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# Essential oil composition and variability of *Artemisia herba-alba* Asso. growing in Tunisia: comparison and chemometric investigation of different plant organs

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**ABSTRACT**

This study was conceived to investigate the composition of four essential oils (EOs) extracted by hydrodistillation from four parts (leaves, stems, leaves/stems, roots) of *Artemisia herba-alba* growing wild in the Center of Tunisia. For this, *Artemisia herba-alba* aerial and roots parts were shade dried with ventilation at room temperature. Then, plant different parts were cut into small pieces and subjected to hydrodistillation using a Clevenger-type apparatus. The gas chromatography (GC) analyses were accomplished with a HP-5890 Series II instrument. The main results showed a total of 152 compounds detected and identified by GC and GC-MS and accounting for 91.3-99.7% of the whole oil. The four oils were characterized by the predominance of monoterpene derivatives (68.2-99.5%) and the major volatile constituent was *α*-thujone (18.2-45.5%). Qualitative and quantitative differences between the four essential oils have been noted for some compounds. The main compounds of leaves essential oil were α-Thujone (45.5%), β-Thujone (11.4%), trans-sabinyl acetate (10.1%), 1,8-Cineole (7.4%) and camphor (6.8%). α-Thujone (27.5%) was also the main compound in the essential oil of leaves/stems, followed by camphor (22.9%), 1,8-cineole (8.3%), β-thujone (8.2%) and camphene (5.6%). The essential oil of stems was dominated by α-Thujone (28%) followed by β-Thujone (11.4%) and chrysantenone (11%). In the essential oil of roots, α-thujone was less represented (18.2%), followed by camphor (14.6%) and curcumen-15-al (14.3%). It is important to mention that curcumen-15-al has been reported for the first time in *Artemisia herba-alba* oil Our results revealed avariability in the chemical composition and the yield of the EOs from *Artemisia herba-alba*. Moreover, curcumen-15-al is a new chemotype first found in *Artemisia herba-alba* from Tunisia.

## INTRODUCTION

The genus *Artemisia* is one of the largest and most widely distributed genera of *Asteraceae* family includes 400 species (Judd *et al.*, 2002). *Artemisia* species are of great socio-economic thanks to their antioxidant potential besides antimicrobial and anti-parasital activities of their essential oils (Eos). *Artemisia herba-alba* is a medicinal and aromatic dwarf shrub that grows wild in arid and semi-arid areas of the Mediterranean basin, extending into northwestern Himalayas (Mohamed *et al.*, 2010). In Tunisia, *Artemisia herba-alba* is found from the mountains around Jebel Oust to the south of the country (Mighri *et al.*, 2009). Herbal tea from this plant has been used as analgesic, antibacterial, antispasmodic and hemostatic agents (Laid *et al.*, 2008).

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Further, the plant is widely used in traditional medicine for the treatment of diabetes, bronchitis, diarrhea, hypertension, and neuralgias (Tahraoui *et al.*, 2007; Mahomoodally *et al.*, 2013). EOs of this species are known for its therapeutic disinfectant, anthelminthic and antispasmodic virtues (Hatimi *et al.*, 2001). The aim of this paper is to provide more information on the chemical composition of the EOs extracted from different parts of *A. herba-alba* collected in the Center of Tunisia.

## MATERIAL AND METHODS

### Plant material

*Artemisia herba-alba* aerial (leaves and stems) and roots parts were collected from Chrarda locality in the Center of Tunisia (Fig. 1). Plant identification was carried out by Pr. MA Nabli, botanist at the Faculty of Sciences of Tunis-Tunisia. All samples were shade dried with ventilation for 15 days at room temperature. The plant used parts was cut into small pieces and subjected to hydrodistillation using a Clevenger-type apparatus (Clevenger, 1928) for 4 h. The oil was collected and stored at 4°C in amber vials before analysis.


