Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Suppl 1, 2011 ISSN - 0974-2441

Research Article

IDENTIFICATION OF THE VOLATILE CONSTITUENTS OF THE ESSENTIAL OIL OF *JUNIPERUS OXYCEDRUS (*CUPRESSACEAE) FROM THE NORTH CENTRE REGION OF MOROCCO

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ABSTRACT

This study was designed to examine the phytochemistry of the essential oil obtained from aerial parts of *Juniperus oxycedrus* collected in Atlas median region from Morocco. The essential oil was extracted by hydro-distillation and analysed by gas chromatography equipped with flame ionisation detector (GC-FID) and gas chromatography coupled to mass spectrometry system (GC/MS). 48 Constituents were identified in leaves oil representing 84.05% of the total oil and the yield was 1.66%. The *Juniperus oxycedrus* leaves oil was characterised by high contents of α-pinene (31.25%) followed by sabinene (5.21%), limonene (5.02%), β–pinene (4.58%), caryophyllene oxide (4.12%), myrcene (3.56%), ρ-cymene (3.21%), β phellandrene (3.01%), γ–terpinene (2.19%), terpinen-4-ol (2.01%), germacrene-D (1.57%), (E)-caryophyllene (1.25%) and σ-ocimene (1.09%).

Keywords: *Juniperus oxycedrus,* Essential oil, GC/MS, α-pinene.

INTRODUCTION

Juniperus oxycedrus is the species found in Morocco and is widely used as traditional folk medicine for treatment of different infectious diseases. It extends to Turkey [1, 2], Colombia [3], Spain [4] and Greece [5, 6]. The leaf essential oil of *Juniperus oxycedrus* has been reported in varying details from Lebanon [7], Corsica [8] and from Croatia [9] .Aromatic oils from junipers have been used since antiquity for fragrance, flavouring, medicinal, antimicrobial, insecticidal, and cosmetic purposes [10, 11, 12, 13, 14]. Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value [15]. 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 [16] and the WHO based on publications on pharmacopoeias and medical plants in 91 countries, the number of medicinal plants is nearly 20,000 [17].

Essential oils and their components are widely used in medicine as constituents of different medical products, in the food industry as flavouring additives and also in cosmetics as fragrances [18] and pharmaceutical industries [19]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition [20]. Also the essential oils are used in traditional medicine for their antiseptic action. Juniper is found in soaps and in pomades with the aim of curing alopecia [21]. The oil is also irritating to microbes, so much so that it kills many of them [22]. The oil extracted from *Juniperus oxycedrus* was used in dermatology to treat chronic eczema and other skin diseases while the rectified oil was used as a fragrance component in detergents, soaps, creams and lotions [23]. The boiled fruit extract of *Juniperus oxycedrus* has widely been used in the treatment of gastrointestinal disorders, common colds, as expectorant in cough, to treat calcinosis in joints and as diuretic to pass kidney stone, against urinary inflammations, haemorrhoids, and as hypoglycaemic [24, 25]. The essential oil of *Juniperus oxycedrus* has been the object of several studies antioxidant activities [26, 27, 28], antinociceptive [29], antifungal [30], cytotoxicity [31], anti-cancer [32], abortive [33] and anti-inflammatory [34].

Moreover volatile compounds obtained from plants, have known antimicrobial, antifungal and insecticidal activities [35, 36, 37, 12]. Essential oils have many therapeutic and the aid the distribution of drugs and antiseptics [38]. Juniper 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 [39]. Multiple studies have been reported on the chemical composition of the essential oils of *Juniperus oxycedrus* belonging to different regions in the world [40, 41, 5, 4, 42]. On other hand, several studies have reported the chemical composition of solvent extracts and essential oil obtained by hydro-distillation of leaves and berries of *Juniperus oxycedrus* [43, 44, 9]. Therefore, antioxidants are very important for the defence of a living system against oxidative stress. The addition of antioxidants to food products earns increasing popularity as a powerful means for extending the shelf-life of products and for decreasing the nutritional losses by preventing or slowing the oxidation process [45]. The most commonly applied antioxidants in the food industry are synthetic phenols, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).

