Chemical Composition of Essential Oil from Italian Populations of *Artemisia alba* Turra (Asteraceae)

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**Abstract:** The use of essential oils as chemotaxonomic markers could be useful for the classification of *Artemisia* species and to characterize biodiversity in the different populations. An analysis of the chemical composition of four essential oils from Italian populations of *Artemisia alba* Turra (collected in Sicily, Marche and Abruzzo) was investigated. In this paper an in depth study of the significant differences observed in the composition of these oils is reported.

**Keywords:** *Artemisia alba*; essential oil; biodiversity; α-bisabolone oxide A; davanone D

1. Introduction

*Artemisia* L. is a large, important genus of the Asteraceae family. It comprises more than 500 species [1] although in the past this number has fluctuated depending on authors’ opinions [2,3]. *Artemisia* is a cosmopolitan genus, mainly distributed in temperate areas of mid to high latitudes of the Northern Hemisphere, with only a few representatives in the Southern Hemisphere. Central Asia is its
center of diversification, while the Mediterranean region and North West America are two secondary speciation areas [4,5]. Some species are also reported in Africa and Europe [3,6].

Due to the high number of species, Artemisia is a taxonomically complex genus because some species have different morphological forms and others closely resemble each other. For this reason a correct identification, based only on morphological details, is quite difficult. The genus has been divided in four subgenus: Abrotanum Bess., Absinthium (Miller) DC., Seriphidium Bess. and Dracunculus Bess [7] although more recently the subgenera Abrotanum, Absinthium, Seriphidium have been joined in the subgenus Artemisia [6].

Artemisia alba Turra is found in the southern part of Europe and is widespread in Italy with the exception of Sardinia [8], and due to its morphological variability has an uncertain botanical placement since some authors have included it in several different subgenus: Absinthium [9], Abrotanum [10] or Artemisia [6]. As confirmation of this complexity, the Sicilian population of this species, due to its peculiar morphological characters, was assigned, in the past, to a differently named intraspecific taxon: A. camphorata Vill. var. subcanescens Ten. [11], A. alba var. incanescens (Jord.) Fiori [9].

Previous chemical studies indicate that patterns of secondary metabolites present in plants of the genera Artemisia include triterpenes, steroids, hydrocarbons, polyacetylens, flavonoids, coumarins, mono and sesquiterpenoids with a wide range of biological activities such as antimalarial, cytotoxic, antihepatotoxic, anti-bacterial, antifungal and antioxidant properties [12,13].

Concerning phytochemical investigations of A. alba, only four papers have been published on the non-volatile components; three papers have been published considering its synonyms A. lobelii All. [14–16], A. biasoletiana Vis., A. suavis Jord., A. incanescens Jord., A. camphorata Vill. listed in the European Flora database [17]. Santonin was isolated from the aerial parts [18], whereas the roots were shown to contain a sesquiterpene-coumarin ether [10]. Studies on the aerial parts of A. alba collected in Calabria showed the absence of sesquiterpenoids and the presence of several nerolidol derivatives [19]. This latter data are in agreement with recent studies [20] according to which the population occurring in Calabria is to be assigned to a diploid subspecies (A. alba subsp.chitachensis Maire). Artalbic acid, a sesquiterpene with an unusual skeleton, was isolated from the aerial parts of A. alba collected in Sicily [21], corresponding to a tetraploid population of this species [22].

The use of essential oils as chemotaxonomic markers could be useful for the classification of Artemisia species and to characterize the biodiversity of the different populations. The GC-MS analysis of essential oils of 14 Artemisia species collected in the North West Italian Alps has allowed us to draw some interesting considerations on the classification of the genus Artemisia. In particular, A. alba is characterized by a high content of camphor like A. vallesiaca, A. glacias and A. vulgaris collected in the same region [23]. Camphor and isopinocamphone were particularly high in A. alba. The same chemical components were found in some Belgian populations of A. alba [24]. The content of monoterpenic aldehydes is high too and cuminaldehyde is the second most important component in the oil [23]. An interesting paper compared the essential oil compositions of two populations of A. alba wild growing on calcareous and serpentine substrates and pointed out the fact that the type of soil could have an important influence on the biosynthesis of A. alba volatiles, especially in the case of populations grown on serpentine rock, characterized by deficiency of water and indispensable mineral elements. The camphor content is high in A. alba from a calcareus habitat, whereas germacrene D is the major component in serpentinophyte A. alba [25].
2. Results and Discussion

