Rosmarinus officinalis* | *Juniperus Phoenicia* |
| 719 | (136),93,91,79,41,39,77,121,136,27 | KI, GC/MS | tricyclene | - | 0.21 |
| 942 | (152),95,41,81,39,55,69,108,67,83,27 | KI, GC/MS | Camphor | 6.02 | - |
| 943 | (136),93,91,69,39,77,92,79,53,41,27 | KI, GC/MS | β-Pinene | 4.58 | 3.58 |
| 945 | (136),93,79,91,77,41,121,67,27,107,39 | KI, GC/MS | Camphene | 5.02 | 0.28 |
| 946 | (136),93,91,39,121,77,92,79,43,41,105 | KI, GC/MS | α-Pinene | 18.25 | 49.15 |
| 948 | (136),41,93,69,39,27,53,79,77,67,91 | KI, GC/MS | myrcene | - | 5.24 |
| 954 | (136),93,77,91,136,79,94,41,80,92,39 | KI, GC/MS | α -phellandrene | - | 7.39 |
| 964 | (136),93,77,91,136,79,94,41,80,92,39 | KI, GC/MS | β-Phellandrene | 0.10 | 0.98 |
| 973 | (136),93,41,91,77,79,39,27,69,94,43 | KI, GC/MS | α-thujene | 0.28 | - |
| 983 | (136),93,41,91,77,79,39,27,69,94,43 | KI, GC/MS | Sabinene | 0.35 | - |
| 988 | (136),93,91,121,77,92,79,43,41,105 | KI, GC/MS | γ-terpinene | - | 1.28 |
| 998 | (136),93,91,136,121,77,92,79,43,41,105 | KI, GC/MS | α-Terpinene | 0.45 | - |
| 1005 | (136),93,91,79,77,92,121,80,136,94,105 | KI, GC/MS | 3-carene | 0.78 | 1.05 |
| 1018 | (136),68,93,39,67,41,27,53,79,94,92 | KI, GC/MS | Limonene | 3.56 | - |
| 1042 | (134),119,134,91,120,117,41,77,39,65,115 | KI, GC/MS | Cymene | 2.02 | 1.10 |
| 1052 | (136),93,121,91,136,79,77,105,39,41,107 | KI, GC/MS | Terpinolene | 0.12 | 0.91 |
| 1059 | (154),43,93,81,71,69,84,68,108,41,55 | KI, GC/MS | 1.8-Cineole | 5.25 | - |
| 1082 | (136),71,41,43,93,55,69,80,39,121,27 | KI, GC/MS | Linalool | 0.20 | 2.54 |
| 1122 | (152),109,41,94,81,39,69,55,97,43,57 | KI, GC/MS | Cis-Verbenol | 0.24 | 0.31 |
| 1137 | (154),71,111,93,43,86,41,69,55,68,154 | KI, GC/MS | Terpinene-4-ol | 1.98 | - |
| 1138 | (154),95,41,110,93,55,67,139,121,96,69 | KI, GC/MS | Borneol | 3.10 | 0.36 |
| 1174 | (154),59,93,121,136,81,43,68,95,67,41 | KI, GC/MS | α-terpineol | 2.89 | 1.02 |
| 1262 | (196),95,43,121,93,136,41,108,110,55,82 | KI, GC/MS | Isobornyl acetate | 0.11 | - |
| 1277 | (196),95,43,93,436,121,41,80,55,108,69 | KI, GC/MS | Bornyl acetate | 4.35 | 0.53 |
| 1321 | (152),92,91,70,55,41,83,79,134,69,119 | KI, GC/MS | Trans-pinocarveol | - | 1.23 |
| 1430 | (204),161,189,204,105,91,119,133,27,55 | KI, GC/MS | γ-cadinene | - | 1.01 |
| 1458 | (204),41,93,69,107,55,79,91,119,77,123 | KI, GC/MS | α -Farnesene | 0.58 | - |
| 1494 | (204),93,133,91,41;79,69,105,107,120,77 | KI, GC/MS | β-Caryophyllene | 2.56 | - |
| 1506 | (220),43,41,79,93,91,95,69,55,67,81 | KI, GC/MS | caryophyllene oxide | - | 0.45 |
| 1515 | (204),161,105,91,41,119,79,81,93,77,27 | KI, GC/MS | Germacrene-D | 1.02 | 0.68 |
| 1548 | (152),82,110,39,41,27,95,137,109,54,152 | KI, GC/MS | piperitone | - | 1.56 |
| 1572 | (204),121,93,41,107,67,79,81,105,91,119 | KI, GC/MS | germacrene B | - | 0.65 |
| 1583 | (222),59,149,43,41,108,93,79,81,67,164 | KI, GC/MS | β-eudesmol | - | 0.36 |
| **Total Identified Compounds** | | | | **63.81** | **81.87** |
| **Essential oil (%)** | | | | **0.54** | **1.62** |

**KI: Kovats Index was determined by GC-FID on CPSIL- 5CB column.*
***Mass range (m/z) was determined by mass spectrometry (PlarisQ).*
****Area (%) was determined by mass spectrometry (PlarisQ).*

## RESULTS AND DISCUSSION

### Chemical composition of the essential oil

The chemical composition of essential oils of *Rosmarinus officinalis* and *Juniperus Phoenicea* are presented in Table 1.

