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Essential oil composition of *Tetraclinis articulata* (Vahl.) Masters. Leaves from Algeria

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Abstract
In order to contribute to the development of the Algerian flora and to identify new substances potentially interesting in the biological and therapeutic plans, we study the phytochemical screening of *Tetraclinis articulata* leaves essential oil from the north-western region of Algeria. The study by GC / MS and GC / FID of the *Tetraclinis articulata* essential oil leaves has identify forty compounds, representing 89.7% of the total chemical composition of this oil. Camphor (19.6%), bornyl acetate (18.7%), germacrene D (7.6%) and borneol (6.3%) were the major constituents. Others are made present but in small quantities. And the phytochemical screening allowed us to report the presence of two chemicals families that are respectively: terpene hydrocarbons represent 23.6% of the essential oil and oxygenated terpenes representing 28.3% of the essential oil.

Keywords: *Tetraclinis articulata* (Vahl.) Masters, essential oil, phytochemical screening, Algeria.

1. Introduction
Medicinal plants have been traditionally used for pharmaceutical and dietary therapy in long history. The Mediterranean region is known for usages of panoply of plants. Among these plants we cite the *Tetraclinis articulata* (Vahl.) Masters. Which is largely used in Algeria on ethno-pharmacology maters. The Thuya, belongs to the Cupressaceae family; and has two synonyms: *Thuya articulata* Desf. And *Callitris quadrivalvis* Rich [1]. It is native to northwestern Africa in the Atlas Mountains of Morocco, Algeria and Tunisia, with two small outlying populations on Malta, and near Cartagena in southeast Spain. It grows at relatively low altitudes in a hot, dry subtropical Mediterranean climate [1]. Our study lies within the scope of the valorization of Algerian medicinal and aromatic plants having the aim of discovering new bioactive natural products. Le Floc'h (1983) reported about their use in human and veterinary medicine in Algeria and in other North African countries, in particular against intestinal and respiratory ailments [1]. Many studies have investigated the biochemical profile of the essential oil composition of some *Tetraclinis articulata* organs [2, 1, 3]. In Tunisia, recent research has revealed that essentials oils prepared from woody terminal branches contained more monoterpene hydrocarbons, representing 60.2% of the total identified compounds. The main objective of this study is to check if the semi-arid bioclimatic conditions have an influence on the biochemical composition of the essential oil isolated from the aerial parts of *Tetraclinis articulate*?

2. Materials and Methods
2.1. Plant material
Fresh leaves of *Tetraclinis articulata* (Vahl) Masters were collected from trees taken randomly in January 2013 from district of Messer, area of Sidi Bel Abbes from western of Algeria. The voucher specimen (LBV/H/ N°105) was deposited in the herbarium of biodiversity laboratory. These leaves were then dried in the dark at room temperature (25 °C) for ten days. The coordinates: latitude: 35° 07 '24'98" N longitude 0°36'02 09" O; altitude: 562 m. The bioclimatic is semi-arid.

2.2. Essential oil extraction
The leaves of examined plants were hydro-distilled (100 g) for 3 h using a modified Clevenger-type apparatus. The yellowish oil (1 mL) for leaves was dissolved in dichloromethane and then dried over anhydrous sodium sulfate. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator at 35 °C and the pure oil kept at 4 °C in the dark.

2.3. Gas Chromatography Analysis (GC-FID & GC/MS)

The chemical composition of leaf oil from *Tetraclinis articulata* in Algeria was determined by GC- FID (TRACE GC-ULTRA S/N 20062969, Thermo Fischer) and GC-MS (TRACE GC-ULTRA S/N 20062969-PolarisQ S/N 210729, Thermo Fischer) equipped with TRIPLUS AS S/N 20063460 in the light of the following experimental protocol.

2.3.1 Gas chromatography analysis (GC-FID)

The quantitative analysis was done with the help of a chromatographer in gas phase equipped with flame ionization detector (GC-FID), Varian capillary column (5% poly diphenyl 95% dimethylsiloxane, TR5- CPSIL- 5CB; 50 m length, 0.32 mm of diameter & Film thickness 1.25 µm). The column temperature was programmed from 40 to 270 °C for 4 °C/min and finally held at that temperature for 10 min. The temperature of the injector was fixed to 250 °C and the one of the detector (FID) to 280 °C. The debit of gas vector (nitrogen) was fixed to 1 mL/min and split injection with split ratio 1:40. The volume of injected was 1 µL of diluted oil in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

2.3.2. Gas chromatography-mass spectrometry analysis (GC/MS)

The identification of different chemical constituents was done by gas phase chromatography (TRACE GC-ULTRA) coupled with spectrometer (PolarisQ). The utilized column was; Varian capillary column (TR5- CPSIL- 5CB; 50 m length, 0.32 mm of diameter & Film thickness 1.25 µm). The column temperature was programmed from 40 to 280 °C for 5 °C/min. The temperature of the injector was fixed to 260 °C and the one of the detector (PolarisQ) to 200 °C. The ionization mode was Electron Impact E I. (70 eV). The scan range was between 40 and 650 amu. The debit of gas vector (Helium) was fixed to 1.5 mL/min. The volume of injected specimen was 1 µL of diluted oil in hexane. The software utilized was x-caliber (Thermo Fisher) with NIST-MS library. 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 (C4-C28) with those of reference products and in comparison with their kovats Index with those of the chemical constituents gathered by Adams [4] and in comparison with their specters of mass with those gathered in a library of (NIST-MS) type and with those reported in the literature.