Hydrodistillation of the aerial parts of *A. alba* Turra collected in Madonie (A), Marche (B), Majella (C) and Mt. Velino (D) yielded 1.5%, 0.4%, 0.16% and 0.03% (w/w) of essential oils, respectively, all characterized by a pale yellow colour. In Table 1 the compounds identified are listed according to their retention indices on a HP-5MS column, and are classified in seven classes on the basis of their chemical structures. The composition of the oils is different, both qualitatively and quantitatively. The oil obtained from *Artemisia alba* from Madonie (A) is characterized by a high concentration of sesquiterpenes that represents more than 60% of the composition of the oil, while in the oils of other populations the presence of monoterpenes and sesquiterpenes is roughly equivalent.

| Component | Ident. | A   | B   | C   | D   |
|-----------|--------|-----|-----|-----|-----|
| **Monoterpene Hydrocarbons** |        |     |     |     |     |
| Santolina triene | 1, 2 | 7.3 | 1.2 |
| α-Thujene | 1, 2 | 0.1 |
| α-Pinene | 1, 2, 3 | 1.7 | 0.3 |
| Camphene | 1, 2 | 1.2 | 0.1 |
| Sabinene | 1, 2 | 0.6 |
| β-Pinene | 1, 2, 3 | 0.5 | 2.7 | 0.3 |
| p-Cymene | 1, 2, 3 | 0.5 | 0.2 |
| Limonene | 1, 2, 3 | 1.0 |
| γ-Terpinene | 1, 2, 3 | 0.2 |
| **Total** |     | 11.3 | 5.8 | 0.9 | -   |
| **Oxygenated Monoterpenes** |        |     |     |     |     |
| Santolina alcohol | 1, 2 | 2.6 | 0.2 |
| 1,8-Cineole | 1, 2, 3 | 1.6 | 0.7 | 0.1 |
| cis-Sabinene hydrate | 1, 2 | 0.2 |
| Artemisia ketone | 1, 2 | 4.6 |
| Artemisia alcohol | 1, 2 | 6.0 |
| trans-Sabinene hydrate | 1, 2 | 1.1 | 0.3 | t |
| Linalool | 1, 2, 3 | 0.6 | 0.1 |
| Hotrienol |     | 3.3 |
| β-Thujone | 1, 2 | 0.7 |
| trans-p-Menth-2-en-1-ol | 1, 2 | 0.3 |
| Chrysanthene | 1, 2 | 1.1 | 3.1 |
| α-Campholenal | 1, 2 | 0.2 |
| trans-Pinocarveol | 1, 2 | 0.1 |
| Camphor | 1, 2, 3 | 1.6 | 3.4 | 0.7 |
| Neolyratol | 1, 2 | 0.3 |
Table 1. Cont.