The constituents of *Rosmarinus officinalis* from Morocco are listed in order of their elution on the column Test Report CP 7770 (CP-SIL- 5 CB) Figure (1). In total, twenty three volatile compounds, representing 63.81% of the total composition, were identified in the leaves oils (Table 1). Monoterpene hydrocarbons were found to be the major group of compounds, the main one being α-pinene (18.25%) followed camphor (6.02%). The most abundant components found in the leaf oil were α-pinene (18.25%), other predominant components were camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%)**.** The essential oils yield of *Rosmarinus officinalis* collected from mountainous region (Tafardoust) (Morocco) was 0.54%. It is relatively low than other plants industrially exploited as a source of essential oils: *Artemisia herba-alba* (0.59%) and *Artemisia absinthium* (0.57%) (Derwich et al, 2009), *Rosmarinus officinalis* (1.9%) (Ozcan et al., 2008), Thymus (1%) (Imelouane et al, 2009), lavender (0.8-2.8%), menthe (0.5-1%), néroli (0.5-1%) and Laurel (0.1-0.35%) (Edward, P., 1987) and *Rosmarinus officinalis* (0.48-1.75%) (Angion et al,. 2004).

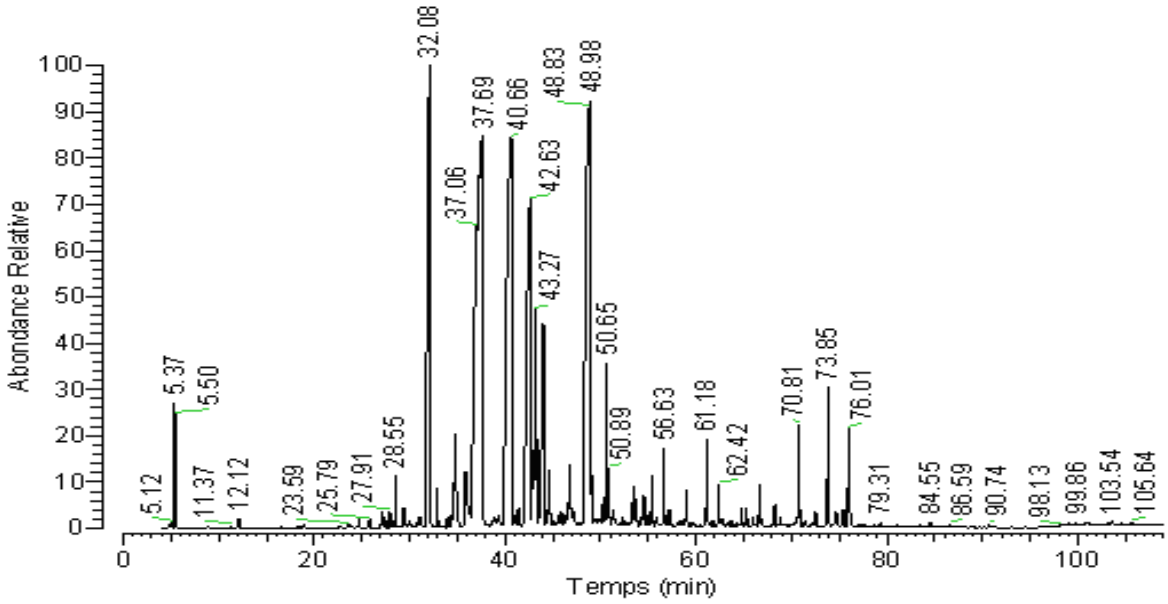


**Figure. 1.** Chromatogram of *Rosmarinus officinalis*

The chemical compositions revealed that this leaves had compositions similar to those of other *Rosmarinus officinalis* essential oils analyzed by (Jaymand, 2003), which the major constituent in lab sample have been reported as α-pinene (30.3%) and 1.8-cineole (15.2%) and in main combinations in semi-industrial essential oil are α-pinene (30%) and 1.8-cineole (12.2%). Phytochemical studies have reported the occurrence of α-pinene (43.9-46.1%), 1.8-cineole (11.1%) and camphor (24-5.3%) in Rosemary essential oil (Azfali, 2009). Other studies by Derwich et al, (2010) reported the major constituent in leaves of *Juniperus phoenicea* collected from Atlas median in the region of Boulmane (Morocco) as α-pinene (49.15%). Contrary it's different to the composition of essential oil of leaves of *Lavandula dentata* study in Sardinia which the major component were verbenone (21.8%) 58 Sachetti, 2005. These differences in oil composition are correlated with different regions or countries where the plant is cultivated (Diab, 2002; Serrano, 2002).

