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Essential Oil Composition of Terminal Branches, Cones and Roots of *Tetraclinis articulata* from Tunisia

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Abstract: The volatiles obtained separately from the terminal branches (woody and non-woody), cones and roots of *Tetraclinis articulata* gathered from the area of Zaghouan (Tunisia) have been analyzed by GC and GC-MS. Remarkable differences were found between the constituent percentages of the different studied organs. The main components in the non-woody branches volatile oil were naphthalen (6.6%), Z-muurolene (29.0%) while Camphene (43.2%), Z- β -ocimene (11.7%), nonanol (5.3%) and a non identified compound (12%) were the main components in the wood branches volatile concentrate. In the cones oil, the most important compounds found were trans-pinacarveol (6.1%), p-cymene-8-ol (10.4%), fenchyl acetate (5.1%), β -phellandrene (8.1%) and carvone (5.3%). The major constituent in the oil of roots was found to be Camphene (70.2%). A comparison of these data with previous results reported in the literature showed surprising differences.

Key words: *Tetraclinis articulata*, different organs, essential oil composition, GC, GC-MS

INTRODUCTION

Tetraclinis articulata (Vahl) Masters belongs to the Cuprecaceae family. It has two synonyms: *Thuya articulata* Desf and *Callitris quadrivalvis* Rich. It is wide spread in North Africa. In Tunisia, it is distributed in the north east but never can be found in the south (Nabli, 1989).

Le Floch reported about its possible use in popular and veterinary medicine in Tunisia and in other countries of North Africa, in particular against intestinal and respiratory ailments as well as skin conditions (Le Floch, 1983; Buhagiar *et al.*, 2000). The wood and its veneer are also highly prized in the handicraft industry because of the smooth finish it can take. Gum sandarac, a type of resin, was used for varnishes, pounce and in dental fillings (Buhagiar *et al.*, 2000; Bellakhdar, 1978; Bellakhdar *et al.*, 1982). The branches of *Tetraclinis articulata* emitted an agreeable odour, they are used as compresses for children to treat fever and as drinks to treat diarrhoea (Ait Igri *et al.*, 1990). The tar obtained from *Thuya* serves to treat several diseases of came (Le Floch, 1983).

The oil composition of some organs of *Tetraclinis articulata* has been the subject of two previous studies (Buhagiar *et al.*, 2000; Ait Igri *et al.*, 1990). There are no investigations on oil roots' chemical composition of this plant.

Oils from the aerial parts of *T. articulata* from Malta were found to contain eighteen main

compounds from which α -pinene (31.0-68.2%), limonene (3.8-25.3%), germacrene D (1.6-5%), camphor (1.1-18.1%) and bornyl acetate (1.8-19.9%) were the major components (Buhagiar *et al.*, 2000).

The wood and the branches oils of *T. articulata* from Morocco were found to contain twenty-five compounds from which the most important components were α -pinene (3.8-9.7%), limonene (1.5-8.5%), camphor (only in branches, 23.5%), borneol (only in branches, 13.2%) and bornyl acetate (only in branches, 31.1%) (Ait Igri *et al.*, 1990).

Recently, further investigations were made on the essential oils of same organs of *T. articulata* from Morocco and showing lack of α -pinene and limonene and different proportions of camphor (only in branches 19.1%), borneol (only in branches 9.6%) and bornyl acetate (only in branches 16.5%). The cedrol (28.2%) and the 1, 7-di-epi-cedrol (17.9%) were found in the wood (Barrero *et al.*, 2005).

As a part of our research on *T. articulata* from Tunisia, and because we did not find any report about the oil composition of its roots, therefore this study was carried out.

MATERIALS AND METHODS

Plant material: *Tetraclinis articulata* was identified according to the flora of Tunisia (Nabli, 1989). A specimen was collected, dried and a voucher specimen (Cuprecaceae n° 1) was deposited at our laboratoire de

Chimie des Substances Naturelles et de Synthèse Organique, Faculté des Sciences de Monastir, Université de Monastir. The plant was gathered from the forestry station of Zaghouan in March 2005. The fresh plant was separated in four parts: leaves, stems, cones and roots. Each part was divided in a little peaces and weighted before isolation of essential oils.