3. Result and Discussion

Tetraclinis articulata essential oil yield is in the order of 0.11 %. This is lower than that noted in several works [5, 6, 7]. This oil was light yellow, their odor is agreeable. The composition of the volatile oil isolated by hydro-distillation from leaves is reported in Table 1. Forty compounds were identified representing 89.7% of the total chemical composition of the essential oil. Camphor (19.6%), bornyl acetate (18.7%), germacrene D (7.6%), trans-caryophyllene (7.1%) and borneol (6.3%) are major compounds. Other compounds such as α-pinene and limonene are also present, but in small quantities. This composition is mostly similar to that of Morocco and Tunisia except for some compounds as camphene, limonene. The variability of these chemical compounds generally would be depended to the ecological, genetic and environmental factors.

Table 1: Essential oil composition of *Tetraclinis articulata* leaves

		Algeria
Compounds	KI	HE%
α-Pinene	933	3.2
Camphene	946	0.2
β- Pinene	973	0.2
Myrcene	983	1.6
p-Cymene	1014	0.1
Limonene	1024	2.8
1.8-Cineol	1024	0.1
Linalool	1086	0.2
α-Campholenal	1107	0.3
Camphor	1125	19.6
Trans-Pinocarveol	1125	0.4
p-Mentha1.7, 2diene 8-ol	1131	1.0
Camphene hydrate	1135	0.9
Borneol	1153	6.3
Terpinen-4-ol	1164	1.1
p-Cymene-8-ol	1164	0.4
α-Terpineol	1175	0.6
Verbenone	1183	0.7
Trans-Carveol	1201	0.5
Carvone	1218	0.2
Bornyl acetate	1273	18.7
α- Terpinyl acetate	1335	1.3
α-Copaene	1377	0.9
Trans-Caryophyllene	1419	7.1
α-Humulene	1451	2.2
γ-Muurolene	1472	0.3
Gemacrene D	1478	7.6
α-Muurolene	1495	0.3
γ-Cadinene	1508	1.4
δ-Cadinene	1517	1.4
Nerolidol-E	1550	0.3
Caryophyllene oxide	1571	2.3
β-Oplopenone	1591	0.3
Humulene epoxide II	1595	0.6
epi-Cubenol	1618	2.1
Caryophylla- 4(14), 8(15) dien-5α-ol	1623	0.2
T- Muurolol	1628	0.4
T-Cadinol	1628	0.6
δ-Cadinol	1628	0.1
α-Cadinol	1641	1.2
TOTAL		89.7

Oil obtained from non woody terminal branches was characterized by the predominance of oxygenated terpenes (28.3%) followed by hydrocarbon terpenes (23.6%) and esters (20%) as shown in Table 2. We can also note the presence of small amounts of alcohols (9.9%), and ketones (0.2%). In addition, it should be noted that the essential oil of our samples could be a source of sesquiterpenes alcohol. This is also one of the characteristic in the majority of Cupressaceae [8].

Table 2: Chemical compounds classes identified in our *Tetraclinis articulata* essential oil.

compounds Classes	Number of compounds identified	%
Hydrocarbons terpenes	16	23.6%
oxygenated terpenes	14	28.3%
Alcohols	7	9.9%
Esters	2	20%
ketones	1	0.2%

When comparing our major compounds results obtained from *Tetraclinis articulata* leaves with the same organs gathered from Tunisia and Morocco [2, 5, 3, 1] we noted a significant difference in composition and yield; in the case of Tunisia and Morocco; α-pinene, camphor and limonene were considered as the principal constituents whereas for Algeria these components were present at 3,2%, 19,6% and 2,8%, respectively (Table 3). Bornyl acetate, germacrene D and trans-caryophyllene which were found to be the major components in our sample are present in small quantities to trace in Tunisia and Morocco.

Several studies have shown that the Camphor, bornyl acetate and borneol present an interesting larvicidal activity [9]. Additionally, it's important to note that the biochemical composition of our essential oil sample confer an interesting insecticidal and larvicidal activity [10]. At the opposite, all the others studies emphasizes the antimicrobial and antifungal activities of the thuya essential oils [2, 5, 11].

Table 3: Comparison of the major components (%) of non woody terminal branches from Algeria, Tunisia and Morocco

Compounds	Algeria	Tunisia	Morocco
α-Pinene	3.2	23.54	16.8
Camphor	19.6	17.27	17.6
Limonene	2.8	5.98	5.7
Borneol	6.3	4.57	4.7
Bornyl Acetate	18.7	0.12	-
α-Terpineol	0.6	0.4	0.4
Trans-Caryophyllene	7.1	-	-
Germacrene D	7.6	0.04	0.3

4. Conclusion

In this study, we performed the extraction of *Tetraclinis articulata* plant essential oil. The extract was subjected to preliminary phytochemical screening by CPG/SM and CPG/FID for detection of natural compounds present in them. The result of phytochemical screening of extracts revealed the presence of forty constituents where there are the predominance of the hydrocarbons and oxygenated terpenes. Camphor (19.6%), bornyl acetate (18.7%), germacrene D (7.6%), trans-caryophyllene (7.1%) and borneol (6.3%) are major the compounds. In another hand; The results of the present study clearly indicate an variability of chemical composition of *Tetraclinis articulata* essential oil in the same organs between Algeria, Tunisia and Morocco that is demonstrate the existence of chemotype notion.

5. References

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