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Research Article

Intraspecific Chemical Variability in the Essential Oils of *Tetraclinis articulata Vahl* Masters

Mbarek Bourkhiss[1]*, Mohammed lachkar[1], Noureddine Ameziane[2] and Tahar Lakhlifi[3]

[1]Engineering Laboratory of Organometallic, Molecular Materials and Environment, Faculty of Sciences, University Sidi Mohammed Ben Abdellah, Morocco

[2]Natural Resources Management and Development Team, Laboratory of Health and Environment, Faculty of Sciences, Moulay Ismail University, Meknes, Morocco

[3]Molecular Chemistry and Natural Substances Laboratory, Faculty of Science, University Moulay Ismail, Morocco

***Corresponding Author:** Mbarek Bourkhiss, Engineering Laboratory of Organometallic, Molecular Materials and Environment, Faculty of Sciences, University Sidi Mohammed Ben Abdellah, Morocco.

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Abstract

The purpose of this work is to explore the chemical variability of essential oils of leaves *Tetraclinis articulata (Vahl)* originating in five localities distributed within the Moroccan Tetraclinis. A significant variation is observed between the outputs according to the forest source of the samples going from 0, 22% to 0, 84%. The chemical composition shows a notable quantitative variation in particular between the majority compounds mainly α-pinene, camphor, bornyl acetate and borneol, without however being able to define distinct chemical races.

Keywords: *Tetraclinis articulata (Vahl)*, Morocco, Essential Oil, Leaves, Chemical Variability, Forest Tree Provenance, Yield, Chemical Composition

Introduction

Tetraclinis articulata Vahl masters (Thuya of Barbary), a member of the cupressaceae family, is an endemic North African tree species that is widely distributed in Morocco, where it ranges from the eastern part of the country to the western high-Atlas region. The surface area of *Tetraclinis* woodlands is estimated at 560 670 ha, which represents approximately 10% of the total forest cover in Morocco [1,2].

Different parts of the thuya of Barbary are used in Moroccan traditional medicine principally against childhood [3], respiratory and intestinal infections, gastric pains, diabetes and hypertension [4].

The phytochemical studies dealing productivity and quality of essential oils of *Tetraclinis articulata* report that there are significant changes in yields and in the chemical composition of these oils especially at leaf biomass [5-7].

In the context of the valorization of biodiversity spontaneous aromatic and medicinal plants in Morocco, this work aims to explore the chemical variability of the essential oils of adult leaves of Thuya of Barbary from five well representative forests in the Moroccan *tetraclinis*.

Materials and Methods

Plant material

Samples of the adult leaves of *Tetraclinis articulata (Vahl) Masters* were collected in March 2013 at different localities distributed within the Moroccan *Tetraclinis* forest to know: Central Plateau (Ait Hatim), the southern region (Essaouira), North (Ben Karrich), High Atlas (Ida-O-Guelloul) and Oriental (Debdou). The trees were randomly chosen.

Essential oils extraction

The dried vegetal material (100g) were water-distillated (3h) using a Clevenger-type apparatus according to the method recommended in the (European Pharmacopoeia,1997) [8]. The yield of the oil was calculated based on the dried weight of the plant material.

Chromatographic analysis

Chromatographic analyses of the essential oils were carried out on a Hewlett-Packard electronically controlled gas chromatograph (HP 5890 series), equipped with a fused silica capillary column type DB-5 of 25 m length, 0.25 mm diameter and 0.25 m film thickness, a flame ionization detector (FID) set at 260°C and fed by a H2/air gas mixture and a split-splitless injector set at 240°C. The injection mode is Split (leakage ratio: 1/50, flow rate: 66 ml/min). The carrier gas used is nitrogen with a flow rate of 1 ml/min. The temperature of the column is programmed from 50 to 250°C at a rate of 4°C/min. The apparatus is controlled by a computer system of type HP "ChemStation", managing the operation of the apparatus and allowing to follow the evolution of the chromatographic analyses.

The identification of the constituents was carried out on the basis of their Kovats index (KI) and on the gas chromatography coupled to mass spectrometry (GC/MS). The latter is performed on a gas chromatograph of the Hewlett-Packard type (HP 5980 series) coupled with a mass spectrometer (HP 5772 series). The fragmentation is performed by electronic impact under a field of 70 eV. The temperature of the column (identical to the one used for CG) is programmed from 50 to 250°C at a rate of 4°C/min and is then maintained at 250°C for 20 min. The carrier gas is helium whose flow rate is fixed at 2 ml/min. The injection mode is Split (leak ratio: 1/70, flow rate: 112 ml/min). The apparatus is connected to a computer system managing a library of NIST 98 mass spectra.