**Fig 1.** Origin of *Artemisia herba-alba* plant grown in Tunisia.

### Gas Chromatographic-Mass Spectral Analysis

The GC analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 µm film thickness). The temperature program was as follows: 60°C for 10 min, ramp of 5°C/min up to 220 °C; injector and detector temperatures 250°C; carrier gas nitrogen (2 mL/min); detector dual FID; split ratio 1:30; injection of 0.5 µL. The identification of the constituents was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by mean of their linear retention indices (L.R.I) relative to the series of *n*-hydrocarbons. The relative proportions of the essential oils constituents were percentages obtained by FID peak-area normalization. GC–EIMS analyses were performed with a Varian CP- 3800 gas chromatograph equipped with a DB-5 capillary column (30 m×0.25 mm, coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperature 220 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 µL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of the authentic samples, comparing their L.R.I. relative to the series of *n*-hydrocarbons and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra, built up from pure substances and components of known oils and MS literature data (Adams, 2009).

## RESULTS AND DISCUSSION

The EO yields were respectively of 1.86%, 0.42%, 0.25% and 0.1% for the leaves, the leaves/stems, the stems and the roots, respectively. According to Haouari and Ferchichi (2009), the oil yield varied between 0.68% and 1.93% in EOs of *Artemisia herba alba* growing in the South of Tunisia. The chemical composition of the analyzed oils is reported in Table 1. Altogether, 152 compounds were identified in these four EOs, accounting for 99.7%, 99.7%, 98.7% and 91.3% of the whole oils, respectively. All the EOs obtained from the different parts were characterized by a high content of *α*-Thujone. It is important to announce that in the EOs of roots, α-Thujone was less represented (18.2%), followed by camphor (14.6%) and curcumen-15-al (14.3%). Curcumen-15-al has been reported for the first time in *Artemisia herba-alba* oil and the correspondent oil should be considered as a new chemotype. *α*-Thujone has been reported as the major constituent of the *Artemisia herba-alba* essential oil originating from Tunisian semi-arid and south region (Akrout, 2004; Kadri *et al.*, 2011), Jordan (Hudaib and Aburjai, 2006), Algeria (Belhatta *et al.*, 2014), Morocco (Paolini *et al.*, 2010) and Israel (Fleisher *et al.*, 2002). This monoterpenic cetone confers to this plant its characteristic smell of Mentha and its bitter taste. Furthermore, leaves EO was characterized by the highest *α*-Thujone amount (45.5%). *β*-Thujone (11.4%) was the second component followed by *trans*-sabinyl acetate (10.1%), 1,8-cineole (7.4%), camphor (6.8%) and isoborneol (3.4%). The main components of this oil differed from those reported by Akrout et *al.* (2010), and according to them, *β*-Thujone was the main component of the oil extracted from the leaves of *Artemisia herba-alba* growing in the South of Tunisia. Furthermore, in this oil, oxygenated terpenes were the most represented compounds (93.8%), but oxygenated monoterpenes prevailed over oxygenated sesquiterpenes (93.3% vs. 0.5%, respectively). Terpene hydrocarbons were present in lower amount (5.7%) divided between monoterpene (4.6%) and sesquiterpene hydrocarbons (1.1%).

Haouari and Ferchichi (2009) reported that the main components were cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone in Eos of Artemisia herba alba growing in the South of Tunisia. They also reported Twelve samples characterized by monoterpenes as major components amounting to more than 57% of the total oil, three had last three samples had approximately the same percentage of monoterpenes and sesquiterpenes.

In addition, non-terpene oxygenated compounds were scarcely detected (0.2% for (*z)*-Jasmone) and phenylpropanoids were present only in traces (eugenol and methyl eugenol). In the EO of leaves/stems, the main compounds were *α*-Thujone (27.5%) and camphor (22.9%) followed by 1,8-cineole (8.3%), *β*-Thujone (8.2%) and camphene (5.6%). It should be noted that camphor had the highest percentage in this oil. Also, in this oil, oxygenated monoterpenes represented the main constituents (87.4%) whereas monoterpene hydrocarbons were more represented, in particular bicyclic monoterpenes (9.7%), but their percentage (12.1%) was considerably lower than that of oxygenated monoterpenes. On the other hand, sesquiterpenes were particularly absent, either as hydrocarbons (only present as trace amounts) or oxygenated derivatives (0.2% for globulol). Non-terpene oxygenated compounds were detected only in trace amounts.