Isolation of essential oils: Hydrodistillation of the fresh material was performed in a Clevenger-type apparatus for 4h up to the point at which the oil contained in the matrix was exhausted. The oil obtained was collected and stored in sealed glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on fresh weight of the sample was calculated.

Analyses of the essential oil

Gas Chromatography (GC): Gas Chromatograph: HP 5890-series II equipped with: Flame Ionization Detectors (FID), HP-5 (5% phenyl 95% dimethylpolysiloxane) 30 mx 0.25 mm, 0.52 µm film thickness fused silica capillary column: The carrier gas was nitrogen (1.2 mL⁻¹ min). The oven temperature programming was 1 min isothermal at 50°C, then 50-280°C at rate of 5°C/min and held isothermal for 1 min. The injection port temperature was 250°C, detector 280°C. Volume injected: 0.1 µL of 1% solution (diluted in hexane). Percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction.

GC/MS: The analyses of the volatiles were run on a Hewlett-Packard GC-MS system (GC: 5890 series II; MSD 5972). The fused-silica HP-5 capillary column (30 mx 0.25 mm, film thickness of 0.25 µm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.2 mL⁻¹ min. Oven temperature was programmed (50°C for 1 min, then 50-280°C at 5°C/min) and subsequently, held isothermal for 20 min. Injector port: 250°C, detector: 280°C, split ratio 1:50. Volume injected: 0.1 µL of 1% solution (diluted in hexane). Mass spectrometer: HP 5972 recording at 70 eV; scan time 1.5s; mass Range 40-300 amu. Software adopted to handle mass spectra and chromatograms was a ChemStation.

Identification of the compounds: The components of the oil were identified by comparison of their mass spectra with those of a computer library (Wiley 275 library). Further confirmation was done from Retention Index data generated from a series of alkanes retention indices (relatives to C₉-C₂₈ on the HP-5 column) (Shibamoto, 1987; Adams, 1995).

RESULTS AND DISCUSSION

T. articulata does not yield the same amount of oil from its different parts. The total yield of essential oil obtained from branches (woody and non woody), cones and roots was 0.13; 0.22; 0.05 and 0.09 (w/w), respectively.

Those oils were light yellow excepted cones oil was translucent, liquid at room temperature, their odours are agreeable. The composition of the volatile oils isolated by hydrodistillation from the different parts of *T. articulata* is reported in Table 1 together with the Retention Indices (RI) calculated for each compound, the percentage composition and the identification methods. The constituents are arranged according to their elution on the apolar BP-1 capillary column. A total of 113 constituents were found from which 7 are Not Identified (NI).

Roots oil analyzed for the first time presented a great difference in the percentages of its constituents compared to those of the aerial parts (Table 1). Monoterpene hydrocarbons represented the important fraction (74.1%) of all the identified constituents from which camphene (70.2%) found to be major. Consequently, roots of *T. articulata* could be an interesting source of camphene. The content progressively increased from the woody terminal branches (43.2%) to the roots (70.2%).

The number of the no identified compounds (7) found in the different analysed essential oils and their weak proportions except which was identified in the woody branches (11.9%) need more amonts for the preparatives chromatographic separations (CG, TLC) then spectroscopic analyses.

Oils obtained from non-woody terminal branches were characterised by the predominance of oxygenated monoterpenes (36.4%) and sesquiterpene hydrocarbons (38.4%) (Table 1), it was principally rich in 4,6-dimethyloctane-3,5-dione (22,4%), Z-muurolene (29,03%) and naphthalene (6.6%).

Oils isolated from woody terminal branches contained more monoterpene hydrocarbons, representing 60.2% of total identified compounds, by comparison constituents to the rest (Table 1). This oil also contained a significant amount (18.8%) of non terpenic compounds. Camphene (43.2%), Z-β-ocimene (11.6%), nonanol (5.3%) and non identified compounds (12%) were the major constituents of this oil. As shown in Table 1, these latter were minor or absent in the non-woody terminal branches oil.