Intensive research on the chemical characteristics has been conducted on this species (Chalchat, 1993; Domokos, 1997). The leaves essential oil of *Rosmarinus officinalis* has been reported in varying detail (Touafek, 2004). In this study the yield and total oil composition of essential oils of *Rosmarinus officinalis* collected from region of Boulmane (Morocco) where 0.54% and 63.81%. This total oil composition (63.81%) is low than other *Rosmarinus officinalis* study in Iran (99.74%) (Moghtader and Afzali, 2009), in Brazil (90.6%) (Takaki et al, 2008) and in Algeria (98.2%) (Touafek et al, 2004). (Ozcan et al., 2008), studied the composition and antifungal activity of *Rosmarinus officinalis* L oil from Turkey, they reported that the yields and the total oil obtained were 1.9%% and 99.93% respectively and the composition is characterized by a high content of *p*-cymene (44.02%), linalool (20.5%), γ-terpinene (16.62%), thymol (1.81%), β-pinene (3.61%), α-pinene (2.83%) and eucalyptol (2.64%). In our previous the essential oils obtained from leaves of *Rosmarinus officinalis* in Spain contained: α-pinene, 1.8-cineole, camphor, verbenone, and borneol (Santoyo et al, 2005). Furthermore, in Egypt (Soliman et al, 1993) studies on the chemistry of the essential oils extracted from the fresh leaves of *Rosmarinus officinalis* collected from Sinai and Giza. The yields were 0.14 and 0.40% respectively. These components represented 82% of the total composition of the oil identified in Sinai. Verbenone (12.3%), camphor (11.3%), bornyl acetate (7.6%) and limonene (7.1%) were the major constituents and 86% of the total composition of the oil, were identified in Giza: Camphor (14.9%), α-pinene (9.3%), and 1.8-cineole (9.0%) were the main constituents.

- The constituents of *Juniperus phoenicea* from Morocco are listed in order of their elution on the column Test Report CP 7770 (CP-SIL- 5 CB; 50m length, 0.32mm of Inside diameter, 0.45mm Outside diameter and Film thickness 1.20 µm) (Figure 2). In total, twenty tree volatile compounds, representing 81.87 % of the total composition, were identified in the leaves oils (Table I). Monoterpene hydrocarbons (70.19%) were found to be the major group of compounds, the main one being α pinene (49.15%) followed by α -phellandrene (7.39%). The most abundant components found in the leaf oil were α-pinene (49.15%) followed by α-phellandrene (7.39%), mycene (5.24%), β-pinene (3.58%), linalool (2.54%), piperitone (1.56%), γ-terpinene (1.28%), Trans-pinocarveol (1.23%) ρ-cymene (1.10%), α terpineol (1.02%) and γ-cadinene (1.01%). The essential oils yield of *Juniperus phoenicea* collected from region of Boulmane (Morocco) was 1.62%. It is relatively higher than other plants industrially exploited as a source of essential oils: *mentha piperita* (Derwich et al, 2010) (1.02%) and Tetraclinis (0.22%) (Bourkhiss et al., 2007).