**Table 1:** Composition (in% of the total identified EO) [a] of the essential oils of leaves, leaves/stems, stems and roots of *Artemisia herba-alba* from center Tunisia.

| Constituents | L.R.I.[b] | Leaves | Leaves/stems | Stems | Roots |
|---|---|---|---|---|---|
| *(E)*-2-Hexenal | 855 | -[c] | - | 0.2 | 0.2 |
| *(Z )*-Salvene | 856 | 0.7 | - | - | - |
| *(E)*-Salvene | 867 | tr | - | - | - |
| *n*-Hexanol | 868 | - | tr[d] | tr | 0.2 |
| Heptanal | 900 | - | - | - | tr |
| Tricyclene | 928 | 0.2 | 0.1 | tr | tr |
| *α*-Thujene | 932 | tr | 0.3 | tr | - |
| *α*-Pinene | 939 | - | 0.4 | 0.3 | - |
| Camphene | 954 | 1.5 | 5.6 | 0.8 | 1.5 |
| Thuja-2,4(10)-diene | 957 | tr | Tr | - | - |
| Benzaldehyde | 962 | - | - | tr | 0.2 |
| Heptanol | 970 | - | Tr | tr | tr |
| Sabinene | 977 | 1.2 | 3 | 0.6 | 0.7 |
| *β*-Pinene | 980 | 0.2 | 0.4 | 0.1 | - |
| 6-methyl-5-hepten-2-one | 986 | - | - | tr | tr |
| 3-Octanone | 988 | tr | Tr | - | - |
| *cis*-meta-mentha-2,8-diene | 987 | - | - | - | 0.3 |
| dehydro-1,8-Cineole | 991 | tr | - | - | - |
| Myrcene | 993 | 0.2 | 0.2 | - | - |
| 3-Octanol | 994 | tr | Tr | - | - |
| Mesitylene | 996 | - | - | tr | - |
| Yomogi alcohol | 999 | tr | - | - | - |
| α-Phellandrene | 1005 | - | - | tr | - |
| *α*-Terpinene | 1019 | 0.2 | 0.4 | 0.1 | Tr |
| *p*-Cymene | 1028 | 0.4 | 1 | 0.5 | 0.4 |
| 1,8-Cineole | 1034 | 7.4 | 8.3 | 1.7 | 3 |
| Santolina alcohol | 1040 | tr | - | - | - |
| Lavender lactone | 1042 | - | - | - | Tr |
| Phenyl Acetylaldehyde | 1044 | - | Tr | tr | 0.3 |
| *(E)-β*-Ocimene | 1051 | - | Tr | - | - |
| *γ*-Terpinene | 1062 | 0.4 | 0.6 | 0.2 | 0.2 |
| Artemisia ketone | 1065 | tr | - | - | - |
| *cis*-Sabinene hydrate | 1069 | 0.2 | 0.5 | 0.4 | 0.4 |
| *n*-Octanol | 1071 | - | tr | - | Tr |
| *cis*-Linalool oxide | 1075 | tr | tr | tr | 0.4 |
| Artemisia alcohol | 1086 | tr | - | - | - |
| Terpinolene | 1089 | tr | 0.1 | 0.1 | Tr |
| 6,7-epoxy Myrcene | 1090 | - | tr | - | - |
| *trans*-Linalool oxide | 1094 | tr | - | - | - |
| *trans*-Sabinene hydrate | 1098 | 0.2 | 0.5 | 0.6 | 0.7 |
| Linalool | 1100 | tr | tr | - | - |
| *α*-Thujone | 1103 | 45.5 | 27.5 | 28 | 18.2 |
| 1-Octen-3-yl acetate | 1113 | - | tr | - | - |
| *β*-Thujone | 1116 | 11.4 | 8.2 | 11.4 | 5.7 |
| *cis-p*-menth-2-en-1-ol | 1122 | - | 1.5 | 1.4 | 1.7 |
| *trans*-Pinene hydrate | 1123 | tr | - | - | - |
| Chrisanthenone | 1125 | 0.5 | 0.7 | 11 | 1.6 |
| Dihydro Sabina ketone | 1126 | 0.7 | - | - | - |