Oil obtained from cones was mainly rich in oxygenated monoterpenes (45.2%) and monoterpene hydrocarbons (14.5%) (Table 1). Trans-pinacarveol

Table 1: Essential oil composition of different organs

Constituents	RI (HP-5)	Terminal branches				Identification
		Non-woody	Woody	Cones	Roots	
α - Pinene	932	-	0.60	-	-	MS,RI
α - Fenchene	933	-	-	-	0.22	MS,RI
Camphene	953	0.57	43.23	-	70.22	MS,RI
3-Methyle nonane	965	-	0.80	-	-	MS,RI
Sabinene	967	-	0.15	-	0.42	MS,RI
β - Pinene	973	-	-	-	0.34	MS,RI
β -Myrcene	990	1.46	0.38	-	0.14	MS,RI
n-Decane	999	-	0.78	-	0.72	MS,RI
δ -3-Carene	1006	-	2.38	-	1.16	MS,RI
α -Terpinene	1010	-	tr	-	-	MS,RI
α -Phellandrene	1008	-	tr	-	-	MS,RI
p-Cymenene	1013	-	0.17	-	tr	MS,RI
Limonene	1023	0.33	tr	-	-	MS,RI
p-Cymene	1027	-	-	-	-	MS,RI
Z- β -Ocimene	1035	1.64	11.66	0.5	tr	MS,RI
σ -Terpinene	1059	0.09	0.14	-	tr	MS,RI
Terpinolene	1080	0.20	0.04	0.17	-	MS,RI
Linalool	1085	0.18	0.73	0.16	tr	MS,RI
Undecane	1100	0.53	-	0.94	-	MS,RI
NI	1102	0.56	-	-	-	MS,RI
n-Nonanal	1104	0.94	0.19	0.44	tr	MS,RI
exo-Fenchol	1112	-	-	-	0.16	MS,RI
Allo-Ocimene	1118	0.27	-	0.18	-	MS,RI
Camphor	1122	-	tr	-	1.42	MS,RI
α -Campholenol	1125	0.19	tr	0.18	tr	MS,RI
Camphene hydrate	1130	0.27	-	0.48	-	MS,RI
Trans-Pinocarveol	1136	1.73	0.60	6.08	0.35	MS,RI
Nerol oxide	1138	-	-	-	tr	MS,RI
Limonene oxide	1143	-	0.08	0.31	-	MS,RI
Borneol	1150	0.24	-	6.86	tr	MS,RI
Nonanol	1153	-	5.33	-	tr	MS,RI
p-Cymene 8-ol	1158	-	-	10.41	-	MS,RI
4,6-Dimethyl-octane-3,5-dione	1160	22.42	0.46	0.36	tr	MS,RI
p-Mentha1-7, 2diene 8-ol	1168	1.64	-	-	-	MS,RI
Myrtenal	1171	1.46	0.36	2.24	0.19	MS,RI
Terpinen-4-ol	1178	-	2.02	1.92	-	MS,RI
Naphtalene	1179	6.62	-	-	-	MS,RI
α -Terpineol	1185	1.62	0.77	3.20	tr	MS,RI
Octanoic acid	1192	-	0.21	-	0.71	MS,RI
Nerol	1196	1.39	0.58	3.85	0.56	MS,RI
Fenchyl acetate	1203	1.16	0.29	5.06	0.17	MS,RI
β -Phellandrene	1209	1.73	0.14	8.09	0.70	MS,RI
Carvone	1215	-	0.42	5.31	0.26	MS,RI
Piperitone oxide	1230	3.37	tr	1.12	0.16	MS,RI
Geraniol	1234	1.86	0.31	0.34	0.13	MS,RI
Linalyl acetate	1239	0.65	0.15	-	0.36	MS,RI
NI	1247	-	0.10	-	-	MS,RI
Decanol	1254	0.12	0.26	2.45	0.04	MS,RI
2-Decanol	1256	1.30	-	0.38	1.02	MS,RI
NI	1261	0.15	tr	-	-	MS,RI
Thymol	1266	0.16	-	-	-	MS,RI
Bornyl acetate	1270	0.12	tr	0.38	0.12	MS,RI
NI	1283	-	-	0.24	-	MS,RI
Undecanal	1286	0.22	tr	0.30	-	MS,RI
NI	1290	-	11.99	0.86	-	MS,RI
Z-Muurolene	1307	29.03	tr	-	tr	MS,RI
Piperitenone	1315	tr	tr	-	-	MS,RI
NI	1320	0.10	-	-	-	MS,RI
Citronellyl acetate	1332	0.13	tr	0.32	-	MS,RI
γ -Cadinene	1341	-	tr	-	-	MS,RI
Neryl acetate	1345	-	0.57	0.12	tr	MS,RI
Undecanol	1356	1.07	-	1.21	tr	MS,RI
α -Ylangene	1370	0.11	-	-	0.38	MS,RI
β -Patchoulene	1377	0.32	tr	0.60	0.24	MS,RI
β -Elemene	1387	-	0.42	0.17	tr	MS,RI
Geranyl acetate	1399	0.28	-	0.35	tr	MS,RI
Dodecanol	1401	-	0.27	0.12	tr	MS,RI
Caryophyllene	1415	-	0.70	-	tr	MS,RI

