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ORIGINAL ARTICLE

Extraction, yield and chemical composition of essential oils of *Juniperus oxycedrus* L. from Tiaret region (Algeria)

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Three forest stations in the Tiaret forest were chosen as sampling sites for *Juniperus oxycedrus* L: Guertouffa, Mekhatria and Manège, depending on exposure and altitude, in order to study the yield and chemical composition of the essential oils of this shrub. The results found for yield showed a percentage of the order of 0.23 to 0.32% in the leaves; on the other hand, it can go up to 0.80% in the berries. The GC/MS chromatographic analysis of G. oxycèdre essential oils allowed the definition of 49 chemical species, divided into 32 families, of which the *Carenes (2-Carene, delta-3-Carene*), and the *Mycenes* (*beta.-Myrcene*), presented high percentages, i.e., 24.72% and 13.84 respectively. A distribution of the chemical species found, according to the exposure and the altitude of the sampled stations, is remarkable, and has made it possible to note the effect of the station factors on the quantity and nature of these oils.

Keywords: Essential oils, Yield, Carenes, Myrcenes, GC-MS chromatograph, Tiaret.

Introduction

Algeria is one of the countries that presents a great diversity of biotope occupied by an important floristic wealth, constitutes in some cases ecosystems of world interest. However, the severe, irregular and sudden climatic constraints, with in particular the phases of prolonged drought influence negatively the growth of trees and the natural regeneration.

The *J. oxycedrus* is a very common species in degraded forests of semi-arid regions in Algeria (Fig. 1). According to Boudy (1950), Maire (1952) and Quezel & Santa (1962), it covers an area of 112 000 ha, from the coastal dunes to the limits of the great Sahara, much more often as a smaller bushy shrub. Moreover, this taxon has a considerable ecological role because it is resistant to drought (Riou-Nivert, 2001) and therefore to soil degradation and anthropic pressure, especially in the most arid regions.

This species can appear either in for-mations in which dominates the tree and shrub layer, or in mixed masses with individ-uals of *Quercusfaginea* subsp. *Faginea.* Lam., *Quercus ilex.* subsp. *ballota* (Desf) Samp., *Quercuspyrenaica, Pinusnigra* Arnold. subsp. *salzmannii, Pinus pinaster.*Ait. and*Pinus sylvestris.*L

In Algeria, various studies have been conducted on essential oils oxycèdre (Bensegueni-Tounsi, 2001), chemotaxonomy (Dob et al., 2008), the antibacterial and antifungal effect of some vegetative organs (Foudil-Cherif &Yassaa, 2012), mono-terpenes and anti-oxidant properties of needles and galls (Fadel et al., 2016).

Fig. 1. Photos of *Juniperus oxycedrus* (Photo: KHENE, 2021).

This work aims to identify mainly the chemical composition of essential oils that can be derived from this species (*Juniperus oxycedrus* L.) in the region of Tiaret, in order to study the effect of altitude and exposure on the variability of different chemical species found by GC-MS.

Presentation of the study area

The study area is located in the pine forest of the Wilaya of Tiaret (154200Ha) in the North-West of Algeria between the Tellian chain in the North and the Atlassian chain in the South, at an average altitude of 980 m (Fig.2).

