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GC MS Analysis of the Volatile Constituents of Essential Oil and Aromatic Waters of *Artemisia annua* L. at Different Developmental Stages

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Artemisia annua L. (Asteraceae) still represents the only source of artemisinin, considered as one of the most important drugs for the treatment of malaria and which, more recently, has been shown to be effective against numerous types of tumors. The foliage and inflorescence of *A. annua* also yield an essential oil upon hydrodistillation. This oil has been evaluated at different development stages (pre-flowering and flowering) by GC/MS. The volatile oil from plants at full blooming showed numerous constituents, with germacrene D (21.2%), camphor (17.6%), β-farnesene (10.2%), β-caryophyllene (9%), and bicyclogermacrene (4.2%) among the main ones. Aromatic waters, after extraction with *n*-hexane, showed the presence, among others, of camphor (27.7%), 1,8-cineole (14%), artemisia ketone (10.1%), α-terpineol (6.1%), *trans*-pinocarveol (5.4%), and artemisia alcohol (2%). From plants at the pre-flowering stage, aromatic waters were obtained with camphor (30.7%), 1,8-cineole (12.8%), artemisia alcohol (11.4%), artemisia ketone (9.5%), alpha-terpineol (5.8%), and *trans*-pinocarveol (3.0%) as the main constituents. The qualitative and quantitative profiles of the two aromatic waters were similar. These results permitted the conclusion to be made that *A. annua* could be harvested a long time before the onset of flowering to obtain higher yields of artemisinin or could be allowed to attain maturity to obtain valuable yields of volatiles.

Keywords: *Artemisia annua*, Asteraceae, volatile constituents, essential oil, aromatic waters, pre-flowering and blossom time, GC-MS analysis.

Artemisia annua L.(family Asteraceae), indigenous to south east Asia, is an annual herb/shrub, which has become naturalized or is cultivated as a horticultural or medicinal plant in many parts of Asia, Africa, Europe, America and Australia. It has been used in Traditional Chinese Medicine for many centuries for the treatment of fever and malaria due to the presence in the leaves and capitula of a unique sesquiterpene endoperoxide called artemisinin. Nowadays, *A. annua* still represents the only source of artemisinin [1,2], considered to be one of the most important drugs for the treatment of malaria and, more recently, it has been shown to be effective also against numerous types of tumors, including breast cancer, human leukemia, colon, and small-cell lung carcinomas [3]. It is well known that natural populations and genetic resources of *A. annua* from different areas have considerable variability in the accumulation of artemisinin in the leaves and the capitula of the plant, with an artemisinin content ranging from 0.001 to 0.8% [1].

The foliage and inflorescence of *A. annua* plants also yield an essential oil upon hydrodistillation, which could represent another potential commercially valuable product [4,5]. In this current study the volatile constituents of both the essential oil and aromatic waters obtained by hydrodistillation of

Table 1: Identified constituents of the investigated samples.