Table 1: Continued

Constituents	RI (HP-5)	Terminal branches				Identification
		Non-woody	Woody	Cones	Roots	
E-Caryophyllene	1423	0.40	-	-	0.10	MS.RI
NI	1431	-	-	-	0.34	MS.RI
(E)- α -Bergamolene	1435	0.20	0.39	0.89	tr	MS.RI
Aromadendrene	1440	-	tr	0.22	0.50	MS.RI
α -Humulene	1453	0.56	-	1.47	0.47	MS.RI
allo-Aromadendrene	1459	0.11	tr	-	tr	MS.RI
ar-Curcumene	1472	0.17	0.30	0.13	tr	MS.RI
4-Nonanol	1479	-	-	-	0.68	MS.RI
Germacrene D	1485	-	tr	0.13	tr	MS.RI
Valencene	1490	-	0.35	-	tr	MS.RI
Bicyclogermacrene	1500	tr	-	0.21	1.18	MS.RI
Daucene	1504	0.12	-	0.18	tr	MS.RI
Calamenene	1510	-	0.20	-	tr	MS.RI
δ -Cadinene	1515	0.13	tr	0.22	0.57	MS.RI
Hexyl benzene	1532	-	0.61	0.33	0.45	MS.RI
α -Cadinene	1536	0.35	-	0.64	0.16	MS.RI
α -Calacorene	1542	0.11	-	-	1.11	MS.RI
β -Calacorene	1548	tr	0.10	-	1.07	MS.RI
(Z)-3-Hexenyl-benzoate	1570	-	-	-	0.23	MS.RI
Spathulenone	1571	-	0.5	-	-	MS.RI
Caryophyllene oxide	1576	0.15	-	-	0.23	MS.RI
Pinocarvone	1586	0.55	tr	1.44	0.47	MS.RI
Guarol	1589	-	tr	-	tr	MS.RI
Humulene _{6,7} -epoxide	1601	4.24	1.20	2.46	tr	MS.RI
β -Caryophyllene	1612	-	-	-	-	MS.RI
Tetradecanol	1613	0.14	tr	0.21	0.10	MS.RI
T-Cadinol	1629	0.23	0.34	0.42	tr	MS.RI
δ -Muurolol	1638	-	-	0.78	0.14	MS.RI
α -Cadinol	1641	1.22	-	0.29	0.12	MS.RI
β -Eudesmol	1644	0.37	0.78	0.26	0.18	MS.RI
γ -Elemene	1651	-	-	-	tr	MS.RI
Bulnesol	1656	0.55	0.31	0.77	tr	MS.RI
α -Bisabolol	1668	-	-	0.20	0.15	MS.RI
Cadalene	1674	0.76	0.26	0.87	0.25	MS.RI
(E-E) Farnesol	1700	0.26	0.19	0.37	0.26	MS.RI
Heptadecane	1709	0.15	-	0.64	0.03	MS.RI
α -Cyperone	1724	0.60	0.08	0.20	0.06	MS.RI
Eicosane	2000	0.13	0.44	0.14	0.10	MS.RI
Heneicosane	2100	0.13	0.62	0.94	0.27	MS.RI
Docosane	2200	0.12	0.76	0.35	0.81	MS.RI
Tricosane	2300	0.15	0.04	0.20	0.03	MS.RI
Tetracosane	2400	0.10	0.03	0.21	0.03	MS.RI
Heptacosane	2700	0.09	-	0.23	-	MS.RI
Nonacosane	2900	0.46	-	0.22	-	MS.RI
Monoterpene hydrocarbons	5.81	60.16	14.48	74.09		
Oxygenated monoterpenes	36.43	6.2	45.17	4.93		
Sesquiterpenes hydrocarbons	38.39	2.62	5.73	4.96		
Oxygenated sesquiterpenes	7.02	3.5	6.99	1.08		
Hydrocarbons	1.86	1.97	2.93	1.27		
Others	6	18.85	5.82	2.99		