| *cis*-Limonene oxide | 1135 | - | tr | - | - |
| *(iso)*-3-Thujanol | 1138 | tr | tr | tr | - |
| *trans*-Pinocarveol | 1140 | 2.4 | 2.7 | 4.3 | 3.6 |
| Terpinen-1-ol | 1143 | - | - | - | Tr |
| Camphor | 1144 | 6.8 | 22.9 | 8 | 14.6 |
| *(neo-iso)*-3-Thujanol | 1152 | tr | - | - | - |
| *(neo)*-3-Thujanol | 1154 | - | tr | tr | Tr |
| Sabina ketone | 1156 | 0.2 | 0.3 | 0.2 | 0.2 |
| Isoborneol | 1158 | - | 2.8 | 2.7 | 2.5 |
| Pinocarvone | 1166 | 0.5 | 1.1 | 2.3 | 1.1 |
| Borneol | 1169 | 3,4 | - | - | - |
| 1-Nonanol | 1172 | - | - | tr | Tr |
| *cis*- Pinocamphone | 1174 | - | tr | tr | Tr |
| 4- Terpineol | 1178 | 0.9 | 1.4 | 0.9 | 1.3 |
| Naphtalene | 1180 | - | - | - | 0.7 |
| a-Thujenal | 1182 | tr | 0.2 | 0.3 | Tr |
| *α*-Terpineol | 1190 | tr | 0.2 | 0.2 | 0.3 |
| *cis*-Piperitol | 1194 | - | - | 0.7 | 0.4 |
| Myrtenal | 1195 | 0.4 | 0.6 | - | 0.5 |
| Myrtenol | 1196 | tr | - | - | - |
| *trans*-Piperitol | 1205 | 0.3 | 0.9 | 2 | 1.7 |
| *γ*-Terpineol | 1207 | tr | - | 0.1 | 0.2 |
| Vebrenone | 1214 | tr | - | - | - |
| *trans*-Carveol | 1221 | tr | tr | tr | Tr |
| Citronellol | 1229 | - | - | - | 0.3 |
| *cis*-Carveol | 1233 | tr | - | - | - |
| Phenol,2-ethyl-6-methyl | 1236 | tr | - | - | - |
| *cis*-Ascaridole | 1237 | - | tr | tr | - |
| Cuminaldehyde | 1240 | 0.2 | 0.2 | 0.3 | Tr |
| Carvone | 1243 | tr | 0.3 | 0.3 | 0.7 |
| Carvotanacetone | 1247 | tr | tr | tr | - |
| Piperitone | 1258 | 0.4 | 1.5 | 2.1 | 3.3 |
| Bornyl acetate | 1260 | 0.2 | - | - | - |
| *cis*-Chrysanthenyl acetate | 1263 | 0.5 | 0.1 | 1.1 | 0.3 |
| Geranial | 1278 | - | tr | - | - |
| Isobornyl acetate | 1285 | 0.7 | 0.7 | 0.8 | 0.4 |
| *trans*-Sabinyl acetate | 1291 | 10.1 | 4.3 | 7.2 | 2.4 |
| *trans*-Pinocarvyl acetate | 1296 | tr | - | - | - |
| Terpinen-4-ol acetate | 1300 | - | tr | 0.1 | - |
| Carvacrol | 1301 | tr | tr | 0.1 | Tr |
| Iso-Ascaridole | 1303 | tr | tr | tr | - |
| 6-Hydroxy carvotanacetone | 1311 | - | tr | 0.3 | Tr |
| *cis*-Pinocarvyl acetate | 1312 | tr | - | - | - |
| Myrtenyl acetate | 1327 | tr | tr | - | - |
| *α*-Terpinyl acetate | 1352 | tr | - | - | - |
| Eugenol | 1361 | tr | - | tr | - |
| *α*-Copaene | 1376 | tr | tr | tr | - |
| *β*-Maaliene | 1380 | - | - | tr | Tr |
| *α*-Isocomene | 1386 | - | - | tr | Tr |
| *(E)*-Jasmone | 1391 | - | - | tr | - |
| *(Z )*-Jasmone | 1393 | 0.2 | tr | 0.6 | 0.2 |
| Methyl Eugenol | 1404 | tr | - | tr | - |
| *β*-Isocomene | 1407 | - | - | tr | - |
| α-Gurjunene | 1409 | - | - | - | Tr |