In this study we report the chemical composition determined by GC/MS and GC- FID, of *Juniperus oxycedrus*, oils growing wild in Morocco.

MATERIALS AND METHODS

Plant material

The leaves of *Juniperus oxycedrus* were collected during April 2010 in Atlas median region (Taferdoust) from Morocco, 15 km in the south east of Boulmane city (latitude: 25° 31 '11" longitude: 5° 22' 21"; altitude: 2100 m). The climate was semi-desertic with strong continental influence with an annual average temperature of 20°C. Specimens were then dried in the open air for sixteen days. The plant was identified by Dr. Elhoussine Derwich and was then isolated from the other specimen and was deposited in Faculty of Medicine and pharmacy, University Sidi Mohamed Ben Abdellah. The amount of oil obtained from each plant material was calculated as:

Oil (% v/w) = observed volume of oil (ml)/ weight of sample (g) x 100

Extraction of essential oils

The essential oils were extracted by hydro-distillation using an apparatus of Clevenger type [46] in Faculty of Sciences of Fez (Morocco). The extraction took 2.5 hours for mixing 200g of plants in 1400 ml of distilled water. The yellowish oil (0.5 ml) for leaves was dissolved in hexane and then dried over anhydrous sodium sulfate. After determining the yield and after filtration the solvent was eliminated by pressure distillation reduced in rotary evaporator at 35°C and pure oil stored at 4°C in obscurity till the beginning of analysis.

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

The essential oils from leaves of *Juniperus oxycedrus* were analysed by gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC/MS) using a CP-SIL- 5 CB column in Unity of GC/ MS and GC, Regional Center of Interface, Sidi Mohamed Ben Adellah University, Fez, Morocco. The GC (TRACE GC-ULTRA, S/N 20062969,

Thermo-Fischer) analysis equipped with flame ionisation detector (GC-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). Column temperature was initially kept at 40 °C for 2 min, then gradually increased to 260 °C at 5 °C/min rate and finally held for 10 min at 260 °C. The temperature of the injector was fixed to 250°C and the one of the detector (FID) to 270°C. The debit of gas vector (nitrogen) was fixed to 1ml/min.The volume of injected specimen was 0.5µl 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 compounds was realised by gas phase chromatography (TRACE GC-ULTRA, S/N 20062969, Thermo-Fischer) coupled with mass spectrometry (PolarisQ, S/N 210729, Thermo Fischer) (GC/MS). 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 260°C for 5°C/min. The temperature of the injector was fixed to 250°C and the one of the detector (PolarisQ) to 200°C.

Ionisation of the sample components was performed in electron impact mode (EI, 70 eV). The debit of gas vector (Helium) was fixed to 1ml/min. Transfer line temperature was 300°C. The mass range from 40 to 650 amu was scanned at a rate of 2.9scans/s. 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 retention indices, calculated in relation to the retention time of a series of lineary alkanes (C_4- C_{28}) with those of reference products and in comparison with their retention indices with those of the chemical components gathered by [47] and in comparison with their spectres of mass with those gathered in a library (NIST-MS Search Version 2.0) and with those reported in the literature [48, 49].

RESULTS AND DISCUSSION

The retention time and chemical composition of essential oils of *Juniperus oxycedrus* are presented in Figure (1) and Table (1).