| $K_i^a$ | $K_i^b$ | Component          | Ident. | A  | B  | C  | D  |
|---------|---------|--------------------|--------|----|----|----|----|
|         |         | **Oxygenated Monoterpenes** |        |    |    |    |    |
| 1149    | 1685    | trans-Verbenol     | 1, 2   | 0.2| 1.8|    |    |
| 1164    | 1684    | trans-Chrysanthenol| 1, 2   |    | 0.4|    |    |
| 1165    | 1587    | Pinocarvone        | 1, 2   |    | 1.6|    |    |
| 1167    | 1719    | Borneol            | 1, 2, 3| 2.1| 9.3| 0.7|    |
| 1174    | 1565    | cis-Pinocamphone   | 1, 2   |    | 14.9| 1.1|    |
| 1176    | 1611    | Terpinen-4-ol      | 1, 2, 3| 1.5| 0.6| 1.2|    |
| 1183    | 1757    | cis-Piperitol      | 1, 2   |    |    | 0.2|    |
| 1185    | 1856    | p-Cymen-8-ol       | 1, 2   |    | 0.3| 0.1|    |
| 1189    | 1706    | $\alpha$-Terpineol | 1, 2, 3| 0.5| 1.2| 0.7|    |
| 1193    | 1648    | Myrtenal           | 1, 2   |    | 0.7| 0.2|    |
| 1197    | 1805    | Myrtenol           | 1, 2   | 0.6| 1.2| 1.4| 0.5 |
| 1201    | 1618    | Safranal           | 1, 2   |    |    | 0.1|    |
| 1217    | 1845    | trans-Carveol      | 1, 2   | 0.5| 1.6| 0.5|    |
| 1226    | 1878    | cis-Carveol        | 1, 2   | 0.7| 0.3| 0.4|    |
| 1238    | 1694    | Neral              | 1, 2   |    |    | 0.4|    |
| 1241    | 1752    | Carvone            | 1, 2   |    | 0.2| 0.4|    |
| 1268    | 1741    | Geraniol           | 1, 2   |    |    | 0.3|    |
| 1293    | 2198    | Thymol             | 1, 2, 3|    |    | 0.4|    |
| 1299    | 2239    | Carvacrol          | 1, 2, 3|    |    | 0.1|    |
| 1343    | 1748    | Piperitone         | 1, 2   | 2.2| 12.6| 32.8|    |
|         |         | **Total**          |        | 12.8| 37.5| 37.7| 41.6|
|         |         | **Sesquiterpene Hydrocarbons** |        |    |    |    |    |
| 1352    | 1466    | $\alpha$-Cubebene  | 1, 2   |    | 0.2|    |    |
| 1377    | 1497    | $\alpha$-Copaene   | 1, 2   |    | 0.3|    |    |
| 1385    | 1535    | $\beta$-Bourbonene | 1, 2   |    | 0.3| 0.1|    |
| 1387    | 1594    | $\beta$-Elemene    | 1, 2   |    | 1.0| 0.4| 0.4 |
| 1415    | 1612    | $\beta$-Caryophyllene | 1, 2, 3| 0.9| 0.3| 0.6|    |
| 1437    | 1530    | $\alpha$-Guaiene   | 1, 2   |    |    | 0.2|    |
| 1453    | 1673    | (E)-$\beta$-Farnesene | 1, 2   | 1.2|    |    |    |
| 1455    | 1689    | $\alpha$-Humulene  | 1, 2   | 1.3| 0.2| t  |    |
| 1463    | 1667    | allo-Aromadendrene | 1, 2   | 0.9|    | 0.5|    |
| 1474    | 1682    | $\gamma$-Gurjunene | 1, 2   | 6.4| 1.0|    |    |
| 1477    | 1726    | Germacrene D       | 1, 2   | 2.1| 4.9| 10.2|    |
| 1478    | 1704    | $\gamma$-Muurolene | 1, 2   |    | 0.6|    |    |
| 1482    | 1741    | $\beta$-Eudesmene ($\beta$-Selinene) | 1, 2   |    | 0.3|    |    |
| 1486    | 1733    | $\alpha$-Selinen | 1, 2   | 7.6| 0.5|    |    |
| 1487    | 1679    | $\alpha$-Amorphene | 1, 2   |    |    | 0.4|    |
| 1489    | 1729    | (Z,E)-$\alpha$-Farnesene | 1, 2   | 2.7|    |    |    |
| 1490    | 1694    | $\beta$-Guaiene    | 1, 2   | 0.2| 0.3|    |    |
| 1491    | 1756    | Bicyclogermacrene  | 1, 2   |    | 2.5|    |    |
| 1506    | 1760    | (E,E)-$\alpha$-Farnesene | 1, 2   | 1.5|    |    |    |
| 1509    | 1746    | cis-(Z)-$\alpha$-Bisabolene | 1, 2   | 0.8| 2.7|    |    |
| 1510    | 1743    | $\beta$-Bisabolene | 1, 2   |    |    | 0.6|    |
Table 1. Cont.