| | | | | | |
|---|---|---|---|---|---|
| *β*-Caryophyllene | 1418 | tr | - | - | - |
| *p*-Cymen-7-ol acetate | 1423 | - | tr | tr | - |
| *(E)*-Geranyl acetone | 1454 | - | - | - | Tr |
| Alloaromadendrene | 1461 | - | tr | tr | - |
| β-Chamigrene | 1475 | tr | tr | - | Tr |
| Germacrene D | 1480 | 0.7 | tr | 0.2 | Tr |
| *(E)-β*-Ionone | 1485 | - | - | - | 0.2 |
| β-Silinene | 1489 | tr | - | - | - |
| Valencene | 1492 | - | - | - | Tr |
| Bicyclogermacrene | 1494 | 0.4 | tr | 0.2 | - |
| *trans*-γ-Cadinene | 1513 | - | tr | - | Tr |
| Cubebol | 1515 | - | - | 0.2 | - |
| *β*-Curcumene | 1516 | tr | - | - | - |
| *δ*-Cadinene | 1524 | tr | tr | - | - |
| Italicene epoxide | 1549 | - | - | tr | - |
| Ledol | 1565 | - | tr | - | - |
| *trans*-Nerolidol | 1566 | tr | - | 0.3 | - |
| Germacrene D-4-ol | 1574 | - | - | tr | - |
| Spathulenol | 1576 | 0.5 | tr | 1.5 | 0.6 |
| Caryophyllene oxide | 1581 | tr | tr | 0.5 | 0.6 |
| Globulol | 1583 | - | 0.2 | - | 0.5 |
| Viridiflorol | 1590 | - | - | 0.3 | - |
| *β*-Copaen-4-*α*-ol | 1591 | tr | tr | 0.4 | 0.2 |
| Carotol | 1594 | - | - | 0.4 | - |
| epi Cedrol | 1596 | - | - | 0.2 | 0.4 |
| Humulene epoxide II | 1607 | tr | - | - | - |
| *β*-Himachalene oxide | *1616* | - | - | 0.2 | - |
| epi-10-*γ*-Eudesmol | 1620 | - | - | - | 0.2 |
| 1-epi-Cubenol | 1628 | tr | tr | - | 0.2 |
| 5-Cedranone | 1630 | - | - | 0.1 | - |
| *γ*-Eudesmol | 1631 | - | - | - | 0.2 |
| *β*-Cedren-9-one | 1632 | - | - | 0.4 | - |
| Epoxy-Allo-Aromadendrene | 1634 | tr | - | - | 0.2 |
| Cubenol | 1641 | - | - | 0.3 | - |
| Tau-Cadinol | 1642 | - | tr | 0.3 | 0.3 |
| *β*-Eudesmol | 1649 | tr | - | tr | 0.4 |
| Selin-11-en-4*α*-ol | 1652 | - | - | - | 0.2 |
| *α*-Eudesmol | 1654 | tr | - | tr | - |
| *α*-Cadinol | 1655 | - | - | - | 0.6 |
| Valerianol | 1658 | - | - | 0.2 | - |
| Valeranone | 1675 | - | - | 0.3 | 0.5 |
| Ishwarone | 1682 | - | - | 0.2 | - |
| Acorenone | 1693 | - | - | 0.2 | 0.4 |
| Germacrone | 1694 | - | - | 0.3 | 0.2 |
| Mayurone | 1710 | - | - | - | 0.5 |
| Curcumen-15-al | 1713 | - | - | 0.3 | 14.3 |
| Cyclocolorenone | 1761 | - | - | tr | 0.3 |
| Benzyl benzoate | 1763 | - | - | tr | - |
| 14-oxy-*α*-Muurolene | 1769 | - | - | tr | - |
| Heneicosane | 2100 | - | - | - | 0.3 |
| *Total identified(%)* | | 99.7 | 99.7 | 98.7 | 91.3 |