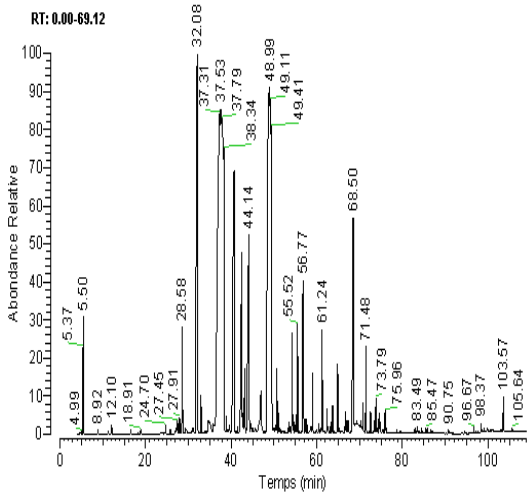

Fig. 1: Chromatogram of *Juniperus oxycedrus*

The constituents of *Juniperus oxycedrus* from Morocco are listed in order of their elution on the CP-SIL- 5 CB column, Figure 1. In total, 48 volatile compounds, representing 84.05 % of the total composition, were identified in the leaves oils Table (1). The most abundant components found in the leaf oil were α-pinene (31.25%) followed by sabinene (5.21%), limonene (5.02%), β-pinene (4.58%), caryophyllene oxide (4.12%), myrcene (3.56%), ρ-cymene (3.21%), β-phellandrene (3.01%), γ-terpinene (2.19%), terpinen-4-ol (2.01%), germacrene D (1.57%), (E)-caryophyllene (1.25%) and σ-ocimene (1.09%). The essential oils yield of *Juniperus oxycedrus* collected in Atlas median region (Taferdoust) from Morocco is of 1.66%. It is relatively higher than other plants industrially exploited as a source of essential oils: *Tetraclinis articulata* (0.22%) [50], *Juniperus thurifera* (0.8%) [51], *Juniperus oxycedrus* (1.14%) [52], *Artemisia herba-alba* (0.59%), *Artemisia absinthium* (0.57%) and *Artemisia pontica* (0.31%) [53], lavender (0.8-2.8%), menthe (0.5-1%), néroli (0.5-1%), Laurel (0.1-0.35%) [54], Artemisia (0.65%) [55] and its low from yield of *Juniperus occidentalis* study by [56] which is (2.3%) and of *Juniperus oxycedrus* in Pindos from Greece which the yield is (2.21%) [6].

The chemical compositions revealed hat this leaves had compositions similar to those of other *Juniperus oxycedrus* essential oils analyzed in Lebanon by [7], Espagne [57], Egypt [26, 58], Tunisia [59] and in Europe by [9], which the major component was α-pinene. [60], studied the Cryptic speciation between *Juniperus deltoids* and *Juniperus oxycedrus* in the Mediterranean collected from the Morocco, Portugal, Spain, France, Italy , Southern Greece, Northern Greece and Turkey, they reported that the major compounds were α-pinene (45.3%, 47.3%, 40.9%, 53.2%, 19.3%, 19.7%, 27.4% and 32.7%) respectively. Contrary it's different to the composition of essential oil of wood of *Juniperus oxycedrus* study in Spain, France and Italy which the major component were δ-cadinene [61].

The berries oil of *Juniperus oxycedrus* study in Greece from two different locations: Holomontas and Pindos which the major components were α-myrcene (23.4%) and citronellol (26.8%) [6] and of *Juniperus occidentalis* which the major commercially important compounds identified as α-cedrene (8.8 %), β-cedrene (2.6 %), thujospene (18.9 %), cuparene (1.5 %), cedrol (38.9 %) and widdrol (1.6%). [62] Have analysed samples collected in Sardinia and they observed the presence, as the most abundant components, besides δ-cadinene, of 1-epi-cubenol (12.5%), cubenol (10.5%), α-muurolol (4.8%), α-cadinol (3.7%) and α-humulene (3.2%). Intensive research has been conducted on this species [63, 64, 65, 66, 51]. In this study the yield and total oil composition of essential oils of *Juniperus oxycedrus* collected in Atlas median region from Morocco where 1.66% and 84.05%. The yield of essential oils of leaves of *Juniperus oxycedrus* is relatively higher than other plants study in Italy (Sardinia) (0.04- 2.54%) [67], Algeria (0.1%) [68] and in Holomontas from Greece (0.97%) [6].