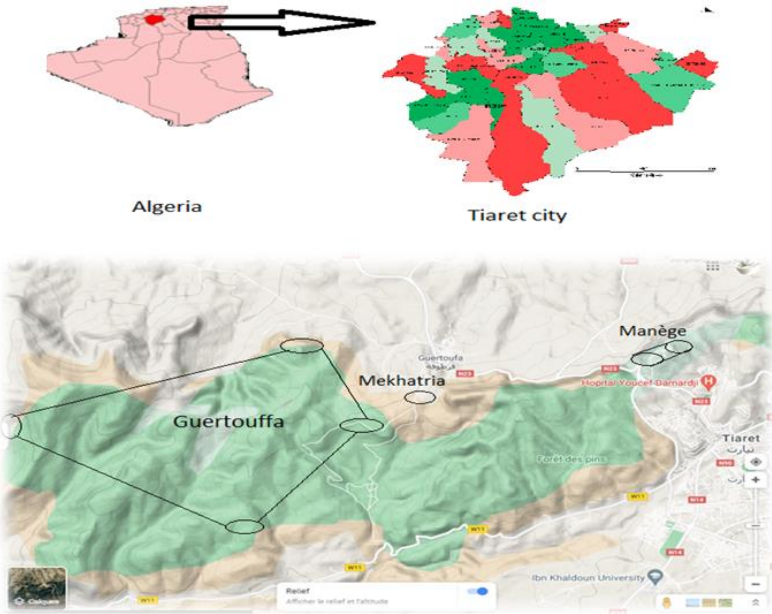

Fig. 2. Geographical location of the study stations.

In the whole of the Tiaret forest, three stations were randomly selected, each measuring 200 m x 100 m. These were:

- Guertouffa station, at an altitude of 950 m and with four exposures: North, South, East and West,
- Manège station, at an altitude of 920 m and two exposures North and West,
- Mekhatria station, at an altitude of 890 m and two exposures North and East.

The plant formation is composed of *Pinus helepensis* Mill. in the tree layer and *Juniperus oxycedrus* L. in the shrub layer. The herbaceous stratum is composed of *Stippa tenacissima* L. and *Ampelodesmos mauritanicus* (Poir.) indicating the aridity of the environment. The climate is semi-arid Mediterranean with an average annual rainfall of 400 mm/year. The predominant winds come from the west and northwest, with average speeds of 3 to 4 m/s.

The samples were taken in such a way as to limit contamination, neither loss nor pollution, by avoiding the use of tools or containers likely to decontaminate the sample (steel or stainless steel tools, containers with walls containing pigments based on trace elements, such as PVC). The geographical coordinates (x, y) of each sample were recorded using a GPS. The samples of the species are collected, registered and transported in paper bags on the day of collection.

Methodology

The hydrodistillation method is used to extract essential oils from *J. oxycedrus*, using a Clevenger. In addition, the starting sample contained 200 g of crushed *Juniperus oxycedrus* leaves with 600 1.5 L of distilled water for 24 hours (Fig.3.). The samples underwent hydro-distillation, at least 02h 45mn. The recovered essential oil was stored at +4°C until use.

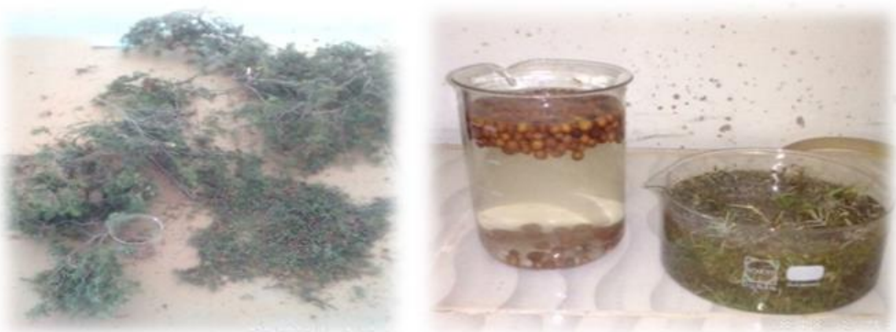

Fig. 3. Maceration of samples (twigs and leaves, berries) (Photo: KHENE,2021).

Results and Discussion

Essential oil yields of *Juniperus oxycedrus*

The yields of essential oils obtained by hydro-distillation are presented in Table 1.

We find that the yields from hydro-distillation of berries are twice those obtained with leaves and twigs of *J. oxycedrus* for the three sites, with an average ranging from 0.26 ± 0.057% to 0.82 ± 0.20%. Several authors cited that the yield of essential oil varied from

0.1% to 0.2% (Dob et al., 2006; Mansouri et al., 2010) in *Juniperus oxycedrus* harvested in the region of Amassine-Ourika, at an altitude of 1300 m, in Marrakech (Morocco), reached 0.15% and the yield of berries reached 0.72% (Achak, 2006).