| \( K_1 \) a | \( K_1 \) b | Component | Ident. | A  | B  | C  | D  |
|------------|------------|-----------|--------|----|----|----|----|
| 1515       | 1776       | \( \gamma \)-Cadinene | 1, 2   | 0.5|    |    |    |
| 1520       | 1839       | 1-S-\( cis \)-Calamene | 1, 2   | 0.1|    |    |    |
| 1526       | 1773       | \( \delta \)-Cadinene | 1, 2   | 1.5| 0.4| 0.3| 0.4|
| 1554       | 1856       | Germacrene B | 1, 2   | 0.3|    |    |    |
|            |            | **Total**   |        | 15.7| 21.0| 7.8| 13.1|

**Sesquiterpene Hydrocarbons**

|          |          |          |
|----------|----------|----------|
|          |          | Oxygenated Sesquiterpenes |
|          |          | 1234     | 1641   | \( nor \)-Davanone | 1, 2 | 0.1 |
|          |          | 1457     | 1712   | Cabreuva oxide B | 1, 2 | 0.9 |
|          |          | 1476     |         | Davana ether | 1, 2 | 0.3 |
|          |          | 1534     | 1991   | Artedo Douglasia oxide A | 1, 2 | 0.9 |
|          |          | 1559     | 1967   | Davanone B | 1, 2 | 0.8 |
|          |          | 1563     | 2065   | Artedo Douglasia oxide D | 1, 2 | 0.6 |
|          |          | 1564     | 2050   | (E)-NEROLDOL | 1, 2 | 0.6 | 6.4 |
|          |          | 1564     | 2056   | Ledol | 1, 2 | 0.2 |
|          |          | 1578     | 2150   | Spathulenol | 1, 2, 3 | 1.6 | 4.2 | 0.4 | 2.1 |
|          |          | 1580     | 2008   | Caryophyllene oxide | 1, 2, 3 | 1.8 | 1.1 | 2.0 |
|          |          | 1587     | 2108   | Dihydroneolidol | 1, 2 |    |    |    | 2.9 |
|          |          | 1588     | 2025   | Davanone D | 1, 2 | 10.5 |
|          |          | 1591     | 2104   | Viridiflorol | 1, 2 | 0.4 |
|          |          | 1598     | 2107   | Guaiol | 1, 2 | 2.8 |
|          |          | 1638     | 2223   | Isopathulenol | 1, 2 | 0.7 | 0.1 |
|          |          | 1640     | 2185   | T-Cadinol | 1, 2 | 2.8 |
|          |          | 1641     | 2209   | T-Muurolo | 1, 2 | 0.9 |
|          |          | 1648     | 2399   | Aromadendrene oxide | 1, 2 |    |    | 1.0 |
|          |          | 1653     | 2252   | \( \alpha \)-Eudesmol | 1, 2 |    |    | 42.2 |
|          |          | 1655     |         | \( a \) C15H22O | 1, 2 | 1.1 |
|          |          | 1657     | 2217   | \( \alpha \)-Bisabolone oxide A | 1, 2 | 16.4 |
|          |          | 1658     | 2156   | \( \alpha \)-Bisabolol oxide B | 1, 2 | 2.2 |
|          |          | 1675     | 2213   | \( Z \)-\( \alpha \)-Bisabole epoxide | 1, 2 | 0.5 |
|          |          | 1682     | 2246   | Bisabolone oxide | 1, 2 | 9.0 |
|          |          | 1682     | 2232   | \( \alpha \)-Bisabolol | 1, 2 | 0.8 | 1.7 | 4.5 |
|          |          | 1687     | 1896   | \textit{allo}-Aromadendrene oxide | 1, 2 | 0.4 | 0.2 | 0.8 |
|          |          | 1689     | 2359   | 8-Cedren-13-ol | 1, 2 | 10.3 |
|          |          | 1692     | 2342   | (2Z,6E)-Farnesol | 1, 2 | 1.9 |
|          |          | 1692     | 2245   | \( \textit{epi-}\alpha \)-Bisabolol | 1, 2 | 0.8 | 4.7 |
|          |          | 1738     | 2162   | \( \alpha \)-Bisabolol oxide A | 1, 2 | 1.4 |
|          |          | 1765     | 2518   | \textit{cis}-Lanceol | 1, 2 | 0.4 | 0.2 |
|          |          | **Total** |        | 47.0| 23.2| 44.5| 30.9|