The essential oil content shows variations in plants of different geographical origin and also in different part of the tree: [9]; studied The essential oils composition in fresh needles and green and mature berries of *Juniperus oxycedrus* collected from Croatia they reported that the number of compounds were 36, 15 and 22 and the total oil obtained were 94.90%, 94.33 and 90.94% respectively. In Portugal [30], studies the Composition and variability of the essential oils of the leaves and berries from *Juniperus navicularis* they reported that the composition is characterized by α-Pinene (6.3-38.0%), limonene (7.0-34.6%), α-phellandrene (2.2–13.1%) and *p*-cymene (4.8–10.3%) were the major constituents of the oils from leaves and β-myrcene (25.8%) and α-pinene (24.4%) were the major ones of the oil from berries. In others studies on the chemistry of *Juniperus oxycedrus* From Lebanon [7], considerable differences were observed in the essential oil composition between berries and wood: α-pinene (27.4%) and δ-cadinene (14.5%) 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%) [69]. On others studies on the chemistry of three Artemisia from Morocco [53], considerable differences were observed in the total oil composition between

Artemisia herba-alba (83.10%), *Artemisia absinthium* (80.72%) and *Artemisia pontica* (43.95%). On the other hand, the essential oils, obtained from berries and leaves of *Juniperus excelsa* in Turkey were 56.1% of the oil and the major compounds identified were α-pinene (34.0%), cedrol (12.3%), L-verbenol (5.4%), and D-verbenol (4.4%) from berries and while in the leaves for 63.2% of the oil and the major constituents were α-pinene (29.7%), cedrol (25.3%), α-muurolene (4.4%), and 3-carene (3.8%) [70]. Intense research reveals that the variation in the quantitative and qualitative composition of the leaf, and mainly the berry oil, has been the subject of previous studies [40, 43, 71, 72].

Table 1: Chemical composition of leaves of essential oils of *Juniperus oxycedrus* from Morocco