Table 1. (%) of the yields of essential oils of *Juniperus oxycedrus*.

Exposition	Type of sample	N	P_A (gr)	P_B (gr)	(yields %)	Moy ± SD%
Guertouffa West	berries	3	200	2.10	1.05	0.82 ± 0.20%
			200	1.47	0.74	
			200	1.34	0.67	
Guertouffa North	Twigs and leaves	3	200	0.30	0.15	0.23 ± 0.068%
			200	0.49	0.25	
			200	0.56	0.28	
Guertouffa South	berries	3	200	1.20	0.60	0.60 ± 0.045%
			200	1.10	0.55	
			200	1.28	0.64	
Guertouffa East	Twigs and leaves	3	200	0.61	0.31	0.32 ± 0.026%
			200	0.70	0.35	
			200	0.59	0.30	
Manège West	berries	3	200	1.37	0.69	0.66 ± 0.089%
			200	1.12	0.56	
			200	1.46	0.73	
Manège North	Twigs and leaves	3	200	0.33	0.17	0.20 ± 0.03%
			200	0.46	0.23	
			200	0.39	0.20	
Mekhatria North	Twigs and leaves	3	200	0.64	0.32	0.26 ± 0.057%
			200	0.48	0.24	
			200	0.41	0.21	
Mekhatria East	berries	3	200	1.30	0.65	0.66 ± 0.05%
			200	1.22	0.61	
			200	1.42	0.71	

The yield of essential oils from *Juniperus oxycedrus* berries is around 2.12%, according to Alan et al. However, Dob, et al., (2006) point out that the yield of *Juniperus oxycedrus* berries growing spontaneously in Djelfa, Algeria, is around 0.10% on average, while the yield of berry samples collected in Corsica is also low, varying between 0.10 and 0.22% (Ottavioli 2009). Furthermore, *J. oxycedrus* berries collected in Sardinia and Greece provided yields in the order of 0.49% and 0.40%, respectively (Angioni, et al., 2003, Stassi, et al., 1995). These values are very close to those of the samples taken from Guertoufa South (0.60%). In *J. oxycedrus* leaves, a slightly lower yield than our results of 0.3% was obtained by several authors (Alberto, et al., 2003, Ghanmi, et al., 2010), Boti, et al., (2006) found 0.04-0.26% in the leaves of *J. oxycedrus* from Corsica (Amri, et al.,2011), Guendouzen and Haddouche, (2006) confirm comparable rates varying between 0.20 and 0.42%" in the leaves and 0.28-1.53% in the cones of *J. oxycedrus* from Tunisia. Ces variations de rendement en huiles essentielles peuvent être attribuées à la variabilité génétique de l'espèce, mais aussi à la localisation géographique spécifique de chaque espèce (Boufares, et al., 2019).

4-Chemical composition of the essential oils of *Juniperus oxycedrus*

The oils obtained by extraction were passed through a Shimadzu GC-MS, which subsequently gave a base file. The chromatographic peaks were processed by Labsolutions software (ver 4.50), using the database from two libraries: *Wiley7n.L* and *NIST11.L.* This software allows in particular to give the chemical name of each compound, the% of its area and its rention times.

The Fig. 4 represents a chromatogram of the sample taken in Guertouffa Ouest analysed by GC/MS. The chemical species found are classified by families (https://fr.wikipedia.org/) and the calculation of the percentage of each chemical species in each essential oil sample was done according to the global Aera and Aera taking into account the retention time.