**Others**

|          |          |          |
|----------|----------|----------|
|          |          |          |
| 977      | 1452     | 1-Octen-3-ol | 1, 2 | 0.2 |
| 1123     | 1570     | Isophorone | 1, 2 |    | t   |
| 1206     | 1510     | Decanal | 1, 2 | 0.2 |
| 1397     | 1959     | \textit{cis}-Jasmarone | 1, 2 | 0.1 |
Table 1. Cont.

| Ki a | Ki b | Component Ident. | A  | B  | C  | D  |
|------|------|------------------|----|----|----|----|
|      |      | Others           | 1,2| 0.6| 0.3|    |
| 1405 | 2031 | Methyleugenol    |    |    |    |    |
|      |      | Total            | 0.7| 0.5| 0.2|    |
|      |      | Esters           |    |    |    |    |
| 1235 | 1583 | trans-Chrysanthenyl acetate | 1,2 | 1.4| 2.1| 1.6|
| 1241 |      | Linalyl formate  | 1,2 |    | 0.1|    |
| 1264 | 1561 | cis-Chrysanthenyl acetate | 1,2 | 0.6| t  | 1.5|
| 1286 | 1567 | Bornyl acetate   | 1,2,3| 0.5|    | 0.2|
| 1325 | 1678 | Myrtenyl acetate | 1,2 |    |    | 0.3|
| 1362 | 1729 | Neryl acetate    | 1,2 |    |    | 0.5|
| 1818 | 1716 | (2Z,6E)-Farnesyl acetate | 1,2 |    | t  |    |
|      |      | Total            | 2.5| 2.2| 0.5| 3.6|
|      |      | Oxygenated diterpenes |    |    |    |    |
| 2135 | 2625 | (E)-Phytol       | 1,2 |    |    | 1.1|
|      |      | Total compounds  | 47 | 57 | 41 | 38 |
|      |      | TOTAL            | 90.0| 90.2| 91.6| 90.3 |

a: Ki = Kovats index; HP-5 MS column; b: Ki = Kovats index; HP Innowax column; 1: retention index, 2: mass spectrum, 3: co-injection with authentic; compound t: traces, less than 0.05%.

All oil extracts from the populations of Marche, Majella and Monte Velino have a content of monoterpenes (43.3%, 38.6% and 41.6%, respectively), which is about twice as high compared with the same class of compounds identified in the oil from Madonie (24.1%).

Among the monoterpenic hydrocarbons in the oil from Madonie, santolinatriene (7.3%), an irregular monoterpane, predominates and it is also present in low concentrations in B, but absent in C and D. On the other hand in the oil from Marche irregular oxygenated monoterpenes are found in higher concentrations. In fact, santolina alcohol, artemisia alcohol, artemisia ketone and chrysanthenone represent about one third (13.9%) of the fraction while in the oil from Madonie santolina alcohol, despite being the most abundant oxygenated monoterpane, accounts for only 2.6%, the remaining (10.2%) of this fraction being constituted by regular oxygenated monoterpenes. The most abundant oxygenated monoterpenes of oil from Marche are borneol (9.3%), artemisia alcohol (6.0%) and artemisia ketone (4.6%); the last two being absent in A, C and D. In the oils from Abruzzo (Majella, C and Monte Velino, D) monoterpenic ketones (cis-pinocamphone, piperitone) are prevalent instead and they account for more than half of the content of monoterpenes.