[a] Percentages obtained by FID peak –area normalization.
[b] Linear retention indices (HP-5 column).
[c] Not detected.
[d] tr < 0.1%

Stems EO was characterized by the predominance of *α*-Thujone (28%). *β*-Thujone and chrysanthenone were detected in similar amounts (11.4% and 11%, respectively). camphor and *trans*-sabinyl acetate were detected at significant amounts (8 and 7.2% respectively). Chrysanthenone was found at its highest percentage; however it was scarcely represented in the other EOs with an amount varying from 0.5% to 1.6%. Also, in the stems, monoterpenes were the main class of volatiles (91.2%), followed by sesquiterpenes (6.7%) and no terpenic oxygenated (0.8%). Among monoterpenes, oxygenated ones were more abundant than hydrocarbons (88.5% vs. 2.7%, respectively). Also among sesquiterpenes, oxygenated derivatives were detected in higher percentage (6.3%) than hydrocarbons ones (0.4%). Phenylpropanoids and no terpenic hydrocarbons were represented in traces.

The EO of roots was dominated by *α*-Thujone whose amount was lower than in the other oils (18.2%). Besides, this oil was characterized by high camphor and curcumen-15-al percentages (14.6% and 14.3%, respectively). It seems that curcumen-15-al was exclusive of EO; however it was detected only in very small amount in the stems (0.3%). It should be noted that this oil type was not reported in literature because. It is reported at the first time that such codominance of 3 main components of *α*-thujone, camphor and curcumen-15-al has been reported in *Artemisia herba-alba* oils. Curcumen-15-al should be considered as a new chemotype of *Artemisia herba-alba*. Like *α*-thujone, *β*- thujone was found with a lower percentage than that found in the others examined oils (5.7% vs. 11.4% and 8.2%).

Comparing the four EOs, monoterpenes had the highest amounts in leaves/stems (99.5%), followed by leaves (97.9%), stems (91.2%) and roots (68.2%). Among studied oragns, leaves accumulated the highest amount of oxygenated monoterpenes (93.3%). For sesquiterpenes, the most important percentages were detected in roots (20.8%), followed by stems (6.7%), leaves (1.6%) and leaves/stems (0.2%). Furthermore, the highest value of oxygenated sesquiterpenes was found in the roots (20.8%).

## CONCLUSION

*Artemisia herba-alba* EOs were characterized by qualitative and quantitative differences depending on the part of the plant. The variability was especially related to the proportions of constituents and relatively to the presence of new compounds or the absence of particular ones. It has been suggested that the variation in EO yield and the composition could be due to the activity of enzymes responsible for the biosynthesis of volatile oils (Hendawy and Khaled, 2005).

According to our results, it seems that chemical composition of *Artemisia herba-alba* essential oil varied significantly with the part of the plant. This characteristic should contribute to the understanding of the pharmacological activities of the herb. Furthermore, it must be taken into account when the plant could be used as aroma source and also in its valorization in many industrial sectors in relation to the type of volatiles accumulated.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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