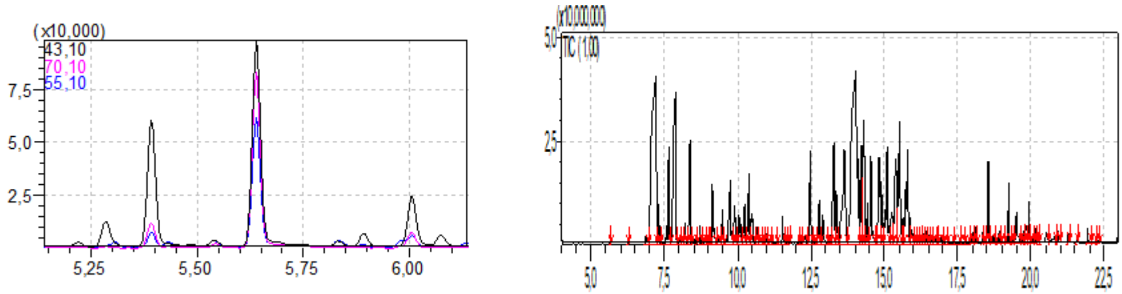

Fig. 4. GC/MS chromatograms of a sample taken in Guertouffa West.

From all the GC/MS chromatograms, 49 chemical species were defined, divided into 32 families. The percentages of the areas found are shown in Table 2.

Table 2. Percentage of areas of different essential oil compounds.

Familly	Chimicals Species	Nbr Species	Area	% Area	RT moy(min)
Pyrazole	*1,2,5-Oxadiazole, 2,2',5,5'-Tetrabromo-4,4'-bithiazole,5-[2'-Hydroxy-4'-chlorophenyl]-3-(2",4"-dichlorophenyl)-1-decylpyrazole, 5-[2'-Hydroxy-4'-chlorophenyl]-3-(2",4"-dichlorophenyl)-1-decylpyrazole, 1H-Carbazole, 2,3,4,9-tetrahydro-7-nitro-, 4-(4-Methylphenyl)-4H-1,2,4-triazole,3-Methyl-1,5-diphenyl-2-pyrazoline*	7	1482604	0,083	12,69±3,56
Thujene	*3-Thujene,4(10)-Thujene,4(10)-Thujene*	3	10268847	0,572	7,33±0,38
Alpha-pinene	*2-Pinene, 2(10)-Pinene,2(10)-Pinene, 2(10)-Pinene, 2(10)-Pinene.*	5	73089006	4,070	7,25±0,34
Cymene	*o-Cymene, o-Cymene, o-Cymene.*	3	9102687	0,507	8,18±0,02
Imidazole	*1H-Imidazole, 4-nitro-2-Hydroxybenzimidazole, 2TBDMS derivative, 2-Hydroxybenzimidazole, 2TBDMS derivative, 1H-Imidazo[4,5-h]quinoline, 5-hydrazino-2-methyl.*	4	5772105	0,321	16,72±3,82
Copaene	*Copaene, Copaene, Copaene, Copaene, Copaene, Copaene, (1) alpha.-Copaene*	7	29577538	1,647	12,98±1,00
Caryophyllene	*Caryophyllene, Caryophyllene, Caryophyllene oxide*	3	3131406	0,174	14,43±2,08
Cubebene	*alpha.-Cubebene, alpha.-Cubebene, alpha.-Cubebene, Cubenene, Cubenene*	5	68737336	3,828	13,78±1,39
Humulene	*alpha.-Humulene, alpha.-Humulene, alpha.-Humulene, alpha.-Humulene, alpha.-Humulene Humulene epoxide I, Humulene epoxide I*	7	9320529	0,519	13,97±0,68
Muurolene	*gamma-Muurolene, alpha.-Muurolene, beta Muurolene*	3	51560210	2,871	13,95±0,17
Germacene	*(-)-Germacrene D, (-)-Germacrene D, (-)-Germacrene D, (-)-Germacrene D, (-)-Germacrene D*	5	492237351	27,413	13,88±0,08
Cardienne	*delta-Cadinene, delta-Cadinene, delta-Cadinene, delta-Cadinene, delta-*	8	108290402	6,031	14,12±0,20