Concerning the content of oxygenated sesquiterpenes, although the total percentages are similar in the four populations, the proportion of the various types of compounds changes drastically. In fact in A ketones (11.5%) and oxides (31.4%) are prevalent with davanone D (10.5%) and α-bisabolone oxide A (16.4%) as main compounds, while alcohols represent only 4.3%. On the other hand in B, C and D, the content of sesquiterpene alcohols is very high (19.9%, 42.2% and 26.2%, respectively). The main compounds among the sesquiterpene alcohols are: 8-cedren-13-ol (10.3%) in the oil from Marche, α-eudesmol (42.2%) in the oil from Majella and epi-α-bisabolol (4.7%), α-bisabolol (4.5%) and (E)-nerolidol (6.4%) in the oil from Monte Velino.
According to the literature [17] α-thujone and camphor are two markers allowing a distinction of *Artemisia* in two groups. Our four oils are characterized by the absence of α-thujone, whereas camphor and its biogenetic precursor, borneol are present in A, B and C.

3. Experimental

3.1. Plant Material

The aerial parts of the four populations of *Artemisia alba* Turra, were collected from blooming plants in Sicily, pastures on carbonate soils at Pizzo Carbonara (Madonie), in spring of 2011 (A); Marche, pastures on carbonate soils between Fabriano (Ancona) and Matelica (Macerata), in spring of 2011, Abruzzo: pastures on carbonate soils at Mt Majella (C) and Mt Velino (D), in summer of 2011 (Figure 1). Samples of the studied material, identified by the authors F. M. Raimondo and V. Spadaro, are kept in the Herbarium Mediterraneum of the Palermo University [Raimondo & Spadaro (PAL)].

**Figure 1.** Map of the samples’ origins: Madonie (A), Marche (B), Majella (C) and Mt. Velino (D) are indicated.

3.2. Isolation of the Essential Oil

The air-dried samples were ground in a Waring blender and then subjected to hydrodistillation for 3 h using *n*-hexane as solvent, according to the standard procedure of the *European Pharmacopoeia* [26]. The extracts were dried over anhydrous sodium sulphate and then stored in sealed vials, at −20 °C, ready for the GC and GC-MS analyses. The samples yielded 1.5% (A), 0.40% (B), 0.16% (C) and 0.03% (D) (w/w) of pleasant smelling yellow oils.
3.3. Gas Chromatography-Mass Spectrometry

Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph (Napoli, Italy) equipped with a HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness), a split-splitless injector heated at 250 °C and a flame ionization detector (FID) at 280 °C. Column temperature was initially kept at 40 °C for 5 min, then gradually increased to 250 °C at 2 °C/min, held for 15 min and finally raised to 270 °C at 10 °C/min. The injection volume was 1.0 μL (split ratio 1:20). A fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm, 0.25 μm film thickness) was also used for analysis. In both cases helium was the carrier gas (1 mL/min). GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus (Napoli, Italy), fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm, 0.33 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; source temperature 250 °C. Mass spectra were scanned in the range 35–450 amu, scan time 5 scans/s. Gas chromatographic conditions were the same as those for GC; transfer line temperature, 295 °C.

3.4. Identification of Components

Most of the constituents were identified by GC by comparison of their retention indices (K_r) with either those in the literature [27,28] or with those of authentic compounds available in our laboratories. Retention indices were determined in relation to a homologous series of n-alkanes (C_8–C_28) under the same conditions. Whenever possible, co-injection with authentic substances was also performed. Component-related concentrations were calculated based on GC peak areas without using correction factors. Further identification of oil components was achieved by comparing their mass spectra on both columns, either with those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature [28,29] and our personal library.

4. Conclusions

The differences in composition between the four oils makes it possible to hypothesize that the Italian populations of Artemisia alba Turra growing on the Madonie (Sicily), in the Marche region, on the Majella and Monte Velino (Abruzzo)—in part related to different cytotypes [19]—surely express from climatic as well as genetic differences. Furthermore, the differences of the oil of the population of the Artemisia alba Turra from Madonie—the most southerly of the species—let us consider that this belongs to a different chemotype from the other ones.

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Sample Availability: Samples of the oils are available from the authors.

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