Chemical formula	Compounds	*RI	Area (%)	**Mass range (m/z)
C10H16	β-pinene	924	4.58	(136),93,91,136,121,77,92,79,43,41,105
C10H16	camphene	933	0.61	(136),93,79,91,77,41,121,80,94,107,39
C10H16	α-pinene	938	31.25	(136),93,91,136,121,77,92,79,43,41,105
C10H16	myrcene	948	3.56	(136),41,93,69,39,27,53,79,77,67,91
C10H16	α -phellandrene	954	0.89	(136),93,77,91,136,79,94,41,80,92,39
C10H16	σ-ocimene	958	1.09	(136),93,41,27,39,79,80,77,43,29,91
C10H16	β phellandrene	964	3.01	(136),93,77,91,136,79,94,41,80,92,39
C10H16	β -thujene	973	1.07	(136),93,41,91,77,79,39,27,69,94,43
C10H16	sabinene	983	5.21	(136),93,41,91,77,79,39,27,69,94,43
C10H16	γ-terpinene	988	2.19	(136),93,91,121,77,92,79,43,41,105
C9H14O	sabina ketone	1001	1.06	(138),81,96,95,55,41,67,43,39,68,82
C10H16	3-carene	1004	0.62	(136),93,91,79,77,92,121,80,136,94,105
C10H16	limonene	1018	5.02	(136),68,93,39,67,41,27,53,79,94,92
C10H14	ρ-cymene	1032	3.21	(134),119,134,91,120,117,41,77,39,65,115
C15H24O	caryophyllene oxide	1506	4.12	(220),43,41,79,93,91,95,69,55,67,81
C10H16	terpinolene	1042	0.09	(136),93,121,91,136,79,77,105,39,41,107
C10H18O	1,8-Cineole	1059	0.30	(154),43,93,81,71,69,84,68,108,41,55
C10H16O	β-thujone	1062	1.05	(152),110,81,95,67,68,41,69,109,55,70
C10H14O	carvone	1190	0.01	(150),82,54,39,93,108,53,107,41,79,91
C10H14O	verbenone	1119	0.01	(150),107,91,39,135,41,80,150,27,79,55
C10H18O	α- terpineol	1133	0.19	(154),59,93,121,136,81,43,68,95,67,41
C10H18O	terpinen-4-ol	1137	2.01	(154),71,111,93,43,86,41,69,55,68,154
C10H14O	myrtenal	1136	1.04	(150),79,107,108,106,77,91,41,105,39,27
C10H16O	myrtenol	1191	1.03	(152),79,91,108,41,93,43,119,77,39,67
C10H16O	verbenol	1126	1.02	(125),109,41,94,81,39,69,55,91,43,57
C10H14O	pinocarvone	1114	1.01	(150),81,53,108,41,69,107,79,39,27,150
C10H18O	borneol	1128	0.83	(154),95,41,110,93,55,67,139,121,96,69
C10H16O	carveol	1206	0.95	(152),91,119,77,134,117,92,39,109,65,93
C15H24	β –copaene	1221	0.44	(204),161,119,105,93,41,91,92,81,120,204
C12H18O2	sabinenyl acetate	1224	0.58	(194), 92,91,81,41,134,55,109,79,43,53
C12H20O2	bornyl acetate	1267	0.53	(196), 95,43,93,436,121,41,80,55,108,69
C12H20O2	α -terpinyl acetate	1333	0.46	(196),43,121,93,136,68,41,59,67,81,79
C15H24	cadinene-3,9-diene	1440	0.40	(204),161,189,204,105,91,133,119,95,41,81
C10H18O	geraniol	1228	0.32	(154),69,41,68,29,93,123,67,70,84,55
C10H16O	trans-pinocarveol	1321	0.21	(152),92,91,70,55,41,83,79,134,69,119
C15H24	γ-cadinene	1430	0.11	(204),161,189,204,105,91,119,133,27,55
C15H28	β-muurolane	1419	0.02	(208)109,95,41,55,81,165,83,69,67,164
C15H28	selinane	1432	0.01	(208),109,95,81,55,96,69,83,67,165,97
C15H26O	α-cedrol	1543	0.26	(222),95,150,151,43,41,81,69,55,107,93
C15H24	β –humulene	1578	0.42	(204),93,80,41,121,92,43,55,67,91,147
C15H28	humulene	1579	0.02	(204),93,80,41,121,92,43,55,67,91,147
C15H24	germacrene D	1505	1.57	(204),161,105,91,41,119,79,81;93,77,27
C15H26O	β -cubenol	1645	0.51	(222),161,105,119,41,81,93,79,93,55,59
C15H18	cadalene	1706	0.01	(198),183,198,168,184,153,165,152,167,169,141
C15H26O	farnesol	1710	0.36	(222),69,81,41,93,95,68,109,67,55,107
C20H34O	manoyl oxide	1978	0.02	(290),275,257,81,192,55,137,177,95,67,43
C15H24	E-caryophyllene	1984	1.25	(204),93,133,91,41;79,69,105,107,120,77
CH36O2	ethyl linoleate	2193	0.03	(308),67,81,41,55,95,54,45,68,82,69
Total Identified Compounds (%)		**84.05**		
Yields (%v/w)		**1.66**		

* RI: Retention indices was determined by GC-FID on a CP-SIL- 5 CB column

** Mass range (m/z) was determined by mass spectrometry (PlarisQ).

CONCLUSION

This study has been concerned with determining the chemical composition of essential oils extracted from the leaves of *Juniperus oxycedrus*, collected in Atlas median region (Taferdoust) from Morocco. The chemical analyses, by GC/MS, GC-FID, have allowed us to identify around 84.05% of the total volatile products for *Juniperus oxycedrus* and 48 volatile compounds were identified. The major constituent in aerial parts was α-pinene (31.25%) and the yield of essential oils was 1.66%. This yield of the plants essential oil that has been studied was important.

ACKNOWLEDGEMENT

The authors thank the Unity of GC/MS and GC, Regional Center of Interface, University Sidi Mohamed Ben Abdellah, Fez, Morocco for the gas chromatography coupled with mass spectrometry (GC/MS) and gas chromatography with flame ionization detection (GC-FID) analysis.

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