Results and Discussion

The yield essential oil

Average yields in essential oils of five provenances of *Tetraclinis articulata* were calculated based on the dry plant materialof the aerial part of the plant. The results obtained are summarized in the table 1.

Forest	Average yields (%)
Essaouira	0,22
Ben Karrich	0,41
Ait Hatim	0,53
Debdou	0,59
Ida-O-Guelloul	0,84

Table 1: Yields essential oils obtained by hydro-distillation of five provenances of *Tetraclinis articulata Vahl Masters*.

The obtained average yields vary based on four sources. Indeed, samples of *Tetraclinis articulata* from Ida-O-Guelloul gave a better yield in essential oil (0.84%) compared to those of Ait Hatim, of Debdou, of Ben Karrich and Essaouira with respectively 0,53% concentrations of 0,59%, 0,41% and 0,22%.

These results are similar to those found by Benali Toumi., *et al*. [10] and found that the yields of essential oil of *Tetraclinis articulata* of three Algerian origins Ouled Mimoun (Wilaya of Tlemcen), El Haçaiba (wilaya of Sidi Bel Abbes) and Frenda (wilaya of Tiaret) are 0,78%, respectively, 0,75% and 0,35%. Barrero., *et al*. [7] indicated that the essential oil yield of Amsa (North of Morocco) is 0,70%. However, other studies have yields that do not exceed 0,3%. Indeed Larabi., *et al*. [11], found that the performance of *Tetraclinis articulata* of Sidi Bel Abbes (Algeria) the region is 0.11% and Achak., *et al*. [12], found that Thuya Tensift- Al Haouz, Marrakech region (Morocco) have yields range from 0.06% to 0,28%.

Chemical composition of essential oil

The results for the chemical composition of essential oils of *Tetraclinis articulata* five provenances (Ait Hatim, Ida-O-Guelloul, Ben Karrich, Essaouira and Debdou) are summarized in the table 2.

KI	Compounds	Formula	Percentage				
			Ait Hatim	Ida-O-Guelloul	Ben Karrich	Essaouira	Debdou
931	α-thujene	$C_{10}H_{16}$	2,26	1,30	-	1,21	-
939	α-pinene	$C_{10}H_{16}$	23,54	11,01	0,03	15,11	0,19
953	camphene	$C_{10}H_{16}$	2,59	1,60	0,01	1,83	0,06
980	β-pinene	$C_{10}H_{16}$	0,56	0,51	0,01	0,47	0,02
1011	δ-3-carene	$C_{10}H_{16}$	0,02	0,07	0,02	0,28	0,03
1018	α-terpinene	$C_{10}H_{16}$	0,27	0,43	0,21	0,51	1,80
1026	p-cymene	$C_{10}H_{14}$	0,91	0,03	0,02	-	0,05
1031	limonene	$C_{10}H_{16}$	5,98	6,04	0,18	5,44	0,73
1125	α-campholenal	$C_{10}H_{16}O$	0,31	0,18	0,20	0,51	0,10
1134	(Z)-limonene oxide	$C_{10}H_{16}O$	0,29	0,25	0,48	0,21	0,15
1139	(*E*)-pinocarveol	$C_{10}H_{16}O$	0,63	0,66	2,55	0,71	-
1143	Camphor	$C_{10}H_{16}O$	17,27	22,93	13,07	31,60	8,20
1156	Isoborneol	$C_{10}H_{18}O$	0,12	0,14	-	0,16	0,06
1165	Borneol	$C_{10}H_{18}O$	4,57	5,39	31,08	5,28	1,96
1177	4-terpineol	$C_{10}H_{18}O$	0,71	1,48	1,74	1,24	0,46
1180	Cymene-8-ol	$C_{12}H_{16}O_2$	0,64	0,50	0,60	0,30	0,05
1189	α-terpineol	$C_{10}H_{18}O$	0,40	0,56	1,16	0,75	0,28
1204	verbenone	$C_{10}H_{14}O$	1,74	1,17	0,91	0,77	0,16
1217	(*E*)-carveol	$C_{10}H_{14}O$	0,72	0,55	2,17	0,76	0,05
1229	(*Z*)-carveol	$C_{10}H_{14}O$	0,10	0,12	0,38	0,13	0 ,19
1262	Cis-chrysanthenyl acetate	$C_{12}H_{18}O_2$	0,04	0,05	0,14	0,04	0,10
1285	Bornyl acetate	$C_{12}H_{20}O_2$	30,74	37,23	20,38	25,38	47,00
1298	carvacrol	$C_{10}H_{14}O$	0,03	0,18	3,44	0,28	7,31
1350	α-terpenyle Acetate	$C_{12}H_{20}O_2$	0,62	1,10	2,72	0,62	2,64
1351	α-longipinene	$C_{15}H_{24}$	0,04	0,21	0,05	0,35	0,08
1376	α-copaene	$C_{15}H_{24}$	0,06	0,18	0,21	0,31	0,54
1433	γ-elemene	$C_{15}H_{24}$	0,03	-	0,07	0,03	0,06
1480	Germacrine d	$C_{15}H_{24}$	0,04	0,15	-	0,28	1,18
1500	(E)-ß-guaiene	$C_{15}H_{24}$	0,13	0,10	0,92	0,08	2,08
1513	γ-cadinene	$C_{15}H_{24}$	0,14	0,16	0,31	0,23	1,00
1581	Caryophyllene oxide	$C_{15}H_{24}O$	0,23	0,29	6,14	0,51	2,15
1597	Widdrol	$C_{15}H_{26}O$	0,38	0,47	1,85	0,51	5,21
1709	α-humulene	$C_{15}H_{24}$	0,04	0,05	0,07	0,04	-
	Total (%)		96,61	96,20	92,86	96,00	83,82