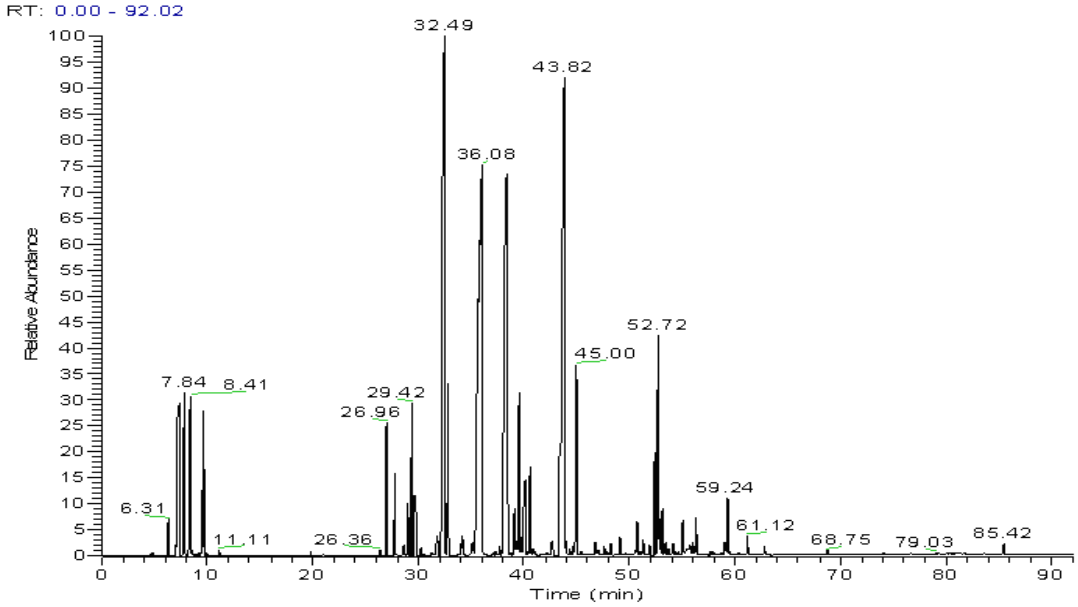


**Figure 2.** Chromatogram of *Juniperus phoenicea*

The chemical compositions revealed that this leaves had compositions similar to those of other *Juniperus phoenicea* essential oils analyzed in USA by Robert et al, (1996) which the major component was α-pinene. Contrary it's different to the composition of essential oil of leaves of *Lavandula dentata* study in Morocco which the major component were 1, 8 cineol (41.28%) and sabinene (13.69%) (Imeloane et al., 2009). Intensive research on the chemical characteristics has been conducted on this species (Robert et al., 1996; Adams, 2001; LeBreton, 1983; San Feliciano et al., 1993; Afifi et al., 1992). The leaves essential oil of *Juniperus phoenicea* has been reported in varying detail (Banthrope et al., 1973). In this study the yield and total oil composition of essential oils of *Juniperus phoenicea* collected from region of Boulmane (Morocco) where 1.62% and 81.87%.

The essential oil content shows variations in plants of different geographical origin, periods and also in different part of the tree:
Emadi et al, (2007), reported the rate of constituents in leaves of *Rosmarinus officinalis* plant being collected in three periods (before, after and during blooming) as α-pinene (20.08%, 27.65% and 17.82%), 1.8-cineole (7.32%, 7.55% and 9.99%) and camphor (9.11%, 8.84 and 15.68%). Furthermore, studies on the chemistry of Iran (Lorenzo et al, 2002), considerable differences were observed in the essential oil composition between *Cuminum cyminum* and *Rosmarinus officinalis*: a-pinene (29.1% and 14.9%), 1.8-cineole (17.9% and 7.43%) and linalool (10.4% and 14.9%) respectively (Gachkar et al, 2007).

- Robert et al, (1996), studied the composition of *Juniperus phoenicea* oil collected from the Portugal, Spain and Greece, they reported that the yields and the total oil obtained were (0.41% and 98.3%), (0.66% and 99%) and (0.58% and 88%) respectively and the composition is characterized by a high content of α-pinene (34.1%, 53.5% and 41.8%), β-phellandrene (19.2%, 5.9% and 3.5%) and β-caryophyllene (0.22%, 1.0% and 0.5%). In our previous studies on the chemistry of Egypt *Juniperus phoenicea* (Elsawi et al., 2007), considerable differences were observed in the essential oil composition between leaves and berries: α-pinene (38.22% and 39.30%), (α -cedrol 31.23% and sabinene 24.29%) respectively. Furthermore, the essential oils, obtained from flower, leaves and stems from basil (*Ocimum basilicum L.)* from Mersin province (Bu¨yu¨keceli-Gu¨ lnar) in Turkey contained: estragole (58.26%, 52.60% and 15.91%), limonene (19.41%, 13.64% and 2.40%) and p-cymene (0.38%, 2.32% and 2.40%) (Jean- Claude et al., 2008). In our previous studies on the chemistry of Uruguay (Lorenzo et al., 2002), considerable differences were observed in the essential oil composition between *Mentha rotundifolia* and *Mentha pulegium*: Piperitenone (80.8%) and Pulegone (73.4%) and the total constituents identified is 93.5% and 99.3% respectively.

## CONCLUSION

This study has been concerned with determining the chemical composition characteristics of essential oils extracted from *Rosmarinus officinalis* and *Juniperus phoenicea* collected in the middle Atlas region of Morocco. The leaf oil obtained from *Rosmarinus officinalis* and *Juniperus phoenicea* grown in Morocco was characterized by GC-MS, GC-FID and twenty three volatile compounds were identified which made up 63.81% of the total essential oil *of Rosmarinus officinalis* and 23 volatile compounds were identified which made up 81.87% of the total volatile products for *Juniperus phoenicea.* The essential oil yields of the studies were 0.54% and 1.62% for *Rosmarinus officinalis* and *Juniperus phoenicea* respectively. The major constituent in aerial parts was α-pinene (49.15%)

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