Constituents	l.r.i. (A)	l.r.i. (B)	*n*-Hexane extract of aromatic waters. Plants at pre-flowering	*n*-Hexane extract of aromatic waters. Plants at full blooming	Volatile oil -full blooming
(E)-3-Hexen-1-ol	853	1373	0.2	0.1	tr
α-Pinene	941	1027	tr	0.4	0.7
Camphene	956	1071	0.1	0.2	1.5
Sabinene	978	1116	tr	0.2	0.2
β-Pinene	982	1108	0.1	0.1	0.5
Myrcene	992	1145	nd	0.5	0.1
2,3-Dehydro-1,8-cineole	994	-	0.1	nd	tr
Yomogi alcohol	998	1401	2.5	1.2	nd
(E)-3-Hexen-1-yl acetate	1008	-	nd	nd	0.1
α-Terpinene	1020	1183	nd	nd	0.1
p-Cymene	1029	1245	tr	0.1	1.7
Santolina alcohol	1033	1413	0.4	0.2	nd
1,8-Cineole	1035	1208	12.8	14.0	1.4
γ-Terpinene	1064	1249	nd	nd	0.3
Artemisia ketone	1065	1355	9.5	10.1	nd
cis-Sabinene hydrate	1071	1462	0.7	0.2	0.2
Artemisia alcohol	1084	1504	11.4	2.0	nd
Linalool	1101	1557	1.1	3.9	0.2
trans-Sabinene hydrate	1103	1458	1.0	0.6	0.2
Dehydrosabinaketone	1123	-	0.8	0.9	nd
α-Campholenal	1127	-	tr	tr	tr
cis-p-Menth-2-en-1-ol	1129	-	0.2	0.2	nd
trans-Pinocarveol	1141	1641	3.0	5.4	nd
trans-p-Menth-2-en-1-ol	1142	1560	nd	nd	1.3
Camphor	1148	1522	30.7	27.7	17.6
Camphene hydrate	1153	-	nd	nd	0.1
β-Pinene oxide	1159	-	0.7	2.5	nd
Sabinaketone	1161	1611	0.2	0.4	nd
Pinocarvone	1166	1549	3.2	3.8	0.3
δ-Terpineol	1169	1676	0.9	1.0	nd
Borneol	1171	1796	3.9	1.8	0.9
Terpinen-4-ol	1180	1607	2.4	3.4	0.6
p-Cymen-8-ol	1186	1835	0.2	0.4	nd
α-Terpineol	1192	1688	5.8	6.1	0.5
Myrtenol	1197	1791	0.1	0.2	nd
Verbenone	1207	1715	0.2	tr	nd
(E,E)-2,4-Nonadienal	1220	1705	0.8	0.6	nd
trans-Carveol	1222	1874	0.8	0.5	0.3
Thymyl methyl oxide	1233	1593	0.1	0.7	nd
cis-Carveol	1234	1845	0.2	0.3	tr
Carvone	1245	1742	0.3	0.3	nd
cis-Chrysanthenyl acetate	1263	-	nd	nd	0.4
Lavandulyl acetate	1286	1596	nd	nd	0.9
Isobornyl acetate	1286	1582	0.7	0.2	nd
Thymol	1293	2186	nd	nd	1.5
trans-Carvyl acetate	1339	1756	nd	nd	1.0
Eugenol	1358	2173	1.1	1.1	tr
cis-Carvyl acetate	1363	1793	nd	nd	0.4
α-Copaene	1377	1475	nd	0.2	0.9
β-cubebene	1389	1545	nd	nd	0.7
Benzyl valerate	1391	-	0.3	0.3	nd
(E)-β-Caryophyllene	1419	1604	tr	0.6	9.0
(E)-β-Farnesene	1456	1660	nd	0.3	10.2
α-Humulene	1457	1666	tr	tr	0.1
β-Chamigrene	1476	1757	tr	0.1	1.4
Germacrene D	1483	1709	tr	0.4	21.2
β-Selinene	1489	1713	tr	0.3	0.8
Bicyclogermacrene	1496	1736	nd	nd	4.2
δ-Cadinene	1524	1731	nd	nd	0.5
trans-Nerolidol	1563	1996	nd	nd	0.4
Spathulenol	1578	2136	0.5	0.7	1.3
Caryophyllene oxide	1583	2070	0.3	0.4	0.6
Globulol	1586	2054	nd	nd	0.2
epi-Cedrol	1599	2139	nd	nd	0.5
β-Acorenol	1640	-	0.2	nd	nd
Cubenol	1643	2045	nd	nd	2.3
T-Muurolol	1645	2150	nd	nd	0.7
Kongol	1662	2236	nd	0.1	0.6
α-Cadinol	1655	2187	nd	nd	0.5
Elemol acetate	1663	-	nd	nd	0.5
(*Z*)-α-Santalol	1680	2310	nd	nd	1.3
Total identified			97.5	95.0	91.2

Table 1 (*Contd.*)

(A): linear retention index (l.r.t) obtained with a phenylmethylsilicone column (apolar)
(B): linear retention index (l.r.t) obtained with a PEG column (polar)

A annua collected at different developmental stages (pre-flowering and full blooming) have been evaluated by GC/MS. The aim of this study was the evaluation of the essential oil and aromatic waters as ingredients of food, pharmaceutical and cosmetic products, depending on the composition of the volatile constituents.

Fresh, wild plant materials were collected near Sesto Fiorentino (FI, Italy) at pre-flowering and full blooming in August and September 2007 and submitted to hydrodistillation. Only samples in full bloom gave essential oil, which was analysed by GC/MS. Aromatic waters obtained from both samples were also analysed after extraction with *n*-hexane. Seventy-two compounds were identified in the different samples, accounting for 91.2%-97.5% of the total compositions.