Order of elution and percentages are given on apolar column (HP-5), all relative response factors being taken as one, RI, retention indices measured on apolar column, tr. Trace < 0.01%, values in bold correspond to major compounds.

Table 2: Comparison of the major components (%) of woody terminal branches from Tunisia, Malta and Morocco

Constituents	Tunisia	Malta (Buhagiar <i>et al.</i> , 2000)	Morocco Station E ₄ (Shibamoto, 1987; Adam, 1985)
α -Pinene	0.6	31.0	9.7
Camphor	tr	18.1	21.2/19.1
Limonene	tr	3.8	8.0
Bornyl acetate	tr	19.1	28.0/16.5
Borneol	-	2.8	7.6/9.6
Germacrene D	tr	1.6	-
α -Terpineol	0.77	-	2.4
Camphene	43.2	2.3	-
Z- β -Ocimene	11.6	-	-
Nonanol	5.3	-	-
Cedrol	-	-	28.2

(6.1%), p-cymene 8-ol (10.4%), fenchyl acetate (5.1%), α -phellandrene (8.1%) and carvone (5.3%) were preponderant in this oil. These constituents were minor or absent in both terminal branches (woody and non-woody) oils indicated above.

As shown in Table 1, oxygenated sesquiterpenes and hydrocarbons were not representative in the chemical composition of the four studied organs. We also noted noticeable differences between the chemical compositions of the different organs volatiles.

When comparing our results obtained from woody branches with the same organs of *T. articulata* gathered in Morocco (Ait Igri *et al.*, 1990; Barrero *et al.*, 2005) and Malta (Buhagiar *et al.*, 2000). we noted a significant difference in composition and yield. In the case of Morocco and Malta, -pinene, camphor and bornyl acetate were considered as principal constituents in the woody terminal branches oil, whereas, for Tunisia these components were present at 0.6% to trace, respectively (Table 2). In the previous studies, the authors indicated the presence of borneol at 2.8% in Malta and at (7.6-9.6%) in Morocco (Ait Igri *et al.*, 1990; Barrero *et al.*, 2005), whereas in our sample, this compound is absent (Table 2).

In Morocco (Ait Igri *et al.*, 1990), they reported the presence of α -terpineol in woody branches oil and cedrol in wood oil (Barrero *et al.*, 2005) but no germacrene D. In Malta (Buhagiar *et al.*, 2000) the reverse situation was true, while present results showed the presence of both at 0.8% and in trace, respectively (Table 2).

Camphene which was found to be major 43.2% in the woody branches oil from Tunisia (Table 1), is present in the same oil from Malta at 2.3% but absent in the case of Morocco, (Ait Igri *et al.*, 1990; Barrero *et al.*, 2005) (Table 2).

Additionally, our results reported finding *Z*- α -ocimene (11.6%) and nonanol (5.3%), both absent in the previous studies (Buhagiar *et al.*, 2000; Ait Igri *et al.*, 1990; Barrero *et al.*, 2005).

In addition to the important number of compounds (113) identified in our samples, the marked difference observed between our results and those of the previous

studies on the same plant reinforces the interpretation of (Buhagiar *et al.*, 2000). However, the authors explained the variation of composition in the parts investigated as evidence for the presence of chemical polymorphism due to the influence of environmental and ecological factors, (Ait Igri *et al.*, 1990; Barrero *et al.*, 2005).

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