Table 2: Chemical composition of essential oil from five provenances of *Tetraclinis articulata Vahl Masters.*

The results for the chemical composition of essential oils of Thuya five sources have identified twenty- three compounds representing a total of 96.61% for the *Tetraclinis* Ait Hatim, twenty-two compounds representing a total of 96.20% for the *Tetraclinis* Ida-O-Guelloul, twenty compounds representing a total of 92,86% for *Tetraclinis* Ben Karrich, twenty compounds representing 92.86% of the essential total oil of *Tetraclinis* from the Essaouira and twenty compounds with a total of 83.82% of *Tetraclinis* from Debdou.

It is apparent from the analysis of these results that the majority compound are:

- The Bornyl Acetate (30,74%), the α-Pinene (23.54%), the Camphor (17.27%), the Limonene (5,98%) and the Borneol (4.57%) for the *Tetraclinis* of Ait Hatim.
- The Bornyl Acetate (37,23%), the Camphor (22,93%), the α-Pinene (11,01%), the Limonene (6,04%) and the Borneol (5,39%)for the *Tetraclinis* from Ida-O-Guelloul.
- The Borneol (31,08%), the Bornyl Acetate (20,38%), the Camphor (13,07%), the Caryophyllene Oxide (6,14%) and the Carvacrol (3,44%) for the *Tetraclinis* Ben Karrich.
- The Camphor (31,60%), the Bornyl Acetate (25,38%), the α-Pinene (15,11%), the Limonene (5,44%) and the Borneol (5,28%) of *Tetraclinis* of Essaouira.
- The Bornyl Acetate (47,00%), the Camphor (8,20%), the Carvacrol (7,31%), the Widdrol (5,21%) and the α-Terpenyle Acetate (2,64%) for *Tetraclinis* Debdou.

Barrero., *et al*. [7] and Achak., *et al*. [12] confirm the wealth of essential oils of Amsa (North of Morocco) and Tensift- Al Haouz (Marrakech region Morocco) bornyl acetate, α-pinene, camphor, and borneol. Buhajiar., *et al*. [13] showed that *Tetraclinis* from the Malte consists mainly of α-pinene (46,4%), bornyl acetate (19,9%), camphor (7,3%) and borneol (3,6%). Benali Toumi., *et al*. [10] showed that Algerian *Tetraclinis* have a high content of one of five compounds: camphor (23,41 to 31,60%), bornyl acetate (17,12 to 25,79%), borneol (6,64 to 14,27%), α-pinene (3,65% to 11,34%) and limonene (2.67 to 10,09%).

These results of the present study clearly indicate an variability of yield and of chemical composition of *Tetraclinis articulata* essential oil from different regions, without however being able to define distinct chemical races.

Conclusion

During our study, we determined and compared yields and chemical compositions of essential oils of *Tetraclinis articulata* from five different regions in Morocco (Ait Hatim, Ida-O-Guelloul, Ben Karrich, Essaouira and Debdou).

We found that the average yields obtained vary according to five sources. In fact, samples of *Tetraclinis* from Ida-O-Guelloul gave a better yield in essential oil (0,84%) compared to those of the Debdou, Ait Hatim, Ben Karrich and Essaouira with values respectively 0,59%, 0,53%, 0,41% and 0,22%.

Chromatographic analysis of these essential oils showed that the chemical compositions are qualitatively consistent with varying levels especially between the major compounds mainly α-pinene, camphor, bornyl acetate and borneol.

These results clearly indicate an variability of yield and chemical composition of *Tetraclinis articulata* essential oils, without however being able to define distinct chemical races.

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