Volatile oil from plants at full bloom showed numerous constituents, with germacrene D (21.2%), camphor (17.6%), β-farnesene (10.2%), β-caryophyllene (9%), and bicyclogermacrene (4.2%) among the main ones. Aromatic waters, after extraction with *n*-hexane, showed the presence, among others, of camphor (27.7%), 1,8-cineole (14%), artemisia ketone (10.1%), alpha-terpineol (6.1%), *trans*-pinocarveol (5.4%), and artemisia alcohol (2%). From plants at the pre-flowering stage, only aromatic waters were obtained with camphor (30.7%), 1,8-cineole (12.8%), artemisia alcohol (11.4%), artemisia ketone (9.5%), α-terpineol (5.8%), and *trans*-pinocarveol (3.0%) as their main constituents. Artemisinin was never detected in either the essential oil or aromatic waters. The qualitative and quantitative profiles of the two aromatic waters were similar.

In the literature there are several studies reporting the GC analysis of the essential oil obtained from different parts of *A. annua* of different origins [4-20], but none concerning the analysis of aromatic waters. A great variability in the qualitative and quantitative composition has been evidenced and apart from ecological factors, plant part and development stage, a main source of variability was the wild plant material or selected cultivar. The majority of the studies have been performed on plant material from India and the main compounds identified in the essential oils from the aerial parts were camphor (0-44.4%), 1,8 cineole (1.7-28.6%), artemisia ketone (0-52.9%), 2,5-dihydro-3-methylfuran (0-68.5%), camphene (0-28.4%), and germacrene D (0-10.9%) [4, 6-12].

The principal constituents detected in the essential oil of plants from Iran, depending on the flowering stage, were camphor (14.3-48.0%), germacrene D (2.0-18.5%),1,8-cineole (5.8-17.3%), α-pinene (0-13.3%), β-selinene (0-10.4%), and β-caryophyllene (0-9.4%) [13-14]. Two studies from the USA took into account the two subspecies, the "glanded" and "glandless" ones. Artemisia ketone was found as the main component (0-35.6% in leaves, up to 56% in flowers). Other important volatiles were germacrene D (0-49.8%), 1,8-cineole (0-28.1%), α-pinene (0-26.7%), camphor (0-20.5%), and pinocarvone (0-15.8%) [5,15].

A few studies with European plant material were performed in France [16], the Netherlands [17] and Hungary [18-19]. All of them reported that artemisia ketone was the main constituent (11.9-63.9%), with the exception of plants grown from Vietnamese seeds, where it was completely absent. Other main constituents were artemisia alcohol (11.0-56.0%) and camphor (21.8%) in Hungarian plant material, germacrene D (2.0-18.5%) in plants collected in France and the Netherlands, and 1,8-cineole (5.1-14.7%) in plants from France. Another study on the essential oil of the fruits reported that sesquiterpenes were the most abundant chemicals, i.e. caryophyllene oxide (9.0%), caryophyllene (6.9%), *(E)*- β-farnesene (8.2%) and germacrene D (4.0%). However, this oil was only partially characterized, with only 52% of the components being identified [20].

Constituents found in our sample of essential oil were very similar to those reported in the literature, namely camphor, germacrene D, and artemisia ketone. In the aromatic waters 1,8 cineole was also found and can represent a good source of volatiles, to be used for different applications, including cosmetic, alimentary and pharmaceutical ones.

Furthermore, the results obtained permitted us to conclude that *A. annua* could be harvested either a long time before onset of flowering to obtain higher yields of artemisinin or the crop could be allowed to attain maturity to obtain valuable yields of the essential oil.

Experimental

Plant material and samples: *Artemisia annua* subspecies "glanded" was identified by Dr Lia Pignotti of the Department of Vegetal Biology, University of Florence, where an authentic specimen is also deposited. About 1 kg fresh, wild plant material was collected near Sesto Fiorentino and immediately submitted to hydrodistillation. Only samples in full bloom gave essential oil (0.5%), which was analysed by GC/MS. Aromatic waters obtained from both samples were also analysed after extraction with *n*-hexane.

GC-EIMS analysis: GC-EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 mm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures at 220 and 240°C respectively; oven temperature was programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection of 0.2 mL (10% *n*-hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their Linear Retention Indices relative to the series of *n*-hydrocarbons, and by computer matching against commercial [21] and home-made library mass spectra built up from pure substances and components of known essential oils and MS literature data [21-26]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

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