	Cadinene, delta-Cadinene, gamma-Cadinene, beta-Cadinene				
Cubenol	*Cubenol*	1	56963	0,003	14,40
Muurolol	*tau.-Muurolol, tau.-Muurolol*	2	1988910	0,111	15,38±0.08
Carotene	*beta. Carotene*	1	6550	0,004	21,78
Polygermanes	*Hexagermane, Hexagermane*	2	61715	0,003	14,34±2.66
14-beta-H-pregna	14-.beta.-H-pregna,	4	1185605	0,066	19,51±1,81
Himachalene	*gamma.-HIMACHALENE*	1	984460	0,055	15,45
Coleon F	*dimer of Coleon F*	2	36136	0,002	18,00±0,16
Ergost-5-en-3-beta-ol	*Ergost-5-en-3-beta-ol, Ergost-7-ene-3,6,23-triol, triacetate, (3.beta.,5.alpha.,6.alpha.,23R)-*	3	2161101	0,120	18,67±6,79
Camphene	*(2) Camphene*	2	21376551	1,190	7,25±0,07
Verbenene	*Verbenene*	1	19056	0,001	7,24
Myrcene	*beta.-Myrcene, beta.-Myrcene*	2	248667565	13,847	7,82±0,10
Carene	*2-Carene, delta-3-Carene*	2	44013348	24,727	8,16±1,34
Linalool	*Linalool, Linalool*	2	11442888	0,637	9,17±0,03
Cadinol	*tau.-Cadino, tau.-Cadino*	2	106946694	5,956	15,35±0,05
Camphorene	*m-Camphorene, m-Camphorene*	2	7455437	0,415	18,08±0,01
Tocopherol	*alpha.-Tocopherol-.beta.-D-mannoside, dl-.alpha.-Tocopher*	3	5880884	0,328	20,55±0,01
Limonene	*D-Limonene*	1	75710023	4,216	8,37

The analysis of Table 2 shows that:

The *Pinene, Cubebene, Muurolene, Cadinene* families recorded an area varying from 1.64 to 6.03% (all exposures combined). However, the largest percentage is recorded for the *Carene family (2-Carene, delta-3-Carene*), i.e., 24.7% and *Myrcene* (*beta.-Myrcene*), i.e., 13.84%. Indeed, Carenes are a group of terpenes, These oxygenated *terpenes* constitute essentially a minority of the oil, which is mainly composed of limonene and other terpene hydrocarbons, these include *terpene* hydrocarbons (monoterpenes, sesquiterpenes), alcohols, esters, aldehydes, ketones and volatile organic acids (Bousbia, 2013). *Beta-myrcene*, also as a monoterpene, is present as a yellowish liquid with a strong aromatic smell. Furthermore, rosemary harvested in Portugal (Serrano, et al., 2002) is rich in *myrcene* (25%).

Relation: geographical parameters-chemical composition of essential oils

In order to study the effect of exposure and altitude on the distribution of the nature and chemical compounds of the essential oils in the *Juniperus oxycedrus* tree, we carried out a canonical correspondence analysis (CCA), taking into account these parameters for the three sampling stations: Guartoufa, Manège and Mekhatria. The result of this CCA is shown in Fig. 5.

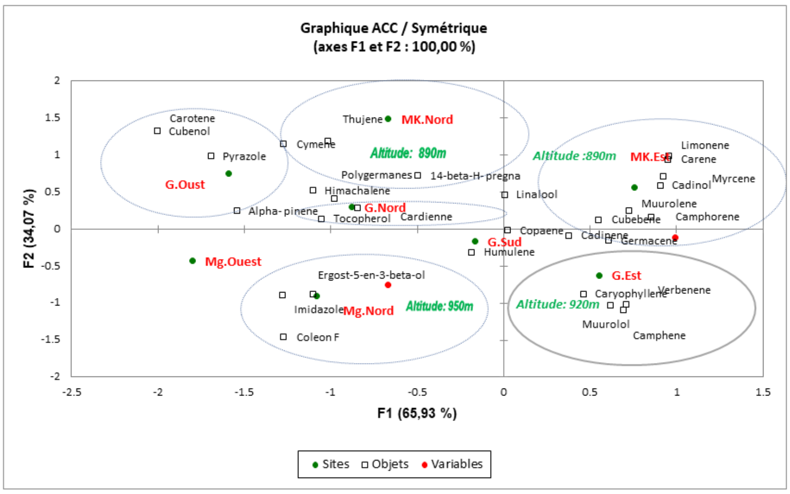

Fig. 5. Canonical analysis of the CCA correspondences between the station parameters "exposure and altitude" and the % of chemical species in the essential oil of *Juniperus oxycedrus*. Legend: MK: Mekhatria station; G: Guertouffa station, Mg: Manège station.

Two dimensions can be interpreted

- F1 which represents 66% of scatterplots (66% of information can be explained in this dimension).
- F2 which represents 34% of scatterplots can be explained in this axis.

On the F1 on the positive side, we find: Alt 890 m, Mekhatria East which corresponds with the species: *Limonene, Myrcene, Alpha-Carene, Camphene, Cymene, Linalool, Cubebene, Germacene, Muurolene, Cadinene, Humulene, Cadinol,Camphorene.*

In the same axis, on the negative side, we find: Mekhatria North; Guertouffa North; Guertouffa West; Alt 890 m, correspond with the compounds: *Cymene, Thujene, Polygermene,, Pyrazole, 14-beta-H-pregna, Tocopherol. Pyrazole, Alpha-pinene, Cymene, Cardienne.*

In the F2 axis, on the negative side, the projection of the information on this axis allows to define:

• The Manège North station and Altitude 950 m, with *Caleon F, Ergost-5-en-3-beta-ol, Imidazole.*

• The Guertouffa East, station at Altitude 920 m, and the compounds *Caryophylène, Muurolol, Camphène.*

This distribution of species according to exposure and altitude shows the effect of stationary factors on the quantity and nature of the oil. Some compounds are present at certain exposures and altitudes, while others are absent.

The study of the composition of an essential oil requires the correct identification of the plants. Indeed, several species or even genera can be confused. Moreover, subspecies and varieties can be found within the same species which may have a different composition in active principles or substances (phenolic compounds, flavonoids, tannins, essential oils, etc.) and consequently different therapeutic properties. It is therefore necessary to determine the species before starting any phytochemical study.

Since the chemical composition of essential oils can vary according to the species (even within the same genus) and according to the organ (leaf, fruit, etc.), after deciding which essential oil to extract (according to the properties and purpose of use), and to obtain the best yield, the age of the plant and the organs, the extraction process, etc., must be taken into account.

Conclusion

The purpose of this study was to determine the yield and chemical composition of essential oils of *J. oxycedrus* in three stations in the forest of Tiaret (western Algeria), which are differentiated by exposure and altitude.

The yield of essential oils in *J. oxycedrus* berries harvested in southern exposures is 0.60%. It is of the order of 0.32% in leaves and branches found in eastern exposures.

Chromatographic analysis identified 49 chemical species in 32 families in the essential oil of *J. oxycedrus*, of which *Carene (2-Carene, delta-3-Carene), and Mycene (beta.-Myrcene*), are the main constituents.

The effect of the exposure and altitude factor on the distribution of the chemical compounds of the extracted oils is noted. We find that: *Myrcene, Cymene, Thujene, Polygermene,,Pyrazole, Tocopherol. Alpha-pinene,Limonene, Cymene, Cardienne, Cymene*, were obtained only in the altitude 890 m. Moreover, *Caleon F, Ergost-5-en-3-beta-ol, Imidazole* are obtained in the northern exposures at 950m; however the compounds *Caryophylene, Muurolol, Camphenes* are obtained in the eastern exposure at 920 m altitude.

In spite of the very strong adaptation of this species to the environmental conditions, the absence of despite the very strong adaptation of this species to environmental conditions, the absence of silvicultural action greatly favors negative adaptations as to the yield and quality of essential oils that could be obtained from this shrub, which absolutely means a very strong coherence in the policy of constituting marketable batches for the various uses of these oils in the ethnopharmacological field.

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